NKX2.1 mutation revealed by a lymphoid interstitial pneumonia in an adult with rheumatoid arthritis

Received: 7 Dec 2022
Accepted: 2 March 2023

To the Editor:

NKX2.1 encodes the thyroid transcription factor 1 (TTF1), which is implicated in lung development and control of the expression of surfactant proteins [1]. This transcription factor is also expressed in the brain and the thyroid, and *NKX2.1* heterozygous mutations are responsible for the "brain–lung–thyroid syndrome". This rare syndrome is characterised by central nervous system abnormalities, interstitial lung disease (ILD) and hypothyroidism, though the triad is inconstant with heterogeneity of organs involved and clinical presentation. A severe ILD can be the only manifestation in up to 25% of *NKX2.1* mutation carriers [2]. Heterogeneous phenotypes may be present in the same family without a clear explanation for the variable penetrance [3]. Most of the cases have been reported in newborns with fatal disease due to neonatal respiratory distress syndrome or ILD in childhood [2–5], and only a few cases are described in adulthood [2, 3, 5]. Recently it has been shown that familial pulmonary fibrosis and rheumatoid arthritis (RA)-associated ILD (RA-ILD) share the same genetic background, but no association with an *NKX2.1* mutation has been reported to date [6, 7]. We describe the case of an adult patient with hypothyroidism and RA-ILD with *NKX2.1* heterozygous pathogenic variation.

A 37-year-old nonsmoker female was referred to our department for the evaluation of ILD. A diagnosis of RA was given 6 years earlier. She also reported hypothyroidism of unknown aetiology (treated with synthetic thyroxin), obesity, hypertension and diabetes. ILD was detected at RA diagnosis (figure 1a). RA treatment required low-dose steroids with different disease-modifying antirheumatic drugs including methotrexate, tocilizumab, rituximab and abatacept. At time of referral, she complained of severe shortness of breath (modified Medical Research Council (mMRC) dyspnoea scale 3) and dry cough. Clinical examination revealed bilateral crackles. Neurological examination was normal. Chest high-resolution computed tomography (HRCT) showed diffuse ground-glass opacities and thickening of the bronchovascular bundles and small nodules in a centrilobular and subpleural location, associated with traction bronchiectasis and honeycombing predominating in the left upper lobe (figure 1b). These features had developed in areas with previous ground-glass opacities. Diffuse enlargement of mediastinal and hilar lymph nodes was also present. Pulmonary function tests showed a severe restrictive defect with reduced forced vital capacity (forced vital capacity (FVC) 2020 mL, 54% predicted) and reduced diffusion capacity for carbon monoxide (D_{LCO}, 25% of the predicted value) with normal forced expiratory volume in 1 s (FEV_1)/FVC ratio (0.87, 107% predicted). During the 6-min walk test she walked 380 m (76% predicted) with desaturation at 80%.

HIV testing was negative. C-reactive protein was 13 $mg \cdot L^{-1}$. Immunological investigations showed positive anti-citrullinated peptide antibodies (35 $U \cdot L^{-1}$) and rheumatoid factor (>200 $IU \cdot L^{-1}$). A search for anti-extractable nuclear antigen (ENA), myositis-specific antibodies and thyroiditis antibodies was negative. Serum protein electrophoresis showed restrictions of gamma heterogeneity but serum protein immunoelectrophoresis was normal. Cytological examination of the bronchoalveolar lavage fluid revealed 410 000 $cells \cdot mL^{-1}$ with 68% alveolar macrophages, 10% lymphocytes and 22% neutrophils. Microbiological evaluation was negative. Endobronchial ultrasound-guided mediastinal lymph node sampling showed normal lymph node cytology. After multidisciplinary discussion and due to the very atypical HRCT pattern with suspicion of pulmonary lymphoma, surgical lung biopsy was performed. Three biopsy specimens were taken from the left lung (apex, lingula and lower lobe). Histology showed a diffuse infiltration of the pulmonary interstitium by small lymphocytes (mainly T-cells) and polytypic

Shareable abstract (@ERSpublications)
This is the first case of a 37-year-old female patient carrier of a heterozygous *NKX2.1* mutation associated with RA-ILD with a histological pattern of LIP. This case illustrates the wide panel of ILD subtypes associated with *NKX2.1* mutations. https://bit.ly/3F49OTS

Cite this article as: Le Guen P, Borie R, Legendre M, *et al*. *NKX2.1* mutation revealed by a lymphoid interstitial pneumonia in an adult with rheumatoid arthritis. *ERJ Open Res* 2023; 9: 00682-2022 [DOI: 10.1183/23120541.00682-2022].

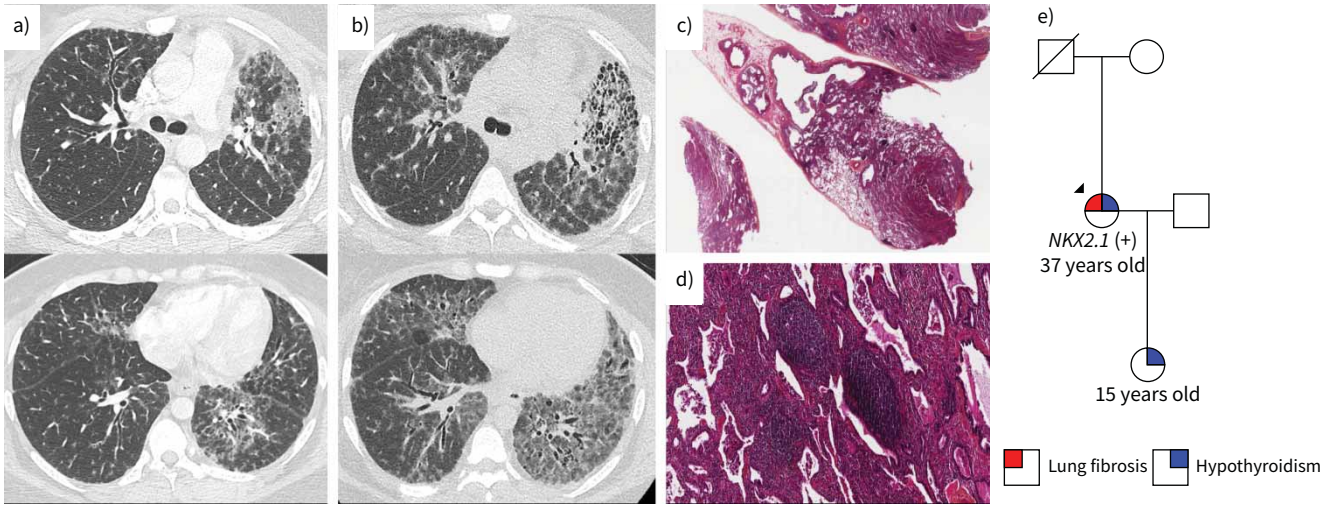

FIGURE 1 a) First thoracic computed tomography (CT) scan for diagnosis of rheumatoid arthritis, showing ground-glass opacities and traction bronchiectasis. b) Evolution of thoracic CT scan 6 years later showing extension of ground-glass opacities and thickening of the bronchovascular bundles and small nodules, associated with traction bronchiectasis and honeycombing in the left upper lobe. c) Histology of surgical lung biopsy: diffuse lymphoid infiltrate expanding the pulmonary interstitium and distorting architecture with cysts; haematoxylin, eosin, saffron (HES) stain; scanning magnification; d) ×20 objective. e) Index case (black arrowhead) was diagnosed with the heterozygous p.(Gln317*) mutation. Her daughter has hypothyroidism.

plasmocytes. Some reactive B-cell follicles were present. A molecular analysis failed to detect any monoclonal population (figure 1c, d). The histological pattern was consistent with lymphocytic interstitial pneumonia (LIP) with some fibrotic remodelling. Histology of the minor salivary glands was normal. We proposed treatment with methylprednisolone pulses (250 mg·day^{-1} for 3 days) then prednisone 60 mg·day^{-1} with progressive tapering, and rituximab (1000 mg on day 1 and day 15). After 6 months of follow-up, dyspnoea was stable (mMRC 3) as was her 6-min walk test with a distance of 380 m (76% predicted). Lung function test results remained stable (FVC 1900 mL, 52% predicted; D_{LCO} 24% predicted).

Because of the early onset of the lung disease and the association with hypothyroidism, a germline genetic analysis was prescribed. A heterozygous nonsense mutation was identified in *NKX2.1* (NM_001079668) (c.949C>T p.(Gln317*)). No mutation was detected in other genes associated with genetic ILD, including telomere-related genes (*TERT*, *TERC*, *PARN*, *RTEL1*, *DKC1*, *TINF2*, *NOP10*, *NHP2)* and other surfactant-related genes (*SFTPC*, *STFPA1*, *SFTPA2*, *ABCA3*).

The *NKX2.1* p.(Gln317*) nonsense mutation was considered as likely pathogenic. This mutation creates a premature stop codon in the last exon of the gene, and the corresponding transcripts probably escape nonsense-mediated mRNA decay (transcripts degradation before protein production), but it probably leads to the production of a non-functional truncated protein lacking its last 85 amino acids (out of 401). It is not described in the gnomAD database that encompasses about 15 500 control subjects with sequencing data at this genomic position. The patient did not have any siblings and there was no parental history of miscarriage. Neither her mother nor her mother's sibling had a history of ILD or neurological disease. Her father died with myocardial infarction without a history of lung disorder, whereas her 15-year-old daughter had an abnormal thyroid test (figure 1e). In conformity with French legislation, her minor daughter was not tested because she had no respiratory or neurological involvement with a normal clinical evaluation. A genetic diagnosis will be offered to her once she gets her majority. Unfortunately, we did not have access to the DNA of our patient's parents to determine if the mutation was *de novo*.

This is the first report of a pathogenic *NKX2.1* mutation associated with RA-ILD revealed in adulthood with a confirmed diagnosis of LIP. This case illustrates the variable phenotypic expression associated with *NKX2.1* mutations and expands the lung phenotype associated with *NKX2.1* mutations to secondary ILD.

In paediatric patients, genetic analysis of surfactant-related genes is recommended in the diagnosis of cryptogenic ILD [8], but guidelines do not recommend any genetic analysis in ILD adult patients. In our

centre, we propose genetic analysis for patients with fibrotic ILD before 50 years of age or presenting with extrapulmonary manifestation suggestive of specific genetic syndrome or before lung transplantation [9, 10], in accordance with the recent European Respiratory Society statement on familial pulmonary fibrosis [11]. According to the laboratory and the phenotype, the genetic analysis may include telomere-related genes, surfactant-related genes analysed through next generation sequencing panel or clinical whole exome sequencing. In this case, the genetic analysis was indicated because of: 1) the unusually young age for severe RA-ILD; 2) the fibrotic ILD pattern; and 3) the association with hypothyroidism of unknown aetiology.

The other interesting point concerns the histological pattern. LIP is a very rare entity in adults. LIP can be idiopathic or associated with HIV infection or with autoimmune diseases, especially Sjögren's syndrome [12]. Cases of LIP with RA without Sjögren's syndrome are very rare [13]. One case of LIP associated with *NKX2.1* mutation has been suspected in a 5-year-old child but without histological confirmation [2]. There is no lung histology reported in adult cases associated with *NKX2.1* mutation [2, 3, 5]. In children, nonspecific interstitial pneumonia and desquamative interstitial pneumonia have been observed [2].

The link between RA, LIP and *NKX2.1* mutation is still unclear. Our main hypothesis is that RA just happened to overlap with the *NKX2.1* gene mutation; RA triggered the development of the ILD and the genetic predisposition facilitated the development of a particularly severe and fibrotic ILD. However, some authors found surfactant proteins in the synovial membrane of RA patients and have discussed the role of surfactant protein in RA inflammation [14, 15]. This case report raises the question that dysfunction of TTF1 protein may affect synovial homeostasis and facilitate the development of RA. Alternatively, dysfunction of TTF1 may promote a pathological immune response targeting the lung, triggering a diffuse lymphocytic infiltration with secondary protein citrullination in the lung, a condition that could promote the development of RA [16, 17].

In conclusion, we report the first case of a 37-year-old female carrier of a heterozygous *NKX2.1* mutation associated with RA-ILD with a histological pattern of LIP. This case illustrates the wide panel of ILD subtypes associated with *NKX2.1* mutations.

Pierre Le Guen [1], Raphael Borie [1], Marie Legendre [2], Clairelyne Dupin [1], Laetitia Dunogeant[3], Sébastien Ottaviani[4], Marie-Pierre Debray[5], Aurélie Cazes[6], Philippe Dieudé [4], Caroline Kannengiesser [7] and Bruno Crestani [1]

[1]Université de Paris, INSERM 1152, Assistance Publique-Hôpitaux de Paris, Hôpital Bichat, Service de Pneumologie A, Centre de Référence des Maladies Pulmonaires Rares, DHU APOLLO, Paris, France. [2]Sorbonne Université, INSERM U933 Maladies génétiques pédiatriques, Assistance Publique-Hôpitaux de Paris, Hôpital Armand-Trousseau, Unité de Génétique moléculaire, Paris, France. [3]Centre hospitalier du pays d'Aix, Service de rhumatologie et médecine interne, Aix-en-Provence, France. [4]Université de Paris, INSERM 1152, Assistance Publique-Hôpitaux de Paris, Hôpital Bichat, Service de Rhumatologie, DHU APOLLO, Paris, France. [5]Assistance Publique-Hôpitaux de Paris, Hôpital Bichat, Service de Radiologie, Paris, France. [6]Université de Paris, INSERM 1152, Assistance Publique-Hôpitaux de Paris, Hôpital Bichat, Département d'anatomie pathologique, DHU APOLLO, Paris, France. [7]Université de Paris, INSERM 1152, Assistance Publique-Hôpitaux de Paris, Hôpital Bichat, Service de génétique, DHU APOLLO, Paris, France.

Corresponding author: Pierre Le Guen (pierre.leguen@aphp.fr)

Provenance: Submitted article, peer reviewed.

Conflict of interest: P. Le Guen has nothing to disclose. R. Borie has received grants and personal fees from Roche and Boehringer Ingelheim, and personal fees from Savara, outside the submitted work. M. Legendre has nothing to disclose. C. Dupin has served on an advisory board for, and received a congress invitation and a speaker fee from AstraZeneca; received speaker fees and congress invitations from Boehringer Ingelheim, Chiesi and Novartis; served as a study investigator for, and received a speaker fee and a congress invitation from GSK; served as an advisory board member and received a congress invitation from Sanofi; and received a congress invitation from Roche, all outside the submitted work. L. Dunogeant has nothing to disclose. S. Ottaviani has nothing to disclose. M-P. Debray has received personal fees and nonfinancial support from Boehringer Ingelheim, and nonfinancial support from Roche, outside the submitted work. A. Cazes has received personal fees from Roche, Boehringer and

AstraZeneca, outside the submitted work. P. Dieudé has nothing to disclose. C. Kannengiesser has nothing to disclose. B. Crestani has received speaker honoraria and support for a congress from AstraZeneca; received grants, speaker honoraria and support for congresses from Boehringer Ingelheim and Roche; received speaker honoraria, honoraria to consultancy and support for a congress from Sanofi; served on an advisory board for Genzyme; and served on an advisory board and received support for a congress from BMS, all outside the submitted work.

References

1 Attarian SJ, Leibel SL, Yang P, *et al.* Mutations in the thyroid transcription factor gene NKX2-1 result in decreased expression of SFTPB and SFTPC. *Pediatr Res* 2018; 84: 419–425.
2 Hamvas A, Deterding RR, Wert SE, *et al.* Heterogeneous pulmonary phenotypes associated with mutations in the thyroid transcription factor gene NKX2-1. *Chest* 2013; 144: 794–804.
3 Jovien S, Borie R, Doummar D, *et al.* Respiratory distress, congenital hypothyroidism and hypotonia in a newborn. *Respiration* 2016; 92: 188–191.
4 Iwatani N, Mabe H, Devriendt K, *et al.* Deletion of NKX2.1 gene encoding thyroid transcription factor-1 in two siblings with hypothyroidism and respiratory failure. *J Pediatr* 2000; 137: 272–276.
5 Nattes E, Lejeune S, Carsin A, *et al.* Heterogeneity of lung disease associated with NK2 homeobox 1 mutations. *Respir Med* 2017; 129: 16–23.
6 Juge PA, Borie R, Kannengiesser C, *et al.* Shared genetic predisposition in rheumatoid arthritis-interstitial lung disease and familial pulmonary fibrosis. *Eur Respir J* 2017; 49: 1602314.
7 Juge PA, Lee JS, Ebstein E, *et al.* MUC5B promoter variant and rheumatoid arthritis with interstitial lung disease. *N Engl J Med* 2018; 379: 2209–2219.
8 Bush A, Cunningham S, de Blic J, *et al.* European protocols for the diagnosis and initial treatment of interstitial lung disease in children. *Thorax* 2015; 70: 1078–1084.
9 Borie R, Kannengiesser C, Nathan N, *et al.* Familial pulmonary fibrosis. *Rev Mal Respir* 2015; 32: 413–434.
10 Borie R, Kannengiesser C, Dupin C, *et al.* Impact of genetic factors on fibrosing interstitial lung diseases. Incidence and clinical presentation in adults. *Presse Med* 2020; 49: 104024.
11 Borie R, Kannengiesser C, Antoniou K, *et al.* European Respiratory Society statement on familial pulmonary fibrosis. *Eur Respir J* 2023; 61: 2201383.
12 Swigris JJ, Berry GJ, Raffin TA, *et al.* Lymphoid interstitial pneumonia: a narrative review. *Chest* 2002; 122: 2150–2164.
13 Cha SI, Fessler MB, Cool CD, *et al.* Lymphoid interstitial pneumonia: clinical features, associations and prognosis. *Eur Respir J* 2006; 28: 364–369.
14 Christensen AF, Sorensen GL, Junker K, *et al.* Localization of surfactant protein-D in the rheumatoid synovial membrane. *APMIS* 2018; 126: 9–13.
15 Hartjen N, Bräuer L, Reiß B, *et al.* Evaluation of surfactant proteins A, B, C, and D in articular cartilage, synovial membrane and synovial fluid of healthy as well as patients with osteoarthritis and rheumatoid arthritis. *PLoS One* 2018; 13: e0203502.
16 Aubart F, Crestani B, Nicaise-Roland P, *et al.* High levels of anti-cyclic citrullinated peptide autoantibodies are associated with co-occurrence of pulmonary diseases with rheumatoid arthritis. *J Rheumatol* 2011; 38: 979–982.
17 Fischer A, Solomon JJ, du Bois RM, *et al.* Lung disease with anti-CCP antibodies but not rheumatoid arthritis or connective tissue disease. *Respir Med* 2012; 106: 1040–1047.