Surfactant Dysfunction (SD) Panel

Disorder also known as: Surfactant deficiency; Surfactant metabolism dysfunction; Interstitial lung disease (ILD); Pulmonary alveolar proteinosis (PAP); Neonatal respiratory failure; Postnatal respiratory distress

SD Panel Gene List: *ABCA3, CSF2RA, CSF2RB, SFTPB, SFTPC*

Clinical Features:
Genetic disorders of surfactant deficiency and metabolism dysfunction are lung disorders with clinical presentations ranging from neonatal respiratory failure to childhood- or adult-onset interstitial lung disease. Pulmonary surfactant is a mixture of lipids and proteins that lines the inner surface of the lungs, reducing surface tension and preventing lung collapse (Wert et al., 2009; Suzuki et al., 2016). Surfactant is made in the alveoli, the tiny air sacks in the lungs where exchange of oxygen and carbon dioxide occurs. Although normal surfactant must be present to facilitate breathing, if too much surfactant builds up it can block air from entering the alveoli resulting in hypoxemic respiratory failure (Suzuki et al., 2016). Surfactant dysfunction disorders can result from problems in production, processing, transport, and clearance of surfactant components (Wert et al., 2009; Suzuki et al., 2016).

The signs and symptoms of surfactant dysfunction can vary in severity. The most severe form causes respiratory distress syndrome in the neonatal period. Shortly after birth affected infants have extreme difficulty breathing, and the subsequent lack of oxygen can damage the infant's brain and other organs. Most infants with this form of surfactant dysfunction do not survive longer than a few months (Wert et al., 2009; Kurath-Koller et al., 2015; Somaschini et al., 2018).

Less severe forms of surfactant dysfunction disorders can cause lung disease in infancy, childhood, or as late as in adulthood. Some symptoms of these milder surfactant dysfunction disorders are tachydyspnea, failure to thrive, hypoxia, and possibly repeated infections (Wert et al., 2009; Kroner et al., 2015).

Genetics:
Pulmonary surfactant metabolism dysfunction disorders are genetically heterogeneous.

The *SFTPB* gene encodes the pulmonary surfactant-associated protein SP-B. Pathogenic variants in *SFTPB* cause SP-B deficiency in an autosomal recessive manner. Hereditary SP-B deficiency is usually associated with a more severe form of surfactant dysfunction which presents in full-term infants with unexplained respiratory distress and is fatal at 3 to 6 months of age. The c.397delCinsGAA pathogenic variant is present in more than two-thirds of patients,

and it has an allele frequency of 1 per 1,000 to 3,000 individuals (Wert et al., 2009; Kurath-Koller et al., 2015). Other types of pathogenic variants reported in the SFTPB gene include nonsense, missense, frameshift, and splice-site variants, as well as small insertions and deletions throughout the gene and a large deletion comprising exons 7 and 8 (Stenson et al., 2014; Kurath-Koller et al., 2015). Pathogenic variants in *SFTPB* usually cause loss-of-function or complete absence of SP-B. Milder disease with more prolonged survival has been reported in some children with variants that allow for some residual SP-B production (Kurath-Koller et al., 2015). Variation in *SFTPB* may also confer an increased risk for idiopathic pulmonary fibrosis and chronic obstructive pulmonary disease in smokers (Selman et al., 2003; Baekvad-Hansen et al., 2010).

The *SFTPC* gene encodes the pulmonary surfactant-associated protein SP-C. Pathogenic variants in *SFTPC* result in a reduction or absence of mature SP-C and the accumulation of abnormal SP-C (Whitsett et al., 2010). Lung disease caused by pathogenic variants in *SFTPC* is inherited in an autosomal dominant manner with variable expressivity and reduced penetrance (Bullard and Nogee, 2007; Guillot et al., 2009). The phenotype associated with *SFTPC* pathogenic variants is quite variable, including neonatal forms with early mortality, childhood and adult forms with chronic respiratory disease, and asymptomatic adult carriers (Guillot et al., 2009). Pathogenic variants in the BRICHOS domain of the protein are often associated with an earlier, more severe presentation (Kroner et al., 2015). Missense variants account for the majority of published variants in the *SFTPC* gene, with I73T being the most common, although frameshift and splice-site variants, as well as small insertions and deletions, have been identified (Wert et al., 2009; Stenson et al., 2014). Pathogenic variants are found to be de novo in 55% of affected individuals and inherited from a parent in the remaining 45% (Wert et al., 2009). Heterozygosity for an *ABCA3* pathogenic variant may modify the severity of lung disease associated with *SFTPBC* variants (Bullard and Nogee, 2007; Wambach et al., 2014).

The *ABCA3* gene is critical for pulmonary surfactant synthesis and processing. Pathogenic variants in *ABCA3* are associated with autosomal recessive surfactant dysfunction that can present as lethal neonatal respiratory distress as well as pediatric and adult interstitial lung disease (Wambach et al., 2014; Kroner et al., 2017). Genotype-phenotype correlation suggests homozygous or compound heterozygous frameshift and/or nonsense pathogenic variants are associated with the most severe phenotype, whereas individuals with null/other or other/other genotypes were more variable and less predictable (Wambach et al., 2014; Kroner et al., 2017). Additionally, research suggests that single (heterozygous) *ABCA3* pathogenic variants may increase the risk for nonlethal neonatal respiratory distress syndrome (Wambach et al., 2012; Naderi et al., 2014).

The *CSF2RA* and *CSF2RB* genes are involved in surfactant clearance in order to maintain a normal surfactant level in alveoli. Pathogenic variants in *CSF2RA* and *CSF2RB* lead to an

accumulation of surfactant in the lungs called hereditary pulmonary alveolar proteinosis (PAP), a recessive disorder which results in progressive respiratory insufficiency (Suzuki et al., 2008; Suzuki et al., 2011; Tanaka et al., 2011; Suzuki et al., 2016). *CSF2RA* is located in the pseudo-autosomal regions of the X and Y chromosomes, and pathogenic variants are more commonly found in *CSF2RA* than *CSF2RB* (Hildebrandt et al., 2014). Reported variants in the *CSF2RA* gene include missense, nonsense, frameshift, splice-site, small insertions and deletions, and gross deletions which can encompass multiple exons or the entire gene; thus far, only missense and frameshift variants have been reported in the *CSF2RB* gene (Stenson, et al., 2014).

Test Methods:
Using genomic DNA from the submitted specimen, the complete coding regions and splice site junctions of the genes on this panel 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. Gene specific exclusions for exon-level deletion/duplication testing for this panel are: SFTPC gene, no copy number testing. Alternative sequencing or copy number detection methods are used to analyze regions with inadequate sequence or copy number data. 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.

Test Sensitivity:
Genetic disorders of surfactant deficiency and metabolism dysfunction are rare, and the clinical sensitivity for the associated genes is not well defined. SP-B deficiency has an estimated incidence of 1 in 1 million live births in the United States. Surfactant dysfuntion due to SFTPC and ABCA3 is thought to be less common than SFTPB-related surfactant dysfunction (Wert et al., 2009). Less than 1% of PAP is hereditary, with the majority of PAP being autoimmune PAP (about 90%) or secondary to other underlying diseases (about 8-9%) (Suzuki et al., 2016).

Gene	Protein	Inheritance	Disease Associations
ABCA3	ATP-BINDING CASSETTE SUB-FAMILY A MEMBER 3	Autosomal Dominant/Autosomal Recessive	Surfactant Metabolism Dysfunction, Interstitial Lung Disease
CSF2RA	GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR RECEPTOR SUBUNIT ALPHA	X-linked recessive (PARI)	Pulmonary Alveolar Proteinosis
CSF2RB	GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR RECEPTOR SUBUNIT BETA	Autosomal Recessive	Pulmonary Alveolar Proteinosis
SFTPB	SURFACTANT PROTEIN-B	Autosomal Recessive	Surfactant Metabolism Dysfunction
SFTPC	SURFACTANT PROTEIN-C	Autosomal Dominant	Surfactant Metabolism Dysfunction

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