Case Report

Pulmonary Pathology in Thyroid Transcription Factor-1 Deficiency Syndrome

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Thyroid transcription factor-1 (TTF-1) deficiency syndrome is characterized by neurologic, thyroidal, and pulmonary dysfunction. Children usually have mild-to-severe respiratory symptoms and occasionally die of respiratory failure. Herein, we describe an infant with a constitutional 14q12–21.3 haploid deletion encompassing the TTF-1 gene locus who had cerebral dysgenesis, thyroidal dysfunction, and respiratory insufficiency. The clinical course was notable for mild hyaline membrane disease, continuous ventilatory support, and symmetrically distributed pulmonary cysts by imaging. He developed pneumonia and respiratory failure and died at 8 months. Pathologically, the lungs had grossly visible emphysematous changes with “cysts” up to 2 mm in diameter. The airway generations and radial alveolar count were diminished. In addition to acute bacterial pneumonia, there was focally alveolar septal fibrosis, pneumocyte hypertrophy, and clusters of airspace macrophages. Ultrastructurally, type II pneumocytes had numerous lamellar bodies, and alveolar spaces contained fragments of type II pneumocytes and extruded lamellar bodies. Although immunoreactivity for surfactant protein SP-A and ABCA3 was diminished, that for SP-B and proSP-C was robust, although irregularly distributed, corresponding to the distribution of type II pneumocytes. Immunoreactivity for TTF-1 protein was readily detected. In summation, we document abnormal airway and alveolar morphogenesis and altered expression of surfactant-associated proteins, which may explain the respiratory difficulties encountered in TTF-1 haploinsufficiency. These findings are consistent with experimental evidence documenting the important role of TTF-1 in pulmonary morphogenesis and surfactant metabolism.

Keywords: alveolar growth abnormality; cerebral dysgenesis; thyroid transcription factor-1; bronchopulmonary dysplasia; surfactant protein

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Awareness of TTF-1 deficiency syndrome and its pulmonary manifestations is limited. Studies describing pulmonary imaging and pathologic features are scarce.

What This Study Adds to the Field

We provide detailed pulmonary histopathologic, ultrastructural and immunohistochemical findings in an infant with this syndrome and describe abnormal airway and alveolar morphogenesis as well as abnormalities in surfactant protein expression in TTF-1 haploinsufficiency, which may account for the respiratory problems in this syndrome.

Thyroid transcription factor-1 (TTF-1, also known as TITF-1, NKX2-1, or TEBP), a member of the NKx2 homeodomain transcription factor family, is essential for the morphogenesis of thyroid, lungs, and brain (3).

TTF-1 knockout mice fail to develop these organs and die at birth, whereas the heterozygous mice reach term (4).

TTF-1 haploinsufficiency in humans is responsible for a rare syndrome (TTF-1 deficiency syndrome) characterized by neurological, thyroidal, and pulmonary dysfunction (5–9). In a recent series that also reviewed the clinical and pathologic findings of 46 patients with TTF-1 deficiency, pulmonary disease occurred in 54%, with “infant respiratory distress syndrome” being the most common (7). The pulmonary pathology has been described briefly in one patient, whereas only one other case report illustrates the pathologic findings (10, 11).

We identified an infant with cerebral dysgenesis, thyroidal dysfunction, respiratory insufficiency, and a karyotype showing a 14q13–21.3 deletion encompassing the TTF-1 gene locus. The infant died at 8 months with pneumonia and respiratory insufficiency. We describe his pulmonary pathology, including morphometric, ultrastructural, and immunohistochemical findings.

CASE REPORT

Clinical and Radiologic Findings

This male infant was born at 35-3/7 weeks to a G1, P0, 34-year-old woman whose pregnancy was complicated by premature rupture of membranes and hypertension precipitating preterm

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labor and delivery by Cesarean section. The birth weight was 1,895 g and Apgar scores were 9 at both 1 and 5 minutes (−1 for muscle tone). Within several minutes of delivery, respiratory distress required intubation. An initial chest radiograph was consistent with hyaline membrane disease (HMD), a dose of surfactant was administered, and ampicillin and gentamicin treatment was begun (Figure 1A). The chest radiograph on Day 2 showed improvement of the HMD (Figure 1B). Clonic movements of the head and neck were noted, and phenobarbital was administered. Once stabilized, the infant was transported to Children's Hospital Boston for further evaluation and management.

On transfer, the infant appeared gravely ill. His temperature was 35.4°C, the pulse was 112 and regular, and blood pressure was 43/36 mm Hg. The respiratory rate was 30/min on initial ventilatory settings of an inspiratory pressure of 23/5 and a rate of 30. His weight of 1.90 kg, head circumference of 28.5 cm, and length of 40 cm were small for gestational age. The anterior fontanel was open and flat, pupils were equal, round, and reactive to light, and sclerae were nonicteric. Coarse rales were heard on auscultation. There was a continuous flow murmur with a normal S1 and S2 and without click or rub. The abdominal examination was normal. For the degree of sedation, he was hypotonic. The plantar, biceps, knee, and ankle reflexes, however, were normal.

Shortly after admission, he had profound hypotension requiring multiple boluses of saline and albumin as well as inotropic support. Because of the inability to ventilate on maximal pressure support, high-frequency oscillatory ventilation was begun with maximum settings of mean airway pressure (MAP) of 22 cm H₂O and a delta P of 53. Hyperoxia testing showed an oxygen saturation of 112%. An echocardiogram demonstrated a large patent ductus arteriosus, a patent foramen ovale (both with bidirectional flow), mildly depressed biventricular function, and an estimated pulmonary artery pressure of 2/3 systemic. Nitric oxide treatment was begun at 20 ppm with improvement in oxygenation. Follow-up echocardiogram showed normalized ventricular and pulmonary artery pressures and nitric oxide was discontinued within 1 week of initiation. The hematocrit was 49.2%, the white cell count 8,700/ml with a differential of 48% neutrophils, 46% lymphocytes, and 13% nucleated red blood

cells, and the platelet count 265,000/ml; the prothrombin and partial thromboplastin times were within normal range. The levels of urea nitrogen, creatinine, glucose, conjugated and total bilirubin, total protein, albumin, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were normal. Magnesium was elevated at 5.4. Newborn screening was normal except for a diminished thyroxine level (T4) of 10.7, which in association with a normal thyrotropin was consistent with resolving euthyroid sick syndrome. A random cortisol level was diminished at 6.5 and a subsequent corticotropin stimulation test was normal.

He developed transient pulmonary interstitial emphysema and continued to require oxygen. He was weaned to normal mechanical ventilation within 2 weeks and by Day 23 no longer required ventilatory support. He gained weight (7 g/kg/d on 140 kcal/kg/d of enteral feeds). Over time, his oxygen requirement increased to an FI_O₂ of 50 to 60% to maintain a saturation of 96%. A "bubble" study was negative for heart/lung arteriovenous malformation. A chest radiograph at 11 weeks showed diffuse, coarse, slightly irregular opacification with mild subsegmental atelectasis in the right upper and lower lobes suggestive of chronic lung disease of prematurity/bronchopulmonary dysplasia (CLD/BPD) (Figure 1C). A chest computed tomography (CT) scan at the same age was most notable for bilateral 1- to 2-mm cysts, predominantly posteriorly and symmetrically distributed (Figures 1D and 1E). Because the CT appearance was not characteristic of CLD/BPD, a lung biopsy was performed (*vide infra*).

Myoclonic jerking prompted a magnetic resonance imaging of the brain, which showed cortical dysgenesis with decreased gyri, moderate ventricular enlargement, and truncation of the anterior aspect of the corpus callosum.

A karyotypic analysis of peripheral blood revealed in all metaphases analyzed an interstitial deletion in the long arm of one chromosome 14 encompassing the TTF-1 gene locus (14q12–21.3) (Figure 2). The mother's karyotype showed an inversion in the same region of one chromosome 14 in 1 of 30 cells. The father's karyotype was normal. The infant continued to exhibit hypotonia and global developmental delay. The prognosis was believed to be poor and he was discharged home at 3.5 months of age on nasal canula oxygen, enteral feedings,

Figure 1. (A) Anteroposterior (AP) chest radiograph (CXR) on day of birth reveals diffuse granular opacification and air bronchograms, consistent with hyaline membrane disease (HMD). (B) AP CXR on second day of life, after treatment with exogenous surfactant, shows an improved mild granular interstitial process suggestive of residual HMD. (C) AP CXR at 11 weeks shows diffuse coarse and slightly irregular opacifications and minimal subsegmental atelectasis in the right upper and lower lobes suggestive of chronic lung disease of prematurity. (D, E) Contiguous computed tomography (CT) images of the lung bases at 11 weeks show multiple discrete 1- to 2-mm cysts predominantly posteriorly. There is slight uneven expansion of the left lung without other interstitial changes. (The CT scan was performed with inadvertent selective right main bronchial intubation accounting for the lower volume and atelectasis of the right lung.)

Figure 2. The patient had a deletion of 14q12–21.3, encompassing the thyroid transcription factor-1 (TTF-1) gene locus. Although the father had a normal set of chromosomes 14, the mother showed in 1 of 30 metaphases an inversion of the q13 region in one chromosome 14.

and phenobarbital. He died 4.5 months later from respiratory failure and pneumonia.

Pulmonary Pathology

The lingular biopsy (at 11 wk of age), which contained bronchioles, but not bronchi, showed striking variation in alveolar size; although some were normal, many were markedly enlarged, especially at the acinar periphery, and in particular, subpleurally. The largest measured 400 μm in diameter, approximately twice expected (12), and the alveolar crests were short (Figure 3). The radial alveolar count was lower than expected (4–6; expected 6–7 [13]). Alveolar lining cells were cuboidal in the smaller alveoli,

whereas they were flat in the larger ones. A few airspaces had small collections of macrophages with periodic acid-Schiff (PAS) positive, diastase-resistant cytoplasmic granules. Intraalveolar, extracellular proteinaceous material was not observed. Alveolar walls, particularly of the smaller alveoli, were slightly thickened with increased collagen and fibrocyte-like cells. Although elastic fibers were diminished within alveolar walls, they were increased in interlobular septa. Alveolar capillaries appeared focally decreased. The smooth muscle in the airways was prominent. The external diameter of the pulmonary arteries at the respiratory bronchiolar level was decreased but was within the normal range at the terminal bronchiolar level. The ratio of pulmonary arterial

Figure 3. (A, B) Simplified, enlarged alveoli with short alveolar crests were the dominant feature in a lingular biopsy at age 3 months. (C) Thyroid transcription factor-1 immunoreactivity was demonstrated in nuclei of airway epithelium and type II pneumocytes. (D) Ultrastructurally, alveolar lining cells were mostly type II pneumocytes (arrows) containing lamellar bodies. Alveolar spaces had abundant surfactant (arrowheads).

medial thickness to external diameter was increased (27%; 6% expected [14]). The pulmonary veins appeared normal.

Immunohistochemistry to assess the expression of various functional and structural pulmonary proteins was compared with an age-matched control (Figure 4). In type II pneumocytes, immunostaining for surfactant proteins SP-A and ABCA3 was diminished, whereas that for SP-D was normal. Immunostaining for SP-B, proSP-B, and proSP-C was robust, but was irregularly distributed, reflecting the patchy distribution of type II pneumocytes. Clara cell-specific protein (CCSP, also known as CC10 or secretoglobin 1A1) was abundant (normal) in bronchiolar epithelium. Nuclear TTF-1 protein was detected in airway cells and type II pneumocytes (Figure 3C). Smooth muscle actin (SMA) immunostaining revealed increased muscularization of peripheral airways and alveolar walls (Figure 4).

Ultrastructurally, type II pneumocytes with numerous lamellar bodies were prominent, and alveolar spaces contained fragments of type II pneumocytes and extruded lamellar bodies (Figure 3D). No tubular myelin was observed.

Permission for a complete autopsy (excluding brain) was granted. The lungs were normally shaped and fissured, but were heavy (combined weight: 131 g; 90 g expected [15]). The inflated left lung volume was nearly normal for age (250 ml; 286 ml expected, departmental unpublished data) but was greater than expected for body length (200 ml [16]). Diffuse consolidation was present in the right lower lobe and focally elsewhere. A major finding, more pronounced than in the biopsy, was extensive emphysematous change with variation in severity, being slightly more marked in the posterior aspects of the lungs (Figure 5). The largest airspaces, up to 2 mm in diameter, were round and cyst-like, tended to be in the paraseptal and subpleural zones, and were optimally visualized with the dissecting microscope. The airway generation count (performed in the posterior lateral bronchus of the right lower lobe as a combination of gross dissection and distal serial step sections) was diminished (17 airway generations; 20–25 expected [17]). Microscopically, in addition to the findings observed in the biopsy, there was extensive bronchopneumonia, mild mononuclear interstitial inflammation, and increased airspace macrophages and airway mucus. A karyotypic analysis of postmortem lung tissue showed an interstitial deletion in the long arm of one chromosome 14 similar to the one observed previously in the peripheral blood.

There was agenesis of the left lobe of the thyroid and enlargement of the right lobe for age (1.9 g; 1.5 g expected for entire thyroid) (18); microscopic examination was unremarkable. Other findings included dolichocephaly, enlargement of the right external ear and right hand relative to those on the left, irregular thinning of several right ribs posteriorly, and a partially intrapericardial thymus.

Figure 4. Immunostaining for mature surfactant proteins SP-B, proSP-B, proSP-C, and SPD in type II pneumocytes was robust, whereas that for ABCA3 and SPA was diminished. Clara cell-specific protein (CCSP) was abundant in the airway epithelium. Smooth muscle actin immunoreactivity was increased in airways and extended into alveolar walls.

DISCUSSION

This infant with cerebral dysgenesis, thyroid hemiagenesis, euthyroid sick syndrome, respiratory insufficiency, and a haploid interstitial deletion of 14q12–21.3 encompassing the TTF-1 gene locus is presumed to have had TTF-1 deficiency syndrome. More than half of the 46 patients reported with this syndrome had

Figure 5. (A) The inflated formalin-fixed lung showed patchy emphysema and areas of pneumonia with consolidation and hemorrhage. (B) A close-up of A at interlobar fissure posteriorly showed numerous scattered "cysts." (C) With the dissecting microscope, the pronounced and diffuse emphysematous changes were readily seen with airspaces measuring up to 2 mm. The largest airspaces were subpleural and at the periphery of the acini.

pulmonary disease, most often manifesting in the neonatal period as respiratory distress syndrome requiring mechanical ventilatory support (9). Recurrent pulmonary infection was another common manifestation, with some patients developing chronic interstitial lung disease (9). Several died from respiratory failure at various ages and one young adult died with metastatic lung carcinoma (5–11, 19).

The pulmonary pathology in this syndrome has been illustrated in only one case report and briefly noted in another (10, 11). The first describes a term infant who developed acute respiratory failure and pulmonary hypertension at birth and died at 40 days. The lungs at autopsy showed impaired pulmonary branching with simplification of the architecture and a low alveolar count. Fibrous thickening of septa and alveoli filled with macrophages were interpreted as suggestive of surfactant insufficiency (11). The second report describes a term infant who developed respiratory distress beginning in the second week of life (10). At 11 months, a lung biopsy showed interstitial fibrosis, chronic inflammation, and alveoli filled with macrophages and PAS-positive material, and a diagnosis of alveolar proteinosis was rendered. This child developed restrictive chronic lung disease, had recurrent pulmonary infections, and died at the age of 23 years with metastatic pulmonary large cell carcinoma. The lungs at autopsy also had interstitial fibrosis and severe emphysema.

The pulmonary disease in the infant in our report is unusual in several aspects. First, being near-term, his risk of developing HMD was low and yet the initial pulmonary symptoms, chest radiograph, and improvement with surfactant administration were consistent with mild HMD. Second, although the subsequent clinical course bore some similarity to CLD/BPD, the level of ventilatory support he received does not usually result in CLD/BPD. Third, the CT scan in this infant was most notable for bilateral, discrete “cysts” predominantly posteriorly and symmetrically distributed, unlike the irregularly distributed emphysematous change without cysts in CLD/BPD. Last, the pathologic features were also unusual. Although enlarged alveoli were reminiscent of those seen in CLD/BPD, there was more variation in size and many were exceptionally large, round, and cyst-like. Simplified and enlarged alveoli as seen in this infant may also be observed in Wilson-Mikity syndrome (20, 21), Down syndrome (22), primary pulmonary hypoplasia, and in association with other congenital abnormalities (23). Wilson-Mikity syndrome was described in preterm infants before the use of mechanical ventilation. In this syndrome, symptoms began after the first week of life and progressed to maximum severity within 2 months, with the majority of infants improving slowly over the ensuing months, unlike the clinical course in this infant. Chest radiographs and pathology in Wilson-Mikity syndrome are believed to be within the spectrum of CLD/BPD (24).

TTF-1 has been shown to play an important role in pulmonary branching morphogenesis and surfactant homeostasis. TTF-1 knockout mice have a rudimentary bronchial tree but do not develop lungs (25). Heterozygous mice appear either unaffected (25) or have a predominantly neurologic phenotype (6). However, mice bearing a homozygous mutant allele of TTF-1, in which seven serine phosphorylation sites were mutated, exhibited hypoplastic lungs with dilated or cystic peripheral structures, reduced terminal alveolar saccules, and diminished vasculogenesis, as well as abnormalities in surfactant protein and CCSP expression (26). TTF-1 is also important for type II pneumocyte differentiation, because it is a major activator of functional lung-specific genes and plays a role in the synthesis and regulation of the surfactant-associated proteins A, B, and C, as well as the phospholipid transporter, ABCA3 (27), and CCSP (25, 28, 29).

The pulmonary morphology in this infant included extensive emphysematous change and cyst-like airspaces, similar to findings in mice bearing homozygous TTF-1 phosphorylation mutations (26). In addition, the diminished number of airway generations in this infant indicates that branching morphogenesis of the bronchial tree was disrupted, a process that is normally complete by 16 weeks of gestation (17). Diminished immunoreactivity for SP-A and ABCA3 in this infant's lung, as well as the irregular distribution pattern for SP-B and proSP-C expression, supports experimental observations regarding the role of TTF-1 in type II pneumocyte differentiation and surfactant protein synthesis and regulation (25, 26, 28, 29). The improvement of symptoms with surfactant administration in this infant and in another child with TTF-1 deficiency (7) is clinical evidence supporting a surfactant abnormality component. In contrast to genetic disorders of surfactant function (i.e., SP-B and ABCA3 deficiencies), wherein lamellar bodies are reduced, absent, or abnormally formed (30–33), or alveolar proteinosis, wherein there is accumulation of phospholipid membranes and abnormal tubular myelin (34), the ultrastructure of the lamellar bodies in this infant appeared normal and were abundant in alveolar lumens. Tubular myelin was not seen, but is generally not observed in lung specimens routinely examined by electron microscopy (Reference [35] and personal observations, A.R.P.-A.). In this infant the pulmonary morphology and abnormal surfactant protein expression are consistent with TTF-1 deficiency syndrome.

The intensity of TTF-1 protein immunostaining in this infant's lung (Figure 2C) was similar to an age-matched control except for the irregular distribution of type II pneumocytes. The detection of TTF-1 is not surprising because in haploinsufficiency, a single allele results in a functional, although reduced, amount of protein with the difference generally not detectable by immunohistochemistry. In humans, heterozygous loss of function of the TTF-1 gene occurs with missense mutations, nonsense mutations, and partial or complete deletions and is associated with varying phenotypes (5–9). In some, neurological disease predominates, whereas in others, pulmonary disease is dominant (6, 7). The phenotypic pulmonary abnormalities resulting from TTF-1 haploinsufficiency appear to vary in severity from minimal to marked. Because TTF-1 regulates the expression of many downstream genes, interactions among these target genes may influence the severity of disease (36).

It is not possible to exclude that genes other than TTF-1 encompassed by the chromosomal deletion in this infant might have also played a role in the pathogenesis of the pulmonary disease or other phenotypic abnormalities. For example, the PAX-9 gene, vicinal to TTF-1, which influences the morphogenesis of the vertebral column, limbs, and pharyngeal pouch derivatives (37–39), might have played a role in the ear and limb asymmetry, dolichocephaly, abnormal ribs, and intrapericardial thymic tissue observed in this infant.

In conclusion, we describe the pulmonary pathology in an infant with TTF-1 deficiency syndrome. The abnormal pulmonary morphogenesis and altered expression of surfactant-associated proteins parallels that observed in experimental murine TTF-1 haploinsufficiency and may explain the respiratory difficulties in this syndrome.

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