



Early View

Research letter

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Pulmonary fibrosis linked to variants in the *ACD* gene, encoding the telomere protein TPP1

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Summary: Pulmonary fibrosis is associated with mutations in the *ACD* gene, encoding the telomere protein TPP1. Potentially disease-causing variants were found in 5% of a cohort of 60 unrelated patients with pulmonary fibrosis who were referred for genetic screening.

Telomeres are a repetitive DNA sequence at the ends of chromosomes. Telomeres shorten with every cell division, and thus become increasingly short with age. Stable telomeres are necessary for cellular survival, and critically short or dysfunctional telomeres lead to cellular senescence or apoptosis. Mutations in genes encoding telomere-associated proteins can lead to increased telomere shortening or telomere dysfunction.¹ These mutations can cause various disease manifestations, which are termed telomere syndromes.¹

Disease-causing mutations in genes encoding telomere-associated proteins, most commonly *TERT*, *TERC*, *RTEL1* and *PARN*, have been identified in up to 11% of patients with sporadic idiopathic pulmonary fibrosis, and in up to 25% of patients with familial pulmonary fibrosis (≥ 2 first-degree relatives with pulmonary fibrosis).²⁻⁴ Here we studied the *ACD* gene, which encodes the telomere protein TPP1, in patients with pulmonary fibrosis.

Patients and methods

Between October 2017 and December 2018 60 unrelated patients with pulmonary fibrosis from St. Antonius Hospital in Nieuwegein, a referral center for patients with interstitial lung diseases in The Netherlands, underwent genetic screening. Screening was offered in case of a family history of pulmonary fibrosis, clinical suspicion of a telomere syndrome, or relatively young age at presentation (44, 9, and 7 patients, respectively). Clinical suspicion of a telomere syndrome was based on haematological abnormalities such as bone marrow failure or anaemia in 6 patients and liver cirrhosis in 3 patients. The cohort included 18 females, and the median age at the time of genetic screening was 69 years (range 33 - 84).

DNA was isolated from blood samples. Exome sequencing and processing of the data were performed at the University Medical Center Utrecht. After enrichment of the exome with the Agilent SureSelect CREV2 kit (Agilent Technologies, Santa Clara, California, USA), whole exome sequencing was performed on an Illumina Novaseq 6000 sequencer. The Illumina sequencing data was processed with the in-house pipeline, IAP v2.6.1,⁵ including GATK v3.4-46, according to the best practices guidelines.⁶ Results were filtered for exonic variants with a population frequency below 0.5% in 36 genes related to telomere syndromes or pulmonary fibrosis (*ABCA3*, *ACD*, *AP3B1*, *CSF2RA*, *CSF2RB*, *CTC1*, *DKC1*, *FAM111B*, *HPS1*, *HPS4*, *ITGA4*, *LIG4*, *MARS*, *NAF1*, *NKX2-1*, *NOP10*, *PARN*, *POT1*, *RNF168*, *RTEL1*, *SAMD9L*, *SFTPA1*, *SFTPA2*, *SFTPB*, *SFTPC*, *SFTPD*, *STN1*, *TEN1*, *TERC*, *TERF1*, *TERF2*, *TERT*, *TINF2*, *TMEM173*, *USB1*, and *WRAP53*). Leukocyte telomere length was measured using previously described methods.⁷ Briefly, telomere length was estimated for each sample from the ratio of telomere repeat copy number to a single gene (human β -globin gene) copy number (T/S ratio). Measurements were performed on the Bio-Rad CFX96™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). Reference values were derived from a cohort of 164 healthy controls. Pulmonary fibrosis diagnoses were made after multidisciplinary discussion, in accordance with the 2018 ATS/ERS/JTS/ALAT guideline.⁸ All patients gave written informed consent in research project R05-08A.

Results

In 19 patients potentially disease-causing variants in one or more telomere genes were found, including four patients with pathogenic mutations in *TERT* and four patients with pathogenic mutations in *RTEL1*. Potentially disease-causing variants in *ACD* were identified in three patients (Figure 1). The first patient was a 33-year-old female referred to our centre because of dyspnoea on exertion. She had been diagnosed with bone marrow failure at age 25 and had also experienced several transient episodes of gastro-intestinal mucositis, which had not led to a definitive diagnosis, and had improved without specific treatment. She reported having grey hair since she was 21 years old. The family history was only available for her mother and sister, and was not suggestive of a telomere syndrome (Figure 1A). She had a smoking history of eight packyears, but no other fibrogenic exposures. On physical examination, reticular hyperpigmentation of the skin on the neck was noted, but no oral leukoplakia or nail dystrophy. No hepatic abnormalities were seen on abdominal ultrasound. A high-resolution CT-scan (HRCT) of the thorax showed pulmonary fibrosis with honeycombing, but inconsistent with a Usual Interstitial Pneumonia (UIP) pattern. (Figure 1B) The

patient was given a working diagnosis of pulmonary fibrosis in the context of a telomere syndrome. Genetic analysis revealed a heterozygous deletion in *ACD*: c.508-510del; p.(Lys170del) (NM_001082486.1), and no abnormalities in the other tested genes.

The second patient was a 62-year-old male who presented with dyspnoea on exertion. His past medical history revealed no clues suggestive of telomere disease, and he reported no early greying of his hair. His family history was notable for pulmonary and autoimmune diseases in several family members, including pulmonary fibrosis in an uncle (Figure 1A). The patient did not report any significant fibrogenic exposures and had a smoking history of two packyears when he was in his twenties. No skin abnormalities were noted on physical examination, and laboratory investigations revealed normal platelet, erythrocyte and leukocyte counts and no macrocytosis. Pulmonary fibrosis with a probable UIP pattern (Figure 1C) was seen on HRCT, and the patient was given a working diagnosis of idiopathic pulmonary fibrosis (IPF). Genetic screening revealed a heterozygous variant in *ACD*: c.508A>G; p.(Lys170Glu), and no abnormalities in the other tested genes.

The third patient was a 57-year-old male who presented with dyspnoea on exertion and chronic cough. His past medical history was notable for cryptogenic liver cirrhosis since age 24, and he reported grey hair since age 30. His family history revealed no additional clues for a telomere syndrome (Figure 1A). The patient had never smoked, but he worked as an electrician and reported exposure to rockwool and dust at work. On physical examination, vitiligo of his fingers, toes and right lower leg was seen, but no other skin abnormalities were noted. Laboratory investigations revealed mild thrombocytopenia, with normal erythrocyte and leukocyte counts and no macrocytosis. Pulmonary fibrosis with honeycombing, consistent with a UIP pattern (Figure 1D), was seen on HRCT. The patient was given a diagnosis of IPF. Genetic screening revealed a heterozygous variant in *ACD*: c.215C>A; p.(Ala72Glu), and no abnormalities in the other tested genes.

Leukocyte telomere length was below the first percentile for age in all three patients (Figure 1E). Blood samples were not available for the family members of patients 1 and 2. However, genetic analysis could be performed in the parents of patient 3, and the patient's 82-year-old father was found to have the same *ACD*-variant. The father had no signs or symptoms suggestive of telomere disease, but did not undergo additional investigations such as lung function testing or CT-scanning.

Discussion

This study shows that pulmonary fibrosis patients can have heterozygous mutations in *ACD*. In 5% of patients with pulmonary fibrosis referred for genetic screening, (potentially) disease-causing variants were present. *ACD* encodes the shelterin component TPP1, which is responsible for telomerase recruitment to telomeres.⁹ Disease-causing mutations in *ACD* have been previously identified in patients with aplastic anemia and Hoyeraal-Hreidarsson syndrome, the most severe form of dyskeratosis congenita.^{10,11}

The variants that were identified in patients 1 and 2 are located in the OB-fold domain of TPP1, which is the telomerase-interacting domain. The variant identified in patient 3 is located in the N-terminal domain, the function of which is currently not known. The variant in patient 1 is classified as a pathogenic mutation, as the same variant has previously been functionally characterized in patients with a aplastic anemia and Hoyeraal-Hreidarsson syndrome.¹⁰⁻¹² This showed that it disrupts TPP1 binding to telomerase, and the mutation itself is sufficient for causing telomere dysfunction in a human cell line.¹³ The phenotype of patient 1 is of interest because it fits well within the concept of a telomere syndrome but is not a classical case of dyskeratosis congenita despite the young age at presentation and involvement of multiple organs. Because of the extremely short telomeres in patients 2 and 3, as well as liver cirrhosis and early greying consistent with a telomere syndrome phenotype in patient 3, a telomere gene mutation would be a likely explanation for their disease. The *ACD*-variants from patient 2 and 3 were absent in the Exome Sequencing Project, 1000 Genomes Project and the Exome Aggregation Consortium. Also, PolyPhen-2 analysis (at <http://genetics.bwh.harvard.edu/pph2/index.shtml>) predicted both variants to be probably damaging.

Of note, it has been suggested that the qPCR technique is less reliable for measuring telomere length than other techniques.¹⁴

The variant in patient 2 is classified as likely pathogenic,¹² because it concerns the same amino acid, Lys170, as the pathogenic mutation in patient 1. The variant in patient 3 is classified as a variant of unknown significance.¹² The fact that the father of patient 3 was healthy, despite carrying the same *ACD*-variant, would suggest that the variant is not disease-causing. Alternatively, genetic anticipation, where symptoms of a telomere syndrome become more severe and have an earlier onset in each successive generation, occurs in families with telomere syndrome. This allows for incomplete penetrance of telomere syndrome features in the father.¹ Unfortunately, there was no blood sample available for telomere length measurement.

In conclusion, this study shows that pulmonary fibrosis is associated with mutations in the *ACD* gene, encoding the telomere protein TPP1. Potentially disease-causing variants were found in 5% of the patients in this cohort, including two mutations classified as pathogenic (3% of patients).

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Figures

Figure 1: (A) Pedigrees for patients 1-3. Sequencing data is shown below the pedigree for patients 2 and 3. Partially filled squares indicate specific diagnoses: red represents pulmonary fibrosis, blue represents bone marrow failure, and green represents liver cirrhosis. (B-D) Chest high-resolution CT-scans for patients 1-3, respectively, showing pulmonary fibrosis in the basal fields. In patient 1, there was bilateral subpleural reticulation and honeycombing, as well as minimal traction bronchiectasis on the left. The pattern was inconsistent with a UIP pattern because of central extension of the fibrosis as well as airtrapping. The scan in patient 2 showed subpleural reticulation, predominantly in the basal fields, but without honeycombing, consistent with a possible UIP pattern. In patient 3 there was subpleural reticulation with traction bronchiectasis predominantly in the lower fields, as well as honeycombing, consistent with a UIP pattern. Groundglass opacities were noted, but this was not the dominant pattern. (E) Leukocyte telomere length in patients 1-3 compared to a cohort of 164 healthy controls. The uninterrupted line represents the 50th percentile for age. The dashed lines represent the 99th, 90th, 10th and 1st percentiles for age.

