



Early View

Research letter

First heterozygous *NOP10* mutation in Familial Pulmonary Fibrosis

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First heterozygous *NOPI0* mutation in Familial Pulmonary Fibrosis

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Take home message: We provide first evidence that a heterozygous *NOPI0* mutation (c.17A>G,p.Tyr6Cys) identified in a large family co-segregates with adult-onset familial pulmonary fibrosis (FPF) and predisposes to short telomere syndrome (FPF, liver, hematologic diseases)

Germline telomere -related gene mutations are associated to familial pulmonary fibrosis (PF) 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 are 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 autosomic 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 (Figure 1a, Figure 1b). Two deceased 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 leukemia 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) an heterozygous pathogenic missense mutation in *NOP10* (*NM_018648*) c.17A>G,p.Tyr6Cys according to international recommendations [6] (Fig1A input). 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*, *RTEL1*, *PARN*, *NAF1*, *ACD*, *NHP2*, *WRAP53*, *SHQ1* and *ZCCHC8*) [7, 8, 9]. The variation is absent in 140,000 individuals from 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 organize 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 7,4Kb, 6,3Kb, 8.5Kb and 8.6 Kb respectively. Telomere length for the individuals III,1 and III,2 were found shorter relative to mother 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. [2]. (Figure 1a).

Analysis of the human NOP10 protein 3D 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, 12, 13, 14].

In this family, the combination of familial pulmonary fibrosis, -the extra-pulmonary manifestations including hematologic and liver disease, -the significant telomere shortening, and -the genetic anticipation (II,4:61y; III,4 onset at 26y / II,1:66y; III,2:43y) strongly evokes a diagnosis of telomeropathy [15]. This is supported by the identification of a monoallelic rare variation predicted to be deleterious in the *NOPI0* telomere related gene that co-segregates with disease. Collectively, these results provide the first evidence that mono allelic *NOPI0* mutation can predispose to short telomere syndrome (familial pulmonary fibrosis, liver and hematologic diseases)

Figure legends

Figure 1a). Three generation pedigree of the proband. Numbers below individual symbols are age at death or study. *NOPI0* 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 input of Figure 1a, the presence of *NOPI0* mutation identified by whole exome sequencing was confirmed by Sanger sequencing. The symbol +/- discloses wild type whereas +/c.17A>G heterozygosity for the mutation. TL: telomere length by FLOW-FISH.

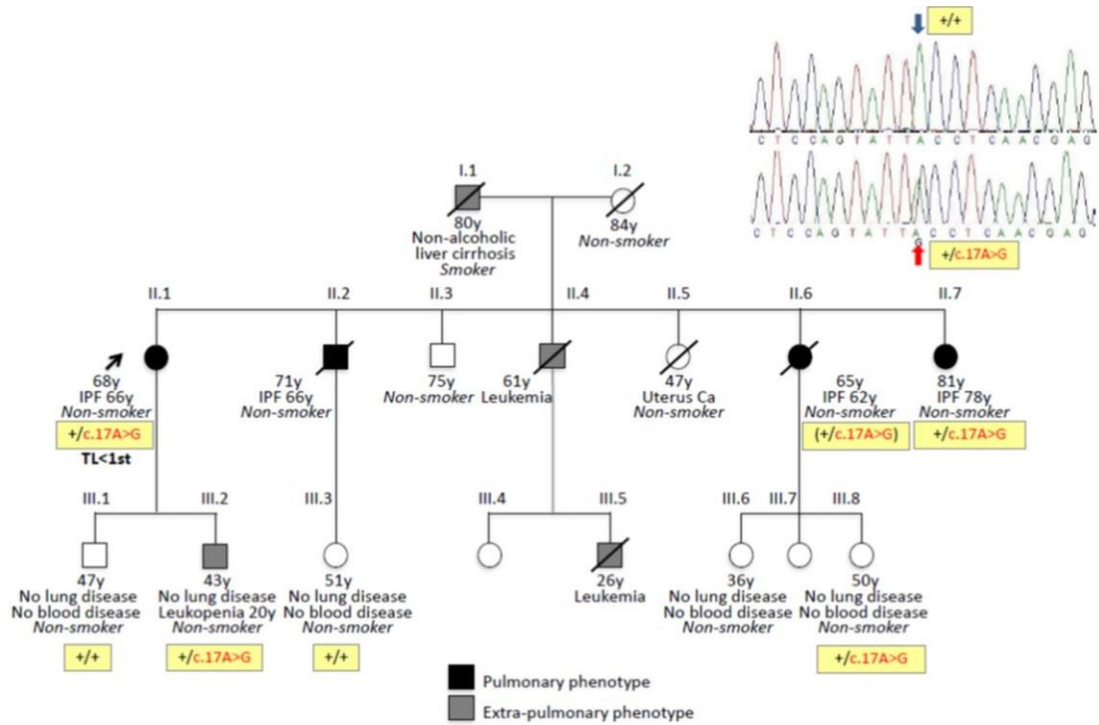
Figure 1b). High resolution CT image at the basal segments of the lower lobes of the proband (II.1) showing peripheral fine reticulation with a microcystic pattern subpleurally. In the inner lung zones intralobular reticulation in association with thin wall traction bronchiectasis are depicted. All features indicate the fibrotic nature of the process.

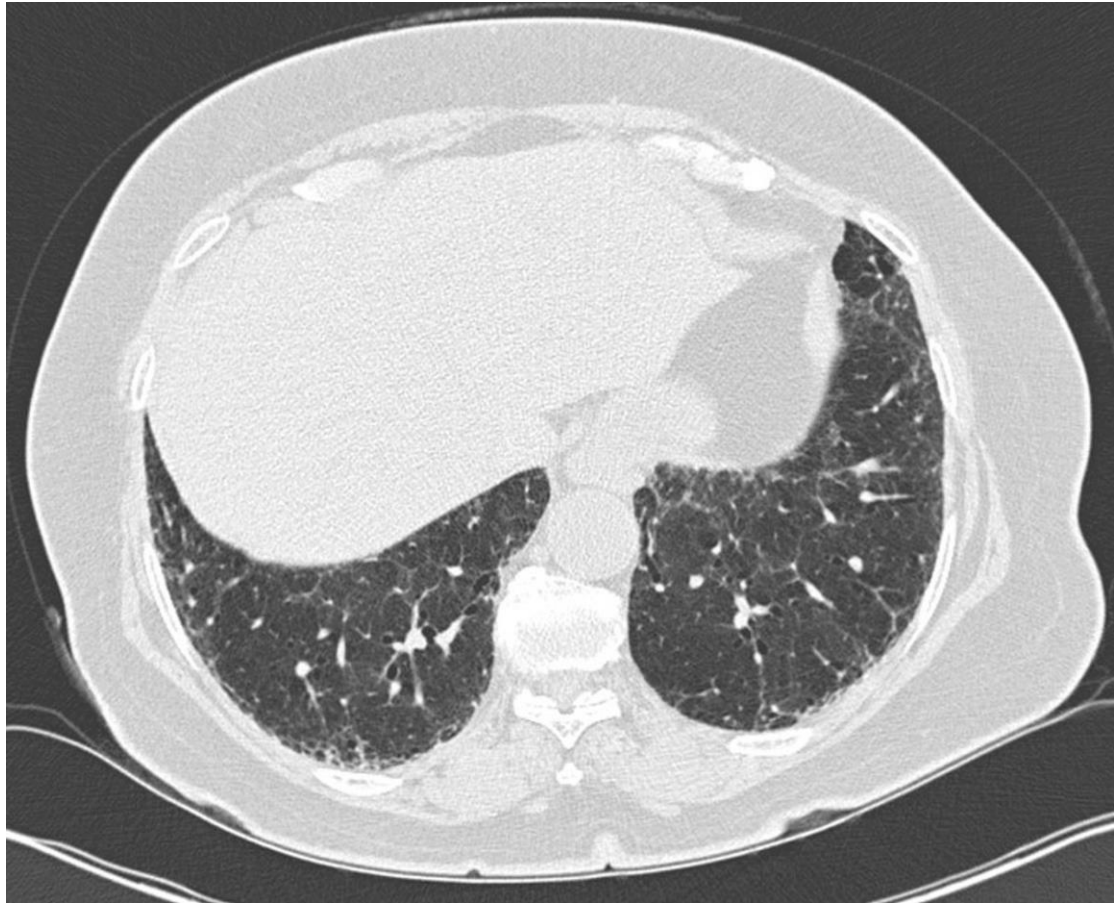
Figure 1c). High resolution CT of the chest at the level of the basal segments of lower lobes of the patient II.7 revealing definite reticulation subpleurally with areas of increased density resulting from coalescence of the reticular opacities and areas of

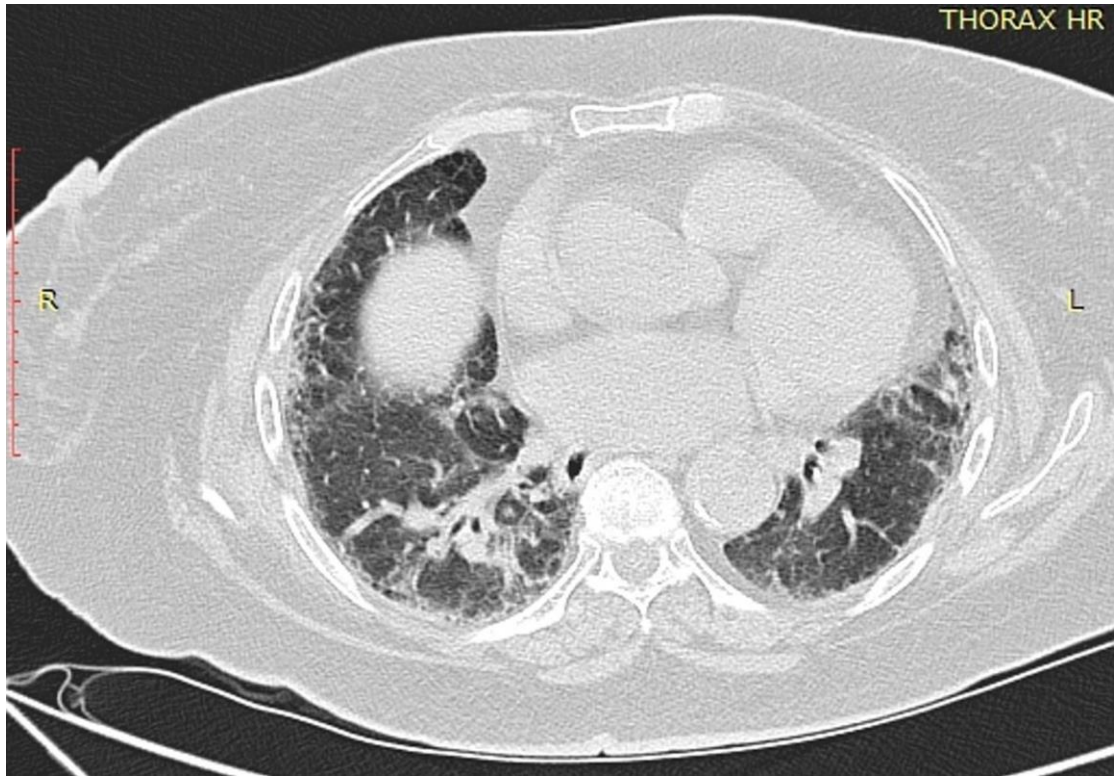
microatelectasis. Reticular changes are also seen more centrally distorting the shape of the vessels, while few areas of macrocystic (honeycomb) changes are seen in contact with the mediastinal pleura. The lungs bilaterally are ill expanded (small volumes)

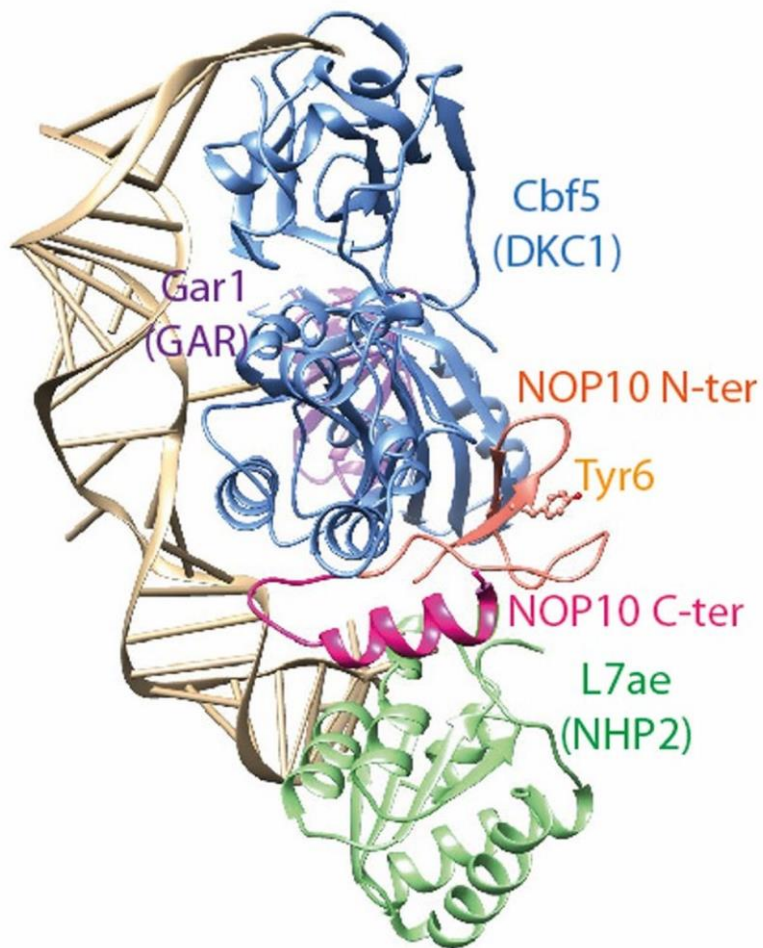
Figure 1d). Ribbon representation of the model of human NOP10, within the frame of a whole H/ACA RNP complex (experimental 3D structure from *Pyrococcus furiosus*, PDB 2EY4). A model of the 3D structure of human NOP10 has been made considering, for its N-terminal region (aa 1-33), the experimental 3D structure of *S. cerevisiae* Nop10 (solved in complex with Cbf5/Dkc1 and Gar1 (11, pdb 3U28)) 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 (12, pdb 2EY4). The 3D structure of human NOP10 could not be modeled as a whole starting from the archaeal Nop10 3D structure, as the N-terminal sequences strikingly diverge between archaeal and eukaryotic species, although still sharing a similar « ribbon » fold, organized 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 organization 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 3D structure were built using Modeller 9v15 (14), according to the alignment presented in Li et al (11).









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