ERJ open research
THE BEST IN OPEN ACCESS BASIC, TRANSLATIONAL & CLINICAL RESPIRATORY RESEARCH

Early View

Original research article

Monogenic gene variants in lung transplant recipients with usual interstitial pneumonia

Christoffer Stark, Juha W. Koskenvuo, Antti Nykänen, Eija H. Seppälä, Samuel Myllykangas, Karl Lemström, Peter Raivio

Please cite this article as: Stark C, Koskenvuo JW, Nykänen A, *et al*. Monogenic gene variants in lung transplant recipients with usual interstitial pneumonia. *ERJ Open Res* 2021; in press (https://doi.org/10.1183/23120541.00583-2021).

Monogenic Gene Variants in Lung Transplant Recipients with Usual Interstitial Pneumonia

Authors and affiliations:

Christoffer Stark, Heart and Lung Center, Helsinki University Hospital and University of Helsinki, Helsinki, Finland

Juha W. Koskenvuo, Blueprint Genetics Ltd, Helsinki, Finland

Antti Nykänen, Heart and Lung Center, Helsinki University Hospital and University of Helsinki, Helsinki, Finland

Eija H. Seppälä, Blueprint Genetics Ltd, Helsinki, Finland

Samuel Myllykangas, Blueprint Genetics Ltd, Helsinki, Finland

Karl Lemström, Heart and Lung Center, Helsinki University Hospital and University of Helsinki, Helsinki, Finland

Peter Raivio, Heart and Lung Center, Helsinki University Hospital and University of Helsinki, Helsinki, Finland

Corresponding author: Christoffer Stark**,** Heart and lung center, Helsinki University Hospital and University of Helsinki, Helsinki, Finland**, address:** Haartmaninkatu 4, 00290 Helsinki, Finland**, phone:** +358504135812**, e-mail:** christoffer.stark@hus.fi

Take home message: A molecular diagnosis is common in lung transplant recipients with usual interstitial pneumonia and are frequently caused by variants in genes related to telomerase function. This finding is not associated with increased risk of allograft dysfunction.

ABSTRACT

Question addressed by the study: The prevalence of monogenic disease-causing gene variants in lung-transplant recipients with idiopathic pulmonary fibrosis is not fully known. Their impact on clinical outcomes before and after transplantation requires more evidence.

Patients and Methods: We retrospectively performed sequence analysis of genes associated with pulmonary fibrosis in a cohort of 23 patients with histologically confirmed usual interstitial pneumonia that had previously undergone double lung transplantation. We evaluated the impact of confirmed molecular diagnoses on disease progression, clinical outcomes and incidence of acute rejection or chronic lung allograft dysfunction after transplantation.

Results: Fifteen patients out of 23 (65 %) had a variant in a gene associated with interstitial lung disease. Eleven patients (48 %) received a molecular diagnosis, of which nine involved genes for telomerase function. Five diagnostic variants were found in the gene for Telomerase reverse transcriptase. Two of these variants, p.(Asp684Gly) and p.(Arg774*), seemed to be enriched in Finnish lung-transplant recipients. Disease progression and the incidence of acute rejection and chronic lung allograft dysfunction was similar between patients with telomere-related disease and the rest of the study population. The incidence of renal or bone marrow insufficiency or skin malignancies did not differ between the groups.

Answer to the question: Genetic variants are common in lung transplant recipients with pulmonary fibrosis and are most often related to telomerase function. A molecular diagnosis for telomeropathy does not seem to impact disease progression or the risk of complications or allograft dysfunction after transplantation.

KEYWORDS

Monogenic, variant, lung, transplantation, rejection, CLAD

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive interstitial lung disease with unknown etiology in most cases [1]. A diagnosis for usual interstitial pneumonia (UIP), the most common type of IPF, is based on typical findings on high-resolution computed tomography (HRCT) and from surgical biopsies [2]. Median survival of patients with IPF is generally less than five years [3]. There is considerable variation in disease progression with sudden exacerbation occurring in some patients [4]. Antifibrotic therapy might improve survival and slow down progression but so far, there are no curative treatment strategies for IPF [5]. Lung transplantation is often the only viable treatment option for these patients. Globally, 22-50 % of lung transplantations are performed for interstitial lung disease [1, 6, 7]. After lung transplantation, rejection and chronic lung allograft dysfunction (CLAD) are the main reasons for graft loss and mortality [8].

The cellular mechanisms responsible for disease progression in IPF are sustained epithelial injury, accelerated epithelial aging, and promotion of interstitial fibrosis by interplay between inflammatory cells and myofibroblasts. Factors such as cigarette smoke, air pollution, microaspiration, and infectious agents are possible triggers for these effects [1, 9]. Genetic factors contribute to the pathogenesis of IPF either via Mendelian inheritance or by increasing disease susceptibility via complex mechanisms. Monogenic disease forms are most commonly associated with genes regulating telomerase function [10]. Telomerase is responsible for maintaining telomere length. Shortened telomeres can lead to several clinical phenotypes and the most common manifestation of adult-onset telomere-disease is pulmonary fibrosis [11].

Utilization of genetic testing is increasing even though it is not routinely used in the clinical work-up of patients with suspected or confirmed IPF nor in patients listed for lung transplantation. Identification of a molecular diagnosis for IPF might allow earlier diagnosis and therapeutic interventions, as well as offer possibilities for genetic counselling and familial risk assessment. Surgical biopsies could also be avoided in patients with a clear molecular IPF diagnosis [12]. A

molecular diagnosis of telomeropathy has been associated with bone marrow failure and liver dysfunction post-transplant, but there is conflicting evidence whether pathological genetic variants affects allograft dysfunction [13, 14]. Our objective was to clarify monogenic disease burden in lung transplant recipients and to identify whether genetics influence disease progression or the incidence of acute rejection and CLAD after transplantation.

MATERIAL AND METHODS

Study subjects, study design and patient management

For this study, we included IPF patients who underwent *de novo* lung transplantation at Helsinki University Hospital between January 2007 and December 2015. Patients had to be alive at the time of recruitment (January 2019) in order to donate DNA samples. We identified 59 transplant recipients meeting these criteria of which 38 patients had histologically confirmed UIP. These patients were invited to participate in the study. Twenty-four patients gave their written informed consent and donated saliva for DNA extraction and analysis. One sample was contaminated, and DNA extraction was not possible. For final analysis, 23 patients were included and followed until December 2019. The ethical review board at Helsinki University Hospital approved the study protocol (permission nr. HUS/2266/2016). All transplantations were performed adhering to the principles of the Declaration of Helsinki.

Patients were managed as previously described [7]. In brief, the patients received triple-drug primary immunosuppression consisting of a calcineurin inhibitor, mycophenolate mofetil (MMF) and corticosteroids. Dosing of the immunosuppressive drugs were adjusted for renal and bone marrow insufficiency during follow-up as necessary. At our institution, we do not routinely use immunosuppressive induction therapy and none of the patients in this study, received induction. Glomerular filtration rates (GFR) were measured by clearance of (99)Tc-DTPA preoperatively and

during follow-up. Routine controls were performed at regular intervals for the first year after transplantation and included spirometry, bronchoalveolar lavage and transbronchial lung biopsies. Rejection and CLAD were classified and treated according to standard protocol [7].

Genetic analysis

A saliva sample was given by all patients for genetic testing. Sequence analysis and copy number variation analysis of 77 genes associated with interstitial lung disease was performed at an independent laboratory (Blueprint Genetics, Helsinki, Finland). In Supplement 1, a gene list including inheritance mode and variant count of each gene in the Human Gene Mutation Database (HGMD) and ClinVar mutation database, is presented. Methods for DNA extraction and sequence analysis is described in Supplement 2.

Genetic variants were classified according to the Association for Molecular Pathology/American College of Molecular Genetics and Genomics guidelines [15]. Relying on the genetic standards, a patient was considered to have a molecular diagnosis when a single pathogenic or likely pathogenic variant was seen as heterozygous in a gene associated with dominant disease or when a pathogenic or likely pathogenic pair was seen as homozygous, or compound heterozygous in a gene associated with recessive disease. Variants of uncertain significance (VUS) were considered highly suspicious based on: 1) a strong and specific correlation between the gene and patient's phenotype, 2) the variant being novel or extremely rare in the genome aggregation database (gnomAD) or control cohorts, and 3) *in silico* predictions supporting pathogenicity or the amino acid position in question being highly conserved in mammals and evolutionary more distant species suggesting that the position does not tolerate variation.

Statistical analysis

For patient data analysis, the following statistical tests were used: Fischer's exact test for single comparative analysis of categorical variables and contingency analysis, unpaired t-test for continuous variables and Log rank test for analyzing event-free survival.

RESULTS

A molecular diagnosis is established in almost half of lung transplant recipients

Fifteen of the 23 patients (65 %) had a total of 20 rare variants that potentially contributed to the patients' phenotypes (Table 1). Of these variants, six were classified as pathogenic, six as likely pathogenic, and three as VUSs. Eleven of the 23 analyzed patients (48 %) received a molecular diagnosis for their IPF (Table 1 – **bolded patients**).

Nine patients with molecular diagnosis had a telomere-related disease; five (45 %) of the diagnostic variants were in the Telomerase Reverse Transcriptase (*TERT*) gene, two (18%) in the Poly(A)-Specific Ribonuclease (*PARN*) gene and one (9 %) each in the Dyskerin Pseudouridine Synthase 1 (*DKC1*) and the Telomeric Repeat Binding Factor 1 Interacting Nuclear Factor 2 (*TINF2*) genes. The patient with a pathogenic variant in *TINF2* had a second mosaic variant in the same gene. One patient had a homozygous frameshift variant, in the gene for Hermansky-Pudlak Syndrome Protein 1 (*HPS1*), confirming its diagnosis. One patient was heterozygous for variants in genes for Endothelin 3 (*EDN3*) and Surfactant Protein A2 (*SFTPA2*).

The remaining cases were heterozygous variants in the genes Serpin Family A Member 1 (*SERPINA1*) and Dynein Axonemal Heavy Chain 9 (*DNAH9*), which are associated with recessive inheritance. None of the patients with *SERPINA1* variants had radiographic signs of emphysema

before lung transplantation. One patient had a VUS in the gene for Sodium Channel Protein type 4

Patient	Gene (inheritance)	Chromosome position	Variant	Genotype	gnomAD (n)	Age at onset	Classification
1	***TERT*** (AD/AR)	**5:1279485**	**c.2051A>G, p.(Asp684Gly)**	**HET**	**29**	**56**	**Pathogenic**
2	***TERT*** (AD/AR)	**5:1279485**	**c.2051A>G, p.(Asp684Gly)**	**HET**	**29**	**56**	**Pathogenic**
	SERPINA1 (AR)	14:94844947	c.1096G>A, p.(Glu366Lys)	HET	3054/25		Pathogenic
3	***TERT*** (AD/AR)	**5:1272362**	**c.2320C>T, p.(Arg774*)**	**HET**	**19**	**57**	**Pathogenic**
	SERPINA1 (AR)	14:94847262	c.863A>T, p.(Glu288Val)	HET	6136/132		Pathogenic
4	**TERT (AD/AR)**	**5:1272362**	**c.2320C>T, p.(Arg774*)**	**HET**	**19**	**55**	**Pathogenic**
5	**TERT (AD/AR)**	**5:1264594**	**c.2768C>T, p.(Pro923Leu)**	**HET**	**1**	**63**	**Likely pathogenic**
6	**DKC1 (XLR)**	**X:153999083**	**c.965G>T, p.(Arg322Leu)**	**HEM**	**0**	**47**	**Likely pathogenic**
	TERT (AD/AR)	5:1279562	c.1974G>A, p.(Val658=)	HET	11		VUS
7	**PARN (AD)**	**16:14704440-14704726**	**c. (388+1_389-1)_(554+1_555-1)del**	**HET**	**0**	**53**	**Likely pathogenic**
8	**PARN (AD)**	**16:14540684-14576744**	**c.(1480+1_1481-1)_(1864+1_1865-1)del**	**HET**	**0**	**55**	**Likely pathogenic**
9	**TINF2 (AD)**	**14:24709846**	**c.840G>C, p.(Lys280Asn)**	**HET**	**0**	**59**	**Pathogenic**
	TINF2 (AD)	14:24709920	c.766C>T, p.(Arg256*)	Mosaic	0		Likely pathogenic
10	**HPS1 (AR)**	**10:100186986**	**c.972del, p.(Met325Trpfs*6)**	**HOM**	**26**	**60**	**Pathogenic**
11	**EDN3 (AD)**	**20:57876506**	**c.95_106delinsCA, p.(Gly32Alafs*174)**	**HET**	**0**	**43**	**Likely pathogenic**
	SFTPA2 (AD)	10:81317087	c.622_624del, p.(Tyr208del)	HET	0		VUS
12	DNAH9 (AR)	17:11597289	c.4719T>G, p.(Tyr1573*)	HET	57	57	Likely pathogenic
13	SCN4A (AD)	17:62022079	c.3866T>C, p.(Leu1289Pro)	HET	0	65	VUS
14	SERPINA1 (AR)	14:94844947	c.1096G>A, p.(Glu366Lys)	HET	3054/25	51	Pathogenic
15	SERPINA1 (AR)	14:94844947	c.1096G>A, p.(Glu366Lys)	HET	3054/25	59	Pathogenic

Subunit Alpha (SCN4A); this patient did not show a phenotype indicating genetic disease.

Table 1. Genetic variants in lung transplant recipients with usual interstitial pneumonia.

AD = Autosomal dominant, AD/AR = Autosomal dominant and recessive, AR = Autosomal recessive, XLR = X-linked recessive, Chr = chromosome. c. = coding DNA sequence, p. = protein sequence, > = substitution, * = termination codon, "=" = synonymous variant, del = deletion, fs = frameshift, delins = deletion/insertion. HET = heterozygote, HOM = homozygote, HEM = hemizygote. gnomAD = genome aggregation database, numbers indicate reported heterozygote/homozygote cases. Classifications follow ACMG criteria: Pathogenic, Likely

pathogenic, VUS = variant of uncertain significance. **Variants considered molecular diagnoses are bolded**. Patients 1-9 had telomere-related disease.

Enrichment of *TERT* variants in patients with telomere-related disease and IPF

Two of the observed *TERT* variants seem to be enriched in Finnish IPF patients. There are 29 individuals heterozygous for the missense variant c.2051A>G, p.(Asp684Gly) among 52188 (0.06%) individuals in gnomAD. The variant is relatively common especially in Finland as there are 27 heterozygous individuals among 9720 (0.28%) Finnish individuals. We found 2 heterozygote cases of this variant, suggesting that it is enriched in lung transplant patients with UIP (OR 32.7, 95 % CI 7,5-124,4, p=0.0021) when compared to the Finnish allele frequency in gnomAD. Previously, the p.(Asp684Gly) variant has been observed as heterozygous in 9.5% of the patients who underwent genetic testing for interstitial lung disease (unpublished results, Blueprint Genetics) suggesting significant enrichment compared to Finnish controls (OR 35.7, 95 % CI 14.6-82.7, p<0.0001).

There are 19 individuals heterozygous for the variant *TERT* c.2320C>T, p.(Arg774*) in gnomAD. Interestingly, the highest allele frequency is seen in Finland as 16 out of 12,775 (0.13%) Finnish controls have the variant. Based on the allele frequency in this study (2/46), there is enrichment of this variant in Finnish lung transplant recipients with UIP (OR 72.5, 95 % CI 16.1-273.8, p=0.0005). Previously, the variant allele was found in 10.5% of Finnish patients with interstitial lung disease (unpublished results, Blueprint Genetics). This data supports that this nonsense variant is significantly enriched in these patients (OR 86, 95% CI 33-224, p<0.00001).

Lung transplant recipients with telomere and non-telomere disease have similar outcomes

We compared patients with telomere disease-causing variants (n=9) and patients with other pathogenic variants or without genetic findings (n=14). Basic clinical characteristics for the two groups are shown in table 2. Patients' ages at the time of diagnosis and transplantation were similar. Telomere-related cases were found in men only, while 64 % of patients were male in the non-telomere

disease group (p=0.12). One patient with *TERT* c.2320C>T, p.(Arg774*) had a brother with confirmed IPF. Two other patients had a positive family history for IPF but no genetic variant finding in this study (p=0.99). The incidence of comorbidities was similar between the groups.

Table 1. Clinical baseline characteristics of lung transplant recipients with telomere-related and non-telomere-related usual interstitial pneumonia.

	Telomere-related (n=9)	Non-telomere-related (n=14)	p-value
Age at Dg, years (min-max)	55±4 (47-63)	55±9 (33-68)	0.88
Age at Tx, years (min-max)	59±5 (50-65)	59±9 (39-70)	0.89
Male gender (%)	9 (100)	9 (64)	0.12
History of Smoking (%)	7 (78)	10 (71)	0.99
Family history for IPF (%)	1 (11)	2 (14)	0.99
Hypertension (%)	4 (44)	5 (36)	0.99
Hyperlipidemia (%)	2 (22)	1 (7)	0.54
Diabetes (%)	2 (22)	0 (0)	0.14
CAD (%)	1 (11)	1 (7)	0.99
Asthma (%)	1 (11)	0 (0)	0.39
Sarcoidosis (%)	1 (11)	0 (0)	0.39
Psoriasis (%)	0 (0)	1 (7)	0.99
Rheumatoid arthritis (%)	0 (0)	1 (7)	0.99
Renal insufficiency (%)*	0 (0)	1 (7)	0.99

Data presented as mean ± SD or n. Dg = diagnosis, Tx = transplantation. CAD = coronary artery disease. * GFR < 60 ml/min/1.73m2 was classified as renal insufficiency.

Outcomes are presented in table 3. Time from diagnosis to lung transplantation was 1100±629 days for patients with telomere-related disease and 1346±684 days for the others (p=0.40). Transplant waiting list times and follow-up times were comparable between the groups. During follow-up all 9 patients (100 %) with telomeropathy developed renal insufficiency. Of these, 8 were mild (GFR 30-60 ml/min/1.73m2) and only 1 severe (GFR < 30 ml/min/1.73m2). In the other group, there were 13 cases (93 %) of new onset mild renal insufficiency of which one was severe. Leukopenia after the transplant hospitalization was observed in 8 (57 %) patients in non-telomere related cases and in 5 (56 %) patients with telomeropathy (p=0.99). Only one patient (7 %) without telomere-related

disease required permanent withdrawal of MMF. The other cases of leukopenia resolved with dose reduction of MMF or were related to cytomegalovirus reactivation and antiviral therapy. The only malignancies observed in our patients were epidermoid carcinomas of the skin. Its incidence was not statistically different between the groups (44 % vs 14 %, p=0.16). Histopathological acute rejection during the first year after transplantation (33 % vs. 57 %, p=0.40) and development of CLAD (33 % vs. 43 %, p=0.99) occurred at a similar rate in both groups. Log-rank test failed to show significant differences for the probability of acute rejection (p=0.32) or CLAD (p=0.42) between the groups.

Table 2. Outcome of lung transplant recipients with telomere-related and non-telomere-related usual interstitial pneumonia.

	Telomere-related (n=9)	Non-telomere-related (n=14)	p-value
Dg – Tx, days (min-max)	1100±629 (439-2460)	1346±684 (87-2766)	0.40
List time, days (min-max)	82±99 (1-321)	82±145 (0-517)	0.99
Follow-up, days (min-max)	2920±791 (1921-4421)	2473±1026 (1509-4624)	0.28
New onset chronic renal insufficiency (%)	9 (100)	13 (93)	0.99
MMF treatment stopped (%)	0 (0)	1 (7)	0.99
Skin malignancies (%)	4 (44)	2 (14)	0.16
Histopathological rejection (%)	3 (33)	8 (57)	0.40
Time to rejection, days (min-max)	59±15 (47-76)	73±49 (31-176)	0.63
CLAD (%)	3 (33)	6 (43)	0.99
Time to CLAD, days (min-max)	1475±329 (1102-1724)	1209±536 (469-1832)	0.46

Data presented as mean ± SD or n. Dg – Tx = time from diagnosis to transplantation, CLAD = chronic lung allograft dysfunction. MMF = mycophenolate mofetil.

DISCUSSION

Our results suggest that almost half of the patients with histologically confirmed UIP receiving lung transplants in Finland have a genetic diagnosis possibly contributing to the development of IPF. Thirty-nine (39 %) percent of the patients had a molecular diagnosis of telomere disease. A recent

report discovered telomere disease-causing variants by using exome sequencing in only 11.8% of lung-transplant recipients [13]. The difference probably highlights globally variable indications for lung transplantation, population differences in genetic contribution and differences between the genetic assays to identify difficult-to-detect genetic variants. We only included patients with confirmed histopathological UIP, and it is possible that genetic variants are overrepresented in this group compared to populations with other types of IPF. In Finland, 50 % of lung transplantations are performed on patients with interstitial lung disease [7], while the number in North America ranges from 22 - 32 % [6, 12]. The penetrance of telomere-disease related variants is variable. In this study, we did not measure telomere lengths, which could have provided further evidence that there is a causative relationship between the observed genetic variant and the patients' phenotypes. It is, however, important to point out that patients can express normal pulmonary phenotypes although their telomeres are shortened [16, 17]. In addition, patients with IPF often have shortened telomeres even without telomere-related genetic disease [17].

Interestingly, only one of the 11 patients with a molecular diagnosis had a positive family history for IPF, whereas the molecular diagnoses of the remaining 10 patients were novel. Sporadic variants have previously been observed in only one out of ten IPF patients [16, 17]. It is possible that some familial cases in this study have previously been undiagnosed or that genetic contribution is underestimated or remains undiscovered in general since genetic testing is not utilized routinely in the work-up of these patients.

Our study provides statistical evidence that *TERT* p.(Asp684Gly) and p.(Arg774*) are the most common missense and nonsense variants related to IPF, and that both of the variants are highly enriched in the Finnish population. This may indicate that telomere-related IPF may be more common in Finland than in other countries, but this hypothesis needs further testing by assessing the penetrance of these variants. *TERT* variants are found in 8-15% of patients with autosomal dominant familial pulmonary fibrosis [16, 18]. Disease caused by mutations in *TERT* are inherited in both autosomal

dominant and recessive manners but is considered dominant with reduced penetrance in relation to pulmonary fibrosis [17]. In the current study, 26% of patients had heterozygous *TERT* variants. The *TERT* p.(Asp684Gly) variant has previously been observed in 1 homozygous and 2 heterozygote individuals out of 135 patients analyzed for suspected telomere-related disease [19].

Genetic variants for *PARN* are associated with impaired telomere maintenance and are found in approximately 5 % of cases with familial pulmonary fibrosis [18]. To our knowledge, the observed *PARN* deletions here, have not been previously published in the relevant medical literature or reported in the disease-related variation databases. There are, however, multiple multi-exon and whole-gene deletions listed in HGMD in patients with telomere shortening related disease. For *PARN* c.(1480+1_1481-1)_(1864+1_1865-1)del, in this study, there is a partially overlapping *de novo* deletion (exons 23-24) identified previously in a patient with bone marrow failure and neurological dysfunction [20].

TINF2 encodes a protein that converts uridine to pseudouridine in ribosomal RNA and is part of a complex protecting telomere ends. In familial pulmonary fibrosis, *TINF2*-variants are observed in 0.6 % of cases. In this study, one patient had two variants for *TINF2*. The missense variant c.840G>C, p.(Lys280Asn) has previously been associated with dyskeratosis congenita [21]. The other c.766C>T, p.(Arg256*) variant was present only in part of the gene reads, suggesting that it was mosaic. Since the DNA sample was extracted from saliva, we could not verify whether the variant was germline mosaic or a somatic mutation. The mosaic variant causes a premature stop codon and loss of normal protein function either through protein truncation or nonsense-mediated mRNA decay from the other allele and might modify the biological mechanism of the other missense variant for this patient as it prevents translation of the other pathogenic missense variant

HPS1 encodes a transmembrane protein that forms a lysosomal complex together with Hermansky-Pudlak syndrome 4 protein. Variants in *HPS1* are associated with autosomal recessive Hermansky-Pudlak syndrome. The syndrome is characterized by albinism, bleeding diathesis, immunodeficiency,

and by pulmonary fibrosis. The variant observed here has previously been found as homozygous in 1 patient with HPS [22]. In HPS, life-threatening manifestations, such as restrictive lung disease occur usually starting from the fourth decade of life, there is, however, large variation in disease severity possibly related to genetic anticipation.

SFTPA2 encodes surfactant protein A2. Surfactant-related genetic variants are thought to increase cellular damage by causing endoplasmic reticulum stress and genetic variants have been associated with autosomal dominant idiopathic pulmonary fibrosis [23]. The observed variant has not previously been reported, but there was strong association between the mutation and the patients' phenotype. The same patient had a variant for *EDN3*, encoding endothelin 3. Variants in this gene have been associated with congenital central hypoventilation syndrome and Waardenburg syndrome. Waardenburg syndrome is characterized by pigmentation abnormalities, hearing loss, musculoskeletal abnormalities and Hirschprung disease [24]. It has not previously been linked to pulmonary fibrosis in the literature but might explain IPF through hypoventilation-related mechanisms. The variant generates a frameshift leading to a premature stop codon in a new reading frame. It is predicted to cause loss of normal protein function either through protein truncation or nonsense-mediated mRNA decay. To our knowledge, the variant has not been published in the relevant medical literature or reported in the disease-related variation databases. Disease caused by *EDN3* variants are inherited in both autosomal recessive and dominant manners. The variant was considered likely pathogenic considering the established association between the gene and patient's phenotype, rarity in control populations and frameshift mutation type.

DNAH9 encodes a microtubule-associated motor protein. Variants in this gene are associated with primary ciliary dyskinesia and several congenital defects. It is however, inherited in an autosomal recessive manner and does not cause disease without another disease-causing variant in the same gene [25]. Variants in *SCN4A*, encoding alpha subunits of sodium channels have been associated with disease that causes myotonia or periodic paralysis, and they are inherited in both autosomal recessive

and dominant manners. The phenotype is highly variable and disease onset usually occurs in infancy or early childhood. Muscle and soft tissue symptoms are the most common manifestations [26]. Inspiratory stridor in early life is the only related respiratory disorder that has been reported previously [27].

The variants for *SERPINA1* in this study were heterozygous and are not able to cause disease without a second disease causing variant in the same gene. The p.(Glu366Lys) variant removes a salt bridge to Lys290 and a hydrogen bond to Thr203, causing misfolding of the protein within the endoplasmic reticulum, which results in a lack of secretion from hepatocytes and a reduction of plasma alpha-1-antitrypsin levels to 10-15% of normal in homozygotes [28] and to 61-77% in heterozygotes [29, 30]. Heterozygous carrier of p.(Glu366Lys) may have moderately decreased to normal serum alpha-1 antitrypsin levels and could therefore influence pulmonary phenotype.

In this study, we analyzed the incidence of monogenetic disease variants and did not include analysis for common gene variants and genes associated with multigenic susceptibility to IPF. Because of the study design, we did not include survival as an endpoint. We investigated possible impact of telomere-related variants on disease progression before transplantation and the incidence of acute rejection and CLAD after transplantation. Previously, these variants have been associated with several complications after transplantation but their impact on CLAD development or survival has been controversial [10, 13, 14]. Based on our results, the risk of developing CLAD and time to CLAD onset was not different between patients with telomere-related and non-telomere-related disease. Patient age at disease onset or transplantation, or the time from diagnosis to transplantation did not differ between the groups. This indicates that disease progression was not related to telomeropathy. Leukopenia or other forms of bone marrow dyscrasia is frequent after lung transplantation [13, 14]. We failed to show any association between bone marrow dysfunction and telomere related IPF. The overall incidence of postoperative renal dysfunction was more common in this study than in many previous reports, but it was not related to telomere disease. The only malignancies observed in this

study, were epidermoid skin carcinomas and they occurred at a similar rate as previously reported [13, 14], with no significant overrepresentation in telomeropathy patients.

This study has several limitations. It was a retrospective study with small sample-size. Patient selection bias is possible because we only included patients that were eligible for lung transplantation and were alive several years after transplantation. These results are necessarily not generalizable to all IPF patients. All patients, in this study, were included based on the same inclusion criteria and the two study groups are thereby comparable.

CONCLUSIONS

Sporadic monogenic variants were found in a higher proportion of lung transplant recipients with IPF than previously reported. We identified two variants in the *TERT* gene that might be enriched in Finnish patients with IPF. This finding could have diagnostic and therapeutic implications in the future but requires further confirmation. In this study, Lung transplant recipients with molecular diagnoses for telomere-related disorders seemed to have similar outcomes as patients without confirmed genetic disease susceptibility or non-telomere-related genetic IPF.

AUTHOR CONTRIBUTIONS

CS recruited the study patients. CS, AN, KL and PR gathered patient data. DNA analysis and interpretation was performed by JK, ES and SM. CS wrote the manuscript. All authors edited and approved the final manuscript.

CONFLICTS OF INTEREST

JK, ES and SM are full-time employees at Blueprint Genetics Ltd. The authors have no others conflicts of interest to declare.

FUNDING

This work was funded by grants from Helsinki University Hospital (Grant n:o Y2019SK017) and Suomen transplantaatiokirurginen yhdistys.

REFERENCES

1. Lederer D, Martinez F. Idiopathic Pulmonary Fibrosis. N Engl J Med 2018;378:1811-23.
2. Raghu G, Remy-Jardin M, Myers J et al. Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. Am J Respir Crit Care Med 2018;198:e44–e68.
3. Ley B, Collard HR, King TE. Clinical course and prediction of survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2011;183:431–440.
4. Kolb M, Bondue B, Pesci A, et al. Acute exacerbations of progressive-fibrosing interstitial lung diseases. Eur Respir Rev 2018;27:180071.
5. Raghu G, Rochwerg B, Zhang Y, et al. An official ATS/ERS/JRS/ALAT clinical practice guideline: treatment of idiopathic pulmonary fibrosis: an update of the 2011 clinical practice guideline. Am J Respir Crit Care Med 2015;192:e3–e19.
6. Christie J, Edwards L, Kucheryavaya A et al. The Registry of the International Society for Heart and Lung Transplantation: Twenty-seventh official adult lung and heart-lung transplant report. J Heart Lung Trans 2010;10:P1104-1118.
7. Nykänen A, Raivio P, Peräkylä L et al. Incidence and impact of chronic lung allograft dysfunction after lung transplantation – single-center 14-year experience. Scand Cardiovasc J 2020;54:192-199.
8. Chambers DC, Cherikh WS, Goldfarb SB, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: thirty-fifth adult lung and heart-lung transplant report-2018; focus theme: multiorgan transplantation. J Heart Lung Transplant 2018;37:1169–1183.
9. Yanagihara T, Sato S, Upagupta C, et al. What have we learned from basic science studies on idiopathic pulmonary fibrosis? Eur Respir Rev 2019;28:190029.
10. Newton C, Molyneaux P and Oldham J. Clinical Genetics in Interstitial Lung Disease. Front Med. 2018;5:116.

11. Arish N, Petukhov D and Wallach-Dayan S. The Role of Telomerase and Telomeres in Interstitial Lung Diseases: From Molecules to Clinical Implications. Int. J Mol Sci 2019; 20:2996.
12. Moua T, Lee A and Ryu J. Comparing effectiveness of prognostic tests in idiopathic pulmonary fibrosis. Expert Rev Respir Med 2019;13:993-1004.
13. Swaminathan A, Neely M,Frankel C et al. Lung Transplant Outcomes in Patients With Pulmonary Fibrosis With Telomere-Related Gene Variants. Chest 2019;156:477-485.
14. Tokman S, Singer J, Devine M et al. Clinical Outcomes of Lung Transplantation in Patients with Telomerase Mutations. J Heart Lung Transplant. 2015;34:1318–1324.
15. Richards S, Aziz N, Bale S et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405-24.
16. Armanios M, Chen J, Cogan J et al. Telomerase Mutations in Families with Idiopathic Pulmonary Fibrosis. N Engl J Med 2007;356:1317-26.
17. Courtwright A and El-Chemaly S. Telomeres in Interstitial Lung Disease: The Short and the Long of It. Ann Am Thorac Soc 2019;16:175–181.
18. Borie R, Kannengiesser C, Sicre de Fontbrune F, et al. Management of suspected monogenic lung fibrosis in a specialised centre. Eur Respir Rev 2017;26:160122.
19. Nordberg A, Rosén A, Raaschou-Jensen K et al. Novel variants in Nordic patients referred for genetic testing of telomere-related disorders. European Journal of Human Genetics 2018;26:858–867.
20. Dhanraj S, Gunja SMR, Deveau AP, et al. Bone marrow failure and developmental delay caused by mutations in poly(A)-specific ribonuclease (PARN). J Med Gen 2015;52:738-748.

21. Savage SA, Giri N, Baerlocher GM, Orr N, Lansdorp PM, Alter BP. TINF2, a component of the shelterin telomere protection complex, is mutated in dyskeratosis congenita. Am J Hum Genet 2008;82:501-9.
22. Oh J, Ho L, Ala-Mello S et al. Mutation Analysis of Patients with Hermansky-Pudlak Syndrome: A Frameshift Hot Spot in the HPS Gene and Apparent Locus Heterogeneity. Am J Hum Genet 1998;62:593–598.
23. Wang Y, Kuan P, Xing C et al. Genetic Defects in Surfactant Protein A2 Are Associated with Pulmonary Fibrosis and Lung Cancer. Am J Hum Gen 2009;84:52–59.
24. Pingault V, Ente D, Dastot-Le Moal F et al. Review and Update of Mutations Causing Waardenburg Syndrome. Hum Mutat 2010;31:391–406.
25. Loges N, Antony D, Mayer A et al. Recessive DNAH9 Loss-of-Function Mutations Cause Laterality Defects and Subtle Respiratory Ciliary-Beating Defects. Am J Hum Gen 2018;103: 995–1008.
26. Dupré N, Chrestian N, Bouchard JP, Rossignol E, Brunet D, Sternberg D, Brais B, Mathieu J, Puymirat J. Clinical, electrophysiologic, and genetic study of non-dystrophic myotonia in French-Canadians. Neuromuscul Disord 2009;19:330-4.
27. Matthews E, Manzur A, Sud Y et al. Stridor as a neonatal presentation of skeletal muscle sodium channelopathy. Arch Neur 2011;68:127-129.
28. Hughes VA, Meklemburg R, Bottomley SP, Wintrode PL (2014) The Z Mutation Alters the Global Structural Dynamics of a1-Antitrypsin. PLoS ONE 9:e102617.
29. Calapoglu M, Deger O, Balaban F et al. Analysis of the alpha-1-antitrypsin deficient alleles M3S, MX, and ZZ by biochemical and molecular methods: a family study. Biochem Genet 2009;47:33-41.
30. Bornhorst J, Greene D, Ashwood E et al. α-1-antitrypsin phenotypes and associated serum protein concentrations in a large clinical population.Chest 2013;143:1000-1008.

Supplement 1.

Gene	Associated phenotypes	Inheritan	ClinVar	HGMD
ABCA3	Interstitial lung disease, Surfactant metabolism dysfunction, pulmonary	AD/AR	11	287
ARHGEF1	Idiopathic bronchiectasis, Immunodeficiencies with antibody defects	AR		1
C11ORF70	Primary ciliary dyskinesia	AR		5
CCDC39	Ciliary dyskinesia	AR	39	47
CCDC40	Ciliary dyskinesia	AR	33	43
CFTR	Cystic fibrosis, Congenital bilateral absence of the vas deferens	AD/AR	518	1803
CHAT	Myasthenic syndrome, congenital	AR	24	73
CHRNA1	Myasthenic syndrome, congenital	AD/AR	28	35
CHRNB1	Myasthenic syndrome	AD/AR	11	11
CHRND	Myasthenic syndrome	AD/AR	18	26
CHRNE	Myasthenic syndrome	AD/AR	48	134
COLQ	Myasthenic syndrome, congenital	AR	23	67
CSF2RA#*	Surfactant metabolism dysfunction, pulmonary	XL	2	17
CSF2RB	Surfactant metabolism dysfunction, pulmonary, 5	AR	2	6
DKC1	Hoyeraal-Hreidarsson syndrome, Dyskeratosis congenita	XL	48	74
DNAAF1	Ciliary dyskinesia	AR	19	38
DNAAF2	Ciliary dyskinesia	AR	13	6
DNAH1	Spermatogenic failure 18	AR	15	32
DNAH11*	Ciliary dyskinesia	AR	66	130
DNAH5	Ciliary dyskinesia	AR	140	197
DNAH9	Primary ciliary dyskinesia	AR		6
DNAI1	Ciliary dyskinesia	AR	17	35
DNAI2	Ciliary dyskinesia	AR	19	6
DNAL1	Ciliary dyskinesia	AR	3	1
EDN3	Hirschsprung disease, Central hypoventilation syndrome, congenital, Waardenburg syndrome	AD/AR	7	21
EFEMP2	Cutis laxa	AR	14	16
ELMOD2	Familial idiopathic pulmonary fibrosis	AD/AR		
ELN	Cutis laxa, Supravalvular aortic stenosis	AD	78	113
FAM111B*	Hereditary Fibrosing Poikiloderma with Tendon Contracture, Myopathy, and Pulmonary Fibrosis, Lung cancer, familial, susceptibilty to	AD	7	7
FBLN5	Cutis laxa, Macular degeneration, age-related	AD/AR	13	22
FLCN	Birt-Hogg-Dube syndrome, Pneumothorax, primary spontaneous	AD	154	210
FOXF1	Alveolar capillary dysplasia with misalignment of pulmonary veins	AD	10	102
GAS2L2	Primary ciliary dyskinesia	AR		3
GAS8	Ciliary dyskinesia, primary, 33	AR	4	6
GLRA1	Hyperekplexia	AD/AR	39	69
HPS1*	Hermansky-Pudlak syndrome	AR	28	55
HPS4	Hermansky-Pudlak syndrome	AR	16	22
ITGA3	Interstitial lung disease with nephrotic syndrome and epidermolysis bullosa	AR	6	11
LTBP4	Cutis laxa with severe pulmonary, gastrointestinal, and urinary abnormalities	AR	10	17
MCIDAS	Primary ciliary dyskinesia	AR	4	3
MECP2	Angelman-like syndrome, Autism, Rett syndrome, Encephalopathy, Mental retardation	XL	506	1039
NAF1		AD		2
NF1*	Watson syndrome, Neurofibromatosis, Neurofibromatosis-Noonan syndrome	AD	1157	2901
NKX2-1	Thyroid cancer, nonmedullary, Choreoathetosis, hypothyroidism, and neonatal respiratory distress, Chorea, hereditary benign	AD	27	137
NME8	Ciliary dyskinesia	AR	1	6
PARN*	Pulmonary fibrosis and/or bone marrow failure, Dyskeratosis congenita	AD/AR	15	29
PHOX2B	Central hypoventilation syndrome, congenital, Neuroblastoma, susceptiblity to, Neuroblastoma with Hirschsprung disease	AD	11	86
PIH1D3	Ciliary dyskinesia, primary, 36	XL	2	12
POLD1	Colorectal cancer, Mandibular hypoplasia, deafness, progeroid features, and lipodystrophy syndrome, Idiopathic bronchiectasis, Immunodeficiency	AD/AR	3	31
RAPSN	Myasthenic syndrome, congenital	AR	26	58
RET	Hirschsprung disease, Central hypoventilation syndrome, congenital, Pheochromocytoma, Medullary thyroid carcinoma, Multiple endocrine neoplasia	AD/AR	122	407
RSPH3	Ciliary dyskinesia, primary, 32	AR	7	5
RSPH4A	Ciliary dyskinesia	AR	18	24
RSPH9	Ciliary dyskinesia	AR	8	12
RTEL1	Pulmonary fibrosis and/or bone marrow failure, Dyskeratosis congenita	AD/AR	58	51
SCN4A	Hyperkalemic periodic paralysis, Myotonia, potassium-aggravated, Paramyotonia congenita, Myasthenic syndrome, congenital, Normokalemic potassium-sensitive periodic paralysis	AD/AR	57	126
SCNN1A	Pseudohypoaldosteronism, Bronchiectasis with or without elevated sweat chloride	AD/AR	10	44
SCNN1B	Liddle syndrome, Pseudohypoaldosteronism, Bronchiectasis with or without elevated sweat chloride	AD/AR	19	47
SERPINA1	Alpha-1-antitrypsin deficiency	AR	49	80
SFTPA1	Idiopathic pulmonary fibrosis	AD		2
SFTPA2	Pulmonary fibrosis, idiopathic	AD	2	5
SFTPB	Surfactant metabolism dysfunction, pulmonary	AR	5	28
SFTPC	Surfactant metabolism dysfunction, pulmonary	AD	8	82
SLC34A2	Pulmonary alveolar microlithiasis	AR	5	19
SLC6A5	Hyperekplexia	AR	15	33
SLC7A7	Lysinuric protein intolerance	AR	55	67
SMPD1	Niemann-Pick disease	AR	110	249
STAT3	Hyper-IgE recurrent infection syndrome, Autoimmune disease, multisystem, infantile onset	AD	47	152
STK36	Primary ciliary dyskinesia	AR		5
STRA6	Microphthalmia, syndromic, Microphthalmia, isolated, with coloboma	AR	22	33
TERC	Aplastic anemia, Pulmonary fibrosis and/or bone marrow failure, telomere-related, Dyskeratosis congenita	AD	42	73
TERT	Aplastic anemia, Pulmonary fibrosis and/or bone marrow failure, telomere-related, Dyskeratosis congenita	AD/AR	48	156
TINF2	Revesz syndrome, Dyskeratosis congenita	AD	25	42
TMEM173	STING-associated vasculopathy, infantile-onsent (SAVI)	AD	4	10
TSC1	Lymphangioleiomyomatosis, Tuberous sclerosis	AD	177	372
TSC2	Lymphangioleiomyomatosis, Tuberous sclerosis	AD	396	1195
ZEB2*	Mowat-Wilson syndrome	AD	154	287
* Some, or all, of the gene is duplicated in the genome. Read more.				

The gene has suboptimal coverage (means <90% of the gene's target nucleotides are covered at >20x with mapping quality score (MQ>20) reads), and/or the gene has exons listed under Test limitations section that are not included in the panel

The sensitivity to detect variants may be limited in genes marked with an asterisk (*) or number sign (#)

Gene refers to the HGNC approved gene symbol; Inheritance refers to inheritance patterns such as autosomal dominant (AD), autosomal recessive (AR), mitochondrial (mi), X-linked (XL), X-linked dominant (XLD) and X-linked recessive (XLR); ClinVar refers to the number of variants in the gene classified as pathogenic or likely pathogenic in this database (ClinVar); HGMD refers to the number of variants with possible disease association in the gene listed in Human Gene Mutation Database (HGMD). The list of associated, gene specific phenotypes are generated from CGD or Mitomap databases.

Supplement 2.

DNA extraction and sequence analysis

Total genomic DNA was extracted from the saliva samples using a bead-based method. DNA quality and quantity were assessed using electrophoretic methods. After assessment of DNA quality, the qualified genomic DNA sample was randomly fragmented using non-contact, isothermal sonochemistry processing. A sequencing library was prepared by ligating sequencing adapters to both ends of DNA fragments. Sequencing libraries were size-selected with a bead-based method to ensure optimal template size and amplified by polymerase chain reaction. 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.

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 nuclear DNA. Variant data was annotated using a collection of tools (VcfAnno and VEP) with a variety of public and private variant databases including the Genome Aggregation Database (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.