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. The GenomeSeqDx test is different from other types of genetic diagnostic tests in terms of the number of genes that are sequenced simultaneously and the inclusion of non-coding regions of the genome. Genome sequencing 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 GenomeSeqDx test targets both the protein-coding and non-coding regions of the human 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 (Bamshad et al., 2011). 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 allowing for the detection of up to ~3% of coding variants that may be missed by ES (Lelieveld et al., 2015; Belkadi et al., 2015). GS has lower depth of coverage on average compared to ES, but more positions are covered to adequate depth for accurate variant calling than ES.

Using genomic DNA from an individual's submitted specimen, the genome is 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. Phenotype-driven gene lists are generated using Human Phenotype Ontology and HGMD gene-phenotype associations, and 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).

Result Reporting:
GenomeSeqDx analysis is performed on the proband and parental samples, and/or additional relatives as needed, when submitted together for analysis. A single report will be issued for the affected individual 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 will apply.

The GenomeSeqDx report issued for the affected individual in the family will contain variations in genes previously implicated in a human disease similar to that 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 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, 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 genome 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 GenomeSeqDx reports, unless a family opts-out of receiving this information on the Informed Consent and Authorization Form as part of the GenomeSeqDx Test Requisition Form. The status for any secondary finding(s) reported for the affected individual will be provided for all relatives tested by GenomeSeqDx; 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 genome will be sequenced simultaneously by massively parallel (NextGen) sequencing on an Illumina sequencing system with 100 bp or greater paired-end reads. Bi-directional sequence will be assembled, aligned to reference gene sequences based on human genome build GRCh37/UCSC hg19, and analyzed for sequence variants using a custom-developed analysis

tool. Capillary sequencing or another appropriate method will be used to confirm all potentially pathogenic variants identified in the individual and relative samples. Sequence and copy number alterations will be reported according to the Human Genome Variation Society (HGVS) nomenclature guidelines and International System for Human Cytogenetic Nomenclature (ISCN) guidelines, respectively.

Analysis of GenomeSeqDx for the proband includes evaluation of variants that are identified to be de novo, compound heterozygous, 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.

Limitations:

The GenomeSeqDx test attempts to evaluate the complete coding and non-coding regions of the genome which includes ~20,000 genes. 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. It is anticipated that approximately 99% of the coding region of an affected individual's genome (i.e. the exome) will be assessed with the GenomeSeqDx test at 10x coverage, while >98% of the entire genome will be covered at a minimum of 1x.

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 data to incorporate updated clinical information and/or newly emerging gene and variant information is available upon request by the health care provider for a fee. Even if the GenomeSeqDx 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. Lelieveld et al. (2015) Hum. Mutat. 36 (8):815-22 (PMID: 25973577)
3. Belkadi et al. (2015) Proc. Natl. Acad. Sci. U.S.A. 112 (17):5473-8 (PMID: 25827230)
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)