Molecular Medicine

Interstitial Lung Disease and Pulmonary Fibrosis in Hermansky-Pudlak Syndrome Type 2, an Adaptor Protein-3 Complex Disease

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Pulmonary fibrosis develops in Hermansky-Pudlak syndrome (HPS) types 1 and 4. Limited information is available about lung disease in HPS type 2 (HPS-2), which is characterized by abnormal function of the adaptor protein-3 (AP-3) complex. To define lung disease in HPS-2, one child and two adults with HPS-2 were evaluated at the National Institutes of Health on at least two visits, and another child was evaluated at the University of Texas Health Science Center San Antonio. All four subjects with HPS-2 had findings of interstitial lung disease (ILD) on a high-resolution computed tomography scan of the chest. The predominant feature was ground glass opacification. Subject 1, a 14-year-old male, and subject 4, a 4-year-old male, had severe ILD, pulmonary fibrosis, secondary pulmonary hypertension and recurrent lung infections. Lung biopsy performed at 20 months of age in subject 1 revealed interstitial fibrosis and prominent type II pneumocyte hyperplasia without lamellar body enlargement. Subject 2, a 27-year-old male smoker, had mild ILD. Subject 3, a 22-year-old male nonsmoker and brother of subject 2, had minimal ILD. Severe impairment of gas exchange was found in subjects 1 and 4 and not in subjects 2 or 3. Plasma concentrations of transforming growth factor-β1 and interleukin-17A correlated with severity of HPS-2 ILD. These data show that children and young adults with HPS-2 and functional defects of the AP-3 complex are at risk for ILD and pulmonary fibrosis.

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INTRODUCTION

There are eight known human subtypes and multiple murine models of Hermansky-Pudlak syndrome (HPS), an autosomal recessive disorder characterized by defective biogenesis of lysosome-related organelles (1). Clinical manifestations include oculocutaneous albinism, a bleeding diathesis due to a platelet storage pool defect, granulomatous colitis and pulmonary fibrosis (2). Many of these manifestations cause considerable morbidity, and a leading cause of mortality in certain subtypes of HPS is fibrotic lung disease (3,4). HPS-1 and HPS-4 are subtypes associated with pulmonary fibrosis and are characterized by aberrant biogenesis of lysosome-related organelles complex-3 (BLOC-3), which is believed to regulate the intracellular localization of lysosomes and late endosomes (5–7).

Pulmonary fibrosis associated with HPS-1 is a progressive interstitial lung disease. Although lung transplantation has prolonged survival in a few individuals, patients with HPS-1 pulmonary fibrosis typically die of their lung disease in their fourth or fifth decades of life (3,4,8,9). Longitudinal pulmonary function tests demonstrate a decline in forced vital capacity (FVC) and diffusion capacity (DLCO). The diagnosis of HPS pulmonary fibrosis in adults with HPS-1 or HPS-4 is usually established by using a high-resolution computed tomography (HRCT) scan rather than lung biopsy; characteristic findings include ground glass opacification, thickened interlobular septa, reticulation, subpleural

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cysts/honeycombing and traction bronchiectasis (10,11).

HPS-2 is associated with mutations in *AP3B1* (GenBank NM 003664; located on chromosome 5q14.1), encoding the β3A subunit of the adaptor protein-3 (AP-3) complex, leading to abnormal formation and function of AP-3 (12–14). The AP-3 complex is a heterotetramer consisting of δ, β3, μ3 and σ3 subunits or adaptins (15). The AP-3 complex regulates biogenesis of lysosome-related organelles and modulates intracellular trafficking of proteins to late endosomes, lysosomes and lysosome-related organelles.

HPS-2 is a rare subtype of HPS, with 12 cases reported in the literature (12–14,16–20). Clinical manifestations include oculocutaneous albinism, a platelet storage pool defect, and recurrent bacterial and viral infections due to immunodeficiency. Patients exhibit neutropenia that is responsive to granulocyte colony-stimulation factor (G-CSF), deficiency of natural killer and natural killer T-cells, T-lymphocyte dysfunction, and in one case hemophagocytic lymphohistiocytosis. Thus far, interstitial lung disease has been reported in a child and an adult with HPS-2 (19,21). Consistent with human HPS-2, the *pearl* mouse, a model for HPS-2, appears highly susceptible to pulmonary fibrosis induced by bleomycin (22,23). In this report, we define the features of interstitial lung disease in two children and two adults with HPS-2.

MATERIALS AND METHODS

Subjects

Subjects 1–3 were enrolled in protocol 95-HG-0193 (www.clinicaltrials.gov, NCT00001456), "Clinical and Basic Investigations into Hermansky-Pudlak Syndrome," which was approved by the Institutional Review Board of the National Human Genome Research Institute. Written informed consent from these three subjects or their parent was obtained, and subjects underwent at least two evaluations at the National Institutes of Health Clinical Center in Bethesda, Maryland. HRCT scans and pulmonary function tests were performed during each visit. Subject 4 underwent clinically indicated testing at the University of Texas Health Science Center San Antonio.

Genetic Analysis

Mutation analysis of *AP3B1* on all subjects was previously performed and reported (12,13,20). In the current study, we performed further genetic analysis on the 63–base pair deletion, which was reported in the cDNA of subjects 2 and 3 (patients 40 and 42, respectively, in reference 12). Their genomic DNA was polymerase chain reaction (PCR)-amplified with primers spanning exon 12 and its intronic boundaries. Standard PCR amplification procedures were used. All amplified products were directly sequenced by using the BigDye 3 Terminator chemistry (Applied Biosystems, Foster City, CA, USA) and separated on an ABI 3130xl genetic analyzer (Applied Biosystems).

Computed Tomography Scan

At the National Institutes of Health, conventional and HRCT scans of the chest using a 1-mm collimation at 30-mm intervals and a high-spatial-frequency reconstruction algorithm were performed without intravenous contrast during end-inspiration in the supine and prone position, respectively (General Electric Medical Systems, Milwaukee, WI, USA). At CHRISTUS Santa Rosa Children's Hospital, 3-mm axial images were obtained by using lung algorithms with sagittal and coronal reformations. HPS pulmonary fibrosis or interstitial lung disease was diagnosed by characteristic findings on HRCT scans of the chest, as previously described (3,10,11).

Pulmonary Function Testing

Measurements were made by using standard equipment, and 6-min walk tests were performed according to American Thoracic Society and European Respiratory Society guidelines (SensorMedics, Yorba Linda, CA, USA) (24–27). Forced expiratory volume in 1 s (FEV_1), FVC, total lung capacity (TLC) and DLCO were expressed as percentages of predicted values. Exhaled nitric oxide was measured by using a Sievers Nitric Oxide Analyzer 280i at 50 mL/s in accordance with the manufacturer's instructions (GE Analytical Instruments, Boulder, CO, USA).

Blood Protein Analysis

Peripheral blood plasma samples from subjects 1–3 were analyzed for monocyte chemotactic factor-1 (MCP-1), monocyte inflammatory protein-1α (MIP-1α), granulocyte monocyte–colony stimulation factor (GM-CSF) (regulated upon activation), normal T cell expressed and secreted (RANTES), interferon γ, interleukin (IL)-17A, total transforming growth factor-β1, platelet-derived growth factor BB homodimer, matrix metalloproteinase (MMP)-1 and MMP-7 by multiplex enzyme-linked immunosorbent assay (Aushon Biosystems, Billerica, MA, USA). Plasma specimens from three subjects with HPS-3, a subtype of HPS that is not associated with interstitial lung disease, were analyzed as controls. The HPS-3 subjects were an 11-year-old male, a 12-year-old female and a 22-year-old male without lung disease. Data are shown as mean ± standard error of the mean. Analyses were performed by using GraphPad Prism® version 5.0 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

Subject Characteristics

HPS-2 was diagnosed by demonstrating absence of platelet dense bodies on electron microscopic examination in three individuals and by the presence of *AP3B1* mutations in all subjects (12,13,21). Pertinent subject characteristics are summarized in Table 1.

Subject 1 is a male who was evaluated at the National Institutes of Health at 5, 8 and 14 years of age (patient 87 in reference 13). His mother is three-fourths Cajun and one-fourth Houma Indian,

Table 1. Subject characteristics.

	Subject 1	Subject 2	Subject 3	Subject 4
Age (years), initial	5	25	20	4
Age (years), follow-up	14	27	22	
Sex	Male	Male	Male	Male
AP3B1 mutations	c.1525C>T (p.R509X) c.195G>T (p.E659X)	IVS11-1G>C c.1739T>G (p.L580R)	IVS11-1G>C c.1739T>G (p.L580R)	IVS10 + 5G>A IVS10 + 5G>A
Tobacco use	No	Yes	No	No
ANC, initial	3,800[a]	795	568	720[a]
ANC, follow-up	4,354[a]	1,030	611	
Infections	Enterococcal sepsis Skin abscesses Viral pneumonias Otitis media Varicella	Otitis media URIs Cellulitis Viral pneumonias	Otitis media URIs	*Pseudomonas* bacteremia Necrotizing fasciitis

ANC, absolute neutrophil count; URIs, upper respiratory infections.
[a]Subject receiving G-CSF.

and his father is half African American, one-fourth Chitimacha Indian, and one-fourth Houma Indian. There is no known history of consanguinity. Genetic analysis demonstrated compound heterozygous nonsense mutations in *AP3B1* (that is, c.1525C>T [p.R509X] and c.1975G>T [p.E659X]), resulting in the absence of *AP3B1* mRNA and the presence of a truncated AP-3 complex (13). Subject 1 has oculocutaneous albinism, easy bruisability, severe recurrent epistaxis, gingivitis, failure to thrive with poor growth and short stature, neutropenia responsive to G-CSF treatment, enterococcal sepsis, skin abscess, severe influenza A and respiratory syncytial virus pneumonias requiring mechanical ventilation, six pneumothoraces, and gastroesophageal reflux with aspiration requiring a Nissen procedure and placement of a gastrostomy button. He did well for the first few months of life; he had a normal white cell count and differential as well as no respiratory problems. Thereafter, he developed severe respiratory difficulty on several occasions, and coincidently had a low neutrophil count with an absolute neutrophil count of <500. The low neutrophil count was corrected by administration of G-CSF, and he has not had further episodes of severe respiratory infections. However, at intervals, he required supplemental oxygen, and nasal drying resulted in frequent episodes of epistaxis, some of which were quite prolonged and severe, requiring transfusions of both platelets and red cells. One episode was life-threatening with hypovolemic shock and severe anemia. Although he continued to have episodes of epistaxis, the severity lessened dramatically with the immediate home administration of subcutaneous desmopressin, and he has not required transfusions of platelets or red cells. An open lung biopsy was performed for evaluation of severe respiratory distress at 20 months of age; histopathologic examination revealed interstitial pneumonitis with focal evolving interstitial fibrosis, alveolar pneumocyte hyperplasia, alveolar siderosis, foamy alveolar macrophages, fibrovascular pleural adhesions and mild smooth muscle pulmonary arterial hyperplasia. The process was temporally uniform. Ultrastructural findings seen by transmission electron microscopy demonstrated prominent type II pneumocyte hyperplasia without lamellar body enlargement, some type II cells with acicular cytoplasmic spaces, interstitial myofibroblasts and collagen fiber bundles and thickening of capillary basement membranes (Figures 1A–D). His lung disease was treated with systemic corticosteroids, including oral prednisone from 2 to 5 years of age. Supplemental oxygen was used during childhood, and his pulmonary fibrosis remained clinically stable. Secondary pulmonary hypertension was diagnosed by echocardiogram; estimated pulmonary artery pressure improved at age 6 years. At 13 years of age, the patient discontinued supplemental oxygen, and his pulmonary hypertension progressed over a few months; estimated pulmonary artery pressure was 63 mmHg by echocardiogram. At age 14 years, subject 1 experienced dyspnea on mild exertion, did not have chronic cough and used supplemental oxygen intermittently. He was exposed to second-hand smoke at home, but did not have a history of unusual environmental exposures. His height and weight were below the fifth percentile. His physical examination revealed pectus carinatum deformity, scattered inspiratory crackles, prominent P2 heart tones and digital clubbing without cyanosis. He received G-CSF treatment three times weekly, and his white blood cell concentration was 7,520 per microliter; 57.9% of these cells were polymorphonuclear leukocytes.

Subjects 2 and 3 were young adult male brothers who were evaluated twice at the National Institutes of Health (patients 40 and 42 in references 12, 13 and 21). Their parents are of Dutch ancestry, and there is no known consanguinity. Genetic analysis showed compound heterozygous mutations in *AP3B1* (that is, c.1166-1228del [p.390-410del] and

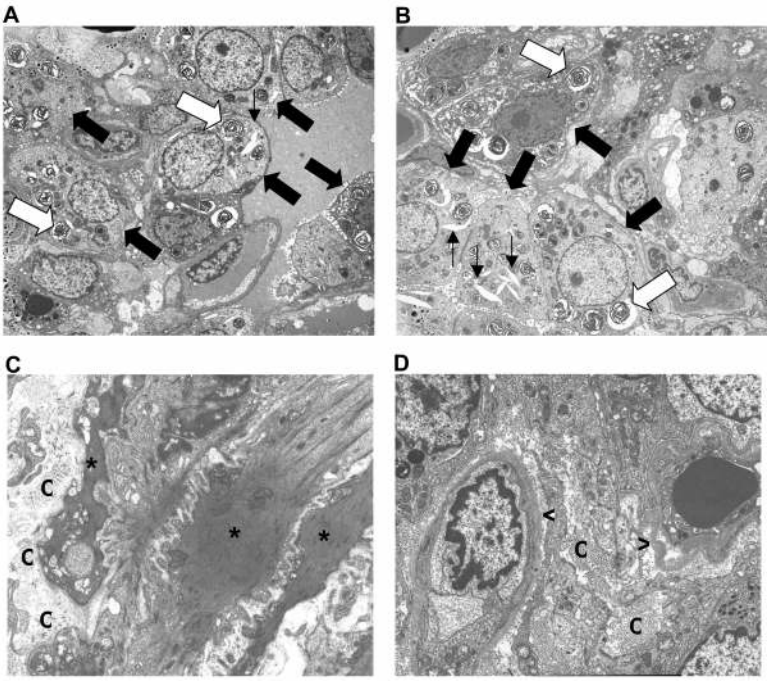

Figure 1. Representative transmission electron micrographs from a subject with HPS-2 and severe interstitial lung disease. (A) and (B) show hyperplasia of type II cells (solid large arrows) with lamellar bodies (open large arrows) in the alveolar interstitial space from subject 1. Some type II cells have acicular spaces (small arrows) in the cytoplasm. Myofibroblasts (*) and collagen fiber bundles (C) are identified in the alveolar interstitium (C). Some areas of the lung contain capillaries with regional thickening of the basement membrane (arrowhead) (D).

c.1739T>G [p.L580R]) (12). Further analysis of their reported 63–base pair cDNA deletion revealed that this was in fact due to a splice site mutation in intron 11, that is, c.1168-1G>C (IVS11-1G>C), resulting in aberrant splicing with skipping of exon 12.

The brothers had a history of oculocutaneous albinism, recurrent epistaxis during childhood, neutropenia, recurrent otitis media and respiratory tract infections and no pneumonias. Subject 2 smoked one-half to one pack of cigarettes daily since age 13 years; he was evaluated at the National Institutes of Health (NIH) at age 25 and 27 years. Subject 3 never smoked, but was exposed to second-hand smoke throughout childhood. His evaluations at NIH were performed at 20 and 22 years of age. Subjects 2 and 3 did not experience dyspnea on exertion or chronic cough, and they did not have a history of significant environmental exposures associated with interstitial lung disease or pulmonary fibrosis. Both subjects were well-developed and well-nourished adults. There were no audible inspiratory crackles, digital clubbing or cyanosis. Without treatment with G-CSF, the white blood cell concentration of subject 2 was 3,540 per microliter with 29.1% polymorphonuclear leukocytes, and the white blood cell concentration of subject 3 was 2,880 per microliter with 21.2% polymorphonuclear leukocytes.

Subject 4 was a 4-year-old male who was evaluated at the University of Texas Health Science Center San Antonio (patient in reference 20). His mother and father are Mexican American, and there is no known history of consanguinity. Genetic analysis demonstrated a homozygous intronic mutation in *AP3B1* (that is, IVS10 + 5G>A). Subject 4 has a history of oculocutaneous albinism, severe failure to thrive, developmental delay, congenital hip dysplasia, congenital adrenal hyperplasia, hypothyroidism and severe gingivitis. Primary cardiac disease includes mild mitral valve insufficiency and untreated Wolff-Parkinson-White syndrome without history of significant tachyarrhythmia. His hematologic issues include recurrent bleeding and easy bruisability as well as neutropenia responsive to G-CSF treatment. He has had multiple infections, including *Pseudomonas* bacteremia, necrotizing fasciitis and abdominal wall infection secondary to a gastrostomy tube infection, cellulitis, otitis media, gastroenteritis, varicella, and influenza A and respiratory syncytial virus pneumonia. Pulmonary disease has manifested as recurrent pneumonia, acute respiratory distress syndrome requiring mechanical ventilation, pulmonary fibrosis, secondary pulmonary hypertension with estimated pulmonary artery systemic pressure of 50 mmHg by echocardiogram, asthma and obstructive sleep apnea. His height and weight were below the third percentile. His physical examination revealed inspiratory crackles and digital clubbing without cyanosis. He was treated with twice-weekly G-CSF, nebulized bronchodilators and nocturnal supplemental oxygen with bilevel positive airway pressure (BiPAP), his white blood cell concentration was 3,600 per microliter and 20% of these cells were polymorphonuclear leukocytes.

Pulmonary Function Measurements

Pulmonary function testing was performed in each subject. Subject 1 had an FVC of 40% predicted at age 8 years, which improved to 71% predicted at age 14 years (Table 2). TLC was normal on both occasions. DLCO, which could not be measured at age 8 years, was 29% predicted at 14 years of age. Consistent with this impairment in gas exchange, a 6-min walk test revealed that oxygen saturation decreased from 96% to 80% while the subject was breathing ambient air. Exhaled nitric oxide measurement was 10.1 parts per billion (normal range, <15–25

Table 2. Pulmonary function testing and blood cytokine concentrations.

	Age (years)	FEV_1[a]	FVC[a]	FEV_1/FVC[b]	TLC[c]	DLCO[c]	NO[d]	TGF-β1 (ng/mL)	IL-17A (pg/mL)
Subject 1	8	42 (0.39)	40 (0.39)	100	88	NA	NA	170.6	8.8
	14	65 (0.94)	71 (1.09)	86	109	29	10.1	36	2.6
Subject 2	25	87 (3.65)	90 (4.70)	78	87	105	NA	39.1	3.7
	27	85 (3.49)	90 (4.62)	75	94	96	NA	72	4
Subject 3	20	82 (3.54)	90 (3.70)	74	91	84	NA	74.8	0
	22	87 (4.79)	90 (4.72)	78	94	108	NA	30.7	0

NA, not available.
[a]Percentage of predicted values (actual values, liters).
[b]Percentage.
[c]Percentage of predicted values.
[d]NO, exhaled nitric oxide (at 50 mL/sec), parts per billion (normal range, <15 to 25 parts per billion).

parts per billion) and is consistent with the absence of clinical evidence of asthma and airway inflammation in subject 1. Pulmonary artery pressure was estimated to be 49 mmHg by transthoracic echocardiogram. Subjects 2 and 3 had normal FVC, TLC and DLCO, and their lung function was stable during a 2-year interval. Arterial blood gas measurements demonstrated normal oxygenation. The FEV_1/FVC ratio was normal in subjects 1, 2 and 3 and indicated that these individuals did not have obstructive lung disease. A 6-min walk test demonstrated that subject 4 desaturated from an oxygen saturation of 95% to 78% while he was breathing ambient air.

Radiographic Imaging

Computed tomography scans of the chest revealed interstitial lung disease of varying severity; subjects 1 and 4 had the most severe radiographic findings (Figures 2A–D). Bilateral ground glass opacifications, thickening of interlobular septa and interstitial reticulations were widespread. These changes showed some improvement between 8 and 14 years of age in subject 1. Milder findings of interstitial lung disease, including ground glass opacities and interstitial reticulations, were seen in subject 2 (Figures 3A, B). The HRCT scans of subject 3 revealed mild ground glass opacification and minimal thickening of interlobular septa in the right middle lobe (Figures 3C, D). These radiographic findings of interstitial lung disease were stable between evaluations for subjects 2 and 3 (data not shown).

Blood Protein Analyses

To determine whether proteins associated with fibrosis correlate with severity of HPS-2 lung disease, concentrations of 10 analytes were measured in longitudinally obtained plasma samples from subjects 1–3. On the basis of lung physiology and radiographic imaging, subject 1 had improved severe interstitial lung disease from 8 to 14 years of age. Subject 2 had mild interstitial lung disease that was stable from 25 to 27 years of age, and subject 3 had minimal interstitial lung disease that was stable from 20 to 22 years of age. Concentrations of transforming growth factor-β1 (TGF-β1) and IL-17A correlated with severity of interstitial lung disease in these subjects with HPS-2 (Table 2). Plasma concentrations of TGF-β1 and IL-17A were highest when subject 1 had the most severe disease (170.6 ng/mL and 8.8 pg/mL, respectively) and were lower in the same subject when his disease was improved (36.0 ng/mL and 2.6 pg/mL, respectively). In subjects 2 and 3, plasma con-

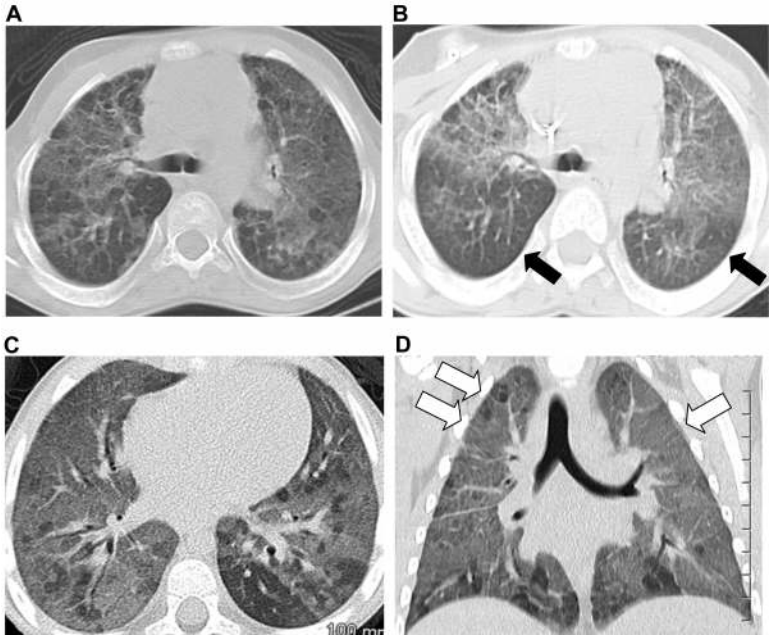

Figure 2. Representative computed tomography scan images from two children with HPS-2 and severe interstitial lung disease. (A) shows severe bilateral ground glass opacities, thickening of interlobular septa and pulmonary fibrosis at the level of the carina for subject 1 at 8 years of age. A computed tomography scan image at the same level shows mild improvement in infiltrates (arrows) at 14 years of age (B). (C) shows extensive bilateral ground glass opacities when subject 4 was 4 years of age. A coronal CT scan image demonstrates diffuse ground glass infiltrates and interstitial reticulations (open arrows) in subject 4 (D).

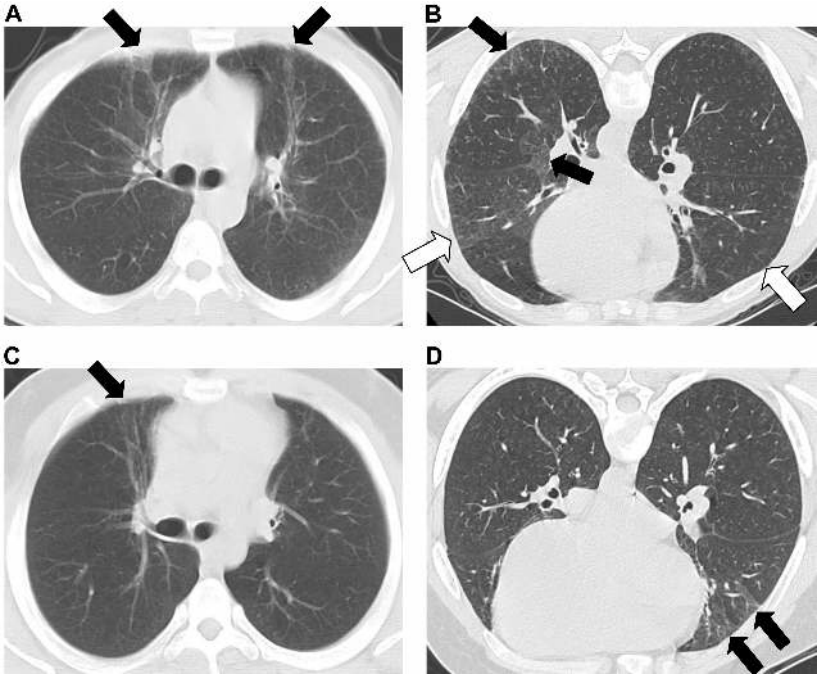

Figure 3. Representative computed tomography and HRCT scan images from two adult brothers with HPS-2 and interstitial lung disease. A computed tomography and HRCT scan image in the prone position (A and B, respectively) show mild bilateral ground glass opacities (arrows) and interstitial reticulations (open arrows) in subject 2 at the age of 27 years. C and D demonstrate computed tomography and prone HRCT scan images from subject 3 when he was 22 years of age. Mild ground glass opacities (arrows) are present in the right middle lobe.

centrations of TGF-β1 and IL-17A were relatively stable. Concentrations of IL-17A were lowest in subject 3 (0 pg/mL), who had minimal interstitial lung disease. For additional controls, concentrations of TGF-β1 and IL-17A were measured in three subjects with HPS-3, which is a subtype of HPS that is not associated with lung disease (39.1 ± 10.8 ng/mL and 0 ± 0 pg/mL, respectively) (data not shown). Concentrations of MCP-1, MIP-1α, GM-CSF, RANTES, interferon γ, platelet-derived growth factor BB, MMP-1 and MMP-7 did not correlate with severity of lung disease in HPS-2 (data not shown).

DISCUSSION

This report defines features of interstitial lung disease and pulmonary fibrosis in four patients with HPS-2, a rare autosomal recessive disorder characterized by defects in the AP-3 complex. Our data demonstrate that interstitial lung disease affects children and young adults with HPS-2 and is associated with radiographic findings of ground glass opacifications, thickening of interlobular septa and interstitial reticulations on HRCT scans of the chest. Consistent with these results, three subjects with interstitial lung disease and HPS-2 had high plasma concentrations of TGF-β1 and IL-17A, and their levels correlated with severity of HPS-2 interstitial lung disease. These findings are relevant, because these profibrotic proteins may have cooperative roles in development of fibrosis in general (28,29). However, further studies are needed to determine whether levels of TGF-β1 and IL-17A correlate with severity of HPS-2 pulmonary fibrosis.

The natural history of HPS-2 lung disease differs from that of HPS-1 pulmonary fibrosis. The pulmonary fibrosis of HPS-1 is a progressive disease usually affecting middle-aged adults and not children (2–4). In contrast, this report shows that children with HPS-2 can develop pulmonary fibrosis and adults can develop subclinical interstitial lung disease. During a 6-year interval, some of the lung function measurements and infiltrates in subject 1 demonstrated improvement, which may be a consequence of resolution of a coexistent occult lung infection, aspiration pneumonitis or pulmonary edema. Longitudinal data from additional patients with HPS-2 are needed to define the natural history of HPS-2 lung disease. It is likely that the differences between the clinical features of HPS-1 and HPS-2 lung disease are due to differences in basic mechanisms of disease. Specifically, HPS-1 is associated with abnormalities in BLOC-3, and HPS-2 is characterized by defects in the AP-3 complex.

The immunodeficiency associated with HPS-2, but not HPS-1, may result in an aggressive form of lung disease that presents at an earlier age than the pulmonary fibrosis of HPS-1. Specifically, recurrent lung injury from pulmonary infection may contribute to the initiation and development of interstitial lung disease in patients with HPS-2. Investigators have studied a possible etiological role of viral infection in idiopathic pulmonary fibrosis, and associations between herpes viruses and idiopathic pulmonary fibrosis have been reported (30). To our knowledge, however, there is no known association between viruses and HPS pulmonary fibrosis. Notably, recurrent infection alone is probably insufficient to cause pulmonary fibrosis, because fibrotic lung disease is not commonly found in patients with primary human immunodeficiency disorders (31).

In addition, pulmonary disease in patients with dysfunction of the AP-3 complex may also result from mistrafficking of proteins leading to aberrant repair mechanisms in the lung. Consistent with these possibilities, it is notable that both the effect of the genetic mutation on protein production and the clinical manifes-

Table 3. Summary of *AP3B1* mutations and pulmonary manifestations in HPS-2.

Patient	Age (years)	Sex	*AP3B1* Mutations[a]	Pulmonary manifestations	Reference
1[b]	14	M	c.1525C>T; p.R509X c.1975G>T; p.E659X	Pulmonary fibrosis, secondary pulmonary hypertension, viral pneumonias, ventilatory failure, pneumothoraces	13
2[b]	27	M	IVS11-1G>C (splice site) c.1739T>G; p.L580R	Respiratory tract infections, interstitial lung disease	12
3[b]	22	M	IVS11-1G>C (splice site) c.1739T>G; p.L580R	Respiratory tract infections, interstitial lung disease	12
4[b]	4	M	IVS10 + 5G>A (splice site) IVS10 + 5G>A (splice site)	Pulmonary fibrosis, secondary pulmonary hypertension, pneumonias, ventilatory failure, asthma, sleep apnea	20
5	21	M	IVS14 + 6T>C (splice site) c.1619insG; p.G540fsX25	None reported	15
6	7	F	c.1063ins-del; p.Q355fsX360 c.1789insC; p.I597fsX608	Pneumonia, respiratory tract infections	16
7	4	M	c.1063ins-del; p.Q355fsX360 c.1789insC; p.I597fsX608	None reported	16
8	2	M	c.904A>T; p.R302X c.904A>T; p.R302X	Pneumonias	17
9	15	F	g.del8168bp; p.del491-550 g.del8168bp; p.del491-550	None reported	18
10	21	M	g.del8168bp; p.del491-550 g.del8168bp; p.del491-550	Pneumonias	18
11	3	F	c.del2078-2165; p.E693fsX13	None reported	19
12	3	M	c.del153-156; p.E52fsX11	Pulmonary fibrosis, respiratory tract infections, bronchiectasis	19

[a]All mutations are converted to the Human Genome Variation Society nomenclature (http://www.hgvsorg/mutnomen).
[b]Subjects 1–4 in this study.

tations are more severe in subject 1 compared with subjects 2 and 3. That is, the genetic mutation in subject 1 is associated with absence of β3A protein, and the compound heterozygous mutations of *AP3B1* in subjects 2 and 3 are associated with reduced production of the β3A subunit (12,13,21). Subject 1 has more severe neutropenia and interstitial lung disease than subjects 2 or 3. In agreement with these data, severe clinical manifestations, including immunodeficiency and pulmonary fibrosis, were reported in a 3-year-old child with a large genomic deletion that generates a premature stop codon in the cDNA and is associated with absence of β3A protein (19). Although the effect of the intronic mutation in subject 4 is undefined (20), it is possible that it may cause a splicing defect, because the intronic mutation results in a weaker 3′ splice donor site of exon 10. These data indicate that severity of HPS-2 may be largely dependent on the effects of genetic mutations in *AP3B1* on protein production and appear to be independent of whether the genetic defects are homozygous or compound heterozygous mutations. The reported mutations in *AP3B1* and pulmonary manifestations of 12 reported cases of HPS-2 are shown in Table 3.

Initial studies focusing on HPS pulmonary fibrosis have improved our understanding of this disease. *Pale ear* and *pearl*, murine models for HPS-1 and HPS-2, do not develop spontaneous pulmonary fibrosis (32). However, they are highly susceptible to pulmonary fibrosis induced by bleomycin (23). These observations indicate that a combination of genetic susceptibility to disease and environmental exposures initiate the fibrotic process in the lung.

Studies have also demonstrated that alveolar macrophages and type II epithelial cells are abnormal in HPS pulmonary fibrosis. Alveolar inflammation is found in animal models and people with HPS pulmonary fibrosis (11,33). Alveolar macrophages in HPS-1 are activated and secrete high concentrations of cytokines, which suggests that a proinflammatory state contributes to HPS lung disease. Type II cells in HPS-1 pulmonary fibrosis are hyperplastic and engorged with distended lamellar bodies, which are lysosome-related organelles (34). We found that in a child with HPS-2 interstitial lung disease, histologic analysis revealed interstitial fibrosis, foamy alveolar macrophages and prominent type II pneumocyte hyperplasia. Transmission electron microscopy demonstrated hyperplastic type II cells without lamellar body enlargement, alveolar interstitial myofibroblasts and collagen accumulation, and areas with capillary basement membrane thicken-

ing. Studies using *pale ear*/*pearl* double mutant mice revealed marked enlargement of type II cells and giant lamellar bodies, impairment of phospholipid secretion from type II cells, accumulation of surfactant protein-B and -C in lung tissue, pulmonary fibrosis and high levels of TGF-β1 in bronchoalveolar lavage samples and alveolar macrophages (35–37). Other investigators demonstrated dysregulated surfactant trafficking and secretion as well as alveolar epithelial type II cell stress and apoptosis in HPS-1 patients and in *pale ear*/*pearl* double mutant mice with spontaneous pulmonary fibrosis (38). These data suggest that alveolar macrophages and/or type II cells may contribute to the pathogenesis of HPS pulmonary fibrosis.

CONCLUSION

Taken together, our results show that children and young adults with HPS-2 are at risk for development of interstitial lung disease and pulmonary fibrosis of varying severity and suggest that pulmonary function tests and HRCT scans of the chest should be considered in the evaluation of patients with HPS-2 to identify and diagnose interstitial lung disease. The young age of these subjects suggests that functional defects of the AP-3 complex are associated with susceptibility to a relatively aggressive form of lung disease. The availability of cellular and murine models for HPS-2 facilitates studies on the pathogenesis of interstitial lung disease, and investigations focusing on the role of the AP-3 complex and/or its cargo proteins in pulmonary fibrosis are indicated.

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DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

REFERENCES

1. Huizing M, Helip-Wooley A, Westbroek W, Gunay-Aygun M, Gahl WA. (2008) Disorders of lysosome-related organelle biogenesis: clinical and molecular genetics. *Annu. Rev. Genomics. Hum. Genet.* 9:359–86.
2. Gahl WA, *et al.* (1998) Genetic defects and clinical characteristics of patients with a form of oculocutaneous albinism (Hermansky-Pudlak syndrome). *N. Engl. J. Med.* 338:1258–64.
3. Brantly M, *et al.* (2000) Pulmonary function and high-resolution CT findings in patients with an inherited form of pulmonary fibrosis, Hermansky-Pudlak syndrome, due to mutations in HPS-1. *Chest.* 117:129–36.
4. Gahl WA, *et al.* (2002) Effect of pirfenidone on the pulmonary fibrosis of Hermansky-Pudlak syndrome. *Mol. Genet. Metab.* 76:234–42.
5. Nazarian R, Falcón-Pérez JM, Dell'Angelica EC. (2003) Biogenesis of lysosome-related organelles complex 3 (BLOC-3): a complex containing the Hermansky-Pudlak syndrome (HPS) proteins HPS1 and HPS4. *Proc. Natl. Acad. Sci. U. S. A.* 100:8770–5.
6. Martina JA, Moriyama K, Bonifacino JS. (2003) BLOC-3, a protein complex containing the Hermansky-Pudlak syndrome gene products HPS1 and HPS4. *J. Biol. Chem.* 278:29376–84.
7. Anderson PD, Huizing M, Claassen DA, White J, Gahl WA. (2003) Hermansky-Pudlak syndrome type 4 (HPS-4): clinical and molecular characteristics. *Hum. Genet.* 113:10–7.
8. Lederer DJ, *et al.* (2005) Successful bilateral lung transplantation for pulmonary fibrosis associated with the Hermansky-Pudlak syndrome. *J Heart Lung Transplant.* 24:1697–9.
9. Thomas de Montpréville V, Mussot S, Dulmet E, Dartevelle P. (2006) Pulmonary fibrosis in Hermansky-Pudlak syndrome is not fully usual. *Ann. Pathol.* 26:445–9.
10. Avila NA, *et al.* (2002) Hermansky-Pudlak syndrome: radiography and CT of the chest compared with pulmonary function tests and genetic studies. *Am. J. Roentgenol.* 179:887–92.
11. Rouhani FN, *et al.* (2009) Alveolar macrophage dysregulation in Hermansky-Pudlak syndrome type-1. *Am. J. Respir. Crit. Care Med.* 180:1114–21.
12. Dell'Angelica EC, Shotelersuk V, Aguilar RC, Gahl WA, Bonifacino JS. (1999) Altered trafficking of lysosomal proteins in Hermansky-Pudlak syndrome due to mutations in the beta 3A subunit of the AP-3 adaptor. *Mol. Cell.* 3:11–21.
13. Huizing M, *et al.* (2002) Nonsense mutations in ADTB3A cause complete deficiency of the beta3A subunit of adaptor complex-3 and severe Hermansky-Pudlak syndrome type 2. *Pediatr. Res.* 51:150–8.
14. Clark RH, *et al.* (2003) Adaptor protein 3-dependent microtubule-mediated movement of lytic granules to the immunological synapse. *Nat. Immunol.* 4:1111–20.
15. Dell'Angelica EC. (2009) AP-3-dependent trafficking and disease: the first decade. *Curr. Opin. Cell. Biol.* 21:552–9.
16. Fontana S, *et al.* (2006) Innate immunity defects in Hermansky-Pudlak type 2 syndrome. *Blood.* 107:4857–64.
17. Enders A, *et al.* (2006) Lethal hemophagocytic lymphohistiocytosis in Hermansky-Pudlak syndrome type II. *Blood.* 108:81–7.
18. Jung J, *et al.* (2006) Identification of a homozygous deletion in the AP3B1 gene causing Hermansky-Pudlak syndrome, type 2. *Blood.* 108:362–9.
19. Wenham M, *et al.* (2010) Two patients with Hermansky Pudlak syndrome type 2 and novel mutations in AP3B1. *Haematologica.* 95:333–7.
20. Chiang PW, Spector E, Thomas M, Frei-Jones M. (2010) Novel mutation causing Hermansky-Pudlak syndrome type 2. *Pediatr. Blood Cancer.* 55:1438.
21. Shotelersuk V, Dell'Angelica EC, Hartnell L, Bonifacino JS, Gahl WA. (2000) A new variant of Hermansky-Pudlak syndrome due to mutations in a gene responsible for vesicle formation. *Am. J. Med.* 108:423–7.
22. Feng L, *et al.* (1999) The β3A subunit gene (*Ap3b1*) of the AP-3 adaptor complex is altered in the mouse hypopigmentation mutant pearl, a model for Hermansky-Pudlak syndrome and night blindness. *Hum. Mol. Genet.* 8:323–30.
23. Young LR, Pasula R, Gulleman PM, Deutsch GH, McCormack FX. (2007) Susceptibility of Hermansky-Pudlak mice to bleomycin-induced type II cell apoptosis and fibrosis. *Am. J. Respir. Cell Mol. Biol.* 37:67–74.
24. Miller MR, *et al.* (2005) Standardisation of spirometry. *Eur. Respir. J.* 26:319–38.
25. Wanger J, *et al.* (2005) Standardisation of the measurement of lung volumes. *Eur. Respir. J.* 26:511–22.
26. ATS Statement. (2002) Guidelines for the six-minute walk test. *Am. J. Respir. Crit. Care Med.* 166:111–7.
27. ATS Workshop Proceedings. (2006) Exhaled nitric oxide and nitric oxide oxidative metabolism in exhaled breath condensate. *Proc. Am. Thorac. Soc.* 3:131–45.
28. Roberts A, *et al.* (1986) Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. *Proc. Natl. Acad. Sci. U. S. A.* 83:4167–71.
29. Wilson MS, *et al.* (2010) Bleomycin and IL-1beta-mediated pulmonary fibrosis is IL-17A dependent. *J. Exp. Med.* 207:535–52.
30. Tang YW, *et al.* (2003) Herpesvirus DNA is con-

sistently detected in lungs of patients with idiopathic pulmonary fibrosis. *J. Clin. Microbiol.* 41:2633–40.
31. Touw CML, *et al.* (2010) Detection of pulmonary complications in common variable immunodeficiency. *Pediatr. Allergy Immunol.* 21:793–805.
32. Tang X, Yamanaka S, Miyagi Y, Nagashima Y, Nakatani Y. (2005) Lung pathology of pale ear mouse (model of Hermansky-Pudlak syndrome 1) and beige mouse (model of Chediak-Higashi syndrome): severity of giant lamellar body degeneration of type II pneumocytes correlates with interstitial inflammation. *Pathol. Int*. 55:137–43.
33. Young LR, Borchers MT, Allen HL, Gibbons RS, McCormack FX. (2006) Lung-restricted macrophage activation in the pearl mouse model of Hermansky-Pudlak syndrome. *J. Immunol.* 176:4361–8.
34. Nakatani Y, *et al.* (2000) Interstitial pneumonia in Hermansky-Pudlak syndrome: significance of florid foamy swelling/degeneration (giant lamellar body degeneration) of type-2 pneumocytes. *Virchows Arch.* 437:304–13.
35. Lyerla TA, *et al.* (2003) Aberrant lung structure, composition, and function in a murine model of Hermansky-Pudlak syndrome. *Am. J. Physiol. Lung Cell Mol. Physiol*. 285:L643–53.
36. Guttentag SH, *et al.* (2005) Defective surfactant secretion in a mouse model of Hermansky-Pudlak syndrome. *Am. J. Respir. Cell Mol. Biol.* 33:14–21.
37. Wang L, Lyerla T. (2010) Histochemical and cellular changes accompanying the appearance of lung fibrosis in an experimental mouse model for Hermansky Pudlak syndrome. *Histochem. Cell Biol*. 134:205–13.
38. Mahavadi P, *et al.* (2010) Epithelial stress and apoptosis underlie Hermansky-Pudlak syndrome associated interstitial pneumonia. *Am. J. Respir. Crit. Care Med.* 182:207–19.