



First heterozygous *NOP10* mutation in familial pulmonary fibrosis

To the Editor:

Germline telomere-related gene mutations are associated with familial pulmonary fibrosis and lead to short telomere syndrome [1, 2]. Most of the short telomere syndrome-related genes, such as *TERT*, *TERC*, *DKC1*, *TINF2*, *NHP2* and *NOP10*, were identified in dyskeratosis congenita patients with early onset mucocutaneous manifestations and/or bone marrow failure [3]. The telomerase complex includes *TERT*, the *TERC* RNA and *DKC1*. *NOP10*, along with *DKC1*, *NHP2* and *GAR1*, is essential for *TERC* stability and telomere maintenance [4]. Homozygous *NOP10* mutation (c.100C>T, p.Arg34Trp) has been reported only once in a consanguineous family with autosomal recessive dyskeratosis congenita without pulmonary fibrosis [5]. Moreover, the p.Arg34Trp *NOP10* mutation caused telomere shortening in homozygous and heterozygous carriers [5]. Here we provide evidence that a heterozygous *NOP10* mutation (c.17A>G, p.Tyr6Cys) identified in a large family co-segregates with adult-onset familial pulmonary fibrosis.

The proband (II.1), a 68-year-old non-smoker female, was diagnosed with pulmonary fibrosis at the age of 66 years (figure 1a and b). Two deceased, a brother (II.2) and sister (II.6), and one alive sister (II.7) were also diagnosed with pulmonary fibrosis (figure 1c). Another brother (II.4) and his son (III.5) died from leukaemia while one of the sons of the proband (III.2) had long-term leukopenia. The deceased father of the proband (I.1) was diagnosed with non-alcoholic liver cirrhosis (figure 1a). Mucocutaneous manifestations were not observed upon examination in this family.

We identified by whole exome sequencing in the proband (II.1) a heterozygous pathogenic missense mutation in *NOP10* (NM_018648) c.17A>G, p.Tyr6Cys according to international recommendations (figure 1a) [6]. No other rare variants predicted to be deleterious were found in whole exome data from the proband in the other genes linked to short telomere syndrome (*TERT*, *TERC*, *DKC1*, *TINF2*, *RTTL1*, *PARN*, *NAF1*, *ACD*, *NHP2*, *WRAP53*, *SHQ1* and *ZCCHC8*) [7–9]. The variation is absent in 140 000 individuals from the gnomAD database. The mutated adenine is conserved at a genomic level (GERP=5). The *in silico* tools Polyphen 2 and CADD predicted a deleterious impact of the amino acid change p.Tyr6Cys with scores of 1 and 24, respectively, reflecting high conservation at a protein level. Flow cytometry and FISH (flowFISH) revealed that telomere length of the proband were shorter than the first percentile [6]. It was not possible to organise flowFISH analysis for all individuals of this family. Thus, telomere length for the individuals II.1, II.7, III.1 and III.2 was also measured through telomeric restriction fragment assay [9] at 7.4 kb, 6.3 kb, 8.5 kb and 8.6 kb, respectively. Telomere length for the individuals III.1 and III.2 were found to be shorter relative to the mother's telomere length [2]. The fact that non-carrier (III.1) and carrier (III.2) brother were found to have the same range of telomere length could be attributed to the "heritability" of telomere length and evokes epigenetic inheritance [10]. The variation co-segregated with pulmonary fibrosis (II.1 II.7; and the obligate carrier II.6) and leukopenia (III.2). Incomplete penetrance observed for the asymptomatic niece (III.8) carrier of the mutation is frequently observed in short telomere syndrome at the age of 50 years (figure 1a) [2].

Analysis of the human *NOP10* protein three-dimensional structure demonstrated that the mutated amino acid Tyr6 is buried with the small, N-terminal globular, ribbon domain of *NOP10*, which is in close contact with Dyskerin *DKC1* (Cbf5 in the archaeal complex) (figure 1d). Hence it is likely that it plays a critical role for the stability of the domain as well as for the integrity of the *DKC1/NHP2/GAR1/NOP10* complex [11–14].

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We provide first evidence that a heterozygous *NOP10* mutation (c.17A>G, p.Tyr6Cys) identified in a large family co-segregates with adult-onset familial PF and predisposes to short telomere syndrome (familial PF, liver, haematological diseases) <http://bit.ly/2wvXsUd>

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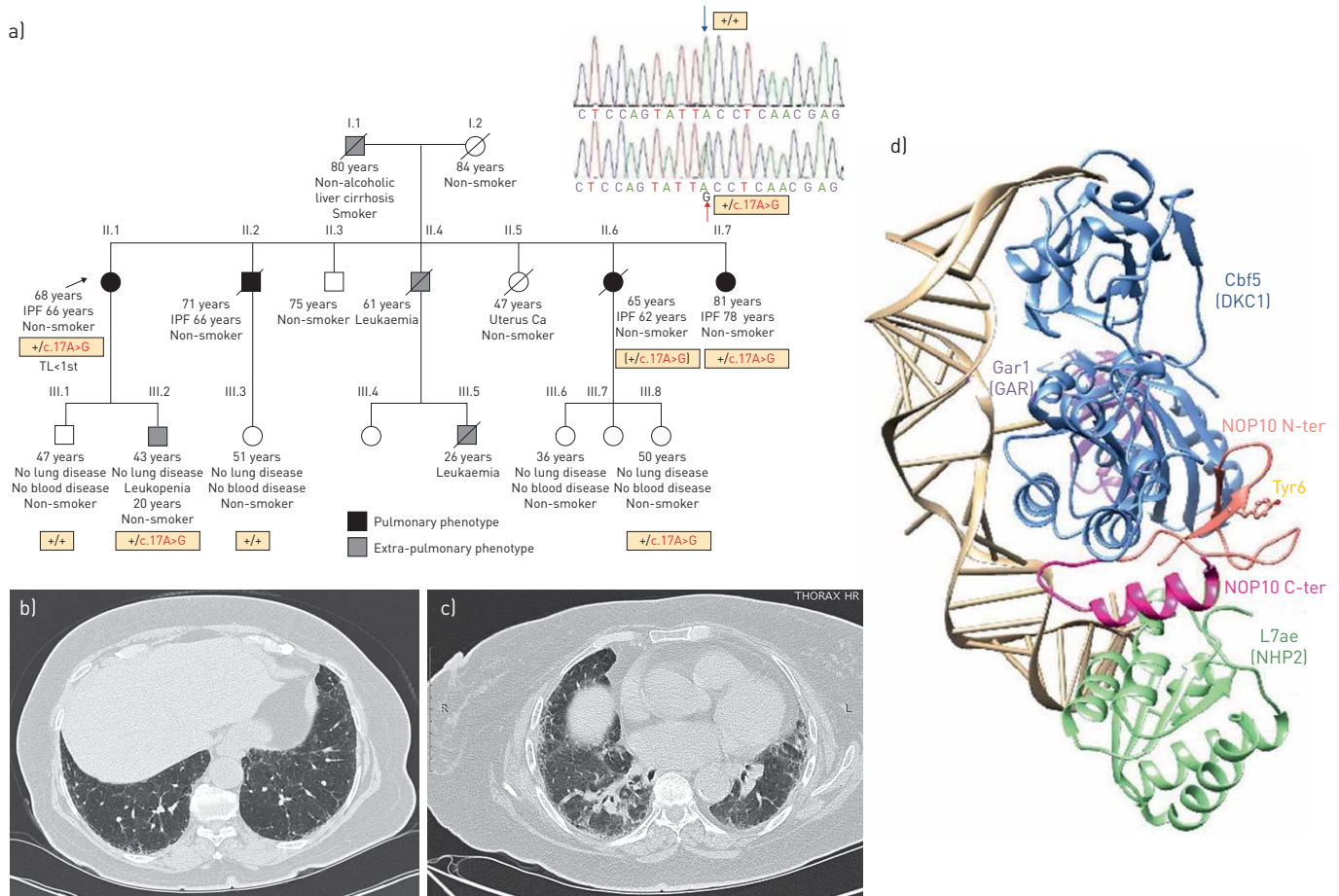


FIGURE 1 a) Three-generation pedigree of the proband. Numbers below individual symbols are age at death or study. *NOP10* mutation status in subjects for whom DNA was available for sequencing and pulmonary and extra-pulmonary phenotype is also depicted. (+/c.17A>G) discloses obligate carrier state. In the insert, the presence of *NOP10* mutation identified by whole exome sequencing was confirmed by Sanger sequencing. The symbol +/- discloses wild type and +/c.17A>G heterozygosity for the mutation. TL: telomere length by flowFISH. b) High-resolution computed tomography (HRCT) image at the basal segments of the lower lobes of the proband (II.1) showing peripheral fine reticulation with a microcystic pattern subpleurally. In the inner lung zones intralobular reticulation in association with thin wall traction bronchiectasis are depicted. All features indicate the fibrotic nature of the process. c) HRCT of the chest at the level of the basal segments of lower lobes of the patient II.7 revealing definite reticulation subpleurally with areas of increased density resulting from coalescence of the reticular opacities and areas of microatelectasis. Reticular changes are also seen more centrally distorting the shape of the vessels, while few areas of macrocystic (honeycomb) changes are seen in contact with the mediastinal pleura. The lungs bilaterally are ill expanded (small volumes). d) Ribbon representation of the model of human NOP10, within the frame of a whole H/ACA RNP complex [experimental three-dimensional structure from *Pyrococcus furiosus*, PDB 2EY4]. A model of the three-dimensional structure of human NOP10 has been made considering, for its N-terminal region [aa 1–33], the experimental three-dimensional structure of *Saccharomyces cerevisiae* Nop10 [solved in complex with Cbf5/Dkc1 and Gar1 [pdb 3U28 [11]] and, for the C-terminal region [aa 34–65], the experimental 3D structure of *Pyrococcus furiosus* Nop10, folded in part into an alpha-helix when contacting both Cbf5 and L7ae/Nhp2 [pdb 2EY4 [12]]. The three-dimensional structure of human NOP10 could not be modelled as a whole starting from the archaeal Nop10 three-dimensional structure, as the N-terminal sequences strikingly diverge between archaeal and eukaryotic species, although still sharing a similar “ribbon” fold, organised like a beta barrel [11]. The model of the human NOP10 3D structure was then superimposed with the frame of the whole H/ACA RNP structure, as observed for archaeal complexes [PDB 2EY4]. This superimposition is supported by the fact that the structure of the individual subunits, overall organisation and interfaces are similar between archaeal and yeast H/ACA RNP complexes [11]. Tyr6 is buried with the small, N-terminal globular, ribbon domain of NOP10 and likely plays a critical for the stability of this domain, in close contact with human Dyskerin DKC1 [Cbf5]. In archaeal proteins, the corresponding position is occupied by one of the four cysteine encapsulating a zinc ion within the ribbon domain [11]. The name of the human subunits of the H/ACA RNP complex are given within brackets. Molecular graphics and analyses were performed using the UCSF Chimera package [13]. Models of the three-dimensional structure were built using Modeller 9v15 [14], according to the alignment presented in Li *et al.* [11].

In this family, the combination of familial pulmonary fibrosis, the extra-pulmonary manifestations, including haematological and liver disease, the significant telomere shortening, and the genetic anticipation (II.4: 61 years; III.4 onset at 26 years/II.1: 66 years; III.2: 43 years) strongly evokes a diagnosis of telomeropathy [15]. This is supported by the identification of a monoallelic rare variation predicted to be deleterious in the *NOP10* telomere-related gene that co-segregates with disease. Collectively, these results provide the first evidence that mono-allelic *NOP10* mutation can predispose to short telomere syndrome (familial pulmonary fibrosis, liver and haematological diseases).

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References

- 1 Kropski JA, Blackwell TS, Loyd JE. The genetic basis of idiopathic pulmonary fibrosis. *Eur Respir J* 2015; 45: 1717–1727.
- 2 Alder JK, Hanumanthu VS, Strong MA, et al. Diagnostic utility of telomere length testing in a hospital-based setting. *Proc Natl Acad Sci USA* 2018; 115: E2358–E2365.
- 3 Niewisch MR, Savage SA. An update on the biology and management of dyskeratosis congenita and related telomere biology disorders. *Expert Rev Hematol* 2019; 12: 1037–1052.
- 4 Armanios M, Blackburn EH. The telomere syndromes. *Nat Rev Genet* 2012; 13: 693–704.
- 5 Walne AJ, Vulliamy T, Marrone A, et al. Genetic heterogeneity in autosomal recessive dyskeratosis congenita with one subtype due to mutations in the telomerase-associated protein NOP10. *Hum Mol Genet* 2007; 16: 1619–1629.
- 6 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: 405–424.
- 7 Borie R, Tabèze L, Thabut G, et al. Prevalence and characteristics of TERT and TERC mutations in suspected genetic pulmonary fibrosis. *Eur Respir J* 2016; 48: 1721–1731.
- 8 Gable DL, Gaysinskaya V, Atik CC, et al. ZCCHC8, the nuclear exosome targeting component, is mutated in familial pulmonary fibrosis and is required for telomerase RNA maturation. *Genes Dev* 2019; 33: 1381–1396.
- 9 Borie R, Bouvry D, Cottin V, et al. Regulator of telomere length 1 (RTEL1) mutations are associated with heterogeneous pulmonary and extra-pulmonary phenotypes. *Eur Respir J* 2019; 53: 1800508.
- 10 Collopy LC, Walne AJ, Cardoso S, et al. Triallelic and epigenetic-like inheritance in human disorders of telomerase. *Blood* 2015; 126: 176–184.
- 11 Li S, Duan J, Li D, et al. Reconstitution and structural analysis of the yeast box H/ACA RNA guided pseudouridine synthase. *Genes Dev* 2011; 25: 2409–2421.
- 12 Rashid R, Liang B, Baker DL, et al. Crystal structure of a Cbf5-Nop10-Gar1 complex and implications in RNA-guided pseudouridylation and dyskeratosis congenita. *Mol Cell* 2006; 21: 249–260.
- 13 Pettersen EF, Goddard TD, Huang CC, et al. UCSF Chimera—a visualization system for exploratory research and analysis. *J Comput Chem* 2004; 25: 1605–1612.
- 14 Marti-Renom MA, Stuart A, Fiser A, et al. Comparative protein structure modeling of genes and genomes. *Annu Rev Biophys Biomol Struct* 2000; 29: 291–325.
- 15 Borie R, Le Guen P, Ghanem M, et al. The genetics of interstitial lung diseases. *Eur Respir Rev* 2019; 28: 190053.

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