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Heterogeneity of lung disease associated with NK2 homeobox 1 mutations

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ABSTRACT

We retrospectively studied the clinical presentation, treatment modalities and outcome in 16 patients with heterozygous *NKX2-1* mutation associated with chronic lung disease.

Twelve different *NKX2-1* mutations, including 4 novel mutations, were identified in the 16 patients. Nine patients presented with brain-lung-thyroid syndrome, 3 had neurological and lung symptoms and 4 had only pulmonary symptoms. Ten patients had neonatal respiratory distress, and 6 of them developed infiltrative lung disease (ILD). The other patients were diagnosed with ILD in childhood (n = 3) or in adulthood (n = 3). The median age at diagnosis was 36 months (IQ 3.5–95). Patient testing included HRCT (n = 13), BALF analysis (n = 6), lung biopsies (n = 3) and lung function tests (n = 6). Six patients required supplemental oxygen support with a median duration of 18 months (IQ 2.5–29). All symptomatic ILD patients (n = 12) benefited from a treatment consisting of steroids, azithromycin (n = 9), and/or hydroxychloroquine (n = 4). The median follow-up was 36 months (IQ 24–71.5). One patient died of respiratory failure at 18 months and another is waiting for lung transplantation.

In summary, the initial diagnosis was based on clinical presentation and radiological features, but the presentation was heterogeneous. Definitive diagnosis required genetic analysis, which should be performed, even in absence of neurological or thyroid symptoms.

Abbreviations: RDS, respiratory distress syndrome; NKX2-1, NK2 homeobox 1; ABCA-3, adenosine triphosphate-binding cassette transporter A3; ILD, infiltrative lung disease; HRCT, high-resolution computed tomography; PCR, polymerase chain reaction; BALF, bronchoalveolar lavage fluid; IQ, interquartile; PaO_2, Partial Pressure of Oxygen; TLC, a total lung capacity; FEV-1, forced expiratory volume in 1 s.

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1. Introduction

Inherited surfactant metabolism diseases represent 10–15% of the cause of neonatal respiratory distress syndrome (RDS) in full term newborns and of infiltrative lung disease (ILD) in older children [1,2]. Pulmonary surfactant is a multi-molecular complex that is secreted into the alveolar airspace to form a thin layer at the air-liquid interface, preventing alveolar atelectasis. It is composed of phospholipids (80–90%) and proteins (10–15%), among which 2–3% are specific proteins called surfactant protein A, B, C and D (SP-A, SP-B, SP-C and SP-D, respectively) [3]. SP-B and SP-C are two hydrophobic proteins, synthesized by alveolar type II cells as pro-peptides (pro-SP-B and pro-SP-C). Their intracellular trafficking includes processing through lamellar bodies where surfactant proteins and lipids are assembled into bilayer membranes by ABCA-3 (adenosine triphosphate-binding cassette transporter A3) [4,5]. The synthesis of surfactant proteins is partly controlled by the transcription factor NK2 homeobox 1 (NKX2-1) [6], a member of the NKX2 transcription factor family. NKX2-1 was initially identified as a nuclear protein binding the promoter of the thyroglobulin gene. However, various studies have later shown that NKX2-1 is also expressed in the brain and the lungs [7]. The NKX2-1 protein is encoded by the *NKX2-1* gene, located on chromosome 14 (locus 14q13), and composed of three exons and two introns [8]. Homozygous mice lacking NKX2-1 expression die shortly after birth, lack lung parenchyma and thyroid gland, and exhibit defects in the ventral region of the forebrain, including the absence of pituitary gland [9]. In humans, autosomal dominant *NKX2-1* mutations are associated with brain-lung-thyroid syndrome, combining varying degrees of congenital hypothyroidism, neurological features (neonatal or early childhood hypotonia evolving to benign hereditary chorea) and pulmonary symptoms [10]. While both neurological and pulmonary features are responsible for the disease morbidity, lung disease is mainly responsible for the mortality. Various pulmonary phenotypes have been described, including RDS in term infants, ILD in children, and more recently in adults [11]. Management of the diseases associated with *NKX2-1* mutations is still a matter of debate, one of the principal issues being the lack of large randomized clinical trials, and treatment is usually based on the severity of the disease and the expertise in each centre [12]. The aim of this study was to describe the pulmonary phenotypes in patients with *NKX2-1* mutations, including the clinical and radiological features, course of treatment and outcome.

2. Materials and methods

2.1. Study population

We retrospectively identified through the French Rare Lung Disease Network (RespiRare®) 16 subjects with *NKX2-1* mutation and associated pulmonary phenotype, who were followed in 8 hospitals throughout France (Centre Intercommunal de Creteil, Creteil; Centre Hospitalier Regional Universitaire, Lille; Hôpital Armand Trousseau, Paris; Hôpital Robert Debré, Paris,; Hôpital Bichat, Paris; Hôpital la Timone, Marseille; Centre Hospitalier Regional Universitaire, Tours, France), and 1 in Argentina (Hospital de Niños Ricardo Gutierrez, Buenos Aires).

Pulmonary phenotypes associated with *NKX2-1* mutations were defined as RDS in newborns at > 37-week gestation with severe hypoxemic respiratory failure and diffuse lung disease on chest radiography, or as ILD in older children or adults with a diagnosis based on clinical features and high-resolution CT (HRCT) scanning. Patients with asthma or recurrent lung infections and carrying a *NKX2-1* mutation were excluded from the study. Four patients including 2 members of a same family, have been reported previously [13–15] (Table 1).

2.2. Genetic analysis

NKX2-1 encodes 2 NKX2-1 protein isoforms: a 371 amino acid peptide (NM_003317.3, 2 exons) and a 401 amino acid peptide (NM_001079668.2, 3 exons). Although the shorter transcript sequence is the most commonly expressed isoform, we choose to use HGVS nomenclature applied to the longer transcript to prevent confusion. *NKX2-1* sequencing was performed by the Genetic Department at each centre. Briefly, EDTA blood samples were collected after obtaining informed consent from the patients or their parents (patients <18 years old), and used to extract leukocyte genomic DNA. Specific primers were used to amplify the coding regions and the intron-exon boundaries of the *NKX2-1* gene (primers sequence available on request) using polymerase chain reaction (PCR). The PCR products were subjected to direct Sanger sequencing. Pathogenicity of novel variants was evaluated depending on the affected domain, the type of mutation (frameshift or deletions vs non-sense), absence in the databases, and in silico analysis by the Alamut software (Interactive Biosoftwares). For each mutation, the *de novo* or inherited status was reported if the parents' DNA was available. The 3 patients harboring a deletion of the whole gene were identified by FISH using a locus-specific BAC mapping to *NKX2-1* at 14q13.3 (RP11-1083E2).

2.3. Design

The following data were retrieved from the patients' medical records and from the RespiRare® database:

- Family history, clinical presentation, association with neurological and/or thyroid symptoms;
- Chest radiography or HRCT reports, lung function tests, bronchoscopy and bronchoalveolar lavage fluid (BALF) reports, lung biopsy report;
- Therapeutic modalities, i.e., corticosteroids (methylprednisolone pulses and oral steroids), oxygen therapy, hydroxychloroquine, azithromycin, enteral feeding;
- Length of follow-up.

Data were expressed as mean ± interquartile (IQ). The study was granted a limited waiver of Authorization requirement, and was approved by our institutional review board.

3. Results

3.1. Genetic features

Twelve different *NKX2-1* mutations were identified in the 16 included patients (Fig. 1). All subjects were heterozygous for their mutation. The 12 mutations comprised 3 deletions of the whole gene and 9 point mutations, including 4 novel mutations (c.175_176del, c.267dup, c.714G>A, c.728G>A) (Fig. 1). Three point mutations (c.583C>T, c.714G>A, c.728G>A) resulted in an amino-acid substitution in the homeodomain (functional domain of NKX2-1 that allows DNA binding), and one (c.572G>T) was located just before the homeodomain. Four mutations induced a frameshift (c.175_176del, c.344dup, c.267dup, c.876_877del), 3 of which being located before the homeodomain. One mutation was an intronic nucleotide substitution (c.463+2T>C), which altered the splice donor site of intron 2, and thus was predicted to cause abnormal splicing. Six mutations were *de novo* (c.344dup, c.267dup, c.876_877del, c.583C>T, c.572G>T, c.728G>A), whereas 3 were inherited (c.175_176del, c.463+2T>C, c.714G>A). The c.344dup was