CFTR Sequencing and Deletion/Duplication Analysis

Disorder Also Known As: Cystic Fibrosis, *CFTR*-Related Disorders, *CFTR*-Related Metabolic Syndrome

Clinical Features:
Cystic fibrosis (CF) is a progressive multi-system genetic disease involving the respiratory tract, pancreas, gastrointestinal tract, hepatobiliary system, sweat glands, and reproductive system. The clinical presentation of CF may range in severity. Respiratory manifestations include persistent lung infections, bronchiectasis, extensive lung damage, recurrent sinusitis, and nasal polyposis.[1,2] Pancreatic issues include pancreatic insufficiency, fat malabsorption, steatorrhea, acute or chronic pancreatitis, and CF-related diabetes. These symptoms stem from thickening of secretions within the pancreatic ducts, prevention of normal release of digestive enzymes, and loss of islet cells.[1] Meconium ileus is present in 15-20% of newborns with CF.[1] Most men with CF have absent or altered vas deferens resulting in infertility, though sperm production is often normal.[1]

Today, most individuals with CF are identified in infancy by newborn screening (NBS) programs. NBS for the identification of CF involves measurement of immunoreactive trypsinogen (IRT). If IRT is elevated, screening proceeds to diagnosis by sweat chloride testing and *CFTR* genetic testing.[3] Although screening or clinical presentation typically leads to the diagnosis of cystic fibrosis during infancy or childhood, some individuals with CF do not present with symptoms until adulthood. [4]

CFTR-related metabolic syndrome (CRMS) is characterized by a positive NBS test in an individual without clinical features of CF.[3,5] CRMS represents those individuals identified by NBS for whom the question of a CF diagnosis is not resolved due to ambiguity in repeat sweat tests and/or genetic testing results. Infants with CRMS are monitored for symptoms as approximately 10-20% of asymptomatic infants can develop clinical features concerning for CF.[5,6]

CFTR-related disorders (*CFTR*-RD) present in childhood or adulthood and often have complex genotype and phenotype relationships. The clinical features of *CFTR*-RD overlap with CF, but are typically isolated. *CFTR*-RD include congenital absence of the vas deferens (CAVD), chronic or recurrent acute pancreatitis, and disseminated bronchiectasis.[7]

CF occurs in all ethnicities, but the disease is most common in individuals of Northern European and Ashkenazi Jewish ancestry, in whom the carrier frequency is 1 in 28 and 1 in 29, respectively.[8,9] The incidence of CF in the highest risk populations is approximately 1 in 2000-4000 live births.[1]

Inheritance Pattern:
CF and CAVD are autosomal recessive disorders. *CFTR*-RD have been reported in individuals with 1 or 2 identified pathogenic CFTR variants.[7] Penetrance for CF is essentially 100% when two CF-causing *CFTR* variants are *in trans*, though there can be considerable variable expressivity.[10]

Test Methods:
Genomic DNA is extracted from the submitted specimen. For skin punch biopsies, fibroblasts are cultured and used for DNA extraction. This DNA is enriched for the complete coding regions and splice site junctions of CFTR, including the poly-T and poly-TG tracts in intron 9, using a proprietary targeted capture system developed by GeneDx for next generation sequencing with CNV calling (NGS-CNV). 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. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications involving coding exons. Alternative sequencing or copy number detection methods are used to analyze regions with inadequate sequence or copy number data by next generation sequencing (NGS). Reported clinically significant variants are confirmed by an appropriate method. 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 but are available upon request.

Test Sensitivity:
The clinical sensitivity of *CFTR* sequencing and deletion/duplication analysis depends in part on the patient's clinical phenotype and family history. In general, the sensitivity is highest for individuals with features suggestive of cystic fibrosis, *CFTR*-related disorders, and *CFTR*-related metabolic syndrome as outlined above. Sequencing and deletion/duplication analysis identify 97-98% and ≤2-3%, respectively, of pathogenic variants in an individual with cystic fibrosis.[2] Approximately 20% of individuals with pancreatitis have at least one identified *CFTR* variant, including CF-causing and bicarbonate deficient (non-CF-causing) variants.[11] In a meta-analysis, 78% of men with CAVD had at least one identified *CFTR* variant.[12] The overall test sensitivity for CRMS or other *CFTR*-RD is unknown.

DNA sequencing will detect nucleotide substitutions and small insertions and deletions, while NGS-CNV analysis, array CGH, or MLPA will detect exon-level deletions and duplications. These methods are expected to be greater than 99% sensitive in detecting pathogenic variants identifiable by sequencing or CNV technology.

Genetic testing using the methods applied at GeneDx is expected to be highly accurate. Normal findings do not rule out the diagnosis of a genetic disorder since some genetic abnormalities may be undetectable by this test. The methods used cannot reliably detect deletions of 20bp to 250bp in size, or insertions of 10bp to 250 bp in size. Sequencing cannot detect low-level mosaicism. The copy number assessment methods used with this test cannot reliably detect mosaicism and cannot identify balanced chromosome aberrations. Rarely, incidental findings of large chromosomal rearrangements outside the gene of interest may be identified. Regions of certain genes have inherent sequence properties (for example: repeat, homology, or pseudogene regions, high GC content, rare polymorphisms) that yield suboptimal data, potentially impairing accuracy of the results. False negatives may also occur in the setting of bone marrow transplantation, recent blood transfusion, or suboptimal DNA quality. In individuals with active or chronic hematologic neoplasms or conditions, there is a possibility that testing may detect an acquired somatic variant, resulting in a false positive result. As the ability to detect genetic variants and naming conventions can differ among laboratories, rare false negative results may occur when no positive control is provided for testing of a specific variant identified at another laboratory. The chance of a false positive or false negative result due to laboratory errors incurred during any phase of testing cannot be completely excluded. Interpretations are made with the assumption that any clinical information provided, including family relationships, are accurate. Consultation with a genetics professional is recommended for interpretation of results.

References:

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