Blueprint Genetics

Comprehensive Pulmonology Panel

THIS IS AN UPDATED REPORT. PLEASE SEE THE FOLLOW-UP REPORT TEXT BELOW. THIS REPORT SUPERSEDES THE REPORT ISSUED ON AUG 26, 2018

FOLLOW-UP REPORT

Follow-up Sep 04, 2018

SFTPC c.361T>C, p.(Cys121Arg) was confirmed using bi-directional Sanger sequencing.

Jonna Tallila, PhD

REFERRING HEALTHCARE PROFESSIONAL

NAME

HOSPITAL

PATIENT

NAME

DOB

AGE
0

GENDER
Female

ORDER ID

CUSTOMER SAMPLE ID

PRIMARY SAMPLE TYPE
DNA

SAMPLE COLLECTION DATE

SUMMARY OF RESULTS

TEST RESULTS

Patient is heterozygous for *SFTPC* c.361T>C, p.(Cys121Arg), which is classified as likely pathogenic.

Del/Dup (CNV) analysis did not detect any known disease-causing copy number variation or novel or rare deletion/duplication that was considered deleterious.

VARIANT TABLE: GENETIC ALTERATIONS

GENE	POS	TRANSCRIPT	NOMENCLATURE	CONSEQUENCE	GENOTYPE	CLASSIFICATION
SFTPC	8:22020985	NM_003018.3	c.361T>C, p.(Cys121Arg)	missense_variant	HET	**Likely pathogenic**
	ID	**gnomAD AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**	
		0/0	probably damaging	deleterious	disease causing	
	OMIM	**PHENOTYPE**		**INHERITANCE**	**COMMENT**	
		Surfactant metabolism dysfunction, pulmonary		AD	-	

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Comprehensive Pulmonology Panel	67	1198	229577	229400	156	99.92

TARGET REGION AND GENE LIST

The Blueprint Genetics Comprehensive Pulmonology Panel (version 3, Mar 01, 2018) Sequence Analysis includes sequence analysis of the following genes: ABCA3, CCDC39, CCDC40, CFTR, CHAT, CHRNA1, CHRNB1, CHRND, CHRNE, COLQ, CSF2RA*, CSF2RB, DKC1, DNAAF1, DNAAF2, DNAH1, DNAH11*, DNAH5, DNAI1, DNAI2, DNAL1, EDN3, EFEMP2, ELMOD2, ELN, FBLN5, FLCN, FOXF1, GAS8, GLRA1, HPS1*, HPS4, ITGA3, LTBP4, MECP2, NAF1, NF1*, NKX2-1, NME8, PARN*, PHOX2B, PIH1D3#, RAPSN, RET, RSPH3, RSPH4A, RSPH9, RTEL1, SCN4A, SCNN1A, SCNN1B, SERPINA1, SFTPA1, SFTPA2, SFTPB, SFTPC, SLC34A2, SLC6A5, SLC7A7, SMPD1, STAT3, TERC, TERT, TINF2, TSC1, TSC2 and ZEB2*. 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) up to 220 bps. This panel should not be used to detect large copy number variations (CNVs; deletions and duplications spanning one or more exons), repeat expansion disorders or diseases caused by mitochondrial DNA (mtDNA) mutations. This test does not recognize balanced translocations or complex inversions, and it may not detect low-level mosaicism.

*Some, or all, of the gene is duplicated in the genome. Read more: https://blueprintgenetics.com/pseudogene/
#The gene has suboptimal coverage when >90% of the gene's target nucleotides are not covered at >20x with mapping quality score (MQ>20) reads.
The sensitivity to detect variants may be limited in genes marked with an asterisk (*) or number sign (#).

STATEMENT

CLINICAL HISTORY

Patient is a 2-month-old girl who born at good condition after full-term pregnancy but soon after birth got quickly progressing severe respiratory failure and pulmonary hypertension. She had also high CRP. Due to disease severity ECMO-treatment was initiated. Now, inflammation has improved but weaning from respirator has not succeeded. There are structural changes in pulmonary tissue, thus primary pulmonary disease is suspected.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Comprehensive Pulmonology Panel identified a heterozygous missense variant c.361T>C, p.(Cys121Arg) in *SFTPC*. It has not been observed in large reference population cohorts of Genome Aggregation Database, (gnomAD, n>120,000 exomes and >15,000 genomes). Database curators have made every effort to exclude individuals with severe pediatric diseases from these cohorts. *In silico* tools PolyPhen, SIFT, and MutationTaster predict the variant as deleterious. Cysteine (Cys) is completely conserved at this position in mammals as well as evolutionary more distant species suggesting that this position may not tolerate variation. Moreover, there is a large physiochemical difference between cysteine and arginine (Arg) demonstrated by radical Grantham score (180).

To our knowledge this variant has not been detected in patients affected with pulmonary surfactant metabolism dysfunction (PSMD). However, there are multiple affected patients with PSMD who have novel (absent in gnomAD) heterozygous variants affecting cysteine-121 residue such as *SFTPC* p.(Cys121Gly) (*de novo*; PMID 23719253), *SFTPC* p.(Cys121Phe) (PMID 25657025, 24648475) and *SFTPC* p.(Cys121Tyr) (PMID 22308375). All of these variants involving residue 121 are radical by Grantham score; Gly (159), Phe (205) and Tyr (194). It has been suggested that residue 121 is important for normal protein function because it is involved in forming a disulfide bridge between Cys121 and Cys189 (PMID 24648475).

SFTPC gene on chromosome 8p21.3 encodes surfactant protein C, which is an extremely hydrophobic surfactant protein essential for lung function and homeostasis after birth. Pulmonary surfactant is a surface-active lipoprotein complex composed of 90% lipids and 10% proteins. Surfactant is secreted by the alveolar cells and it maintains the stability of pulmonary tissue by reducing the surface tension of fluids that coat the lung. Autosomal dominant mutations in *SFTPC* cause pulmonary surfactant metabolism dysfunction type 2 (OMIM *178620, also called pulmonary alveolar proteinosis due to SFTPC deficiency), and are associated with interstitial lung disease in older infants, children and adults. In addition to canonical transcript (NM_003018.3) consisting 197 amino acids within 6 exons, *SFTPC* has multiple other isoforms with RefSeq ID. All 9 pathogenic or likely pathogenic variants submitted to ClinVar (Aug 2018) are either missense (7) or splicing variants (2). In 2015, Kröner *et al.* described a cohort of 17 patients with *SFTPC* mutations, of which 15 carried single variant potential to explain their disease (PMID 25657025). The mutation occurred as *de novo* in ten out of 11 cases where parental samples were available. Two of the cases in the study carried two *SFTPC* variants, other was shown to be compound heterozygous. The pLI value of *SFTPC* gene in ExAC reference population is 0.07 (minimum 0.00 – maximum 1.00) indicating tolerance for loss-of-function variation but missense variant count is slightly lower than expected by gene size (missense constraint +1.11) indicating intolerance for this kind of variation. One per 1402 individuals in gnomAD reference population carries a unique *SFTPC* missense variant (not present in anybody else in this cohort) and one per 9583 individuals carry a high-quality truncating *SFTPC* variant affecting canonical transcript.

Pulmonary surfactant metabolism dysfunction type 2 is generally severe early-onset disease. Half of the patients described Kröner *et al.* presented with neonatal respiratory symptoms (PMID 25657025). The mean age at disease onset was two months (range 0–132). Failure to thrive was the most important extra-pulmonary manifestation (15 out of 17 patients) and treated in subjects by percutaneous endoscopic gastrostomy tube in 10 subjects. Five patients had also cardiac anomalies and two had mental retardation. Imaging by CT have revealed significant changes from initial homogeneous ground glass attenuation to increasing signs of fibrosis with honeycombing, peribronchial, interlobular, and intralobular septal thickening as well as cyst formation. Therapy with hydroxychloroquine moderately improved the clinical course in six patients and had a good effect in another six individuals, shortly after initiation of therapy. Family history revealed lung disease, previous abortions or postnatal death in about one-third of cases.

Mutation nomenclature is based on GenBank accession NM_003018.3 (*SFTPC*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

SFTPC c.361T>C, p.(Cys121Arg) is classified as likely pathogenic considering the current evidence of the variant (established association between the gene and the patient's phenotype, rarity in control populations, *in silico* predicted pathogenicity, multiple disease associated variants affecting the same codon)). However, additional information is still needed to confirm the pathogenicity of the variant. Genetic counseling and family member testing are recommended. Disease caused by *SFTPC* variants is inherited in an autosomal dominant manner, and thus each child of an affected individual has a 50% chance of inheriting the variant. A proband with autosomal dominant pulmonary surfactant metabolism dysfunction type 2 may have the disorder as a result of a *de novo* event. BpG offers targeted variant testing for the family if requested.

YHTEENVETO

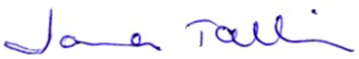
Luokittelemme *SFTPC* c.361T>C, p.(Cys121Arg) -geenivirheen todennäköisesti tautia aiheuttavaksi (likely pathogenic), koska kyseisen geenin ja taudin välille on osoitettu yhteys, geenivirhe on harvinainen verrokkiaineistoissa, muutos on ennusteohjelmien perusteella haitallinen ja samassa kodonissa sijaitsee useita samantyyppiseen kliiniseen kuvaan assosioituvia geenivirheitä. Tarvitaan kuitenkin lisänäyttöä geenivirheen patogeenisyyden varmentamiseksi. Suosittelemme lähisukulaisten perinnöllisyysneuvontaa ja kohdennettua geenitestausta. *SFTPC* geenin virheet aiheuttavat vallitsevasti periytyvän taudin. Potilaalla, jolla on vallitsevasti periytyvä tauti, voi kyseessä olla nk. uusi geenivirhe (*de novo*). Vallitsevasti periytyvää tautia sairastavan henkilön jokaisella lapsella on 50%:n todennäköisyys periä sairautta aiheuttava geenivirhe vanhemmaltaan. BpG tarjoaa sukulaisten geenitestauspalvelua.

CONFIRMATION

SFTPC c.361T>C, p.(Cys121Arg) has been confirmed using bi-directional Sanger sequencing.

STEP	DATE
Order date	Aug 08, 2018
Sample received	Aug 14, 2018
Reported	Aug 26, 2018
Last reviewed	Sep 04, 2018

On Aug 26, 2018 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

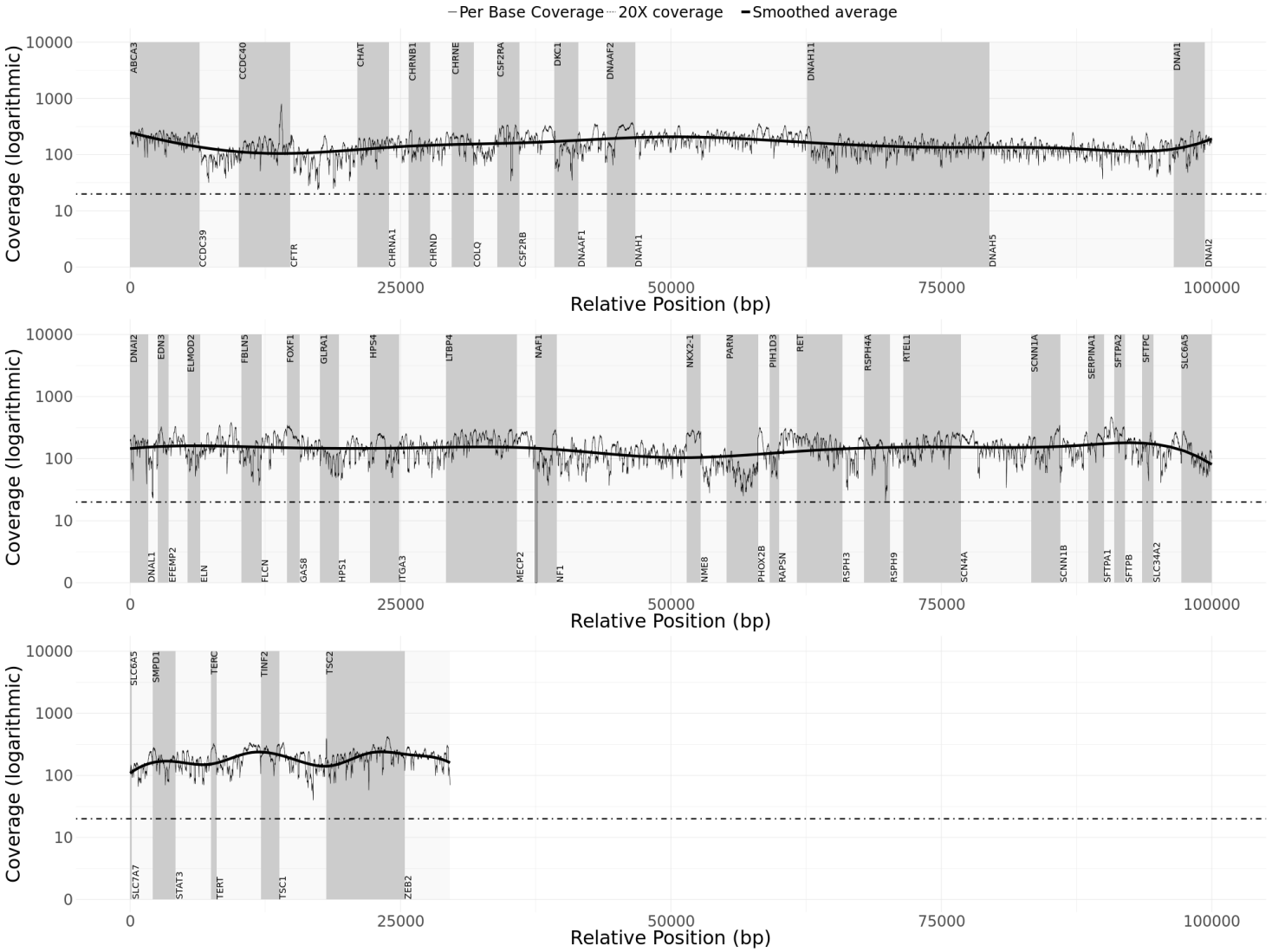


Jonna Tallila, Ph.D.

Senior Geneticist

Juha Koskenvuo, MD, Ph.D.

Lab Director, Chief Medical Officer


Per Base Coverage
20X coverage
Smoothed average
Coverage (logarithmic)
10000
1000
100
10
0
ABCA3
CCDC40
CHAT
CHRNB1
CHRNE
CSF2RA
DKC1
DNAAF2
DNAH11
DNAI1
CCDC39
CFTR
CHRNA1
CHRND
COLQ
CSF2RB
DNAAF1
DNAH1
DNAH5
DNAI2
0
25000
50000
75000
100000
Relative Position (bp)
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EDN3
ELMOD2
FBLN5
FOXF1
GLPA1
HPS4
LTBP4
NAF1
NKX2-1
PARN
PIH1D3
RET
RSPH4A
RTEL1
SCNN1A
SERPINA1
SFTPA2
SFTPC
SLC6A5
DNAL1
EFEMP2
ELN
FLCN
GAS8
HPS1
ITGA3
MECP2
NF1
NME8
PHOX2B
RAPSN
RSPH3
RSPH9
SCN4A
SCNN1B
SFTPA1
SFTPB
SLC34A2
SLC6A5
SMPD1
TERC
TINF2
TSC2
SLC7A7
STAT3
TERT
TSC1
ZEB2

APPENDIX 5: SUMMARY OF THE TEST

SEQUENCE ANALYSIS

Laboratory process: Total genomic DNA was extracted from the biological sample using a spin column 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 and purified with SPRI beads. DNA fragments were then end-repaired and sequencing adapters were ligated to both ends of the resulting fragments. Prepared DNA-Adapter libraries were size-selected with SPRI beads to ensure optimal template size and then amplified by ligation-mediated PCR (LM-PCR). The amplified sequencing library was purified using SPRI beads and a hybridization-capture method was applied for enrichment of whole exome and select non-coding regions (xGen Exome Research Panel with custom-designed capture probes, IDT). The enriched sequencing library was amplified by LM-PCR and purified using SPRI beads. The quality of the completed sequencing library was controlled by ensuring the correct template size and quantity and to eliminate the presence of leftover primer-dimers. Each captured library passing quality control was sequenced using the Illumina sequencing system with paired-end sequencing (150 by 150 bases). Sequencing-derived raw image files were processed using a base-calling software (Illumina) and the sequence data was transformed into FASTQ format.
Bioinformatics and quality control: The bioinformatics analysis began with quality control of raw sequence reads. Clean 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). The panel content was sliced from high-quality exome sequencing data acquired as presented above. The sequencing depth and coverage for the tested sample were calculated based on the alignments. 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 as well, after which raw sequence reads were processed into variants by a proprietary bioinformatics pipeline.
Interpretation: Our variant classification follows the Blueprint Genetics Variant Classification Schemes modified from the ACMG guideline 2015. Minor modifications were made to increase the reproducibility of the variant classification and to improve the clinical validity of the report. Likely benign and benign variants were not reported. The pathogenicity potential of the identified variants were assessed by considering the predicted consequence, the biochemical properties of the codon change, the degree of evolutionary conservation as well as a number of reference population databases and mutation databases such as, but not limited, to the 1000 Genomes Project, gnomAD, ClinVar and HGMD. For missense variants, *in silico* variant prediction tools such as SIFT, PolyPhen, MutationTaster were used to assist with variant classification. The clinical evaluation team assessed the pathogenicity of the identified variants by evaluating the information in the patient referral, reviewing the relevant literature and manually inspecting the sequencing data if needed. Reporting was carried out using HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines.
Confirmation: Pathogenic and likely pathogenic variants that established a molecular diagnosis were confirmed with bi-directional Sanger sequencing unless all of the following criteria were fulfilled: 1) the variant quality score (QS) was above the internal threshold for a true positive call, 2) an unambiguous manual curation of the variant region using IGV was concordant with the variant call and 3) previous Sanger confirmation of the same variant has been performed at least three times in our laboratory. Reported variants of uncertain significance were confirmed with bi-directional Sanger sequencing only if the QS was below our internally defined score of a true positive call.
Analytic validation: This laboratory-developed test has been independently validated by Blueprint Genetics. The sensitivity of this panel is expected to be in the same range as the validated whole exome sequencing laboratory assay used to generate the panel data (sensitivity for SNVs 99.65%, and indels 1-50 bps 99.07%, and specificity >99.99%). 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.
Regulation and accreditations: This test has not been cleared or approved by the FDA. 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.

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
NM_181426.1(CCDC39):c.1167+1248A>G
NM_000492.3(CFTR):c.-495C>T
NM_000492.3(CFTR):c.-249G>C
NM_000492.3(CFTR):c.-165G>A
NM_000492.3(CFTR):c.-85C>G
NM_000492.3(CFTR):c.-34C>T
NM_000492.3(CFTR):c.53+124T>C
NM_000492.3(CFTR):c.1393-18G>A
NM_000492.3(CFTR):c.1585-9412A>G
NM_000492.3(CFTR):c.1585-19T>C
NM_000492.3(CFTR):c.1680-886A>G
NM_000492.3(CFTR):c.1680-883A>G
NM_000492.3(CFTR):c.1680-877G>T
NM_000492.3(CFTR):c.2908+19G>C
NM_000492.3(CFTR):c.2909-15T>G
NM_000492.3(CFTR):c.2988+33G>T
NM_000492.3(CFTR):c.3140-26A>G
NM_000492.3(CFTR):c.3140-11A>G
NM_000492.3(CFTR):c.3469-1304C>G
NM_000492.3(CFTR):c.3717+40A>G
NM_000492.3(CFTR):c.3718-2477C>T
NM_000492.3(CFTR):c.3873+33A>G

NM_000492.3(CFTR):c.3874-4522A>G
NM_000080.3(CHRNE):c.501-16G>A
NM_000080.3(CHRNE):c.-94G>A
NM_000080.3(CHRNE):c.-95G>A
NM_000080.3(CHRNE):c.-96C>T
NM_001363.3(DKC1):c.-142C>G
NM_001363.3(DKC1):c.-141C>G
NM_001363.3(DKC1):c.85-15T>C
NM_000114.2(EDN3):c.-125G>A
NM_000114.2(EDN3):c.-19C>A
NM_001278939.1(ELN):c.2272+20C>G
NM_005501.2(ITGA3):c.1383-11T>A
NM_001042492.2(NF1):c.-273A>C
NM_001042492.2(NF1):c.-272G>A
NM_001042492.2(NF1):c.288+2025T>G
NM_001042492.2(NF1):c.587-14T>A
NM_001042492.2(NF1):c.587-12T>A
NM_001042492.2(NF1):c.888+651T>A
NM_001042492.2(NF1):c.888+744A>G
NM_001042492.2(NF1):c.888+789A>G
NM_001042492.2(NF1):c.1260+1604A>G
NM_001042492.2(NF1):c.1261-19G>A
NM_001042492.2(NF1):c.1392+754T>G
NM_001042492.2(NF1):c.4110+945A>G
NM_001042492.2(NF1):c.4110+1802delA
NM_001042492.2(NF1):c.4173+278A>G
NM_001042492.2(NF1):c.5269-38A>G
NM_001042492.2(NF1):c.5610-456G>T
NM_001042492.2(NF1):c.5812+332A>G
NM_001042492.2(NF1):c.6428-11T>G
NM_001042492.2(NF1):c.6642+18A>G
NM_001042492.2(NF1):c.7190-12T>A
NM_001042492.2(NF1):c.7971-321C>G
NM_001042492.2(NF1):c.7971-17C>G
NM_001042492.2(NF1):c.8113+25A>T
NM_001134477.2(PARN):c.-165+2C>T
NM_005055.4(RAPSN):c.193-15C>A
NM_005055.4(RAPSN):c.-199C>G
NM_005055.4(RAPSN):c.-210A>G
NM_020975.4(RET):c.2392+19T>C
NM_000295.4(SERPINA1):c.-5+1G>A
NR_001566.1(TERC):n.-22C>T
chr3:g.169482906-169482906
NM_198253.2(TERT):c.-57A>C
NM_000548.3(TSC2):c.-30+1G>C
NM_000548.3(TSC2):c.976-15G>A
NM_000548.3(TSC2):c.2838-122G>A
NM_000548.3(TSC2):c.5069-18A>G
NM_014795.3(ZEB2):c.-69-1G>A

GLOSSARY OF USED ABBREVIATIONS:

AD = autosomal dominant
AR = autosomal recessive
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
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.
Transcript = GenBank accession for reference sequence used for variant nomenclature