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Author(s)	Hayasaka, Itaru; Cho, Kazutoshi; Akimoto, Takuma et al.
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Title: Genetic Basis for Childhood Interstitial Lung Disease among Japanese

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Running title: Genetic basis for chILD

Authors: Itaru Hayasaka¹, Kazutoshi Cho¹, Takuma Akimoto¹, Masahiko Ikeda¹, Yutaka Uzuki¹, Masafumi Yamada², Koh Nakata³, Itsuko Furuta⁴, Tadashi Ariga², Hisanori Minakami⁴

¹Maternity and Perinatal Care Center, Hokkaido University Hospital, Sapporo, Japan

²Department of Pediatrics, Faculty of Medicine and Graduate School of Medicine,
Hokkaido University, Sapporo, Japan

³Bioscience Medical Research Center, Niigata University Medical & Dental Hospital,
Niigata, Japan

⁴Department of Obstetrics, Faculty of Medicine and Graduate School of Medicine,

Hokkaido University, Sapporo, Japan

Corresponding author: Kazutoshi Cho, MD, PhD,

Maternity and Perinatal Care Center, Hokkaido University Hospital,

N14W5 Kita-ku, Sapporo, 060-8648, Japan

TEL +81-11-706-5846

FAX +81-11-706-7981

E-mail chotarou@med.hokudai.ac.jp

All authors contributed significantly to this study.

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Disclosure

None of the authors have any conflicts of interest.

Category of study: Clinical Research

Abstract

Background: Genetic variants responsible for childhood interstitial lung disease (chILD) have not been studied extensively in Japanese patients.

Methods: The study population consisted of 62 Japanese chILD patients. Twenty-one and four patients had pulmonary hypertension resistant to treatment (PH) and hypothyroidism, respectively. Analyses of genetic variants were performed in all 62 patients for *SFTPC* and *ABCA3*, in all 21 PH patients for *FOXF1*, and in a limited number of patients for *NKX2.1*.

Results: Causative genetic variants for chILD were identified in 11(18%) patients: *SFTPC* variants in six, *NKX2.1* variants in three and *FOXF1* variants in two patients. No patients had *ABCA3* variants. All three and two patients with *NKX2.1* variants had hypothyroidism and developmental delay, respectively. We found 6 novel variants in this study.

Conclusion: Mutations in *SFTPC*, *NKX2.1*, and *FOXF1* were identified among Japanese infants and children with chILD, whereas *ABCA3* mutations were rare.

Introduction

Respiratory difficulties are often seen in neonates because of poor adaptation to lung respiration. However, some cases of respiratory failure cannot be explained by prematurity or poor adaptation. Childhood interstitial lung disease (chILD) is one such rare pathology that causes respiratory dysfunction in infants and children (1).

Childhood interstitial lung disease (chILD) comprises a group of disorders that cause respiratory dysfunction in infants and children. The chILD disorders includes pulmonary alveolar proteinosis (PAP) diagnosed based on bronchoalveolar lavage (BAL) and/or lung histology (2), interstitial pneumonitis (IP) diagnosed based on lung histology, and alveolar capillary dysplasia with misalignment of pulmonary veins (ACDMPV) diagnosed based on lung histology (3). Widespread ground glass opacification (GGO) and individually irregular consolidation on the dependent side or geographic opacification on high-resolution computed tomography (CT) are helpful for suspicion of chILD (4).

Some cases of chILD have genetic abnormalities, and hereditary chILD usually occurs in early childhood (5). Known genetic abnormalities include mutations in *SFTPB* for surfactant protein (SP)-B deficiency (6), *SFTPC* for SP-C abnormality (7), *ABCA3* for *ABCA3* deficiency (8), and *NKX2.1* for TTF-1 dysfunction among infants with PAP or IP, and *FOXF1* among infants with ACDMPV (9, 10). In addition, known abnormalities responsible for PAP include abnormalities in granulocyte macrophage colony-stimulating factor (GM-CSF) receptor (11, 12) and the presence of antibodies against GM-CSF (13). Therefore, assessment of GM-CSF stimulating phosphorylation of signal transducer and activator of transcription-5 (pSTAT-5) (12) and/or determination of anti-GM-CSF antibody are also helpful to understand the pathogenesis of PAP.

We launched a system to aid Japanese neonatologists and pediatricians to search for genetic causes of unexplained respiratory failure in 2011, and the results of our study in 43 cases during the period between February 2011 and July 2013 were reported previously (14). Here, we report the results of the analyses of genetic abnormalities in

an additional 62 infants and children with respiratory failure between August 2013 and

June 2016.

Materials and Methods

The present system was announced to Japanese neonatologists/pediatricians via the E-mail Network of Neonatologists in February 2011 after receiving approval from the Institutional Review Board of the Faculty of Medicine and Graduate School of Medicine, Hokkaido University. Collaboration with the Japan Society for Neonatal Health and Development (JSNHD), formerly the Japan Society for Premature and Newborn Medicine (JSPNM), was begun in February 2012 to facilitate collection of cases with severe and unexplained lung dysfunction: the JSPNM announced 3300 neonatologists four times annually since February 2012 to prospectively register patients with unexplained sustained respiratory distress due to genetic disorders and of unknown origin. Patients with respiratory failure due to known reasons were excluded. Most Japanese neonatologists also worked as professional pediatricians to treat school-aged children with lung dysfunction, were members of the JSPNM, and were working at approximately 90% of all facilities with neonatal intensive care units in Japan.

1. Patients

The inclusion criteria for study entry were followings; 10 years old or less at onset, severe sustained (> 1 week) lung dysfunction, diffuse pulmonary infiltrate on chest X-ray and/or GGO on CT. Patients with known causative factors, such as infection, congenital heart disease, systemic bone disease, neuromuscular disease, malformations, pulmonary hypertension after birth asphyxia, or bronchopulmonary dysplasia associated with prematurity were excluded. Sixty-two patients with chILD were analyzed in this study. All 62 patients were referred to us for analysis of genetic variants in the 35-month period between August 2013 and June 2016. All 62 families of the 62 patients provided detailed clinical information and blood samples for genetic analyses with written informed consent. These 62 patients were admitted at 48 hospitals located widely throughout Japan . Pulmonary hypertension resistant to treatment (PH) was defined as that evidenced on echocardiography and resistant to treatment, including sedation, diuretics, oxygen administration, and vasodilators, such as inhaled nitric oxide gas and prostaglandin I₂ in this study. Two patients with PH were treated with extracorporeal membrane oxygenation; ECMO.

2. Analyses of the *SFTPC*, *ABCA3*, *FOXF1*, and *NKX2.1*

Genomic DNA was extracted from peripheral blood using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). PCR methods for *SFTPC*, *ABCA3*, and *FOXF1* were described previously (14). Analysis of *NKX2.1* was introduced in February 2015.

Purified products were subjected to nucleotide sequence analysis by a commercial sequencing service (FASMAC, Kanagawa, Japan). Nucleotide sequences were compared with the reported reference sequences: NM_003018.3 (*SFTPC*),

NM_001089.2 (*ABCA3*), NM_001451.2 (*FOXF1*), and NM_001079668.2 (*NKX2.1*).

Analyses of *SFTPC* and *ABCA3* were performed in all patients. Analysis of *NKX2.1* was performed in a limited number of patients referred to us on or after February 2015.

Analysis of *FOXF1* was performed in all patients with PH. No patients showed clinical features compatible with SP-B deficiency, recurrent respiratory distress syndrome (RDS) at birth and following PAP. As SP-B deficiency has been considered to be rare in Japanese child (14), *SFTPB* analysis was not performed in any patient in this study.

Although patient with *ABCA3* deficiency also shows recurrent RDS, there was no information about prevalence of *ABCA3* deficiency in Japan, so we performed *ABCA3*

analysis for all patients. Candidate missense variants were evaluated with SIFT (<http://sift.jcvi.org>), PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>) and MutationTaster (<http://www.mutationtaster.org>). And allele frequencies of the variants were checked in East Asians according to the 1000 Genomes and ExAC/gnomAD.

3. Analyses of GM-CSF-induced phosphorylation of signal transducer and activator of transcription (pSTAT-5) and anti-GM-CSF antibody

Peripheral blood mononuclear cells were suspended in RPMI 10% FBS at a concentration of 1×10^6 cells/mL, and incubated in the presence or absence of 20 ng/mL GM-CSF for 15 minutes. Whole-cell lysates were prepared by homogenization in $1 \times$ SDS sample buffer, separated by glycine/SDS-PAGE according to standard procedures, and transferred onto PVDF membranes. Anti-STAT-5 (pY694) antibody (BD, San Diego, CA) was used at a final concentration of 0.5 μ g/mL, and anti-actin antibody (Sigma, St. Louis, MO) was used at a final concentration of 1 μ g/mL as a loading control. Serum GM-CSF autoantibody was measured using the method described previously (15) by one of the authors (KN) at Niigata University Medical & Dental Hospital.

Results

Lung histology was examined in 14 of patients (Figure 1). Lung specimens were reviewed at the individual institutions and we also confirmed the findings. Other patients of ILD were diagnosed from clinical features. Eight patients were diagnosed of IP, 7 were nonspecific interstitial pneumonia; NSIP and one was desquamative interstitial pneumonia; DIP. Pathological diagnoses were ACDMPV in three, PAP in two, mild non-specific hypertrophy of vascular media in one. Five patients underwent BAL and four were diagnosed with PAP based on typical gross appearance and microscopic findings, with milky white color and massive precipitate, PAS positive proteinous material, respectively. Another patient received BAL was provisionally diagnosed as IP, clinically diagnosed chILD without BAL findings compatible to PAP. Thus, a total of 19 patients had diagnoses based on histology/BAL. Of the 62 study subjects, 21 exhibited PH (Table 1). Thyroid status was known in all 62 patients, and hypothyroidisms of four patients were diagnosed at neonatal period. Median (range) age at DNA sampling was 2 months (0d – 16y).

Profiles of 11 patients with detected causative genetic variants

Genetic variants considered responsible for the disease were detected in 11 (18%) of the 62 patients (Table 2). Median (range) gestational week at birth was 40 (36 – 41) and median birthweight was 3.24 (2.53 – 3.76) kg in these 11 patients. Six patients had diagnoses based on lung histology/BAL (Subjects 1, 5, 6, 7, 9, and 11). Thus, 32% (6/19) of patients with diagnoses based on lung histology/BAL exhibited genetic variants. Of the 9 patients with *SFTPC* or *NKX2.1* variants, none had pulmonary hypertension resistant to treatment.

Among the nine genetic variants detected in the 11 patients (Subjects 1 – 11 in Table 2), three (including *SFTPC* c.218T>C, p.Ile73Thr, *SFTPC* c.134T>G, p.Leu45Arg, and *FOXF1* c.256C>T, p.Arg86Trp) are known to be responsible for lung dysfunction (17-19). To our knowledge, six other mutations in six patients in the present study have not been reported previously.

Analysis of SFTPC and ABCA3

All 62 patients underwent analyses of *SFTPC* and *ABCA3* genes. None exhibited *ABCA3* variants, but six exhibited *SFTPC* variants: c.218T>C, p.Ile73Thr in three (Subjects 1, 2, and 3 in Table 2), and c.541delC, p.Leu181Trpfs*5 (Subject 4); c.134T>G, p.Leu45Arg (Subject 5); and c.181A>G, p.Ser61Gly (Subject 6) in one each.

Lung dysfunction occurred at ages ranging from 1 year 0 months to 1 year 7 months in our three patients with c.218T>C, p.Ile73Thr variant manifesting clinical features of IP.

In three patients with other *SFTPC* variants (Subjects 4, 5, and 6), lung dysfunction occurred immediately after birth. Subject 4 with a novel mutation of c.541delC, p.Leu181Trpfs5X required mechanical ventilation, steroids, and home oxygen therapy until 1, 3, and 9 months of age, respectively. This patient required no supplemental oxygen when last seen at 6 years old. Subject 5 with c.134T>G, p.Leu45Arg mutation exhibited GGO on CT at age 16 days, and was shown to have PAP with BAL at age 16 days. This patient required home oxygen therapy and died from pulmonary aspiration at 2.4 years old with a weight of 6.0 kg while awaiting lung transplantation. Subject 6 with a novel mutation of c.181A>G, p.Ser61Gly was diagnosed with PAP at age 30 days,

required mechanical ventilation until 1.3 years old, and was treated with hydroxychloroquine and steroids, but died 4 months after lung transplantation at 9.3 years old.

The asymptomatic parents of four patients (Subjects 1, 3, 4, and 5) underwent genetic testing: one *SFTPC* variants was derived from *de novo* mutation (Subject 5), and two (Subjects 1 and 4) and one (Subject 3) were inherited from the maternal and paternal family lines, respectively.

Analysis of NKX2.1

Analysis of the *NKX2.1* gene was introduced in February 2015, and was performed in 38 (61%) of the 62 patients. Three patients exhibited *NKX2.1* variants: c.1117C>T, p.Gln373X (Subject 7 in Table 2); c.1016_1017insCCATCTCCGTGGGCAGCGG, p.Gly339fs (Subject 8); and c.954_958GCAGG>CAG, p.Gln318fs (Subject 9) in one each. Thus, the frequency of *NKX2.1* variants was 7.9% (3/38). All three patients with *NKX2.1* variant had hypothyroidism (Subjects 7, 8, and 9). *NKX2.1* analysis was performed in four patients with and in 34 without hypothyroidism, respectively. All

three patients with *NKX2.1* variants presented with cyanosis, and showed high blood levels of KL-6 (a protein expressed in lung epithelial cells (16); range, 5467 – 10000 U/mL), SP-A (33 – 118 ng/mL), and SP-D (111 – 397 ng/mL), as well as GGO on pulmonary CT. Treatment with steroids was ineffective in Subject 9 with PAP, but a combination of steroids and hydroxychloroquine was apparently effective in two patients (Subjects 7 and 8). Azathioprine was ineffective in Subject 7 and surfactant replacement was apparently effective in Subject 8. All three patients required home oxygen therapy. Total anomalous pulmonary venous connection (TAPVC) was corrected surgically in the neonatal period in Subject 9. These patients were followed at individual institutes and our information has been updated. The patient with hypothyroidism and ILD without an *NKX2.1* was born with 38 weeks of gestation and 3110g of birthweight. Lung biopsy at 2y showed NSIP. He had mild hyperactivity but no developmental delay at 3y. He had chronic respiratory failure resistant to steroids, hydroxychloroquine and clarithromycin.

The asymptomatic parents of two patients (Subjects 8 and 9) underwent genetic testing: both were derived from *de novo* mutation. Sequencing strategy in this study did not permit assessment of deletions including *NKX2.1*.

Analysis of FOXF1

Twenty one patients with PH, 3 with histological proven ACDMPV, 17 clinically suspected of ACDMPV with infantile onset PH, one with multiple anomalies underwent *FOXF1* analyses and two exhibited *FOXF1* variants (Figure 1, Table 1): c.256C>T, p.Arg86Trp in one (Subject 10 without available lung histology in Table 2) and c.852_856delTATCA, p.Tyr284X in the other (Subject 11 with ACDMPV). Subject 10 had PH immediately after birth, was treated with mechanical ventilation, inhaled nitric oxide, and prostaglandin I₂, and was still alive as an inpatient at 4 months old. Subject 11 with a novel mutation of p.Tyr284X had anal atresia, and died from PH at age 5 days despite aggressive treatment, including mechanical ventilation, inhaled nitric oxide, and prostaglandin I₂; a diagnosis of ACDMPV was made based on analyses of an autopsied lung specimen. The asymptomatic parents of Subject 10 underwent genetic testing, but neither had c.256C>T, p.Arg86Trp (Table 2). In two of the three patients with

ACDMPV diagnosed based on lung histology in this study, *FOXF1* variants was not detected. Sequencing strategy in this study did not permit assessment of deletions including and surrounding *FOXF1*.

Assessment of pSTAT-5 and anti-GM-CSF antibody

Three of the six patients diagnosed with PAP based on histology/BAL were shown to have genetic variants (Subjects 5, 6, and 9 in Table 2). None of the remaining three PAP patients had *SFTPC*, *ABCA3*, or *NKX2.1* variants. Two PAP patients (Subject 9 with *NKX2.1* variants and one without genetic variant) underwent assessment of pSTAT-5 and anti-GM-CSF antibody; the patient without genetic variant was shown to have significant anti-GM-CSF antibody titer in the blood. Thus, factors considered responsible for the disease were detected in four of the six patients diagnosed with PAP based on histology/PAP in this study. The two patients with neither genetic variants considered responsible for the disease nor abnormalities of pSTAT-5 or anti-GM-CSF antibody had clinical diagnoses other than PAP; primary immunodeficiency disease in one and juvenile idiopathic arthritis (JIA) in the other. The latter patient was diagnosed with JIA at 7 years old, experienced recurrent pneumonia while on steroids with

immunosuppressant, was diagnosed with PAP at 8 years old based on lung histology, and was shown to have *SFTPC* c.115G>T pVal39Leu in this study. This variant was judged as “damaging” on SIFT, “benign” on PolyPhen-2, and “disease causing” on MutationTaster. However, *SFTPC* c.115G>T, p.Val39Leu was considered unlikely to be the causative factor of PAP of this patient based on the allele frequency of *SFTPC* c.115G>T, p.Val39Leu, i.e., 0.7% and 0.05%, in East Asians according to the 1000 Genomes Project and ExAC/gnomAD, respectively.

Discussion

The present system was helpful for determination of genetic variants considered responsible in 11 (18%) of 62 patients with chILD, and suggested that among Japanese chILD patients, *NKX2.1* variants are relatively common. Among the nine genetic variants detected in the 11 patients (Subjects 1 – 11 in Table 2), three (including *SFTPC* c.218T>C, p.Ile73Thr, *SFTPC* c.134T>G, p.Leu45Arg, and *FOXF1* c.256C>T, p.Arg86Trp) are known to be responsible for lung dysfunction (17-19). To our knowledge, six other mutations in six patients in the present study have not been reported previously. These 6 variants were not listed in the East Asian database in 1000 Genome Project and ExAC/gnomAD. However, it was speculated that these six mutations were responsible for the lung dysfunction of six patients based on followings. The pro-SP-C amino acids of codon 61 are well preserved in many mammals, and *SFTPC* c.181A>G, p.Ser61Gly in Subject 6 was judged as “damaging” with SIFT, “probably damaging (0.999)” with PolyPhen-2, and “disease causing” with MutationTaster. Frameshift mutation can cause abnormal protein function, and the frameshift mutation, *SFTPC* p.His142fs, is reported in a neonate with SP-C abnormality

(20). Frameshift mutations were detected in three cases in this study: *SFTPC* p.Leu181Trpfs*5 (Subject 4), *NKX2.1* p.Gly339fs (Subject 8), and *NKX2.1* p.Gln318fs (Subject 9). Nonsense mutation can cause abnormal protein function, and those of *NKX2.1* p.Gln373X (Subject 7) and *FOXF1* p.Tyr284X (Subject 11) were detected in two patients in the present study. Thus, a “damaging” mutation in Subject 6, frameshift mutations in Subjects 4, 8, and 9, and nonsense mutations in Subjects 7 and 11 were considered responsible for lung dysfunction in these six infants.

Of the *SFTPC* variants detected in this study, c.218T>C, p.Ile73Thr detected in three patients (Subjects 1 – 3) accounts for more than 25% of patients with *SFTPC* variants presenting with clinical features of both IP and PAP (21), and varying age at onset (22).

FOXF1 variants were detected in two patients in this study (Subject 10 and 11). As the variant of p.Arg86Trp detected in Subject 10 can cause ACDMPV (19), Subject 10 may have suffered from ACDMPV. However, this patient lacked anomalies in the heart, alimentary tract, and urogenital organs, although approximately 80% of ACDMPV patients have anomalies of other organs, particularly of the cardiovascular, gastrointestinal, and/or genitourinary systems (23). The results of a previous study

substantiated the suggestion that mutations in *FOXF1* led to manifestation of ACDMPV and that this transcription factor is involved in the development of the pulmonary, cardiovascular, gastrointestinal, and genitourinary systems (19). Although rare cases of histologically proven ACDMPV with *FOXF1* variants showed slow onset and could survive with intensive care (24), with affected patients typically developing lung dysfunction and PH a few hours after birth, consistent with the findings in two of our patients (Subjects 10 and 11).

FOXF1 mutation was not detected in two of three patients diagnosed with ACDMPV based on lung histology in this study. We sequenced only the coding region of *FOXF1* in this study. As variants in upstream regions and copy number variants of *FOXF1* can cause ACDMPV (25), the possibility of these abnormalities in the two patients without *FOXF1* mutation in our setting could not be excluded.

Three patients had *NKX2.1* mutations (Subjects 7, 8, and 9); none of these mutations (c.1117C>T, p.Gln373X, c.1016_1017insCCATCTCCGTGGGCAGCGG, p.Gly339fs, and c.954_958GCAGG>CAG, p.Gln318fs) has been reported previously. All three patients with *NKX2.1* mutations had hypothyroidism. Similar to previous findings,

NKX2.1 variants were more common among those with hypothyroidism. TTF-1 encoded by *NKX2.1* is a protein expressed in the thyroid gland, lung primordium, and central nervous system (CNS), and is expressed specifically in the epithelial cells of the lung (26). TTF-1 abnormality caused by *NKX2.1* mutation was first reported in 1998 (27) and is associated with lung dysfunction, hypothyroidism, chorea, and/or psychomotor developmental delay (28). Lung dysfunction, hypothyroidism, and abnormalities in the CNS are seen in 54%, 87%, and 93% of patients with *NKX2.1* mutations, respectively, with all three seen in 50% of patients, and both hypothyroidism and CNS abnormalities are seen in 30% of patients (29). Subject 9 had TAPVC in this study. However, to our knowledge, there have been no literature reports describing TAPVC in patients with *NKX2.1* mutation. The finding of TAPVC was associated with the finding of the mutation, the question is whether it was causally related is what is unknown. Thirty-five (92%) of the 38 patients examined did not show *NKX2.1* mutation in this study. We sequenced only the coding region of *NKX2.1* in this study, and this may explain the *NKX2.1* mutation detection rate of 8% (3/38) in this study.

To our knowledge, a total of 17 Japanese cases of *NKX2.1* mutations have been previously described in seven reports to date (30 – 36). These 17 cases showed various abnormalities: chorea in 14 (82%), hypothyroidism in 11 (65%), recurrent respiratory infections in six (35%), and mental retardation in four (24%). However, none of the 17 cases exhibited RDS, although lung dysfunction associated with *NKX2.1* mutations includes RDS other than IP, PAP, and recurrent respiratory infections; Hamvas *et al.* reported symptoms suggestive of RDS, chILD, and recurrent respiratory infections in 76%, 19%, and 43% of patients, respectively (9). As our system was developed to aid in the determination of genetic variants for chILD patients, it was not clear how many Japanese infants/children with symptoms suggestive of RDS and recurrent respiratory infections had *NKX2.1* mutations.

In this study, genetic abnormalities considered causative of lung dysfunction were found in three of six patients with PAP diagnosed based on histology/BAL. In our previous study (14), genetic variants considered causative of lung dysfunction was detected in three of three patients with PAP diagnosed based on histology/BAL (two *SFTPC* mutations and one *ABCA3* mutation). Thus, our system indicated that the

majority of Japanese patients with infantile and childhood PAP had mutations in *SFTPC*, *ABCA3*, or *NKX2.1*, and suggested that investigation of these abnormalities can efficiently detect causative genetic abnormalities.

None of the 62 and only one of 43 infants tested had an *ABCA3* mutation in the present and our previous studies (14), respectively, while six of the 62 (9.7%) and four of the 43 infants (9.3%) tested had *SFTPC* mutations in the present and our previous studies (14), respectively. Thus, *ABCA3* deficiency was suggested to be rare in the Japanese population. However, *ABCA3* deficiency is relatively common in patients with surfactant protein dysfunction disorders in Western countries (37, 38). These results suggested that there are ethnic differences in the prevalence rates of *ABCA3* deficiency. The allele frequency of Glu292Val, the most common variant of *ABCA3* in Europeans, is 4/1000 according to the European database, while it is 0/1000 according to the East Asian database in 1000 Genome Project. This may explain the low frequency of *ABCA3* abnormalities in our study population.

In conclusion, 62 patients with chILD were analyzed in this study. Genetic variants considered causative of lung dysfunction were detected in 11 of the 62 patients (18%),

consisting of *SFTPC* mutations in six, *NKX2.1* mutations in three, and *FOXF1* mutations in two cases. Among Japanese chILD patients, *NKX2.1* and *SFTPC* variants and *SFTPC* variants appeared to be more common than *ABCA3* variants. We are now planning to establish gene panel for chILD using next-generation sequencer and whole exome analysis for undiagnosed cases.

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Figure legend

Figure 1: Process of the study

Numbers in square brackets refer to subjects listed in table 2, and numbers in round brackets refer those of patients. Histology and BAL were available in 14 and 5 patients, respectively. IP was diagnosed in 8 patients by histology, and PAP was diagnosed in 6 patients (2 patients by histology and 4 patients by BAL). Seventeen infants with PH were clinically suspected of PH and received *FOXF1* analysis. *FOXF1* variant was detected in a patient. Among remaining 26 patients, *SFTPC* variant was detected in patient onset at 1y5m, and *SFTPC* and *NKX2.1* variants were detected in patients with onset at birth.

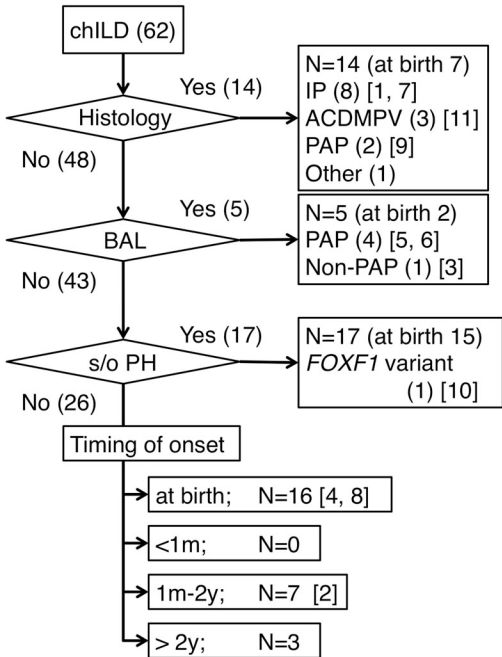


Table 1: Backgrounds of the 62 study subjects

Available lung histology	14 (23%)
Histology-proven IP ^a	8 (13%)
Histology-proven PAP ^b	2 (3.2%)
Histology-proven ACDMPV ^c	3 (4.8%)
Non specific pulmonary hypertension	1 (1.6%)
Available BAL ^d	5 (8.1%)
BAL-proven PAP	4 (6.5%)
IP suspected	1 (1.6%)
Available thyroid status	62 (100%)
Hypothyroidism	4 (6.5%)
Pulmonary hypertension	21 (34%)
Gestational age at birth (weeks)	39 (28 - 41)
Age at onset (day, month, year)	0d (0d - 9y)
at birth	40 (65%)
<1m	2 (3.2%)
1m – 2y	14 (23%)
2.0y≤	6 (9.7%)
Age at DNA sampling	2m (0d - 16y)
Mortality	11 (18%)
<i>SFTPC</i> analysis performed	62 (100%)
<i>ABCA3</i> analysis performed	62 (100%)
<i>NKX2.1</i> analysis performed	38 (61%)
<i>FOXF1</i> analysis performed	21 (34%)

Data are presented as the median (range) or number of patients (% of the starting cohort). a, interstitial pneumonitis; b, pulmonary alveolar proteinosis, c, alveolar capillary dysplasia with misalignment of pulmonary veins; d, bronchoalveolar lavage.

Table 2: Genetic variations detected in 11 patients

Subject, Gender/BW ^a (percentile)/GA ^b	Histology/PH ^c /Complication	Onset/Outcome	Genetic variation/Polyhen-2/Origin	Treatment
<i>SFTPC</i> variation				
1, Male/3.31 (60 th)/41	NSIP ^d /No/No	1y0m/Survive (2y4m)	c.218T>C, p.Ile73Thr/0.855/Maternal	PSL ⁱ 2mg/kg/day, HCQ ^j , HOT ^k
2, Male/3.46 (80 th)/40	NA ^e /No/No	1y5m/Survive (2y8m)	c.218T>C, p.Ile73Thr/0.855/Unknown	PSL 2mg/kg/day, no supplemental oxygen at 2y8m
3, Male/3.40 (70 th)/41	NA/No/No	1y7m/Survive (2y5m)	c.218T>C, p.Ile73Thr/0.855/Paternal	PSL 1mg/kg/day, HCQ, HOT
4, Male/3.24 (33 rd)/41	NA/No/No	0d/Survive (6y)	c.541delC, p.Leu181Trpfs*5-/Maternal	Surfactant, PSL 2mg/kg/day, no supplemental oxygen at 6y
5, Female/2.53 (46 th)/36	PAP ^f /No/No	0d/D (2y5m)	c.134T>G, p.Leu45Arg/1.000/ <i>De novo</i>	Surfactant, HOT
6, Male/3.76 (99 th)/39	PAP/No/No	0d/D (9y)	c.181A>G, p.Ser61Gly/0.999/Unknown	Surfactant, mPSL ^l , HCQ, HOT, Lung transplantation
<i>NKX2.1</i> variation				
7, Female/2.68 (16 th)/40	NSIP/No/hypothyroidism	11m/Survive (11y)	c.1117C>T, p.Gln373X/-/Unknown	PSL 1.5mg/kg/day, HCQ, azathioprine, levothyroxine, HOT
8, Male/2.90 (54 th)/38	NA/No/hypothyroidism, developmental delay	0d/Survive (1y6m)	c.1016_1017insCCATCTCCGT-GGGCAGCGG, p.Gly339fs/-/ <i>De novo</i>	Surfactant, steroids, sivelestat, HCQ, levothyroxine HOT
9, Female/3.65 (96 th)/40	PAP/No/hypothyroidism, TAPVC ^g , developmental delay	3m/Survive (2y9m)	c.954_958GCAGG>CAG, p.Gln318fs/-/ <i>De novo</i>	Surgery for TAPVC, PSL 2mg/kg/day, levothyroxine, HOT
<i>FOXF1</i> variation				
10, Female/2.70 (10 th)/40	NA/Yes/No	0d/Survive (4m)	c.256C>T, p.Arg86Trp/1.000/ <i>De novo</i>	Surfactant, milrinone, sildenafil, hospitalization
11, Female/2.99 (59 th)/39	ACDMPV ^h /Yes/anal atresia	0d/D (5d)	c.852_856delTATCA, p.Tyr284X/-/Unknown	Surfactant, colostomy for anal atresia, mPSL, milrinone

Survive and Death means survival to age and death at age indicated in parenthesis, respectively. a, birthweight (kg); b, gestational age at birth (weeks of gestation); c, pulmonary hypertension; d, nonspecific interstitial pneumonia; e, not available; f, pulmonary alveolar proteinosis; g, total anomalous pulmonary vein connection, h, alveolar capillary dysplasia with misalignment of pulmonary veins; i, prednisolone; j, hydroxychloroquine; k, home oxygen therapy; l, methylprednisolone pulse therapy