Patient is heterozygous for \( \text{TERT} \ c.2812\text{C}>\text{T}, \ p.(\text{Arg938Trp}) \), which is a variant of uncertain significance.

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Pos</th>
<th>Transcript</th>
<th>Nomenclature</th>
<th>Consequence</th>
<th>Genotype</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>TERT</td>
<td>5:1264550</td>
<td>NM_198253.2</td>
<td>c.2812\text{C}&gt;\text{T}, p.(\text{Arg938Trp})</td>
<td>missense</td>
<td>HET</td>
<td>Variant of uncertain significance</td>
</tr>
</tbody>
</table>

**ID**

**Phenotype**
Aplastic anemia, Dyskeratosis congenita, Pulmonary fibrosis and/or bone marrow failure, telomere-related

**Inheritance**
AD&AR

**Comment**
-

### Sequencing Performance Metrics OS-SEQ

<table>
<thead>
<tr>
<th>Panel</th>
<th>Genes</th>
<th>Exons</th>
<th>Bases</th>
<th>Bases &gt; 15X</th>
<th>Median Coverage</th>
<th>Percent &gt; 15X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interstitial Lung Disease Panel</td>
<td>24</td>
<td>404</td>
<td>64667</td>
<td>64345</td>
<td>162</td>
<td>99.5</td>
</tr>
</tbody>
</table>

**Target Region and Gene List**

Blueprint Genetics Interstitial Lung Disease Panel (version 1, March 9, 2016) consists of sequence analysis of genes associated with familial idiopathic pulmonary fibrosis, other interstitial pulmonary diseases and unspecified pulmonary fibrosis: ABCA3, CSF2RA*, DKC1, ELMOD2, HPS1*, HPS4, ITGA3, NF1*, NKF2-1, PARN*, RTEL1, SFTA1, SFTP2, SFTP6, SFTPC, SLC7A7, SLC34A2, SMPD1, STAT3, TERC, TERT, TINF2, TSC1 and TSC2. The panel is targeting all protein coding exons and exon-intron boundaries of all target genes. It also covers a number of mutations located outside these coding regions. This test covers the majority of familial idiopathic pulmonary fibrosis, other interstitial pulmonary diseases and unspecified pulmonary fibrosis mutations known to date and it should be used to detect single nucleotide substitutions and small insertions and deletions (INDELs). In addition, the Interstitial Lung Disease Panel includes OS-Seq Del/Dup (CNV) Analysis (version 1, updated November 15, 2016) for the same genes as listed above. It should be used to diagnose deletions and duplications (e.g. copy number variants) in protein-coding regions of the genes included in the panel. Detection limit of the test varies through the genome from one to six exons depending on exon size, sequencing coverage and sequence content.

* Some regions of the gene are duplicated in the genome leading to limited sensitivity within the regions (link to duplicated regions): http://blueprintgenetics.com/pseudogene/. Thus, low-quality variants are filtered out from the duplicated regions and only high-quality variants confirmed by other
methods are reported out.

The test does not recognise balanced translocations or complex inversions, and it may not detect low-level mosaicism. The exact boundaries of the copy number aberration cannot be determined with this test. The test should not be used for analysis of sequence repeats or for diagnosis of disorders caused by mutations in the mitochondrial DNA.
STATEMENT

CLINICAL HISTORY

Patient is a 45-year-old male with upper airway obstruction. Patient's father and paternal uncle were affected with pulmonary fibrosis, both are deceased.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Interstitial Lung Disease Panel identified a heterozygous missense variant c.2812C>T, p.(Arg938Trp) in TERT. The variant is absent both from the Exome Aggregation Consortium (ExAC) control cohort and the Genome Aggregation Database (gnomAD) consisting in total of 126,216 reference exomes and 15,137 reference genomes. It is predicted damaging by in silico tools. To our knowledge, the variant has not been reported in the literature or databases such as Clinvar, HGMD or Telomerase Database.

Telomerase is a ribonucleoprotein polymerase that maintains telomere ends by addition of the telomere repeat TTAGGG. The enzyme consists of a protein component with reverse transcriptase activity and an RNA component which serves as a template for the telomere repeat. Shortened telomeres can cause a wide variety of clinical features that constitute a phenotypic spectrum (PMID: 19405848). The most severe form is dyskeratosis congenita (autosomal dominant or autosomal recessive, MIM #613989), characterized by early childhood onset of skin abnormalities, bone marrow failure, predisposition to malignancy, and risk of pulmonary and hepatic fibrosis. Adult-onset pulmonary fibrosis (MIM #614742) is the most common manifestation of mutant telomerase genes. Other manifestations include aplastic anemia due to bone marrow failure, hepatic fibrosis, and increased cancer risk, particularly myelodysplastic syndrome and acute myeloid leukemia (PMID: 19405848). Phenotype, age at onset, and severity are determined by telomere length, not just telomerase mutation. Pathogenic mutations in TERT, TERC, SFTPc, and SFTPc2 have been identified in patients with disease related to shortened telomeres. In general, point mutations which lead to a single amino acid substitution, are better tolerated than frameshift and splicing junction mutations, limiting but not abolishing telomerase activity. Progression of symptoms within the next generation is characteristic of telomerase-deficiency diseases.

Mutations in TERT, the gene encoding for the telomerase reverse transcriptase, are the most frequently identified mutations and are present in 18% of cases of autosomal dominant familial pulmonary fibrosis (GeneReviews NBK1230, MIM #614742). Penetrance for the phenotype associated with pathogenic variants in TERT is unknown but thought to be incomplete. Diaz de Leon et al. observed incomplete penetrance (~40%) in families with pulmonary fibrosis and TERT pathogenic variants and variable phenotype ranging from lung disease and liver disease to bone marrow dysfunction (PMID: 20502709). Numerous mutations causing amino acid substitution, additions, deletions, and frameshifts within TERT have been connected to human diseases (Telomerase Database). TERT is composed of three domains, N-terminal extension that contains RNA-interaction domains 1 and 2 (RID1 and RID2), reverse transcription domain (RT) where nucleotide transfer occurs, and a C-terminal extension (CTE) for processivity and localization.

Mutation nomenclature is based on GenBank accession NM_198253.2 (TERT) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

We classify the TERT c.2812C>T, p.(Arg938Trp) as a variant of uncertain significance (VUS), as there is not currently enough evidence to completely support pathogenicity. It should be noted that there is a strong association between the gene and patient's phenotype, the identified variant is rare in control populations and in silico tools used predict the substitution deleterious. At the moment, screening of the variant should not be used for risk evaluation within family members. However, if family member testing is considered, it could help to gain information needed for further classification of the variant. Management of the patient and family should be based on clinical evaluation and judgment.

<table>
<thead>
<tr>
<th>STEP</th>
<th>DATE</th>
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</thead>
<tbody>
<tr>
<td>Order date</td>
<td>Feb 08, 2017</td>
</tr>
<tr>
<td>Sample received</td>
<td>Mar 08, 2017</td>
</tr>
<tr>
<td>Reported</td>
<td>Mar 29, 2017</td>
</tr>
</tbody>
</table>

On Apr 12, 2017 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:
Coverage plot for sample 10358

Panel type: Interstitial Lung Disease Panel
Number genes: 24
Median coverage: 162
Mean coverage: 184
Percent above 15x coverage: 99.5
APPENDIX 5: SUMMARY OF METHODS

OS-SEQ (SEQUENCE ANALYSIS)

Sequencing. Total genomic DNA was extracted from the biological sample. DNA quality and quantity were assessed using a fluorometric electrophoresis method. Extracted total genomic DNA was mechanically fragmented and enzymatically end-repaired. DNA adapters were added using a ligation-based method and the sequencing library was amplified using PCR. Quality and quantity of the sequencing library DNA were assessed through electrophoresis and fluorometric analyses, respectively. A proprietary Oligonucleotide-Selective Sequencing (OS-Seq) method was used for capturing genomic targets and sequencing was performed using an Illumina sequencing system.

Data analysis. Raw sequence reads were filtered to exclude reads with ambiguous base calls and trimmed from the 3' ends based on base call quality and presence of adapter, poly-A or capture oligo sequences. The remaining high-quality reads were mapped to the human genome reference sequence (Hg19). Single nucleotide variants (SNVs) and short insertions and deletions (INDELs) were identified using a proprietary data analysis pipeline. The pathogenicity of the identified variants was predicted based on the biochemical properties of the codon change and the degree of evolutionary conservatism using PolyPhen, SIFT and Mutation Taster. Identified variants were annotated using allelic frequencies from large population studies (e.g. 1000 Genomes Project and the Exome Aggregation Consortium), by matching with various genotype-phenotype association databases (e.g. ClinVar) as well as by searching from an in-house curated database of previously reported variants.

Interpretation. The clinical evaluation team assessed the pathogenicity of the identified SNV and INDEL variants by evaluating allele frequency, in silico predictions, the annotations from public variant databases and matches in the in-house mutation database and related medical literature. Information in the referral about the patient's phenotype was compared with experimental data in the relevant medical literature to link the identified variants to specific clinical phenotypes. Sequencing data was manually inspected to confirm the variant findings.

Confirmation. SNV and INDEL variant(s) classified as pathogenic or likely pathogenic as well as variants of uncertain significance with quality score <500 were confirmed using direct Sanger sequencing of the PCR amplicons.

Reporting. Reporting was carried out using an HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines.

Notes. This laboratory-developed test has been independently validated by Blueprint Genetics. This test has not been cleared or approved by the FDA. A normal result by this test does not rule out the diagnosis of a disorder since some DNA abnormalities may be undetectable by this 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.

Accreditation. 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.

DEL/DUP (CNV) ANALYSIS

Data analysis. Deletions and duplications (Del/Dups) were detected from the sequence analysis data using a proprietary bioinformatics pipeline, which processes aligned sequence reads provided by the Blueprint Genetics OS-Seq data analysis 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. 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. Identified variants were annotated using data from our in-house curated and maintained database and public databases (1000 Genome Project, Database of Genomic Variants, ExAC and DECIPHER).

Interpretation. The clinical evaluation team assessed the pathogenicity of the identified Del/Dups by reviewing the variant annotations. Clinical relevance of the identified variants was evaluated by relating the findings to the information in the patient referral and reviewing the relevant literature and databases.

Confirmation. Del/Dup variant(s) classified as pathogenic or likely pathogenic were confirmed using a quantitative-PCR assay if they cover less than 10 target exons or the sum of on-target exons and off-target bins (200kb) is < 10 (at least one on-target exon is required).

Reporting. Reporting was done using an HGNC-approved gene nomenclature.

Notes. This laboratory-developed test has been independently validated by Blueprint Genetics. This test has not been cleared or approved by the FDA. A normal result by this test does not rule out the diagnosis of a disorder since some DNA abnormalities may be undetectable by this 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.

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GLOSSARY OF USED ABBREVIATIONS:

**POS** = genomic position of the variant in the format of chromosome:position
**ID** = rsID in dbSNP
**Transcript** = GenBank accession for reference sequence used for variant nomenclature
**Nomenclature** = HGVS nomenclature for a variant in the nucleotide and the predicted effect of a variant in the protein level
**ExAC AC/AN** = allele count/allele number in the Exome Aggregation Consortium Database
**AD** = autosomal dominant
**AR** = autosomal recessive
**OMIM** = Online Mendelian Inheritance in Man®
**ExAC** = Exome Aggregation Consortium Database (>60,000 unrelated individuals)
**het** = heterozygous
**hom** = homozygous
**Del/Dup** = Deletion and Duplication
**CNV** = copy number variation

PolyPhen, SIFT and MutationTaster are in silico prediction tools used to evaluate the significance of identified amino acid changes.