Blueprint Genetics

Sample report as of June 1st, 2022. Regional differences may apply. For complete and up-to-date test methodology description, please see your report in Nucleus online portal. Accreditation and certification information available at **blueprintgenetics.com/certifications**

Neonatal Respiratory Distress - Surfactant Dysfunction Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME	HOSPITAL

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
		0		

PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE	CUSTOMER SAMPLE ID
Blood		

SUMMARY OF RESULTS

PRIMARY FINDINGS

The patient is homozygous for *ABCA3* c.3863-98C>T, which is pathogenic.

Del/Dup (CNV) analysis

Negative for explaining the patient's phenotype.

PRIMARY FINDINGS: SEQUENCE ALTERATIONS

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
ABCA3	NM_001089.2	c.3863-98C>T	HOM	intron_variant	AR	**Pathogenic**

	ID	ASSEMBLY	POS	REF/ALT		
	rs189077405	GRCh37/hg19	16:2333457	G/A		

	gnomAD AC/AN	POLYPHEN	SIFT	MUTTASTER	PHENOTYPE
	0/0	N/A	N/A	N/A	Interstitial lung disease, Surfactant metabolism dysfunction, pulmonary

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Neonatal Respiratory Distress - Surfactant Dysfunction Panel	5	51	11326	11326	260	100

TARGET REGION AND GENE LIST

The Blueprint Genetics Neonatal Respiratory Distress - Surfactant Dysfunction Panel Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: *ABCA3, FOXF1, NKX2-1, SFTPB* and *SFTPC*.

This panel targets protein coding exons, exon-intron boundaries (± 20 bps) and selected non-coding, deep intronic variants (listed in Appendix 5). This panel should be used to detect single nucleotide variants and small insertions and deletions (INDELs) and copy number variations defined as single exon or larger deletions and duplications. This panel should not be used for the detection of repeat expansion disorders or diseases caused by mitochondrial DNA (mtDNA) mutations. The test does not recognize balanced translocations or complex inversions, and it may not detect low-level mosaicism.

CLINICAL HISTORY

Patient is a newborn baby with deteriorating respiratory distress since the first day of life. Upon chest CT, a diffuse interstitial pattern was seen. There is no family history of similar disease.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Neonatal Respiratory Distress - Surfactant Dysfunction Panel identified a homozygous intronic variant *ABCA3* c.3863-98C>T.

ABCA3 c.3863-98C>T

This variant is absent in the Genome Aggregation Database control population cohorts (gnomAD, n>120,000 exomes and >15,000 genomes). *ABCA3* c.3863-98C>T is a well-established disease causing variant described in the literature and HGMD database. Originally, this intronic *ABCA3* IVS25-98C>T variant corresponding to c.3863-98C>T was detected in compound heterozygous state with *ABCA3* c.2068G>A, p.Glu690Lys variant in a full-term male infant who developed hypoxemic respiratory failure and pulmonary hypertension shortly after birth (PMID: 22337229). Aberrant splicing of the allele containing *ABCA3* c.3863-98C>T variant was shown by both *in silico* tools, which predicted cryptic donor site and sequencing the RNA extracted from the lungs and blood. In the same study, three other patients had the heterozygous *ABCA3* c.3863-98C>T variant together with another missense variant and four apparently unrelated neonates from South America who had fatal neonatal respiratory disease were homozygous for c.3863-98C>T. A homozygous *ABCA3* c.3863-98C>T has also been reported in a newborn full-term Colombian baby boy who was the son of non-consanguineous parents, who was delivered via caesarean section with severe respiratory depression (PMID: 27670912). This intronic variant has also been reported together with *ABCA3* c.622C>T, p.Arg208Trp in a patient with neonatal RDS (PMID: 27516224).

ABCA3

The ATP-binding cassette subfamily A member 3 (ABCA3) belongs to the ABC transporter family. ABCA3 is predominantly expressed in lung tissue, localized to the membranes of the lamellar bodies of alveolar type II cells, where it is critical for pulmonary surfactant synthesis and processing. *ABCA3* mutations have been associated with lethal neonatal respiratory distress (PMID: 22707629), pediatric and adult interstitial lung disease (PMID: 15976379), and surfactant metabolism dysfunction (MIM #610921). Most of the more than 190 distinct *ABCA3* mutations among patients with lung disease are unique to individuals and families (HGMD professional 2020.1). Mutations associated with ABCA3 deficiency are distributed throughout the gene and include nonsense, small deletions and insertions leading to frameshift, missense, splicing variants and gross deletions. Lung disease resulting from *ABCA3* mutations is expressed in an autosomal-recessive manner, requiring mutations on both alleles. However, among individuals with two *ABCA3* mutations, the pulmonary phenotype varies in terms of age of presentation, disease severity, and progression. Genotype-phenotype correlations exist for homozygous or compound heterozygous mutations in *ABCA3*. Frameshift or nonsense *ABCA3* mutations leading to loss-of-function are predictive of neonatal presentation and poor outcome, whereas missense, splice site, and insertion/deletions are less reliably associated with age of presentation and prognosis (PMID: 24871971).

Mutation nomenclature is based on GenBank accession NM_001089.2 (*ABCA3*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

ABCA3 c.3863-98C>T is classified as pathogenic based on currently available evidence supporting its disease-causing role. Disease caused by *ABCA3* variants is inherited in an autosomal recessive manner. The patient is homozygous for the variant, which is consistent with autosomal recessive inheritance. If the patient's parents are each confirmed to be carriers of this variant, any siblings of the patient will have a 25% chance of being homozygous for the variant and thus affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being an unaffected non-carrier. Genetic counselling and family member testing

are recommended.

| STEP	DATE
Order date	
Sample received	
Sample in analysis	
Reported	

(This statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results.)

Signature

Name

Title

Readability of the coverage plot may be hindered by faxing. A high quality coverage plot can be found with the full report on nucleus.blueprintgenetics.com.
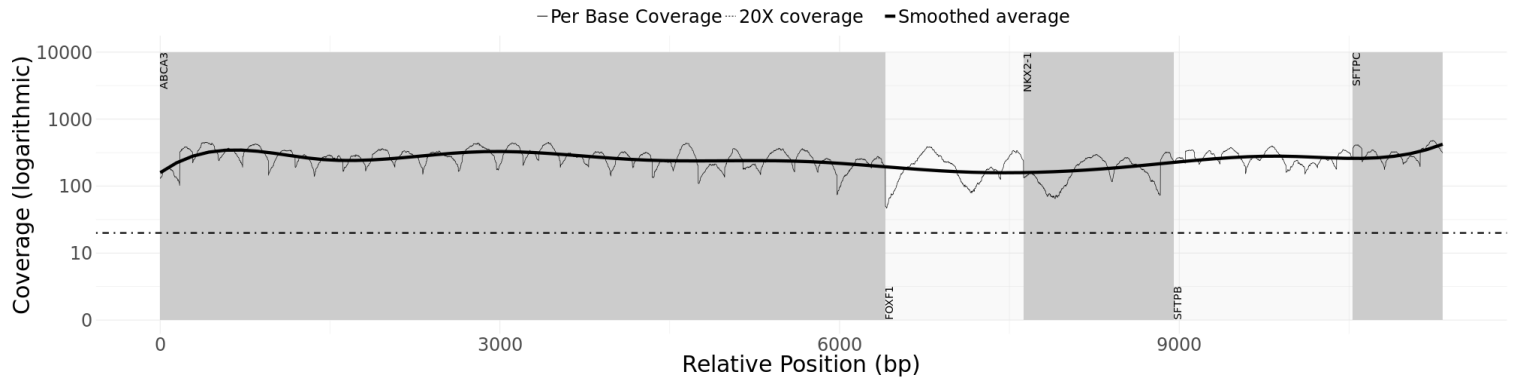
APPENDIX 5: SUMMARY OF THE TEST

PLUS ANALYSIS

Laboratory process: When required, the total genomic DNA was extracted from the biological sample using bead-based method. DNA quality and quantity were assessed using electrophoretic methods. After assessment of DNA quality, qualified genomic DNA sample was randomly fragmented using non-contact, isothermal sonochemistry processing. Sequencing library was prepared by ligating sequencing adapters to both ends of DNA fragments. Sequencing libraries were size-selected with bead-based method to ensure optimal template size and amplified by polymerase chain reaction (PCR). Regions of interest (exons and intronic targets) were targeted using hybridization-based target capture method. The quality of the completed sequencing library was controlled by ensuring the correct template size and quantity and to eliminate the presence of leftover primers and adapter-adapter dimers. Ready sequencing libraries that passed the quality control were sequenced using the Illumina's sequencing-by-synthesis method using paired-end sequencing (150 by 150 bases). Primary data analysis converting images into base calls and associated quality scores was carried out by the sequencing instrument using Illumina's proprietary software, generating CBCL files as the final output.

Bioinformatics and quality control: Base called raw sequencing data was transformed into FASTQ format using Illumina's software (bcl2fastq). Sequence reads of each sample were mapped to the human reference genome (GRCh37/hg19). Burrows-Wheeler Aligner (BWA-MEM) software was used for read alignment. Duplicate read marking, local realignment around indels, base quality score recalibration and variant calling were performed using GATK algorithms (Sentieon) for nDNA. Variant data for was annotated using a collection of tools (VcfAnno and VEP) with a variety of public variant databases including but not limited to gnomAD, ClinVar and HGMD. The median sequencing depth and coverage across the target regions for the tested sample were calculated based on MQ0 aligned reads. The sequencing run included in-process reference sample(s) for quality control, which passed our thresholds for sensitivity and specificity. The patient's sample was subjected to thorough quality control measures including assessments for contamination and sample mix-up. Copy number variations (CNVs), defined as single exon or larger deletions or duplications (Del/Dups), were detected from the sequence analysis data using a proprietary bioinformatics pipeline. The difference between observed and expected sequencing depth at the targeted genomic regions was calculated and regions were divided into segments with variable DNA copy number. The expected sequencing depth was obtained by using other samples processed in the same sequence analysis as a guiding reference. The sequence data was adjusted to account for the effects of varying guanine and cytosine content.

Interpretation: The clinical interpretation team assessed the pathogenicity of the identified variants by evaluating the information in the patient requisition, reviewing the relevant scientific literature and manually inspecting the sequencing data if needed. All available evidence of the identified variants was compared to classification criteria. Reporting was carried out using HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines. Likely benign and benign variants were not reported.

Variant classification: Our variant classification follows the Blueprint Genetics Blueprint Genetics Variant Classification Schemes modified from the ACMG guideline 2015. Minor modifications were made to increase reproducibility of the variant classification and improve the clinical validity of the report.

Databases: The pathogenicity potential of the identified variants were assessed by considering the predicted consequence of the change, the degree of evolutionary conservation as well as the number of reference population databases and mutation databases such as, but not limited to, the gnomAD, ClinVar, HGMD Professional and Alamut Visual. In addition, the clinical relevance of any identified CNVs was evaluated by reviewing the relevant literature and databases such as Database of Genomic Variants and DECIPHER. For interpretation of mtDNA variants specific databases including e.g. Mitomap, HmtVar and 1000G were used.

Confirmation of sequence alterations: Sequence variants classified as pathogenic, likely pathogenic and variants of uncertain significance (VUS) were confirmed using bi-directional Sanger sequencing when they did not meet our stringent NGS quality metrics for a true positive call. In addition, prenatal case with diagnostic findings were confirmed.

Confirmation of copy number variants: CNVs (Deletions/Duplications) were confirmed using a digital PCR assay if they covered less than 10 exons (heterozygous), less than 3 exons (homo/hemizygous) or were not confirmed at least three times previously at our laboratory. Furthermore, CNVs of any size were not confirmed when the breakpoints of the call could be

determined.

Analytic validation: The detection performance of this panel is expected to be in the same range as our high-quality, clinical grade NGS sequencing assay used to generate the panel data (nuclear DNA: sensitivity for SNVs 99.89%, indels 1-50 bps 99.2%, one-exon deletion 100% and five exons CNV 98.7%, and specificity >99.9% for most variant types). It does not detect very low-level mosaicism as a variant with minor allele fraction of 14.6% can be detected in 90% of the cases. Detection performance for mtDNA variants (analytic and clinical validation): sensitivity for SNVs and INDELs 100.0% (10-100% heteroplasmy level), 94.7%(5-10% heteroplasmy level), 87.3% (<5% heteroplasmy level) and for gross deletions 100.0%. Specificity is >99.9% for all.

Test restrictions: A normal result does not rule out the diagnosis of a genetic disorder since some DNA abnormalities may be undetectable by the applied technology. Test results should always be interpreted in the context of clinical findings, family history, and other relevant data. Inaccurate, or incomplete information may lead to misinterpretation of the results.

Technical limitations: This test does not detect the following: complex inversions, gene conversions, balanced translocations, repeat expansion disorders unless specifically mentioned, non-coding variants deeper than ±20 base pairs from exon-intron boundary unless otherwise indicated (please see the list of non-coding variants covered by the test). Additionally, this test may not reliably detect the following: low level mosaicism, stretches of mononucleotide repeats, indels larger than 50bp, single exon deletions or duplications, and variants within pseudogene regions/duplicated segments. The sensitivity of this test may be reduced if DNA is extracted by a laboratory other than Blueprint Genetics. Laboratory error is also possible. Please see the Analytic validation above.

Regulation and accreditations: This test was developed and its performance characteristics determined by Blueprint Genetics (see Analytic validation). It has not been cleared or approved by the US Food and Drug Administration. This analysis has been performed in a CLIA-certified laboratory (#99D2092375), accredited by the College of American Pathologists (CAP #9257331) and by FINAS Finnish Accreditation Service, (laboratory no. T292), accreditation requirement SFS-EN ISO 15189:2013. All the tests are under the scope of the ISO 15189 accreditation (excluding mtDNA testing and digital PCR confirmation).

NON-CODING VARIANTS COVERED BY THE PANEL:

NM_001089.2(ABCA3):c.3863-98C>T
NM_001089.2(ABCA3):c.1112-20G>A
NM_001089.2(ABCA3):c.-26-2A>G

GLOSSARY OF USED ABBREVIATIONS:

AD = autosomal dominant

AF = allele fraction (proportion of reads with mutated DNA / all reads)

AR = autosomal recessive

CNV = Copy Number Variation eg, one exon or multiexon deletion or duplication

gnomAD = genome Aggregation Database (reference population database; >138,600 individuals)

gnomAD AC/AN = allele count/allele number in the genome Aggregation Database (gnomAD)

HEM = hemizygous

HET = heterozygous

HOM = homozygous

ID = rsID in dbSNP

MT = Mitochondria

MutationTaster = *in silico* prediction tools used to evaluate the significance of identified amino acid changes.

Nomenclature = HGVS nomenclature for a variant in the nucleotide and the predicted effect of a variant in the protein level

OMIM = Online Mendelian Inheritance in Man®

PolyPhen = *in silico* prediction tool used to evaluate the significance of amino acid changes.

POS = genomic position of the variant in the format of chromosome:position

SIFT = *in silico* prediction tool used to evaluate the significance of amino acid changes.