Idiopathic pulmonary fibrosis with benign *SFTPC* variant and pathogenic *MARS1* mutations: can't see the forest for the trees!

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To the Editor:

Pulmonary alveolar proteinosis (PAP) is characterised by the accumulation of lipoproteins in the pulmonary alveoli due to defective clearance by alveolar macrophages [1]. It may be associated with myriad immune, metabolic and infectious diseases. In adults, autoimmune PAP is due to anti-granulocyte–macrophage colony-stimulating factor (GM-CSF) antibodies. In children, hereditary PAP may be due to biallelic mutations in *CSF2RA* (Mendelian Inheritance in Man (MIM); www.omim.org identifier 306250) and *CSF2RB* (MIM 138981) encoding the α- or β-chains, respectively, of the GM-CSF receptor.

Recently, mutations in *MARS1* (MIM 156560), encoding the ubiquitously expressed human cytoplasmic methionyl-tRNA synthetase 1, were found associated with interstitial lung and liver disease (ILLD), a polymorphic entity that includes PAP [2–4]. Notably, such forms are due to biallelic mutations of *MARS1* and are inherited as an autosomal recessive trait, whereas single mutations in the same gene can cause the dominant *MARS*-related Charcot–Marie–Tooth 2U disease [5] and other axonal peripheral neuropathies [6].

Here, we report the atypical case and genetic analysis of a patient who was suspected to have PAP in childhood. In adulthood, severe pulmonary fibrosis with pulmonary hypertension developed, requiring lung transplantation. In this patient, we initially identified an *SFTPC* benign variant and subsequently two pathogenic compound heterozygous variants of *MARS1*.

The female patient was born at term to nonconsanguineous parents. She had a history of suspected PAP at the age of 2 years. Despite a moderate clinical impact (*i.e.* hypocapnia due to hyperventilation; moderate pulmonary insufficiency with no failure to thrive; and no liver alterations), she had radiological signs suggesting PAP (images not available). The sweat chloride test result was negative, and metabolic disorders or autoimmunity were excluded. Bronchoalveolar lavage fluid had a milky appearance, but periodic acid–Schiff (PAS) staining was negative. Lung biopsy performed at 3 years showed alveolar proteiform exudate with lymphoplasmocytic infiltrates and interstitial lesions. However, the diagnosis of PAP could not be confirmed because of negative PAS staining. A search for anti-GM-CSF antibodies was negative at that time. The patient received repeated therapeutic whole-lung lavage until adolescence, with only partial radiological improvement. She was then lost to follow-up.

At the age of 33 years, the woman gave birth to a healthy child, but 2 years later, a new pregnancy ended in late pregnancy loss. From then, her lung condition worsened gradually over 2 years, with increased dyspnoea (New York Heart Association class IV), productive cough and exertional and coughing desaturation. Imaging revealed progressively worsening diffuse infiltrative opacities (figure 1a) together with moderate pre-capillary pulmonary hypertension (mean pulmonary arterial pressure 30 mmHg). A search for anti-GM-CSF antibodies was still negative.

The patient's respiratory condition worsened markedly, with requirement for continuous supplemental oxygen; the forced vital capacity (FVC) was 700 mL (*i.e.* 23% pred) and total lung capacity 1.13 L (25% pred), with hypoxaemia and moderate hypercapnia. She finally benefited from bilateral lung transplant. Pathologic examination of the explanted lung revealed intra-alveolar accumulation of amorphous acellular

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Even in the absence of liver disease, *MARS1* screening should be considered in severe lung fibrosis of young individuals. Interpretation of the genetic variants can evolve with improvement of knowledge (databases, bioinformatic tools) over time. https://bit.ly/45OxF5E

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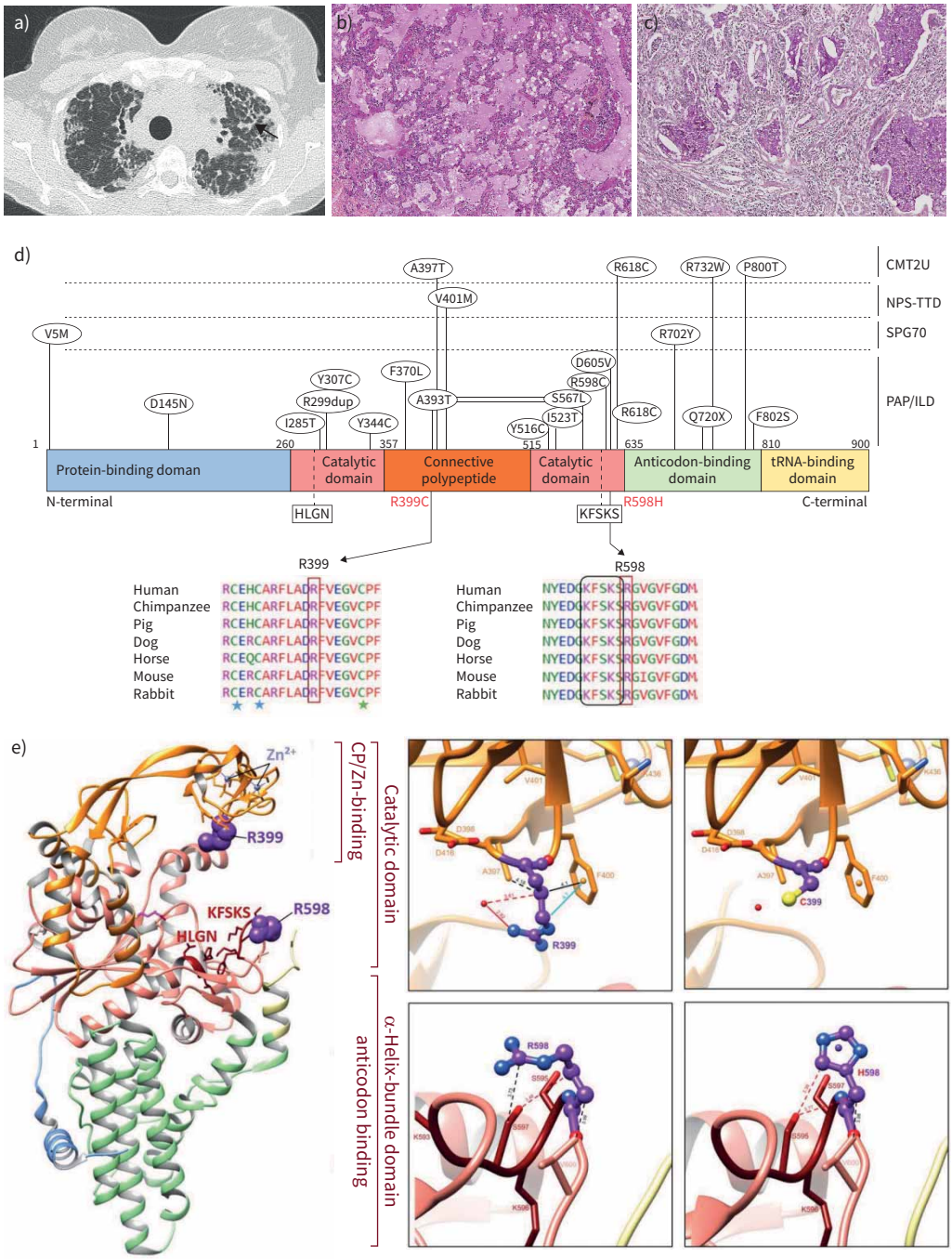

FIGURE 1 a) High-resolution computed tomography scan of the chest of a 37-year-old woman. Reticular opacity predominating in the upper lung zones consisting of mesh-like opacities associated with traction bronchiectasis and irregular bronchiolar dilation (arrow). The imaging pattern suggests fibrotic nonspecific interstitial pneumonia. b) Lung explant histopathology with haematoxylin–eosin–saffron staining (200x magnification) showing alveolar accumulation of amorphous acellular material in the lungs. c) Periodic acid–Schiff (PAS) staining (200x magnification) showing PAS-positive intra-alveolar material and accumulation of PAS-positive material in alveoli with discreet signs of inflammation. d) Schematic representation of the known methionyl-tRNA synthetase 1 (MetRS) (*MARS1*) mutations according to the phenotype and positioning of the p. Arg399Cys and p.Arg598His mutations. The MetRS protein comprises 900 amino acids and contains a protein-binding domain (PBD) (blue), a catalytic core consisting of the connective polypeptide inserted into the Rossmann fold catalytic domain, an anticodon-binding domain (green) and a tRNA binding domain (yellow). The signature sequences HLGN and KFSKS in the Rossmann fold are indicated with dotted lines. The locations of the published mutations are indicated according to the associated phenotype: pulmonary alveolar

proteinosis (PAP)/interstitial lung disease (ILD), spastic paraplegia (SPG70), nonphotosensitive trichodystrophy (NPS-TTD) and Charcot–Marie–Tooth 2U (CMT2U). The mutations R399C and R598H are represented in red. Alignments of the sequences of MetRS from different species are shown using the Clustal Omega software. R399 and R598 are highly conserved. Blue and green stars indicate the cysteine residues involved in Zn binding motifs 1 and 2, respectively. e) Location of the two mutations in the three-dimensional model of human MetRS (5goy) complexed with methionine (pink). The two mutated residues Arg399 and R598 are shown in purple spheres. The PBD is in blue, the catalytic domain in light red with the connective polypeptide in orange, the anticodon-binding domain in green and the tRNA-binding domain in yellow. The HLGN and KFSKS signature sequences are in dark red. In zoomed pictures, mutated amino acids are in purple in ball-and-stick configuration. Oxygen atoms are in red, nitrogen in blue and sulfur in yellow. Interactions are shown in lines: black lines indicate hydrophobic interactions at <5 Å distance between involved atoms (dotted lines for weak carbon–carbon interaction and solid lines for stronger carbon–aromatic interaction), red lines show hydrogen bonds (dotted lines for weak C–H···O interaction and solid lines for strong N–H···O interaction) and blue lines indicate cation-π interaction involving positively charged nitrogen of the arginine residue and aromatic ring of the nearby phenylalanine residue.

PAS-positive material and alveolar septa fibrosis, concluding advanced pulmonary fibrosis (fibrotic nonspecific interstitial pneumonia-like pattern) and pleural fibro-elastosis (figure 1b,c).

2 years after the lung transplant, the woman was in good clinical condition, with FVC 90% pred. Liver and kidney function test results were still in the normal range, as were lymphocyte and granulocyte counts. Neurological examination was unremarkable.

The genetic analysis was performed for the patient and her parents after obtaining informed consent according to French legislation. Sanger sequencing of the *SFTPC* gene (NM_005411.5), encoding surfactant protein-C, revealed first the c.10G>A (p.Gly4Ser) heterozygous variant. Although this *SFTPC* variant was predicted to be benign by several *in silico* tools, it was initially classified as a variant of unknown significance because of its rarity. However, a segregation study in the family identified the heterozygous variant in the asymptomatic father, ruling out a severe pathogenic impact of this variant. Subsequent analysis of *NKX2–1* (NM_001079668.2), *ABCA3* (NM_001089.2), *SFTPB* (NM_00542.3) and *CSF2RA* (*NM_172245.4*) gave negative results.

Finally, we identified two missense variants in *MARS1* (NM_004990.4) in compound heterozygosity. Both variants affected highly conserved amino acids as observed by using the Clustal Omega alignment tool [7] (figure 1d) and were predicted as probably damaging by most *in silico* algorithms, with highly pathogenic combined annotation dependent depletion (CADD) Phred-scores [8]. The maternal inherited variant c.1195C>T (p.Arg399Cys) was located in the connective polypeptide (CADD Phred-score: 32) and the paternal inherited variant c.1793G>A (p.Arg598His) in the catalytic domain (CADD Phred-score: 31) (figure 1d). To better understand the structural defects caused by the variants, we visualised them on the three-dimensional model (PDB: 5goy) by using UCSF ChimeraX [9, 10] (figure 1e). The p.Arg399 residue (R399) had hydrophobic interactions with the surrounding residues p.Ala397 and p.Phe400 because of their close proximity (<5 Å) and thus could be involved in structure maintenance.

The R399 residue was also involved in cation-π interaction with the aromatic residue F400. Of note, the p.Ala397Thr (A397T) was recently found involved in Charcot–Marie–Tooth 2U [11]. The p.Arg399Cys (R399C) variant causes many changes in noncovalent interactions, probably leading to a less stable protein. The p.Arg598His (R598H) variant is contiguous with the ^{593}KFSKS^{597} signature sequence (KMSKS motif) in the Rossmann fold, near the cavity for binding methionine. Histidine residues can be involved in proton transfer reactions and can affect the pH-dependent properties of proteins [12]. This p.Arg598His variant could affect the structure/stability of the cavity or the kinetics of methionine binding. Moreover, the p.Arg598His mutation was recently reported in a compound heterozygous patient with ILLD [13]. At the same position, the p.Arg598Cys has recently been described to decrease MARS1 activity in a family with ILLD [14].

More than 90% of patients with *MARS1* variant-associated PAP have liver disease and systemic inflammation, with very early-onset severe course and poor outcome [2–4, 15]. Our patient had isolated severe lung disease with severe pulmonary fibrosis described at explant histological examination, which probably represents the long-term evolution of an atypical form of PAP that began in childhood. Indeed, in the largest cohort reported by Enaud *et al.* [16], lung fibrosis was present in 60% of the patients at age 13 years and in almost 100% of the patients at age 25 years.

In our patient, the previously identified heterozygous p.Gly4Ser *SFTPC* variant did not cause the disease. It might have acted as a negative modifier epistatic factor causing evolution to severe pulmonary fibrosis at an early age. The severity and clinical course of the phenotype prompted us to continue our investigations and further identify the causal *MARS1* pathogenic variants.

In conclusion, the case highlights the complexity of genotype–phenotype correlations in the field of chronic pulmonary diseases. Interpretation of the genetic variants can evolve with improvement of knowledge (databases, bioinformatic tools) over time. We emphasise the importance of regular reassessment to resolve diagnostic dead-ends in rare lung diseases and initiate targeted therapies.

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