Heterogeneous Pulmonary Phenotypes Associated With Mutations in the Thyroid Transcription Factor Gene *NKX2-1*

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Background: Mutations in the gene encoding thyroid transcription factor, *NKX2-1*, result in neurologic abnormalities, hypothyroidism, and neonatal respiratory distress syndrome (RDS) that together are known as the brain-thyroid-lung syndrome. To characterize the spectrum of associated pulmonary phenotypes, we identified individuals with mutations in *NKX2-1* whose primary manifestation was respiratory disease.

Methods: Retrospective and prospective approaches identified infants and children with unexplained diffuse lung disease for *NKX2-1* sequencing. Histopathologic results and electron micrographs were assessed, and immunohistochemical analysis for surfactant-associated proteins was performed in a subset of 10 children for whom lung tissue was available.

Results: We identified 16 individuals with heterozygous missense, nonsense, and frameshift mutations and five individuals with heterozygous, whole-gene deletions of *NKX2-1*. Neonatal RDS was the presenting pulmonary phenotype in 16 individuals (76%), interstitial lung disease in four (19%), and pulmonary fibrosis in one adult family member. Altogether, 12 individuals (57%) had the full triad of neurologic, thyroid, and respiratory manifestations, but five (24%) had only pulmonary symptoms at the time of presentation. Recurrent respiratory infections were a prominent feature in nine subjects. Lung histopathology demonstrated evidence of disrupted surfactant homeostasis in the majority of cases, and at least five cases had evidence of disrupted lung growth.

Conclusions: Patients with mutations in *NKX2-1* may present with pulmonary manifestations in the newborn period or during childhood when thyroid or neurologic abnormalities are not apparent. Surfactant dysfunction and, in more severe cases, disrupted lung development are likely mechanisms for the respiratory disease.

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Abbreviations: ChILD = childhood interstitial and diffuse lung disease; ILD = interstitial lung disease; RDS = respiratory distress syndrome; RSV = respiratory syncytial virus; SP = surfactant protein; TTF-1 = thyroid transcription factor protein

Mutations in genes encoding surfactant protein-B (*SFTPB*), member A3 of the ATP-binding cassette family of transporters (*ABCA3*), and surfactant protein-C (*SFTPC*) cause neonatal respiratory distress syndrome (RDS) or childhood interstitial and diffuse lung disease (ChILD).¹ Considerable overlap in the clinical and histologic features of the lung disease associated with these mutations exists, which are collectively referred to as surfactant dysfunction disorders. Children with findings of surfactant dysfunction but

without identifiable mutations in the *SFTPB*, *SFTPC*, or *ABCA3* genes have been reported.^{2,3}

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Thyroid transcription factor 1 (TTF-1), encoded by the gene *NKX2-1* (Entrez gene identification number 7080), is expressed in the thyroid gland, brain, and lung. In the lung, it is an early marker of lung differentiation and is important for structural development

of the lung and the expression of surfactant protein (SP)-B, SP-C, and ABCA3, and a large network of other proteins.⁴ Haploinsufficiency for *NKX2-1* due to either complete gene deletions or loss-of-function mutations results in brain-thyroid-lung syndrome (MIM 610978), with affected individuals having variable degrees of pulmonary disease, thyroid dysfunction, and neurologic abnormalities.⁵⁻¹⁸ Neonatal RDS and chronic lung disease in older individuals have been reported in association with *NKX2-1* mutations, but the pulmonary phenotype remains incompletely characterized, particularly in older children. Given the role of *NKX2-1* in the surfactant system, we hypothesized that individuals with mutations in *NKX2-1* may present not only with neonatal RDS, but also with ChILD and evidence of surfactant dysfunction. We sought to further characterize the range of pulmonary phenotypes in children with *NKX2-1* mutations to better define the mechanisms leading to chronic lung disease.

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Drs Hamvas and Deterding contributed equally to this work. A preliminary report of some of these studies was presented at the American Thoracic Society International Conference in May 2010 and published in abstract form (Deterding RR, Uchida DA, Stephan M, et al. Thyroid transcription factor 1 gene abnormalities: an under recognized cause of Children's Interstitial Lung Disease. *Am J Respir Crit Care Med*. 2010;181:A6725). Data from three subjects described in this manuscript were reported in 2001, before the mechanism for their lung disease was identified (Amin RS, Wert SE, Baughman RP, et al. Surfactant protein deficiency in familial interstitial lung disease. *J Pediatr*. 2001; 139[1]:85-92).

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MATERIALS AND METHODS

Patient Selection

We identified subjects both retrospectively and prospectively through institutional review board-approved protocols at Washington University School of Medicine (201103064 and 201106421), Johns Hopkins University School of Medicine (NA_00045539), and University of Colorado School of Medicine (10-0472). DNA specimens from subjects identified at Washington University School of Medicine and Johns Hopkins University School of Medicine were obtained from infants and children without an identified etiology for their lung disease and who had previously tested negative for mutations in *SFTPC*, *SFTPB*, and *ABCA3*. Newborns of >35 weeks' gestation with severe hypoxemic respiratory failure and diffuse lung disease on chest radiographs (n = 82, including three who were found in this study to have mutations in *NKX2-1*), older children with a clinical and/or lung histopathologic diagnosis of interstitial lung disease (ILD) or "chronic lung disease" (n = 136, including six with *NKX2-1* mutations), and individuals who had undergone lung transplantation with clinical and/or histopathologic diagnoses of ILD (n = 15, including four with *NKX2-1* mutations) were included. The parents provided informed consent. Other subjects were identified through a University of Colorado, retrospective, clinical database of children with diffuse lung disease and ILD who came to attention through clinical evaluation (n = 8).

Sequencing Analysis

NKX2-1 encodes two isoforms of TTF-1: a 371 amino acid isoform (NM_003317.3) that is most abundantly expressed in the lung, and a 401 amino acid isoform (NM_001079668.2, "transcript 1") that is expressed in thyroid and brain.^{19,20} Clinical laboratories use the transcript 1 sequence that is consistent with Human Genome Variation Society nomenclature, and this reference sequence is used for this report. *NKX2-1* sequencing and/or microarray-based comparative genomic hybridization were performed through Clinical Laboratory Improvement Amendments-certified laboratories (Signature Genomics, LLC; Nemours Alfred I. duPont Hospital for Children; or the Johns Hopkins DNA Diagnostic Laboratory) or through research protocols.

Histopathology, Immunohistochemistry, and Electron Microscopy

Lung tissue was obtained at transplantation (n = 4), biopsy (n = 5), or autopsy (n = 1), and evaluated by histopathologic examination (n = 10); immunohistochemistry for TTF-1, ABCA3, and SP-A, SP-B, SP-C, and SP-D (n = 7); and electron microscopy (n = 8). See e-Appendix 1 and e-Table 1 for more details.

RESULTS

Variability in Clinical Presentation and Course

Twenty-one subjects with lung disease had mutations identified in *NKX2-1*; their clinical and genetic features are listed in Table 1 and Figure 1. Lung disease was characterized by neonatal RDS (with and without pulmonary hypertension, n = 17), RDS progressing to ILD (n = 1, subject M), ILD manifesting from 4 months to 7 years (n = 3, subjects J, K, and L1), and pulmonary fibrosis at age 26 years (n = 1, subject L2). CT imaging demonstrated a range of findings from mild to diffuse ground-glass opacities, cysts, infiltrates,

Table 1—Phenotype and Genotype Description of Study Cohort

Patient	Sex	Presenting Pulmonary Phenotype (Age)	Neurologic Phenotype (Age of Onset)	Thyroid Phenotype (Age of Onset)	NKX2-1 Mutation	Age at Most Recent Assessment	Pulmonary Status	Neurologic Status	Thyroid Status
Whole gene deletions									
A	F	Hypoxemia; recurrent infections (2 y)	Hypotonia, developmental delay, ataxia, absent corpus callosum	Yes (11 y)	Deletion ^a	14.9 y	Supplemental oxygen; recurrent pneumothoraces	Hypotonia, developmental delay, ataxia	Replacement
B	F	Brief oxygen at birth	Developmental delay	Yes (12 mo)	Deletion ^a	10 y	Recurrent infections; no oxygen since birth	Ataxia, behavior problems	Replacement
C	F	RDS, PH, ECMO (birth)	Hypotonia, developmental delay (18 mo)	Yes (congenital)	Deletion ^a	7 y	Oxygen at night through 5 y; bronchodilator / steroids with URI	Truncal hypotonia, ataxia; fine motor and speech delay	Replacement
D	M	RDS, PH (birth)	Hypotonia, developmental delay (4 mo), microcephaly (1 y)	Yes (congenital)	Deletion ^a	3.5 y	Off oxygen at 1 y; asymptomatic	Gross and fine motor delay, language delay	Replacement
E	M	Severe RDS (birth)	Hypotonia, developmental delay (6 mo)	Yes (congenital)	Deletion exons 1-2	2.8 y	Oxygen, azithromycin; intermittent steroids and hydroxychloroquine	Ataxia; gross motor and language delay	Replacement
Mutations affecting homeodomain									
F	F	Severe RSV/influenza A (12 mo)	Hypotonia, developmental delay, chorea (14 mo)	Yes (12 mo, only during acute illness)	c.384_391del8 (GCCGTACC) p.Pro129GlyfsX307	5 y	Supplemental oxygen until 4 y ^b	Hypotonia, developmental delay, chorea	Normal
G	F	Hypoxemia, recurrent infections, oxygen on mother as a child) (4 mo)	Hypotonia, developmental delay (birth)	Yes (5 mo); mother and maternal grandmother hypothyroid	c.432 C>A p.Tyr144X	Died at age 17 mo (RSV)	N/A	N/A	N/A

(Continued)

Table 1—Continued

Patient	Sex	Presenting Pulmonary Phenotype (Age)	Neurologic Phenotype (Age of Onset)	Thyroid Phenotype (Age of Onset)	NKX2-1 Mutation	Age at Most Recent Assessment	Pulmonary Status	Neurologic Status	Thyroid Status
H	M	RDS (birth); recurrent infections, laryngeal cleft, ECMO for HMPV	Mild hypotonia; (birth)	Yes (congenital)	c.552_556 del (CCCGC) p.Pro185AlaX250	19 mo	Supplemental oxygen ^b	Gross motor delay; abnormal movements	Replacement
I	M	Refractory RDS (birth); recurrent infections	Confounded by pretransplant ICU care	Yes (congenital)	c.583C>T p.Arg195Trp	Died at age 3 y (PH)	Lung transplant (8 mo)	Hypotonia, gross motor delay	Replacement
J	M	RDS (birth) → ILD; recurrent infections	ADHD (10 y), chorea (12 y)	No	c.590T>C p.Leu197Pro	13.8 y	Lung transplant (10 mo)	ADHD, chorea	Normal
K	F	ILD (1 y)	None	No	c.592T>C p.Phe198Leu	Died at age 19 y (rejection)	Lung transplant (13 y)	Normal	Normal
Family ^c L1	F	ILD (7 y)	None	No	c.592 T>C p.Phe198Leu	23 y	PH, obstructive lung disease	Normal	Normal
L2	F	Pulmonary fibrosis (26 y)	None	Not examined	c.592 T>C p.Phe198Leu	Died at age 33 y (MVA)	N/A	Normal	N/A
L3	F	RDS (birth); LIP	None	Not examined	c.592 T>C p.Phe198Leu	Died at age 5 y (respiratory failure)	Recurrent pneumonia	Normal	N/A
M	F	RDS (birth); progression to ILD; RSV at 9 mo; alveolar proteinosis	None	No	c.594 C>G p.Phe198Leu	16 y	Supplemental oxygen	Normal	Normal
Mutations 3' to homeodomain N	M	30 wk gestation; refractory RDS (birth)	Onset confounded by pretransplant ICU care	No	c.804_812 dupCGCGGGGGG p.Gly269_271 dupGlyGlyGly	10.7 y	Lung transplant (7 mo); asymptomatic	Gross motor and speech delay; hearing loss	Normal

(Continued)

Table 1—Continued

Patient	Sex	Presenting Pulmonary Phenotype (Age)	Neurologic Phenotype (Age of Onset)	Thyroid Phenotype (Age of Onset)	NKX2-1 Mutation	Age at Most Recent Assessment	Pulmonary Status	Neurologic Status	Thyroid Status
O	M	30 wk gestation; RDS (birth); recurrent infections	Hypotonia, developmental delay (birth)	No	c.804_812 dupCGCGGGGGG p.Gly269_271 dupGlyGlyGly	30 mo	Supplemental oxygen until 24 mo ^b	Gross motor delay	Normal
P	M	RDS, ECMO (birth)	Absent corpus callosum	Yes (congenital)	c.818_838del121 and c.1034_1047del114 p.273_279delGCPQQQ and p.His346Alafs	Died at age 1 mo	N/A	N/A	N/A
Q	M	RDS, PH (birth)	Hypotonia and seizures; vein of Galen thrombosis (birth), developmental delay (6 mo)	Yes (congenital)	c.1044_1045insC; p.His349ProfsX90	6.3 y	Oxygen until 4 y	Chorea; seizures; gross, fine motor and language delay	Replacement
R	M	RDS; PH (birth)	None	Yes (congenital)	c.1092_1108del17 (CGCCAGCCCCGGCGCGC); p.Ser366GlyfsX67	Died at age 6 mo	N/A	N/A	N/A
S	F	RDS (birth); recurrent infections, chronic lung disease	Hypotonia, developmental delay (1-3 mo)	Yes (congenital)	c.1157_63 dupACTACGG; p.Thr389LeufsX52	4.5 y	Lung transplant 22 mo; asymptomatic	Gross, fine motor; and language delay; ataxia	Replacement

ADHD = attention-deficit/hyperactivity disorder; ECMO = extracorporeal membrane oxygenation; F = female; HMPV = human metapneumovirus; ILD = interstitial lung disease; LIP = lymphocytic interstitial pneumonitis; M = male; MVA = motor vehicle accident; N/A = not applicable; PH = pulmonary hypertension; RDS = respiratory distress syndrome; URI = upper respiratory infection.

^aLong span deletion (Fig 1).

^bAltitude 1,600 m.

^cReported by Amin et al² before NKX2-1 mutation was identified.

FIGURE 1. A, Diagram of the region of chromosome 14q. B, Chromosome 14q region expanded to demonstrate 14q13.1-14q21.1, which includes *NKX2-1* (small black vertical bar at 14q13.3). C, An expanded view of *NKX2-1* localizing each of the mutations found in this report. Coordinates shown are according to the hg18 build of the human genome. The region “HD” in exon 3 represents the region encoding the DNA binding domain (homeodomain, c.565-759 corresponding to amino acids 189-253). Gray bars denote large deletions (subjects A, B, C, and D have approximately 1 Mb or larger deletions, and Subject E has a deletion that spans at least exons 1 and 2.) Δ = deletion; fs = frameshift; i = insertion; O = missense; X = nonsense.

and fibrosis (e-Fig 1). Recurrent pulmonary infections were prominent in nine cases. Five individuals died of their lung disease, four underwent lung transplantation before their *NKX2-1* mutation was identified, and the remaining individuals are alive with varying degrees of respiratory dysfunction. Altogether, 12 (57%) had the full triad of the brain-thyroid-lung syndrome (Fig 2).

Variability in Histopathologic, Immunohistochemical, and Ultrastructural Appearance

The pulmonary histology was heterogeneous, both among and within cases, and in some cases was consistent with previously identified surfactant-dysfunction mutations: interstitial widening and pneumocyte hyperplasia (n = 8), desquamative interstitial pneumonia (n = 4), accumulation of foamy alveolar macrophages (n = 8), and pulmonary alveolar proteinosis (n = 4) (Fig 3, e-Table 2).^{1,21} Homogeneous areas of alveolar septal widening, septal fibrosis, and chronic interstitial inflammation resembled nonspecific interstitial pneumonia (n = 4). In several cases there was pleural fibrosis, lymphatic dilatation, arterial medial-wall thickening, and extension of smooth muscle into alveolar ducts and walls. Some cases with milder disease demonstrated normal or only minimally altered alveolar architecture, demonstrating that microscopic tissue analysis alone may not be sufficient to raise the possibility of a clinically relevant *NKX2-1* mutation (subjects F and H) (Fig 2).

In seven cases, there was mild to extensive enlargement of alveolar spaces, consistent with alveolar simplification and the ChILD classification of a growth disturbance (Fig 3).³ In addition, seven cases had evidence of remodeling independent of the presence or degree of growth disturbance (e-Table 2). The child who died following respiratory syncytial virus (RSV) infection (subject G) had severe airway lobular injury and repair; there were no histopathologic findings in the thyroid or brain.

Immunoreactive ABCA3, SP-A, mature SP-B, and proSP-C were present in all samples evaluated (e-Table 3), and were detected primarily in hyperplastic type 2 pneumocytes (e-Fig 2). Staining for SP-A was also present along the luminal surface of the alveoli and in acellular proteinosis-like material (e-Fig 2). Nuclear staining for TTF-1 in the epithelium was weak or absent only in subject G; staining for both cellular and secreted forms of SP-D was weak in this same individual, whose tissue was obtained at autopsy at age 17 months after an RSV infection. Subject F also had weak or absent SP-D staining of the biopsy specimen obtained 10 months after a severe RSV infection at age 12 months. Immunohistochemistry from Family L was previously reported by Amin et al,² with immunoreactive SP-A and SP-B detected but not proSP-C. Antibodies to ABCA3 were not available at the time of those studies.

Electron microscopy demonstrated variable numbers of lamellar bodies with heterogeneous morphology

FIGURE 2. Venn diagram demonstrating the constellation of neurologic, thyroid, or pulmonary disease present in each of the subjects at any time during their course (denoted by letters corresponding to Table 1). Five individuals from three families had pulmonary symptoms only, but thyroid function was not examined in L2* and L3*. Subject R+ died at 6 mo, before neurologic symptoms were detected. Because pulmonary symptoms prompted the initial evaluation in all these cases, we did not identify any individuals with only neurologic or thyroid abnormalities.

(Fig 3, e-Table 2). Normal lamellar bodies were present in all cases but were of variable size; composite lamellar bodies were numerous, but multivesicular bodies were infrequent and occasionally fused to lamellar bodies. Some cells contained large aggregates of lamellar bodies. Variably sized homogeneous and heterogeneous dense bodies were present, including a few with a “fried egg” appearance similar to that seen with ABCA3 deficiency (subjects I and J).^{3,22}

Variability in Mutations

Mutations consisted of single nucleotide variants (n = 8), small intragenic insertions or deletions (n = 8), and single or multiple gene deletions (n = 5) (Fig 1, Table 1); all subjects were heterozygous for their mutations or deletions. The subjects with whole-gene deletions had milder pulmonary disease that did not result in lung transplantation or death. No other obvious correlation between the type or site of the mutation, the type or severity of lung disease, the histopathologic phenotype, or combination with neurologic or thyroid abnormalities was apparent (Table 1). One mutation, p.F198L, was present in five individuals, including three members of one family (family L) who presented at birth, 7, and 26 years of age. The mutation was reported previously as familial SP-C deficiency without an *SFTPC* mutation identified.² Two different

mutations accounted for this single amino acid change: c.592 T > C (family L and subject K, who were unrelated) and c.594 C > G (subject M). Two unrelated infants with an insertion of three glycine residues at codon 269, subjects N and O, were both born at 30 weeks’ gestation, but had significantly different pulmonary outcomes: lung transplantation vs milder chronic respiratory insufficiency. In the 15 individuals who were tested, no mutations in *SFTPB*, *SFTPC*, or *ABCA3* were identified. None of these *NKX2-1* mutations were found in a cohort of 24 infants with severe RDS or in the Exome Variant Server database²³ (e-Appendix 1, e-Table 4). Other than family L, the mutations were spontaneous in the seven subjects whose parents were tested (subjects A, C, D, F, H, I, and Q).

DISCUSSION

The present study includes the largest number of subjects reported to date with *NKX2-1* mutations. Our subject ascertainment was based upon the presence of pulmonary disease and, therefore, we did not identify any individuals with only neurologic or thyroid abnormalities. Our series further demonstrates that severe pulmonary disease may be the presenting and only manifestation of *NKX2-1* mutations.

Our conclusions differ from those of Teissier et al,²⁴ who selected 24 cases for *NKX2-1* analysis based on the combinations of neurologic, thyroid, and pulmonary phenotypes. Only four of their 24 subjects had *NKX2-1* mutations and those subjects also had neurologic and/or thyroid manifestations along with their pulmonary disease. The authors thus concluded that *NKX2-1* analysis for patients with isolated pulmonary disease was unlikely to be informative. In contrast, in our series, there were clearly individuals whose pulmonary disease either preceded the neurologic phenotype (subject J) or who did or do not have any neurologic and/or thyroid phenotype for up to age 23 years (subjects K and M, and family L).

Since our approach was retrospective, we acknowledge that some subtle neurologic manifestations might have been missed. Despite this limitation, our pulmonary disease-based cohort with diverse clinical phenotypes and ages of presentation demonstrates that a high index of suspicion is necessary in evaluating a child with idiopathic pulmonary disease with or without hypothyroidism, neurologic abnormalities, recurrent severe infections, or airway abnormalities. Finally, the histopathologic and immunohistochemical findings support the hypothesis that disruption of TTF-1 targets necessary for functional and structural lung development and surfactant homeostasis is important in the pathogenesis of the pulmonary findings.

FIGURE 3. Histopathologic variability between patients. A, A growth abnormality with alveolar enlargement and simplification present in lungs from subject N (hematoxylin-eosin stain). B, A growth abnormality with alveolar enlargement and simplification present in lungs from subject I (hematoxylin-eosin stain). C, In contrast, minimal changes without alveolar enlargement are present in lung from subject H, shown at same magnification (hematoxylin-eosin stain). D, Electron micrograph from subject E shows hyperplastic pneumocytes with normal lamellar bodies (long arrow), cytoplasmic heterogeneous dense structures some containing lamellar body like membranes and small vacuoles (short arrow), and composite lamellar body and dense structures (dashed arrow).

Disrupted Lung Development and Growth

TTF-1 is a key regulator of the earliest phases of lung development.²⁰ Its importance is demonstrated by absence of lung development in murine lineages with complete ablation of TTF-1 signaling and by pulmonary hypoplasia associated with mutation of TTF-1 phosphorylation sites.^{25,26} Four of 10 subjects in this report, along with others reported previously, had alveolar simplification and cyst formation, and one subject (H) had a laryngeal cleft, all of which might represent disruption in structural lung development due to aberrant TTF-1 function.^{1,8,13} As the histopathologic specimens were obtained after multiple interventions, including mechanical ventilation, it is also possible that the growth disturbance was a secondary response to those interventions, although the severity of the initial presentations suggests presence of an underlying abnormality at birth.

Altered Surfactant Homeostasis

NKX2-1 is critical for surfactant function and metabolism, and the phenotypes of severe neonatal RDS and histopathologic appearance of a surfactant-dysfunction disorder suggest that altered surfactant homeostasis

contributes to the pathophysiology of the lung disease.^{8,9,11} Several previous studies demonstrated diminished expression or levels of surfactant-associated proteins, and the finding of an *NKX2-1* mutation in the subjects from family L potentially explains the reduced SP-C expression previously observed in those subjects.^{2,8,9,11} In contrast, the p.R195W mutation seen in subject I was also found in a previously reported patient (designated p.R165W in that report), and was associated with increased transactivation of *SFTPC* in A549 cells, suggesting a dominant negative effect.⁹ Variability in immunohistochemical phenotyping suggests that the corresponding mutation may have differential effects on gene expression; however, this variability may also reflect differences in timing of tissue acquisition, interventions, or history of infection. Analysis of *NKX2-1* variants in vitro with interrogation of surrogate cell systems will be needed to address these questions.

Pulmonary Infections

Recurrent pulmonary infections have been reported in children with haploinsufficiency for *NKX2-1*. While we did not systematically compare the frequency of

infections with the number anticipated for children in this age range, infections were a prominent feature in more than half of the subjects in this report, two of which were severe (subjects G and H).^{2,6,12} Two subjects with a distant-past respiratory infection (F and G) had an unanticipated reduction of SP-D expression, and, to our knowledge, are the first subjects reported with such findings in the presence of an *NKX2-1* mutation. No deviations from the known *SFTPD* sequence were found in subject F to account for the lack of SP-D. SP-D, one of the pulmonary collectins, has an important role in local immunity and defense to viral pathogens. Children with severe RSV infection or recurrent pneumonias may have decreased or absent levels of SP-D in BAL fluid during the acute phase of the infection, such that the role of *NKX2-1* in SP-D gene expression is less clear.^{27,28} Murine SP-D gene expression, as assessed by microarray, was actually increased in transgenic mice expressing a phosphorylation mutant form of *Nkx2-1*, suggesting negative regulation by TTF-1; however, no changes in human SP-D gene expression were detected with either gain or loss of function approaches in vitro.^{19,29} Thus, it is difficult to determine the relationship between the *NKX2-1* mutations, SP-D expression, and the recurrent infections in these children, especially in light of other children in this report with *NKX2-1* mutations and recurrent infections but who do not have evidence of decreased SP-D expression.

Whether from hypotonia or disrupted lung growth, surfactant function, or innate immunity, the possibility of recurrent and severe respiratory infections necessitates aggressive preventive measures in young children, such as exposure avoidance, influenza immunization, and RSV immunoglobulin administration.

Genotype-Phenotype Correlation

Eleven different mutations, along with large genomic deletions that spanned up to 20 genes surrounding the *NKX2-1* locus, were identified. While individuals with large deletions had early-onset disease and involvement of all three organ systems, haploinsufficiency for other genes within the deleted regions might have contributed to the phenotypic variability. Mutations were identified at varying times in the subjects' clinical course, and mostly retrospectively, further limiting correlations between clinical or histopathologic phenotype and specific types or location of mutations. Three mutations, p.R195W, p.L197P, and p.F198L, map to the homeodomain, a 60 amino acid, highly conserved region that is critical for DNA binding. These mutations could thus affect DNA binding and gene transactivation, although p.R195W did not appear to affect binding to the *SFTPB* or *SFTPC* promoters by electrophoretic mobility shift assays in a previous report.⁹ This observation does not preclude abnormal

TTF-1 binding to other TTF-1 responsive genes or tissue-specific differences in expression of the normal TTF-1 allele.

NKX2-1 is highly regulated through interaction with other transcription factors, serine phosphorylation in the activation domains (which were probably disrupted in the subjects with frameshift mutations), and epigenetic modification. While beyond the scope of our current study, these factors may also account for the phenotypic variability of our patients.^{14,26,30-33}

In summary, our findings emphasize the variable clinical and histopathologic phenotypes due to diverse *NKX2-1* mutations. Such mutations may result solely in pulmonary disease without manifestations in other organ systems, or the neurologic manifestations may be subtle or present later in life. Thus, clinicians should maintain a high index of suspicion when confronted with children with diffuse lung disease of unknown cause, especially when other endocrine and neurologic system involvement is present. In addition, the autosomal dominant pattern of expression should prompt genetic testing of the parents to determine risks of recurrence and to permit prospective and systematic follow-up to refine the phenotypes and understand the mechanisms of mutations in *NKX2-1*.

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Dr Hamvas: contributed to study conception and design; data acquisition, analysis, and interpretation; drafting of the manuscript; critical input into the final version of the manuscript; and served as principal author.

Dr Deterding: contributed to study conception and design; acquisition, data analysis, and interpretation; drafting of the manuscript; and critical input into the final version of the manuscript.

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Additional information: The e-Appendix, e-Figures, and e-Tables can be found in the "Supplemental Materials" area of the online article.

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