REFERENCE LABORATORY GENETICS

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GENETIC STUDY OF IDIOPATHIC PULMONARY FIBROSIS AND PULMONARY SURFACTANT METABOLISM DYSFUNCTION BY MASSIVE SEQUENCING (NGS)

Request No.:	**000**		
Client:	-		
Analysis code:	30131		
Patient Name:	**xxx**		
Date of Birth:	N/A	**Patient Ref.:**	xxx
Gender:	Male	**Sample Type:**	Whole blood
Sample Arrival Date:	DD/MM/AAAA	**Date of Result:**	DD/MM/AAAA

Clinical information: Neonate with intractable hyaline membrane disease

RESULT AND INTERPRETATION

The presence of a heterozygous likely pathogenic variant that could explain the hypothesis of idiopathic pulmonary fibrosis and pulmonary surfactant metabolism dysfunction, has been identified (see Recommendations)

The complete list of studied genes and coverage in Table 1. (Methodology)

The complete list of studied genes and coverage details is available in Table 1. (Methodology)

Gene	Variant*	Zygosity	Inheritance pattern	Classification^
SFTPC	NM_003018.3: **c.567T>G** NP_003009.2: **p.(Cys189Trp)**	Heterozygosis	Autosomal Dominant	**Likely pathogenic**

* Nomenclature according to HGVS v15.11
^ Based on the recommendations of the *American College of Medical Genetics and Genomics (ACMG)*

The ***SFTPC*** variant **c.567T>G (p.(Cys189Trp))** is a missense that predicts an amino acid change from Cysteine to Tryptophan at position 189 of the protein, affecting a functional domain. It is described in HGMD (CM1515739) database as pathogenic variant associated with Infant acute respiratory distress syndrome. The variant does not appear in the dbSNP database or in the gnomAD population frequency database. The bioinformatic predictors (SIFT, Mutation Taster and Polyphen-2) estimate that the change would have a pathogenic effect. A pathogenic variant (c.566G>A p.(Cys189Tyr) associated with the interstitial lung disease due to SP-C deficiency (PMID: 19443464) has been described at the same position. In the scientific literature, the variant is described in a newborn child with respiratory problems (PMID: 26029841).

Based on these data, the variant is classified as a **Likely Pathogenic variant.**
The *SFTPC* gene (OMIM:178620) is associated with chronic respiratory distress with surfactant metabolism deficiency (OMIM: 610913), entity with an autosomal dominant inheritance pattern.

RECOMMENDATIONS

The study of the variant in the parents would allow to determine if the variant is *de novo* or if any of them presents it. In the event that this is *de novo*, evidence of its possible involvement in the patient's clinical condition would increase.

Genetic counselling should be offered to the patient by the prescriber physician. If additional information regarding the results or genetic counselling is required, the physician can contact our team at genetics@referencelaboratory.es.

METHODOLOGY

DNA extraction and quantitative and qualitative evaluation of the DNA obtained.

Capture and enrichment of exonic regions and flanking intronic areas of genes contained in the REFLAB MedExome (Roche) sequencing panel with the Roche NimbleGen SeqCap EZ HyperCap Library™ technology.

Massive sequencing with the NextSeq™(Illumina) sequencer.

Identification of the variants of interest in regard to the reference genome (hg19) after filtering, according to specific quality criteria. Annotation of the obtained variants with a specific bioinformatic software: Alamut Visual™ (Interactive Biosoftware), Ingenuity Variant Analysis™ (QIAGEN), Variant interpreter™ (Illumina) and VarAFT™. The used reference databases have been the population databases dbSNP, 1000genomes, EXAC and gnomAD; the clinical databases Human Gene Mutation Database (HGMD version 2019.3), ClinVar and LOVD; the disease specific databases, if applicable, and Reference Laboratory Genetics´own databases. The bioinformatic analysis to evaluate the possible impact of the variants of interest on the structure and functionality of the protein has been carried out with the bioinformatic programs Mutation Taster, SIFT and PolyPhen-2. These analyses are only a predictive tool; they were not experimentally proven.

The nomenclature used to define the variants follows the criteria of the *Human Genome Variation Society (HGVS)* (http://www.HGVS.org/varnomen).

Classification of variants based on the recommendations of the *American College of Medical Genetics and Genomics (ACMG)* (Richards S. *et al*., 2015). Only those variants that, based on current information, are considered pathogenic, likely pathogenic or of uncertain clinical significance, are reported. (The complete list of identified variants is available upon request).

The obtained average reading depth was 185,8x being > 20x in 99,4% of the regions analysed.

LIMITATIONS: The results obtained do not exclude variants outside the analysed regions of the genome or genetic anomalies not detectable by massive sequencing such as large rearrangements, large deletions/duplications (Copy Number Variant; CNV), insertions / deletions of> = 10 nucleotides, variants in repetitive regions or with a high percentage of GC, and variants in genes with pseudogenes with highly homologous sequences.
It is not possible to rule out the presence of variants in other unanalysed genes.

Table 1. STUDIED GENES AND COVERAGE DETAILS

Gene	NM	10x %	Exons with coverage < 100%*
ABCA3	NM_001089	100,00	-
AGL	NM_000642	100,00	-
CFTR	NM_000492	100,00	-
CSF2RA	NM_006140	100,00	-

CSF2RB	NM_000395	100,00	-
DMBT1	NM_007329	100,00	-
FOXA1	NM_004496	100,00	-
FOXA2	NM_021784	100,00	-
G6PC	NM_000151	100,00	-
GAA	NM_000152	100,00	-
GBE1	NM_000158	100,00	-
GYS2	NM_021957	100,00	-
LAMC2	NM_005562	100,00	-
MUC5B	NM_002458	100,00	-
NOD2	NM_022162	100,00	-
NPC1	NM_000271	100,00	-
NPC2	NM_006432	100,00	-
PFKM	NM_000289	100,00	-
PHKA2	NM_000292	98,71	5 13 17 25
PYGL	NM_002863	100,00	-
PYGM	NM_005609	100,00	-
SFTPA2	NM_001098668	100,00	-
SFTPB	NM_198843	100,00	-
SFTPC	NM_003018	100,00	-
SFTPD	NM_003019	100,00	-
SLC2A2	NM_000340	99,94	4
SLC37A4	NM_001164277	100,00	-
SMPD1	NM_000543	100,00	-
TERC	NR_001566	100,00	-
TERT	NM_198253	97,32	1 8
TSC1	NM_000368	99,86	14
TSC2	NM_000548	100,00	-

*Due to the current intrinsic limitations associated with massive sequencing technology, some gene exons analysed may be insufficiently covered. If it is considered appropriated by a medical specialist, it would be possible to sequence exons with coverage below 100% using the Sanger method or other alternative molecular technique.

IMPORTANT NOTE

The information contained in this report is based on current scientific knowledge and the results obtained from the application of the technology in this report, are detailed. Due to continuous advances, the documented information may be modified in the future as a result of the emergence of new scientific evidence.

The genetic/genomic studies carried out by Reference Laboratory S.A. are exclusively intended for qualified health professionals for their interpretation. The results obtained are not, per se, a medical consultation, diagnosis or treatment, nor should they be interpreted as such. Only a specialized professional can correctly interpret the results and offer a diagnosis or prescribe a treatment to a patient based on these. Consequently, no information obtained from our studies can be used to replace the advice and diagnosis of a specialized professional.

Signed: Cristina Camprubí, PhD

Head of Diagnosis and Genetic Counseling

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