Combined Lysosomal and Peroxisomal Disorders Panel

Panel Gene List: *ABCD1, ABHD5, ACOX1, ADAMTSL2, AGA, AGPS, AMACR, ARSA, ARSB, ASAH1, ATP6AP1, CLN3, CLN5, CLN6, CLN8, CTNS, CTSA, CTSD, CTSF, DNAJC5, DNM1L, FAR1, FUCA1, GAA, GALC, GALNS, GBA, GLA, GLB1, GNE, GNPAT, GNPTAB, GNPTG, GNS, GPC3, GUSB, HEXA, HEXB, HGSNAT, HSD17B4, HYAL1, IDS, IDUA, LAMP2, LIPA, LYST, MAN2B1, MANBA, MCOLN1, MFSD8, NAGA, NAGLU, NEU1, NPC1, NPC2, PEX1, PEX10, PEX11B, PEX12, PEX13, PEX14, PEX16, PEX19, PEX2, PEX26, PEX3, PEX5, PEX6, PEX7, PHYH, PNPLA2, PPT1, PSAP, SCARB2, SCP2, SGSH, SLC17A5, SMPD1, SUMF1, TPP1, TRIM37, VPS33A*

Lysosomal and Peroxisomal Disorders Overview:
Lysosomes and peroxisomes are organelles involved in the catabolism of various biomolecules within the cell. Lysosomal disorders are a clinically and genetically heterogeneous group of disorders caused by deficiency of lysosomal enzymes, or non-enzymatic proteins involved in lysosomal biogenesis, transport, or maturation. Deficiency of these enzymes or proteins leads to the accumulation of partially degraded products resulting in progressive cellular dysfunction and clinical abnormalities. Most lysosomal disorders can be classified by the nature of the stored material: mucopolysaccharidoses, mucolipidoses, glycoproteinoses, sphingolipidoses, oligosaccharidosis and glycogen storage diseases.[1] Peroxisomal disorders comprise two heterogeneous subgroups of conditions: peroxisomal biogenesis disorders (PBD) which are characterized by defects in peroxisome synthesis, assembly and biochemical functions, and disorders of single peroxisomal enzymes and beta-oxidation deficiencies which are involved in ether lipid biosynthesis, phytanic, pristanic, and pipecolic acid catabolism, fatty acid beta-oxidation and other functions localized to peroxisomes.

Clinical Features of Lysosomal Disorders:
Lysosomal disorders may affect a single organ system or multiple organ systems. The disease course of these conditions is generally progressive, but the age of onset, nature and severity of symptoms can be variable between the disorders. Common symptoms of lysosomal disorders include hepatosplenomegaly, coarsening of facial features, short stature, corneal clouding, dysostosis multiplex, and neurological regression.[2]

Clinical Features of Peroxisomal Biogenesis Disorders:
Peroxisomal biogenesis disorders consist of i) Zellweger spectrum disorders, including Zellweger syndrome, neonatal adrenoleukodystrophy, and infantile Refsum syndrome, and ii) rhizomelic chondrodysplasia punctata. Zellweger spectrum disorder involves a range of symptoms such as hypotonia, liver dysfunction, hearing loss, retinal dystrophy, optic nerve abnormalities, seizures, leukodystrophy, chondrodysplasia punctate, and characteristic facial

features. While children with Zellweger syndrome are typically diagnosed in the neonatal period, individuals with neonatal adrenoleukodystrophy and Refusm syndrome may present with less severe symptoms and may be diagnosed later in childhood. Rhizomelic chondrodysplasia punctata is characterized by rhizomelia, epiphyseal and metaphyseal abnormalities, intellectual disability, seizures, cataracts, coronal clefts, contractures, characteristic facial features, and pulmonary hypoplasia.[4,5]

Clinical Features Peroxisomal Single Enzyme Defects:
Peroxisomal single enzyme defects include adult Refsum syndrome, X-linked adrenoleukodystrophy, rhizomelic chondrodysplasia punctata type 2 and type 3, acyl-CoA oxidase deficiency, D-bifunctional enzyme deficiency, alpha-methylacyl-CoA racemase deficiency, *DNM1L*-related leukoencephalopathy, and mulibrey nanism. The clinical features of peroxisomal enzyme defects vary depending on the specific disorder, however many patients may present with neurological deficits and symptoms similar to Zellweger spectrum disorder. A diagnosis of peroxisomal disorders is based on clinical features, biochemical studies that include analysis of very long chain fatty acids, phytanic acid, pipecolic acid, pristanic acid, plasmalogens and bile acids, and complementation analysis in fibroblasts.[6]

Utility of Molecular Testing:
The diagnosis of peroxisomal and lysosomal disorders is challenging; these disorders have overlapping clinical and biochemical features which, in the absence of complementation analysis in fibroblasts, may make it difficult to identify the underlying molecular etiology. Due to the biochemical complexity, genetic heterogeneity, and the need for a fibroblast biopsy for complementation studies, many clinicians are using Next generation sequencing panels in the diagnosis of patients. In addition, clarification of the underlying genotype may have prognostic value, and is essential for genetic counseling and subsequent family studies.

Genetics:
Most lysosomal and peroxisomal disorders are associated with autosomal recessive inheritance. The *ABCD1, ATP6AP1, GLA, GPC3, IDS, and LAMP2* genes are associated with X-linked inheritance, the *DNAJC5* gene is associated with autosomal dominant inheritance, and the *DNM1L* and *GNE* genes are associated with both autosomal dominant and autosomal recessive inheritance.

Test Methods:
Using genomic DNA extracted from the submitted specimen, the complete coding regions and splice site junctions of the genes tested are enriched 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; however, technical limitations and inherent sequence properties effectively reduce this resolution for some genes. Alternative sequencing or copy number detection methods are used to analyze or confirm regions with inadequate sequence or copy number data by NGS. Concurrently, multiplex ligation-dependent probe amplification (MLPA) was performed to evaluate for an exon-level deletion or duplication of the *ABCD1* gene. 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.

The technical sensitivity of sequencing is estimated to be >99% at detecting single nucleotide events. It will not reliably detect deletions greater than 20 base pairs, insertions or rearrangements greater than 10 base pairs, or low-level mosaicism. The copy number assessment methods used with this test cannot reliably detect copy number variants of less than 500 base pairs or mosaicism and cannot identify balanced chromosome aberrations. Assessment of exon-level copy number events is dependent on the inherent sequence properties of the targeted regions, including shared homology and exon size. This test does not include deletion/duplication testing of exon 9 of *ABCD1*. Recombinations involving the *IDS* gene and its pseudogene that do not involve exon-level deletions will not be detected by this test. For the *GUSB* genes deletion/duplication analysis is limited, and will only detect large (multi-exon) events. This test does not include exon-level deletion/duplication analysis for the *ADAMTSL2* and *GBA* genes.

Test Sensitivity:

The clinical sensitivity of sequencing and deletion/duplication analysis of the genes included in this panel depends in part on the patient's clinical phenotype. In a study of 66 unrelated patients from Spain who were suspected to have an a lysosomal disorder, a next generation sequencing panel of 57 genes identified a diagnosis in thirty eight percent (25/66) of patients.7 Sequence analysis of 14 peroxin genes associated with PBDs is estimated to detect ~96% of affected individuals; ~70% of cases are caused by pathogenic variants in *PEX1*, while ~26% of cases are due to pathogenic variants in *PEX6, PEX10, PEX12*, or *PEX26*.[1,2,3] In a large study of patients with a clinical diagnosis of X-ALD, sequencing analysis of *ABCD1* detected pathogenic variants in 95% of individuals.[6] Deletions have been detected in 3% of individuals with X-ALD.[8]

References:

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