Shared genetic predisposition in rheumatoid arthritis-interstitial lung disease and familial pulmonary fibrosis

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ABSTRACT Despite its high prevalence and mortality, little is known about the pathogenesis of rheumatoid arthritis-associated interstitial lung disease (RA-ILD). Given that familial pulmonary fibrosis (FPF) and RA-ILD frequently share the usual pattern of interstitial pneumonia and common environmental risk factors, we hypothesised that the two diseases might share additional risk factors, including FPF-linked genes. Our aim was to identify coding mutations of FPF-risk genes associated with RA-ILD.

We used whole exome sequencing (WES), followed by restricted analysis of a discrete number of FPF-linked genes and performed a burden test to assess the excess number of mutations in RA-ILD patients compared to controls.

Among the 101 RA-ILD patients included, 12 (11.9%) had 13 WES-identified heterozygous mutations in the *TERT*, *RTEL1*, *PARN* or *SFTPC* coding regions. The burden test, based on 81 RA-ILD patients and 1010 controls of European ancestry, revealed an excess of *TERT*, *RTEL1*, *PARN* or *SFTPC* mutations in RA-ILD patients (OR 3.17, 95% CI 1.53–6.12; $p=9.45\times10^{-4}$). Telomeres were shorter in RA-ILD patients with a *TERT*, *RTEL1* or *PARN* mutation than in controls ($p=2.87\times10^{-2}$).

Our results support the contribution of FPF-linked genes to RA-ILD susceptibility.

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Introduction

Rheumatoid arthritis (RA) is a destructive, systemic inflammatory and autoimmune disorder that affects up to 1% of the general adult population worldwide. Extra-articular disease occurs in nearly 50% of all RA patients, the lung being frequently involved [1]. Indeed, lung disease causes 10%–20% of all deaths in RA patients [2–4]. Specifically, interstitial lung disease (ILD) is the leading cause of mortality, accounting for a mortality rate that is approximately 13% higher in RA patients as compared to the general population [2, 4, 5], with a three-fold higher risk of death for those with ILD than those without [5]. In addition, whereas overall RA mortality rates are decreasing, RA-ILD deaths are increasing [6]. Despite its frequency and prognostic impact, RA-ILD has not been given much attention and we are far from understanding its pathogenesis [7].

In comparison to ILD occurring in other connective tissue diseases, patients with RA-ILD frequently present the usual interstitial pneumonia (UIP) pattern, which is characteristic of pulmonary fibrosis [8]. This pattern might explain the poor outcomes of RA-ILD patients, with survival rates similar to those of pulmonary fibrosis patients [9]. Familial pulmonary fibrosis (FPF) that might display histological patterns other than UIP has been linked to mutations in telomere maintenance-associated [10–15] and surfactant protein genes [16–18]. Most importantly, FPF and RA-ILD share common risk factors, such as cigarette smoking and the male sex [19, 20].

Given the above-cited similarities of RA-ILD and FPF, we hypothesised that RA-ILD and FPF share genetic risk factors. Therefore, we performed whole exome sequencing (WES) in RA-ILD patients to determine the contribution of mutations in genes previously linked to FPF.

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Methods

Study participants

Consecutive RA patients with high-resolution computed tomography (HRCT) chest scans showing ILD were recruited by a French network of ILD-expert pulmonologists and RA-expert rheumatologists from 10 university hospitals during the period 2013–2015. All medical records were centrally reviewed by multidisciplinary discussion that included a pulmonologist (B. Crestani), rheumatologist (P. Dieudé) and radiologist. Medical records were independently reviewed to confirm whether subjects met the American College of Rheumatology criteria for RA [21]. The HRCT chest scans of the subjects were analysed by an experienced reader, blinded to clinical, biologic and genetic data, who scored the scans to ensure that the criteria for ILD were met [9]. In-house subjects (n=1010) without known autoimmune/inflammatory and/or pulmonary diseases served as healthy controls (supplementary material). The relevant ethics committees approved all procedures, and written informed consent was obtained from all participants in agreement with French bioethics laws.

WES followed by restricted analysis of FPF-linked genes in RA-ILD patients

FPF-risk genes implicated in telomere maintenance include telomerase reverse transcriptase (*TERT*) [10], telomerase RNA component (*TERC*) [10, 11], dyskerin (*DKC1*) [12], telomere-interacting factor-2 (*TINF2*) [13], regulator of telomere-elongation helicase-1 (*RTEL1*) [14, 15] and polyadenylation-specific ribonuclease deadenylation nuclease (*PARN*) [15]. FPF mutations are also found in genes that encode the following surfactant proteins: surfactant protein C (*SFTPC*) [16], ATP-binding cassette, subfamily A, member 3 (*ABCA3*) [17] and surfactant protein A2 (*SFTPA2*) [18]. We used WES, followed by an analysis restricted to these nine FPF-linked genes to assess excess mutations in RA-ILD patients. Sanger sequencing independently confirmed the WES-identified candidate disease-associated mutations.

TERT and RTEL1 molecular modelling and three-dimensional structure visualisation

Models of the three-dimensional structure of TERT and RTEL1 were built and analysed to assess the mutation effects.

Genotype–phenotype association analyses

Clinical, demographic, biological, HRCT chest scan and pulmonary function test results were assessed at RA-ILD patient inclusion. All HRCT scans were centrally reviewed and scored by a senior radiologist (M-P. Debray) (supplementary material). A telomeric restriction fragment length (TRFL) assay was used to measure telomere length in RA-ILD patients with mutations in telomere-maintenance candidate genes.

Statistical analyses

Power calculation

The 101 cases and 1010 controls provided a power higher than 70% to detect an overall association with an odds ratio of 3.0 (supplementary material).

Ancestry-inference analysis

Ancestry of all RA-ILD patients and controls was verified by principal component analysis, based on the individuals of the 1000 Genomes Project. To avoid population stratification bias, all outlier patient (*i.e.* those not of European ancestry according to the 1000 Genomes Project) data were excluded from the association analyses (burden test).

Burden test

A classical burden test was used to assess excess-risk mutations in RA-ILD. Significance was assessed using a one-sided Wald test.

Genotype–phenotype association analysis

Continuous variables, expressed as median (range), were compared using the t-test; categorical variables, expressed as n (%), were compared by the Fisher's exact test. Comparisons of RA-ILD patients with mutations were drawn using non-parametric tests because of the small sample size. Generalised additive models were used to evaluate the linearity of the relationship between continuous variables and mutation probability. Telomere lengths for *TERT*/*RTEL1*/*PARN* mutation carriers (n=11) and 15 healthy age-matched controls (TRFL being previously assessed for 13 of them [22]) were compared by logistic regression adjusted for age, using the R 3.1.2 glm function and corresponding figures were created with Graphpad Prism 6.0b. All statistical analyses were performed using the R 3.1.2 software. Levels of significance were defined at $p<0.05$.

Methods and corresponding analyses are detailed in the supplementary material.

Results

Phenotype of RA-ILD patients

We included 101 consecutive independent RA-ILD patients. Mean±SD age at RA onset was 53.54 ±15.40 years; 82.1% were anti-citrullinated peptide antibody-positive, 84.8% were rheumatoid factor-positive and 71.1% had erosive disease. Mean age at ILD onset was 61.42±11.81 years and mean RA duration before ILD detection was 7.93±10.83 years. Overall, 54.5% of all patients were ever-smokers and 65.4% showed the UIP pattern on HRCT. The demographic information and clinical characteristics are summarised in table 1.

Exome sequencing of FPF-linked genes in RA-ILD patients

WES combined with restricted analysis of the nine FPF-linked genes, followed by Sanger sequencing confirmation revealed that 12/101 RA-ILD patients (11.9%) carried 13 heterozygous mutations in the *TERT*, *RTEL1*, *PARN* or *SFTPC* coding regions (table 2, figure S2).

For telomere-maintenance genes, six RA-ILD patients carried six heterozygous *TERT* mutations: c.2383-2A>G, affecting intron splicing, not reported in the Exome Aggregation Consortium (ExAC) database, and c.3323C>T, p.Pro1108Leu, with ExAC minor allele frequency (MAF) of 5.55×10^{-5}. In addition, four RA-ILD patients carried the previously reported FPF recurrent mutation [23]: c.1234C>T, p.His412Tyr. The *TERT* p.His412Tyr MAF is 1.5% in the European population ExAC database, which could suggest that this variant is a common polymorphism. However, taking into account 1) a MAF of 0.6% in the overall ExAC database, 2) evidence of linkage of p.His412Tyr to familial pulmonary fibrosis [24] and 3) the functional consequences including shortened telomere length [24], in addition to decreased catalytic activity *in vitro* [23, 25], we considered p.His412Tyr a low penetrant mutation and included it in our genetic association test. *RTEL1* sequencing revealed four patients with four heterozygous mutations: three new mutations (c.2695 T>C, p.Phe899Leu; c.2824G>A, p.Asp942Asn; and c.2875C>T, p.His959Tyr) and the previously reported pathogenic mutation: c.2890T>C, p.Phe964Leu [22]. The p.Phe899Leu, p.His959Tyr and p.Phe964Leu mutations were not listed in the ExAC database, but p.Asp942Asn had an ExAC MAF of 2.06×10^{-4}. One RA-ILD patient carried a *PARN* heterozygous frameshift mutation

TABLE 1 Demographic and phenotypic characteristics of 101 patients with rheumatoid arthritis (RA)-associated interstitial lung disease (ILD) according to mutation status in familial idiopathic pulmonary fibrosis linked genes

Characteristic	All patients	With mutations	Without mutations	p-value
Female	59/101 (58.4)	6/12 (50)	53/89 (59.6)	0.75
Age at RA onset years	53.54±15.40	45.83±14.76	54.59±15.26	0.07
RA duration years	13.47±10.83	13.08±10.71	13.53±10.91	0.89
Age at ILD onset years	61.42±11.81	53.27±10.21	62.51±11.63	0.015
ILD duration years	5.17±5.99	5±3.28	5.19±6.29	0.87
RA duration preceding ILD detection years	7.93±10.83	8±11.59	7.92±10.80	0.98
Ever-smoker	55/101 (54.5)	7/12 (58.3)	48/89 (53.9)	1.00
Smoking pack years	24.85±18.41	20.14±16.97	25.54±18.67	0.46
Active smoker	9/55 (16.4)	2/7 (28.6)	7/48 (14.6)	0.70
Fibrogenic exposure or airborne contaminants	26/82 (31.7)	2/10 (20)	23/72 (31.9)	0.69
Methotrexate ever	82/101 (81.2)	9/12 (75)	73/89 (82)	0.85
Anti-TNF biologic ever	28/99 (28.3)	6/12 (50)	22/87 (25.3)	0.15
RA manifestations				
ACPA-positive	78/95 (82.1)	10/11 (90.9)	68/84 (80.9)	0.69
RF-positive	84/99 (84.8)	12/12 (100)	72/87 (82.8)	0.26
Erosive disease	69/97 (71.1)	11/12 (91.7)	58/85 (68.2)	0.41
Chest CT scan pattern				
UIP	66/101 (65.4)	8/12 (66.7)	58/89 (65.2)	0.97#
Possible UIP	6/101 (5.9)	0	6/89 (6.7)	
NSIP	8/101 (7.9)	1/12 (8.3)	7/89 (7.9)	
Unclassifiable, other	21/101 (20.8)	3/12 (25)	18/89 (10.2)	
Pulmonary function test results at inclusion				
FVC % pred	85.08±25.78	76±27.85	86.28±25.43	0.27
*D*LCO % pred	55.65±19.47	50.36±23.65	56.41±18.85	0.43
TLC % pred	80.88±20.62	77.47±20.38	81.46±20.78	0.58

Data are presented as mean±SD or (%), unless otherwise indicated. Some values were missing and the denominators are indicated. TNF: tumour necrosis factor; ACPA: anti-citrullinated peptide antibodies; RF: rheumatoid factor; CT: computed tomography; UIP: usual interstitial pneumonia; NSIP: nonspecific interstitial pneumonia; FVC: forced vital capacity; *D*LCO: diffusing capacity of the lung for carbon monoxide; TLC: total lung capacity. #: UIP and possible UIP patterns *versus* other patterns.

TABLE 2 Clinical characteristics of 12 rheumatoid arthritis (RA)-associated interstitial lung disease (ILD) patients with familial idiopathic pulmonary fibrosis (FPF)-linked gene mutations

Case	Sex	Age years	RA duration years	ACPA status	RF status	Erosive disease	Familial history of ILD and/or STS[#]	ILD duration years	Chest CT diagnosis	FVC % pred	D_{LCO} % pred	Locus	c-DNA change	Amino acid change
1	F	61	3	Positive	Positive	Yes	No	3	UIP	49	33	*PARN*	c.1749_1750delAG	p.Ser585fs*5
2	F	66	14	ND	Positive	Yes	No	ND	UIP	44	NA	*RTEL1*	c.2695T>C	p.Phe899Leu
3	M	66	1	Positive	Positive	Yes	No	1	NSIP	64	56	*RTEL1*	c.2824G>A	p.Asp942Asn
4	M	64	10	Positive	Positive	Yes	No	10	UIP	58	26	*RTEL1*	c.2875C>T	p.His959Tyr
5	M	59	14	Positive	Positive	Yes	Yes (brother with IPF)	10	UIP	50	32	*RTEL1*	c.2890T>C	p.Phe964Leu
6	F	56	5	Positive	Positive	Yes	Yes (sister with IPF)	4	UIP	ND	58	*TERT*	c.2383-2A>G	–
7	F	63	38	Negative	Positive	Yes	No	5	Unclassifiable	102	57	*TERT*	c.3323C>T	p.Pro1108Leu
8	M	69	3	Positive	Positive	Yes	Possible (father with cirrhosis)	4	Unclassifiable	97	59	*TERT*	c.1234C>T	p.His412Tyr
9	M	68	24	Positive	Positive	Yes	No	4	UIP	104	103	*TERT*	c.1234C>T	p.His412Tyr
10	F	47	21	Positive	Positive	Yes	No	0	UIP	99	72	*TERT*	c.1234C>T	p.His412Tyr
11	M	48	8	Positive	Positive	Yes	Yes (daughter with IPF)	8	Unclassifiable	30	60	*TERT*	c.1234C>T	p.His412Tyr
												SFTPC	c.218T>C	p.Ile73Thr
12	F	40	16	Positive	Positive	Yes	No	6	UIP	28	57	*SFTPC*	c.180G>A	p.Met60Ile

ACPA: anti-citrullinated peptide antibodies; RF: rheumatoid factor; STS: short telomere syndrome; CT: computed tomography; FVC: forced vital capacity; D_{LCO}: diffusing capacity of the lung for carbon monoxide; M: male; F: female; ND: unknown, could not be determined; IPF: idiopathic pulmonary fibrosis; UIP: usual interstitial pneumonia; NSIP: nonspecific interstitial pneumonia. [#]: familial history of ILD was assessed for all RA-ILD patients carrying mutations in FPF-linked genes; the familial history of STS was assessed for patients carrying mutations in telomere maintenance genes (*i.e. TERT, RTEL1* and *PARN*).

(c.1749_1750delAG, p.Ser585fs*5), not reported in the ExAC database. We found no mutations in *TERC*, *DKC1* or *TINF2* genes.

For genes encoding surfactant-related proteins, one RA-ILD patient carried a previously reported heterozygous *SFTPC* mutation (c.218T>C, p.Ile73Thr) and another carried an unreported *SFTPC* heterozygous mutation (c.180G>A, p.Met60Ile). Both mutations were located at highly conserved positions in the pro-SP-C-linker domain. One RA-ILD patient was a double heterozygote carrying both a heterozygous mutation in *SFTPC* (c.218T>C, p.Ile73Thr) and a heterozygous mutation in *TERT* (c.1234C>T, p.His412Tyr). No mutations were detected in *ABCA3* or *SFTPA2* genes. Details of the identified mutations are in supplementary table S1.

Predicted structural impact of the TERT *and* RTEL1 *mutations*

TERT

His412, located in the telomere RNA-binding domain (TRBD), is predicted to be in a helix involved in the binding of the TERT template-boundary element (TBE), which acts as a molecular guide to position the template in the active site. His412 does not make direct contact with the TBE, but is located on a positively charged TRBD surface (supplementary figure S3), which suggests that the mutation affects binding to structural elements located in the p3 helix and/or pseudoknot. Position 1108 is located on the C-terminal extension (CTE) thumb domain, in the loop that sharply turns the protein chain before the last 24 residues. Amino-acid Pro1108 is located in a local hydrophobic core; leucine substitution at this position preserves the residue's hydrophobic nature and is not predicted to engender major unfolding. However, because proline residues induce a kink in the main chain of the protein, this mutation could destabilise the structure of the terminal residues that interact with the CTE and RNA-template regions, and perhaps the TRBD near His412.

RTEL1

RTEL1 encodes an essential iron-sulfur (FeS)-containing DNA helicase that is critical for telomere maintenance and DNA repair. All mutations affect the first RTEL1 harmonin-like domain (supplementary figure S4a and c). Amino acids Phe899 and His959 occupy highly conserved positions. Leucine substitution at position 899 is predicted to disturb interaction with a yet uncharacterised partner of the harmonin-like domain, whereas tyrosine at position 959, although not directly involved in the putative binding groove, might also alter harmonin-like domain interactions. The effect of the asparagine at position 942, located in the α-3α-4 loop, remains undetermined. Furthermore, eucine replacement of phenylalanine at the highly conserved position 964, located in helix α-5, is predicted to disturb domain folding [22].

Burden test

Principal component analysis of WES data genotypes revealed that 81 patients, excluding the 20 outliers among the 101 RA-ILD patients, were clustered with the 1000-genome (1000G) subjects of European ancestry (supplementary figure S5a). Excess mutations in FPF-linked genes were evaluated in these 81 patients and compared to our 1010 controls of European ancestry. The retained patients had an excess number of risk mutations compared to controls: 12.35% (with at least one candidate disease-associated mutation) *versus* 4.46% (burden test, OR 3.17, 95% CI 1.53–6.12; $p=9.45\times10^{-4}$) (figure 1, table 3; supplementary figure S5a). The association remained significant with more stringent clustering on 1000G individuals of European ancestry: 14.71% of mutations in 68 cases *versus* 4.76% in 903 controls (OR 3.60, 95% CI 1.72–7.04; $p=3.14\times10^{-4}$) (supplementary figure S5b and supplementary table S5).

Genotype–phenotype-association analyses

Clinical phenotype

RA-ILD patients carrying a *TERT*, *RTEL1* or *PARN* mutation showed no other clinical manifestation related to a telomere syndrome, such as skin abnormalities, typical haematological abnormalities (*i.e.*macrocytosis, anaemia and thrombocytopenia), bone marrow failure or liver disease. Mean age at ILD onset was significantly lower for patients with mutations than those without mutations: 53.27±10.21 *versus* 62.51±11.63 years, respectively; p=0.015 (table 1). Plots based on smoothing splines supported a nonlinear association between age at ILD onset and *TERT*/*RTEL1*/*PARN*/*SFTPC* mutations, with higher mutation probability for patients 36–41 years old than those younger or older (p=0.040) (figure 2a). Results remained significant after removal of the patient with a *SFTPC* mutation (p=0.042). No other phenotypic differences were detected; notably, pulmonary function and HRCT chest scan pattern at inclusion were similar for both subgroups (table 1).

Telomere lengths in RA-ILD patients with PARN, TERT or RTEL1 mutations

Consistent with previously reported telomere lengths of similar mutation carriers [14, 26, 27], telomere lengths in genomic DNA isolated from the circulating leukocytes of 11 RA-ILD patients with *TERT*, *RTEL1* or *PARN*

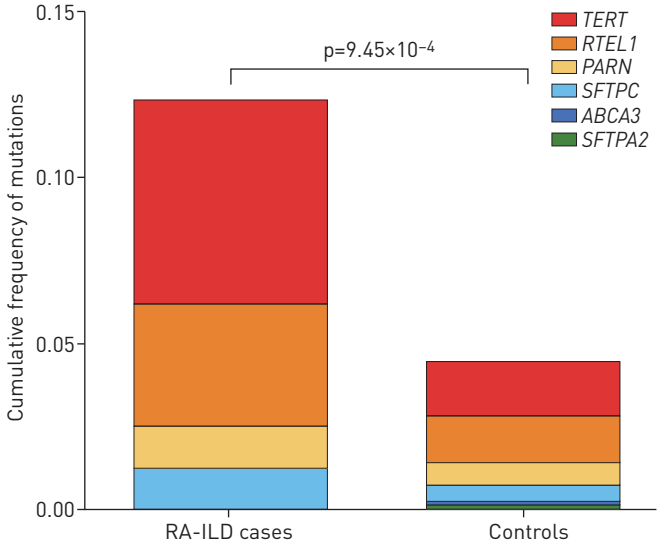

FIGURE 1 Excess mutations in familial pulmonary fibrosis (FPF)-linked genes in rheumatoid arthritis-interstitial lung disease (RA-ILD) patients: burden test. Frequencies of *TERT*, *RTEL1*, *PARN* and *SFTPC* gene mutations in 81 RA-ILD patients and 1010 controls in the French Caucasian population.

mutations were shorter than those from 15 controls (p=0.0114) (figure 2b; supplementary table S2), as confirmed by logistic regression adjusted for age ($p=2.87\times10^{-2}$).

Familial history of ILD and short telomere syndrome in patients with mutations

Among the 12 RA-ILD patients carrying a FPF-linked mutation, three had a family history of interstitial lung disease: case #5 (RTEL1 p.Phe964Leu) had a brother with idiopathic pulmonary fibrosis (IPF) (deceased); case #6 (*TERT* c.2383-2A>G) had a sister with IPF (deceased); and case #11 (*TERT* p.His412Tyr and SFTPC p.Ile73Thr) had a daughter with IPF (deceased). The father of case #8 (*TERT* p.His412Tyr) died of cirrhosis that was compatible with a short telomere syndrome (table 2) [28].

Discussion

To date and to our knowledge, this is the first exome sequencing study of RA-ILD patients. Our findings, from a candidate gene approach, provide evidence of an association between RA-ILD and mutations in FPF-linked genes (*TERT*, *RTEL1*, *PARN* or *SFTPC*). The burden of these mutations was significantly greater in patients than in controls. Moreover, the association was robust after adjustment for more stringent clustering of 1000G subjects of European ancestry. Our results show that RA-ILD and FPF share genetic risk factors, suggesting common pathogenetic mechanisms. The familial aggregation detected in 25% of RA-ILD patients carrying at least one mutation in FPF-linked genes supports this hypothesis.

For telomere-maintenance genes, we detected the *TERT* p.His412Tyr mutation that has been previously linked to FPF and dyskeratosis congenita [23, 25]. Our findings support the theory of *TERT* p.His412Tyr as a low penetrance mutation, with two of the four RA-ILD patients carrying the p.His412Tyr mutation, evidently shortened telomere length (supplementary table S2) and one whose father died of cirrhosis compatible with a

TABLE 3 Burden test for 81 rheumatoid arthritis (RA)-associated interstitial lung disease (ILD) patients and 1010 controls among the French Caucasian population

Gene	Patients			Controls			p-value	Odds ratio
	0	1	2	0	1	2		(95% CI)
ABCA3	81	0	0	1009	1	0		
PARN	80	1	0	1003	7	0		
RTEL1	78	3	0	996	14	0		
SFTPA2	81	0	0	1009	1	0		
SFTPC	80	1	0	1005	5	0		
TERT	75	6	0	993	17	0		
Multigene panel testing	71	9	1[#]	965	45	0	9.45×10^{-4}	3.17 (1.53–6.12)

0: homozygous for the wild-type allele; 1: heterozygous; 2: homozygous for the rare allele. [#]: RA-ILD case carrying one *SFTPC* and one *TERT* mutation was considered homozygous for a familial pulmonary fibrosis-linked gene mutation.

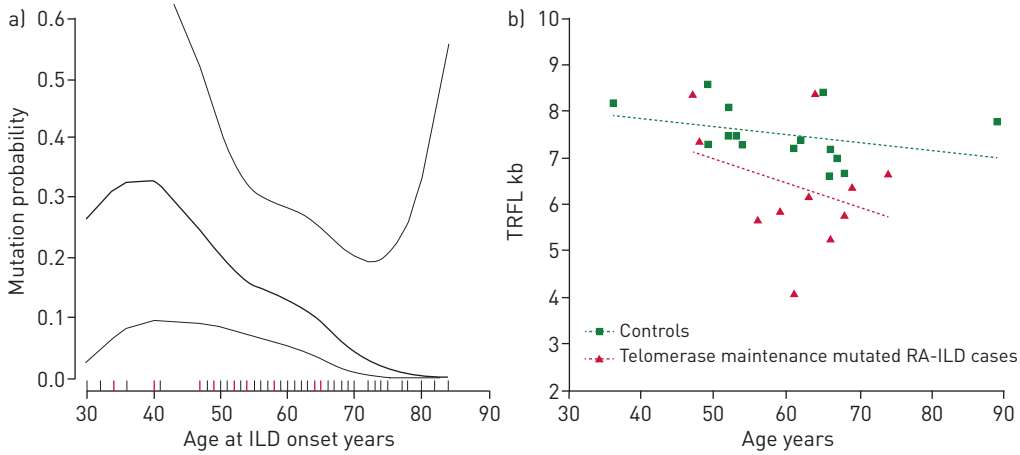

FIGURE 2 Genotype–phenotype association analysis. a) Plots based on smoothing splines of the association between age at interstitial lung disease (ILD) diagnosis and probability of a *TERT*, *RTEL1*, *PARN* or *SFTPC* mutation. Rheumatoid arthritis-associated ILD (RA-ILD) carriers of a mutation in the multigene panel tested are in red. b) Linear regression analyses of telomere lengths in RA-ILD patients (red) with *PARN*, *TERT* or *RTEL1* mutation and controls (green). TRFL denotes the telomeric restriction fragment length.

familial short telomere syndrome [28]. Unfortunately, the affected father was not sequenced for *TERT*, to establish supplementary linkage evidence for *TERT* p.His412Tyr, which represents one limitation of the present study. We also identified p.Pro1108Leu in a highly conserved residue in the functional domain of the protein and a splice mutation that abolishes the acceptor splice site. Among the three new singletons in *RTEL1*, p.Phe899Leu, p.His959Tyr and p.Asp942Asn were predicted to be deleterious by at least two of the three prediction tools used. We previously reported the p.Phe964Leu mutation as a possibly deleterious mutation [22]. Furthermore, the *PARN* mutation leads to a frameshift and premature stop codon. Consistent with previously reported telomere lengths of *TERT*, *RTEL1* or *PARN* mutation carriers [14, 26, 27], we found shorter lengths in the RA-ILD *TERT*, *RTEL1* or *PARN* mutation carriers of the present study than in the controls, which confirms the deleterious effects of these mutations on telomere maintenance. The mechanism linking *PARN* mutations to telomere shortening was recently elucidated: PARN is required for TERC 3′-end maturation [29]. Our RA-ILD patient with the *PARN* frameshift mutation had the shortest telomeres, which further supports PARN participation in telomere maintenance.

We also investigated genes encoding surfactant-related proteins and detected two *SFTPC* mutations. The p.Ile73Thr mutation, located within the proSP-C (surfactant protein C) linker domain, accounts for more than 30% of all *SFTPC* mutations associated with diffuse parenchymal lung disease in patients with sporadic and inherited (autosomal-dominant) disease [16, 30]. Moreover, p.Met60Ile, a new mutation also located in the non-BRICHOS SP-C domain, was identified. Non-BRICHOS mutations within the proximal COOH propeptide (*e.g.* p.Ile73Thr) induce aberrant intracellular trafficking of proSP-C, which eludes cleavage and accumulates in the endosomal system, thereby causing cellular dysfunction [31]. Although we could not examine the functional consequences of the SP-C p.Met60Ile mutation, several lines of evidence favour its pathogenicity: 1) p.Met60Ile is located in a highly conserved region; 2) it has one of the three highest Combined Annotation Dependent Depletion scores; and 3) it is not in the ExAC database.

Our findings demonstrate the usefulness of WES combined with restricted candidate gene analysis in identifying RA-ILD-associated mutations, despite complexities, such as locus heterogeneity and late-onset disease. Because of the small number of available patients, our association study had neither sufficient power nor an appropriate design for gene discovery (*e.g.* no *a priori* hypothesis) [32]. Consequently, WES of larger RA-ILD and control populations, probably with international collaboration, is required to identify new RA-ILD risk genes and to refine the exact contribution of FPF-linked genes to the development of RA-ILD.

In the present genetic case–control association study, we provide evidence for an association between a panel of candidate genes (FPF-linked genes) and the "RA-ILD" phenotype, *i.e.* susceptibility to RA-ILD (RA-ILD *versus* controls). Our results do not provide information about the putative roles of these genes in 1) susceptibility to overall RA (RA *versus* controls) and 2) the risk of ILD in the RA population. These issues suggest that a genetic association study should be performed in RA-ILD cases compared to RA cases without ILD. To date, these issues remain unsolved and therefore support the need for an appropriately designed study facilitated by international collaborations, to test whether FPF-linked genes are also RA modifier genes, thereby increasing the risk of ILD in RA.

From a clinical perspective, the relatively high prevalence of male patients compared to that observed in a recent report of a large multiethnic RA population [33] and the rate of ever smoker patients, are consistent with that previously reported in RA-ILD [19, 34, 35]. Furthermore, consistent with that previously reported for ILD patients with *RTEL1* or *TERT* mutations, ILD occurred earlier in RA-ILD patients with mutations than in those without mutations in telomere-maintenance genes, which might illustrate genetic anticipation, as has been reported in telomere-mediated disorders [36]. Nonetheless, the relatively small sample of RA-ILD patients carrying a mutation limits a genotype–phenotype association analysis, which emphasises the importance of future international collaborative studies on the genetics of RA-ILD.

FPF-risk genes involved in telomere maintenance might be linked to ILD associated with autoimmune diseases, because *PARN* or *RTEL1* mutations have been identified in ILD patients with RA, autoimmune hepatitis, Sjögren's syndrome and more recently systemic sclerosis [22, 26, 37]. This hypothesis is reinforced by diminished telomerase activity and shortened telomere lengths that are apparently connected to premature immunosenescence in various systemic immune-mediated diseases, and more recently by the identification of *TERT* as a risk gene for systemic lupus erythematosus [38, 39]. In addition, we detected two *SFTPC* mutations in RA-ILD patients. To our knowledge, *SFTPC* mutations have only been associated with or linked to interstitial pneumonia, thereby contributing to ILD pathogenesis *via* endoplasmic reticulum stress in alveolar epithelial cells [16]. For the first time, our results provide evidence of an association between *SFTPC* mutations and RA-ILD that might contribute to the hypothesis of a pivotal role of the lung in the pathogenesis of RA [40]. Furthermore, our results were observed in European Caucasian patients and would require replication in other populations.

In conclusion, our findings establish, for the first time, shared genetic risk factors between the RA-ILD phenotype and familial pulmonary fibrosis.

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