Filamin A (FLNA) Mutation – A Newcomer to the Childhood Interstitial Lung Disease (ChILD) Classification

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Keywords:	Interstitial Lung Disease (ILD), Imaging
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Abstract:	Aim: Interstitial lung disease (ILD) in infants represents a rare and heterogenous group of disorders, distinct from those occurring in adults. In recent years a new entity within this category is being recognised, namely filamin A (FLNA) mutation related lung disease. Our aims are to describe the clinical and radiological course of patients with this disease entity to aid clinicians in the prognostic counseling and management of similar patients they may encounter. Method: A retrospective case note review was conducted of all patients treated at our institution (a specialist tertiary referral childrens' centre) for genetically confirmed FLNA mutation related lung disease. The clinical presentation, evolution, management and radiological features were recorded and a medical literature review of Medline indexed articles was conducted. Results: We present a case series of four patients with interstitial lung disease and genetically confirmed abnormalities within the FLNA gene. Their imaging findings all reveal a pattern of predominantly upper lobe overinflation, coarse pulmonary lobular septal thickening and diffuse patchy atelectasis. The clinical outcomes of our patients have been variable ranging from

	infant death, lobar resection and need for supplemental oxygen and bronchodilators. Conclusion: The progressive nature of the pulmonary aspect of this disorder and need for early aggressive supportive treatment make identification crucial to patient management and prognostic counseling.

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17th February 2017

Dear Editor, Deputy Editor and Reviewers,

Thank you for your re-review of our paper entitled 'Filamin A (FLNA) Mutation – A Newcomer to the Childhood Interstitial Lung Disease (ChILD) Classification' Manuscript ID: PPUL-16-0605. We have re-reviewed your additional comments and responded to these below.

1) Reviewer 1:

- Were other genetic causes of infant ChILD ruled out?
  Thank you for the reclarification – the patient in case 3 was also tested for cystic fibrosis and myotonin-protein kinase expansion, which were both negative. This has been added to the description of the case 3, in the fourth paragraph, fourth and fifth lines.

- Was the FLNA variant predicted to be damaging based on analyses using variant effect prediction tools?
  The FLNA variant for case 3 was described by the genetic laboratory as being novel, without any prior report or awareness of pathogenicity in other patients. As such, the genetic report stated that pathogenicity of this variant was 'uncertain' and could not give us a prediction of pathogenic likelihood.

  We have however included this novel mutation in our case series as we felt it was important to highlight the uncertainty that can come with FLNA testing and also to raise awareness of this variant, the symptoms and radiographical abnormalities in our patient, should this be encountered by others in the future.

- Abstract, Results: I would caution using 'interstitial lung pathologies' as some might take this to mean histopathology showing interstitial changes, as biopsy was only done for 2/4 patients.
  The word 'pathologies' has been amended to 'disease' to remove any reader assumption regarding tissue confirmed histology.

- Page 22, line 53: 'patient's parents' should read 'patients' parents'.
- Page 23, line 50: Eliminate double periods.
- Page 25, line 17: 'Child' should read 'ChILD'
- Table 1: 'chocking' should read 'choking'
  Thank you – all typos listed above have been corrected.

Once again I thank the reviewers for their invaluable input, support and interest of our manuscript. We do sincerely hope that you will now find it publishable in the updated state.

Best wishes,
Susan Shelmerdine (on behalf of all co-authors).

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Full Title:

Filamin A (*FLNA*) Mutation – A Newcomer to the Childhood Interstitial Lung Disease (ChILD) Classification

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Disclosures:

The author and co-authors have no financial or commercial conflicts of interest to disclose.

Presentations:

This work has previously been presented as an educational e-poster at the International Pediatric Radiology (IPR) Conference held in Chicago, USA in June 2016. It has not been published, nor is it being considered for publication elsewhere.

Keywords:

Filamin A, *FLNA*, Interstitial Lung Disease, Imaging

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Abbreviations List:

ASD – atrial septal defect

BiPAP - bilevel positive airway pressure

ChILD – Children's Interstitial Lung Disease

CPAP – continuous positive airway pressure

CT – computed tomography

ECHO – echocardiography

ETT – endotracheal tube

FLNA - Filamin A

HFOV – high flow oxygen ventilation

ILD – interstitial lung diseases

PDA – patent ductus arteriosus

PFO – patent foramen ovale

PICU – paediatric intensive care unit

MDT – multidisciplinary team

NIV – non-invasive mechanical ventilation

VSD – ventricular septal defect

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Abstract:

Aim:

Interstitial lung disease (ILD) in infants represents a rare and heterogenous group of disorders, distinct from those occurring in adults. In recent years a new entity within this category is being recognised, namely filamin A (*FLNA*) mutation related lung disease. Our aims are to describe the clinical and radiological course of patients with this disease entity to aid clinicians in the prognostic counseling and management of similar patients they may encounter.

Method:

A retrospective case note review was conducted of all patients treated at our institution (a specialist tertiary referral childrens' centre) for genetically confirmed *FLNA* mutation related lung disease. The clinical presentation, evolution, management and radiological features were recorded and a medical literature review of Medline indexed articles was conducted.

Results:

We present a case series of four patients with interstitial lung disease and genetically confirmed abnormalities within the *FLNA* gene. Their imaging findings all reveal a pattern of predominantly upper lobe overinflation, coarse pulmonary lobular septal thickening and diffuse patchy atelectasis. The clinical outcomes of our patients have been variable ranging from infant death, lobar resection and need for supplemental oxygen and bronchodilators.

Conclusion:

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The progressive nature of the pulmonary aspect of this disorder and need for early aggressive supportive treatment make identification crucial to patient management and prognostic counseling.

Introduction:

Interstitial lung disease (ILD) in infants represents a rare and heterogenous group of disorders, distinct from those occurring in adults. In 2007 the ChILD (Children's Interstitial Lung Disease) research co-operative proposed a new classification scheme for diffuse pulmonary lung diseases encompassing a variety of genetic and developmental aetiologies[1]. The classification was based upon biopsy and imaging results from eleven centres contributing 187 cases of diffuse lung disease in infancy over a 5 year study period. This has been incorporated into the latest 2013 American Thoracic Society Clinical Practice Guideline: Classification, Evaluation and Management of Childhood Interstitial Lung disease in Infancy and serves as a framework by which to build upon, with discovery and delineation of molecular and specific aetiologies adding to this classification with time.[23]. In recent years a new disease entity is being increasingly recognised, namely filamin A (*FLNA*) mutation related lung disease.

Filamin A is an actin-binding protein expressed ubiquitously within the body with multiple roles both in cell signaling and maintenance of cell shape and motility[4,5]. A well-known association already exists between this mutation and disorders of neuronal migration, vascular function, connective tissue integrity

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and skeletal development[6] however pulmonary manifestations are only just being described with four published case reports to date[7],[8],[9], [10] and a further five patients altogether reported within two separate poster abstract publications[11],[12].

In the cases reported, radiographic features have mimicked those of congenital lobar emphysema with hyperlucent pulmonary parenchyma, multi-lobar hyperinflation and pulmonary vascular attenuation. Pathology obtained from lung biopsy or resection have revealed a combination of alveolar simplification, emphysematous changes and pulmonary arteriopathy suggesting that mutations of *FLNA* appear to result in alveolar growth abnormality and thus reflect another causal aetiology within the referenced classifications[1,13]. In nearly all cases treatment has involved either lobar resection or lung transplantation[7,8,11] with only one case reported where supportive therapy was successful[9].

Given the progressive nature of this disease and potentially fatal outcomes, we present our own case series of four patients with genetically confirmed *FLNA* mutation and associated pulmonary manifestations. This case series will focus on clinical and radiographical findings of this condition with the aim of raising awareness and improving identification of this rare entity.

Methods:

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A retrospective case note review was conducted of all patients treated at our institution (a specialist tertiary referral childrens' centre) for genetically confirmed *FLNA* mutation related lung disease. Patients tested for FLNA mutation without lung disease were excluded from this analysis.

At our institution, all genetic testing for FLNA is performed on peripheral blood samples, at an off-site UKAS accredited genetic laboratory by use of bi-directional Sanger sequencing. Determination of pathogenicity of variants are carried out at the laboratory using bioinformatics software Alamut v 2.0 (Interactive Biosoftware, Rouen, France).

The clinical presentation, evolution, management and radiological features were recorded and a medical literature review of Medline indexed articles of previously described cases was conducted. The parents of all patients described in this article have provided consent for discussion of their children's cases.

Results:

Over the last 10 years at our institution, there have been 4 genetically confirmed cases of *FLNA* mutation related lung disease. An overview of the genetic abnormalities, key radiographical features and clinical outcomes are provided in **Table 1**. A detailed case review of each patient is outlined below.

Case 1

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A female infant with a normal antenatal course was delivered via elective Caesarean section at 36 + 5 weeks gestation due to breech presentation. The delivery was otherwise unremarkable and the patient was discharged home on her second day of life. Although initially thriving, her weight gain plateaued at 6-8 weeks of age.

At three months of age the patient was admitted to her local hospital with sudden onset difficulty in breathing. Oxygen saturations were low at 80% in air, improving to 100% with 2 litres of oxygen via nasal cannula. A chest radiograph **(Figure 1)** was performed which demonstrated right upper zone consolidation, mediastinal shift to the right and hyperinflation of the left lung with septal thickening. An empirical course of Ceftriaxone was started, however respiratory symptoms deteriorated overnight requiring intubation and high frequency oscillatory ventilation (HFOV).

The patient was transferred to our institution for further medical input. On arrival the patient showed signs of septic shock requiring aggressive fluid resuscitation and inotropic support. Transthoracic echocardiogram revealed pulmonary hypertension, small patent ductus arteriosus (PDA) and patent foramen ovale (PFO). A right pleural effusion was drained on admission and did not culture any micro-organisms. A bronchioalveolar lavage was initially positive for pneumocystis carinii and a three week course of co-trimoxazole was started. Repeat lavage one week later was negative.

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A computed tomography (CT) of the chest **(Figure 2 a to c)** revealed a right sided pneumothorax, extensive bilateral chronic lung changes with left sided abnormal architectural changes and regions of centrilobar emphysema. A tube oesophagram did not reveal a tracheo-oesophagela fistula.

The patient continued to have a difficult clinical course, with failure to extubate, increasing oxygen requirements and recurrent admissions to paediatric intensive care unit. Repeat thoracic CT studies did not reveal any significant change in appearances apart from resolution of a previous right pneumothorax **(Figure 2 d to f)**.

Given the lack of clear diagnosis for the lung changes, molecular genetic testing was sought. There was no evidence for cystic fibrosis, pulmonary surfactant protein B (SFTPB) or C (SFTPC) ABCA3 gene deficiencies or any immunodeficiencies.

At the age of 5 months, she underwent PDA ligation and right lung wedge biopsy (**Figure 3 a**). The biopsy results demonstrated poor alveolar development and immaturity with mild thickening of the small arteries. A post-operative echocardiogram revealed reduction in pulmonary pressures with only a small residual atrial septal defect (ASD) remaining.

The patient was eventually weaned from ventilation and extubated at age 6 months onto bilevel positive airway pressure (BiPAP) support. She was then

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cycled to continuous positive airway pressure (CPAP) for increasing durations and discharged from the intensive care unit to the ward. Shortly after she developed pyrexia and loose stools rapidly escalating to respiratory distress and arrest. She was re-intubated and ventilated and transferred to the paediatric intensive care unit (PICU). After discussion with family and medical teams, the decision was made to form a tracheostomy at age 8 months.

Unfortunately the patient was unable to recover from the event, requiring continued oxygen and ventilator support, and died shortly after aged 9 months. A postmortem examination was interpreted as atypical congenital alveolar dysplasia. There were no areas of normal lung tissue, nor any large cysts or active infection **(Figure 3 b).** Further genetic testing performed post-mortem revealed a mutation in the *FLNA* gene, felt to be contributory to the severe lung changes.

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Case 2

A term female infant with an unremarkable antenatal course was noted to have poor weight gain during the early months of her life. At the age of 7 months, her parents reported one episode of choking during a swimming outing in a chlorinated pool. There was no concern regarding near drowning or requirement for poolside resuscitation however the parents decided to take the infant to her local hospital for review. An admission chest radiograph was performed and demonstratedemphysematous changes within the right lung,

which would not have been consistent with the clinical history. A chest CT was performed during the same admission which confirmed the radiograph findings of hyperinflation of the right upper and middle lobes with areas of bronchial wall thickening and dilatation in the lower lobes **(Figure 4).**

The patient continued to have multiple episodes of intercurrent pulmonary infections necessitating prolonged hospital admission and escalation in ventilatory support with 100% oxygen via high flow nasal cannulae with a maximal FiO2 of 45%.

During her hospital admission, she was discovered to have a PDA which was ligated. Transpulmonary gradient pressures measured at this time were elevated and a course of sildenafil initiated. A transthoracic echocardiogram revealed a hypertrophied right ventricle with preserved function and no significant chamber dilatation.

Given the destructive lung changes, genetic analysis was sought and a mutation of the *FLNA* gene was identified. Due to the known association between *FLNA* mutation and neurological abnormalities, an MRI of the brain was performed demonstrating periventricular nodular heterotopia (PVNH), although the patient was not suffering from any neurological symptoms at this stage.

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During the course of the following months the chest radiograph changes were progressively deteriorating **(Figure 5)** with increasing right upper lobe hyperinflation, left sided mediastinal shift and reduced aeration within the left lung. After careful consideration and discussion at the respiratory multi-disciplinary team (MDT) meeting, a right upper lobectomy was performed at 18 months of age. The histopathology results of the resected lobe demonstrated emphysematous changes but without significant inflammatory lung disease **(Figure 6).**

The patient made a slow, but successful recovery post-lobectomy with improvements in chest symptoms, saturating up to 98% on room air. She has had minor viral illnesses requiring additional supplemental oxygen but no further respiratory support. She is currently 4 years old and progressing well clinically. Her pulmonary vasodilators are slowly being weaned and she no longer requires diuretic medications. The latest echocardiogram does continue to demonstrate some signs of elevated pulmonary arterial pressure and therefore she continues to be monitored in our institution's outpatient clinic for signs of aortic root dilatation or valvulopathy.

Case 3

This female infant was born via vaginal delivery through meconium stained liquor at 40 +4 weeks gestation. At birth she was transferred and intubated on the neonatal intensive care unit (NICU) due to increased work of breathing and low oxygen saturations. The patient continued to have a difficult neonatal

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course requiring high flow oxygen ventilation, nitric oxide and inotropic support.

After multiple unsuccessful attempts at extubation, the patient was transferred to a tertiary specialist children's hospital for further care. A chest CT at this stage, aged 2 months, revealed pan lobular emphysematous changes within both lungs with hyperinflation of the upper lobes and right middle lobe **(Figure 7).** At three months of age she was successfully extubated and placed on non-invasive mechanical ventilation (NIV) support. A bronchoscopy at this time demonstrated left lower lobe bronchomalacia and multiple bronchial stenoses. A transthoracic echocardiography revealed a structurally normal heart without any evidence of pulmonary hypertension.

After a prolonged course of NIV support and difficulty weaning from CPAP, the decision was made to site a tracheostomy at 6 months of age. The patient was slowly established on a portable ventilator via the tracheostomy with improvement in her baseline respiratory rate and work of breathing. She was then transferred to our institution for long term ventilation management. The admission chest radiograph **(Figure 8)** shows bilateral bronchial wall thickening with persistent hyperlucency and inflation of the upper lobes.

During her inpatient stay, she was difficult to wean from ventilation, developing recurrent episodes of wheezing and bronchoconstriction which were responsive to Ipratropium bromide and Salbutamol. Genetic testing was

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sought based on re-review of the external CT imaging. This was negative for cystic fibrosis and myotonin-protein kinase expansion but confirmed a novel variant missense mutation within the filamin A gene. Given the uncertainty regarding pathogenicity, the patient's immediate family members were all reviewed and tested for FLNA gene mutation. The patient's mother and younger sister were asymptomatic but found to have the identical missense mutation, and the patient's father did not. After prolonged discussion and genetic consultation, given the absence of other explainable lung disease, the consensus hypothesis was that the lung disease may have been manifested by an invoked unfavourable X inactivation in the patient.

Over the next years, the patient slowly improved with bronchodilator nebulization and satisfactory gas exchange on lower CPAP, tolerating periods on the Swedish nose during the day. She is now 3 years old and has had her tracheostomy removed within the last 6 months. She is continuing to gain weight and only requires the usage of bronchodilator inhalers for a recurrent wheeze.

Case 4

This female infant was born at 38 weeks gestation with a normal antenatal and immediate neonatal course. She was admitted to her local hospital with suspected viral bronchiolitis at 3 months of age and required supplemental oxygen. An echocardiogram at this time revealed a secundum ASD with

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bidirectional flow, mildly dilated and hypertrophied right ventricle and right atrium as well as pulmonary hypertension.

The chest radiograph appearances at this stage included right upper lobe hyperinflation with right middle lobe and left lower lobe atelectasis **(Figure 9).** The subsequent chest CT demonstrated dilatation of the central pulmonary artery with bilateral extensive chronic lung disease with nodular change and bronchial wall thickening. In addition, hyperinflation of the upper lobes bilaterally were seen **(Figure 10).**

The patient was admitted to our institution for a hybrid procedure involving both cardiac catheterisation and cardiac MRI. These studies revealed a PDA, confirmed bidirectional flow through the ASD and significant pulmonary arterial hypertension. The systemic and pulmonary venous connections were normal.

A respiratory consultation was sought and the pulmonary hypertension was presumed to be secondary to the diffuse lung disease. The patient was started on Bosentan in addition to her regular dose of Sildenafil. Investigations for aspiration, gastro-oesophageal reflux, immunodeficiency and cystic fibrosis were all negative.

In view of the severe early onset cystic lung disease, she was screened for a Filamin A gene mutation, which was confirmed. She is currently 6 years old,

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and continues on supplementary oxygen support of 1L at home and pulmonary vasodilators.

Discussion:

Our case series has demonstrated a variable outcome and management course for four patients seen with confirmed *FLNA* gene mutations occurring with interstitial lung disease. One patient required surgical intervention in the form of a right upper lobectomy after which a dramatic clinical improvement was observed, two patients were treated with supplementary oxygen and ventilatory support with pulmonary arterial vasodilators and have demonstrated a steady but slow improvement in respiratory symptoms and one patient died in infancy having been treated with maximal medical therapies and multiple episodes of mechanical ventilatory support.

Compared to the limited number of previously published cases (summarised in Table 2), our case series show similar features with our patients having an early age of onset of respiratory symptoms, presence of cardiac co-morbidities, pulmonary arterial hypertension (seen on echocardiography) and in one patient, the confirmatory diagnosis of periventricular nodular heterotopia on brain MRI. On chest imaging, all our patients demonstrated features both on radiography and CT of predominantly upper lobe hyperinflation and segmental basal atelectasis. This echoes previous cases where imaging appearances have been described as those featuring a

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combination of bronchopulmonary dysplasia, bronchomalacia and congenital lobar emphysema[12].

With regards to the cardiac co-morbidities, two of our patients were found to have a patent ductus arteriosis, which was also seen in 5 previously reported FLNA associated lung disease cases[7,11] and was the second commonest associated cardiac abnormality within 17.6% (6 patients) of patients with FLNA associated PVNH (second only to aortic valvular insufficiency)**Error! Bookmark not defined.**. This illustrates the importance of echocardiographic findings in this cohort of patients and the need for future extended medical surveillance from a cardiovascular standpoint.

Although one of our four cases was found to have PVNH on brain imaging, none of our cases reported neurological symptoms. This may be explained by the young age group or case mix of our cohort. Lange et al[14] report in their large case series, of 34 patient with detailed clinical history and FLNA associated PVNH, many patients (n=10) had either none or minor neurological symptoms (e.g. headaches) whilst the remainder had seizure onset during adolescence or adulthood (n = 20). Interestingly, only 2 patients in this cohort had any respiratory symptoms described (these are not detailed further, apart from being described as 'obstructive lung disease'). Whilst we acknowledge a strong association between FLNA mutation and PVNH exists, given the young age of our patients, lack of neurological symptoms and increased risk from general anaesthesia in order to perform the MRI studies

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(due to their lung disease), this was not felt to be clinically indicated at the present stage. The patients' parents have all been extensively counseled on associated anomalies in FLNA mutations, and should neurological symptoms arise, further neurological examinations would be arranged.

In terms of treatment, in contrast to 4 other prior cases in the literature[11], none of the patients in our series required lung transplantation. Whilst one of our patients died at an early age and may not have been a suitable surgical candidate, transplantation may be an alternative treatment in severe cases where supportive therapies do not appear to work. Additionally, the individual *FLNA* mutations identified within our subgroup are all novel and provide a record by which future cases with *FLNA* mutations can be compared to help guide management and provide information on potential patient prognosis. In one of our cases (case 3), the genetic mutation was a missense variant mutation, highlighting a clinical diagnostic dilemma and necessitating genetic testing of several family members. In the absence of other explainable causes for the patient's lung disease, this was assumed to be contributory and has been included in this case series in the hope that our findings may aid in patient counseling and for comparison to future similar cases to emerge in the medical literature.

Genetic analysis and counseling can also aid family members, particularly in the setting of family planning, to determine options for prenatal genetic testing or preimplantation genetic diagnosis for further pregnancies. However there

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are limitations to this, one being the discovery of missense variant mutations as highlighted and described in case 3 which may pose a diagnostic dilemma. In addition, although our case series highlights the clinical and radiological findings of lung disease in patients with confirmed FLNA mutations, the spectrum of such lung disease is still to be established and there may be 'missed cases' of FLNA mutation in patients with potentially milder forms of the disease, being treated and labeled as having 'abnormalities of lung growth'. This may be an interesting area for further research work.

The pathogenesis of lung disease associated with *FLNA* mutations is unclear but a variety of hypotheses have been proposed. One explanation may be due to abnormal binding properties between Filamin A and beta integrin cell adhesion receptors, crucial in airway development[15]. The association between *FLNA* mutation with connective tissue disorders[16] may also suggest interplay with other gene abnormalities within the same functional pathway. These explanations have also been proposed to account for the associated cardiac anomalies in many observed cases of FLNA associated lung disease.

An alternative mechanism may be from atypical interactions of Filamin A with the cystic fibrosis transmembrane regulator (CFTR) thereby destabilizing chloride channel function in the airways[17]. This is however felt to be a less likely cause for the pulmonary features as florid bronchiectasis and impaired mucociliary fluid clearance has only been described in one patient so far with *FLNA* mutation[12]. Furthermore poor immunological function with a super-

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added infective aetiology has been suggested due to the role played by Filamin A in T cell activation and interleukin production[18], however patients with the *FLNA* mutation have not shown susceptibility to any specific organisms despite many of the patients in our case series regularly presenting to hospital in childhood with recurrent pulmonary infections.

Conclusion

We present the clinical radiological, cardiac and histological features of *FLNA* gene mutation ChILd. This diagnosis should be considered for atypical diffuse lung disease with the hallmark feature of marked hyperinflation[19], predominantly in the upper lobe distribution with accompanying pulmonary arterial hypertension in the majority of cases. There may be a potential association with periventricular nodular heterotopia (PNH)[16.] Whilst the treatment options may be predominantly supportive, our case series has shown that some clinical improvements may be achieved and that the prognosis is variable.

Acknowledgements:

None

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Image Legends

Figure 1

Chest radiograph of case 1 at age 3 months demonstrating hyperinflation and lucency within the left lung with mediastinal shift to the right. Fine interstitial thickening and septations are noted in the left lower zone. There is right upper lobe consolidation.

Figure 2 (a-f)

Case 1: Serial axial chest CT images (with lung windows) are displayed demonstrating lung changes with age. The top row (a to c) highlights imaging appearances within the upper, middle and lower zones respectively at 3 months of age. There is a moderate sized right pneumothorax with underlying ground glass change in the lung parenchyma. The left lung is hyperinflated with interlobular septal thickening and areas of air trapping. The bottom row (d to f) demonstrates lung appearances at 5 months of age reflecting on-going left lung hyperinflation and septal thickening. The pneumothorax is no longer present.

Figure 3

Haematoxylin and eosin stained histopathology images of (a) the initial wedge biopsy of lung including the pleura and (b) a representative post-mortem section of the patient's lung from case 1. The wedge biopsy reveals simplification of the alveolar architecture with dilatation of the distal airspaces. There is peribronchial fibrosis and focal haemorrhage. The post-mortem section demonstrates thickened alveolar septa and contain double capillary loops. This appearance was present

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throughout the lung and is the diagnostic feature of atypical congenital alveolar dysplasia. There is marked intra-alveolar oedema. Elsewhere within the lung there was fibrosis and airspace dilatation.

Figure 4

Case 2: Axial chest computed tomography images of the upper (a), middle (b) and lower (c) zones at four months of age. Right upper and middle lobe hyperinflation with patchy ground glass opacification and atelectasis are present within the left lung and right lower lobe.

Figure 5

Serial chest radiographs from case 2 performed at (a) 8 months of age and (b) 15 months of age reveal progressive right sided hyperinflation, mediastinal shift to the left and diffuse ground glass opacification within the left lung.

Figure 6

Histopathology lung biopsy image from case 2 with elastic van Gieson staining. There is marked dilatation of the distal air spaces without thickening of the walls. The included muscular pulmonary arteries are thick walled in keeping with pulmonary arterial hypertension. There is also some artefactual alveolar haemorrhage.

Figure 7

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Case 3: Axial chest CT images of the (a) upper and (b) lower lobes at 2 months of age demonstrate upper lobe hyperinflation with sparse pulmonary vascular markings and bibasal medial atelectatic changes.

Figure 8

A supine chest radiograph of case 3 performed at 10 months of age demonstrates persistent upper lobe hyperinflation, flattening of both hemidiaphragms and perihilar airspace changes. The patient has a tracheostomy in situ at this stage and is fed via a nasogastric tube.

Figure 9

Chest radiograph of patient in case 4 performed at 8 months of age demonstrates right upper lobe hyperinflation and atelectasis within the right midzone and left lower lobe.

Figure 10

Axial chest computed tomography images of case 4 of the (a) upper lobes and (b and c) lung bases reveal marked overinflation of both upper lobes and the right middle lobe with bilateral lower lobe atelectasis

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Full Title:

Filamin A (*FLNA*) Mutation – A Newcomer to the Childhood Interstitial Lung Disease (ChILD) Classification

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Disclosures:

The author and co-authors have no financial or commercial conflicts of interest to disclose.

Presentations:

This work has previously been presented as an educational e-poster at the International Pediatric Radiology (IPR) Conference held in Chicago, USA in June 2016. It has not been published, nor is it being considered for publication elsewhere.

Keywords:

Filamin A, *FLNA*, Interstitial Lung Disease, Imaging

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Abbreviations List:

ASD – atrial septal defect

BiPAP - bilevel positive airway pressure

ChILD – Children's Interstitial Lung Disease

CPAP – continuous positive airway pressure

CT – computed tomography

ECHO – echocardiography

ETT – endotracheal tube

FLNA - Filamin A

HFOV – high flow oxygen ventilation

ILD – interstitial lung diseases

PDA – patent ductus arteriosus

PFO – patent foramen ovale

PICU – paediatric intensive care unit

MDT – multidisciplinary team

NIV – non-invasive mechanical ventilation

VSD – ventricular septal defect

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Abstract:

Aim:

Interstitial lung disease (ILD) in infants represents a rare and heterogenous group of disorders, distinct from those occurring in adults. In recent years a new entity within this category is being recognised, namely filamin A (*FLNA*) mutation related lung disease. Our aims are to describe the clinical and radiological course of patients with this disease entity to aid clinicians in the prognostic counseling and management of similar patients they may encounter.

Method:

A retrospective case note review was conducted of all patients treated at our institution (a specialist tertiary referral childrens' centre) for genetically confirmed *FLNA* mutation related lung disease. The clinical presentation, evolution, management and radiological features were recorded and a medical literature review of Medline indexed articles was conducted.

Results:

We present a case series of four patients with interstitial lung ~~pathologies~~ disease and genetically confirmed abnormalities within the *FLNA* gene. Their imaging findings all reveal a pattern of predominantly upper lobe overinflation, coarse pulmonary lobular septal thickening and diffuse patchy atelectasis. The clinical outcomes of our patients have been variable ranging from infant death, lobar resection and need for supplemental oxygen and bronchodilators.

Conclusion:

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The progressive nature of the pulmonary aspect of this disorder and need for early aggressive supportive treatment make identification crucial to patient management and prognostic counseling.

Introduction:

Interstitial lung disease (ILD) in infants represents a rare and heterogenous group of disorders, distinct from those occurring in adults. In 2007 the ChILD (Children's Interstitial Lung Disease) research co-operative proposed a new classification scheme for diffuse pulmonary lung diseases encompassing a variety of genetic and developmental aetiologies[1]. The classification was based upon biopsy and imaging results from eleven centres contributing 187 cases of diffuse lung disease in infancy over a 5 year study period. This has been incorporated into the latest 2013 American Thoracic Society Clinical Practice Guideline: Classification, Evaluation and Management of Childhood Interstitial Lung disease in Infancy and serves as a framework by which to build upon, with discovery and delineation of molecular and specific aetiologies adding to this classification with time.[23]. In recent years a new disease entity is being increasingly recognised, namely filamin A (*FLNA*) mutation related lung disease.

Filamin A is an actin-binding protein expressed ubiquitously within the body with multiple roles both in cell signaling and maintenance of cell shape and motility[4,5]. A well-known association already exists between this mutation and disorders of neuronal migration, vascular function, connective tissue integrity

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and skeletal development[6] however pulmonary manifestations are only just being described with four published case reports to date[7],[8],[9], [10] and a further five patients altogether reported within two separate poster abstract publications[11],[12].

In the cases reported, radiographic features have mimicked those of congenital lobar emphysema with hyperlucent pulmonary parenchyma, multi-lobar hyperinflation and pulmonary vascular attenuation. Pathology obtained from lung biopsy or resection have revealed a combination of alveolar simplification, emphysematous changes and pulmonary arteriopathy suggesting that mutations of *FLNA* appear to result in alveolar growth abnormality and thus reflect another causal aetiology within the referenced classifications[1,13]. In nearly all cases treatment has involved either lobar resection or lung transplantation[7,8,7,11,10] with only one case reported where supportive therapy was successful[9,8].

Given the progressive nature of this disease and potentially fatal outcomes, we present our own case series of four patients with genetically confirmed *FLNA* mutation and associated pulmonary manifestations. This case series will focus on clinical and radiographical findings of this condition with the aim of raising awareness and improving identification of this rare entity.

Methods:

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A retrospective case note review was conducted of all patients treated at our institution (a specialist tertiary referral childrens' centre) for genetically confirmed *FLNA* mutation related lung disease. Patients tested for FLNA mutation without lung disease were excluded from this analysis.

At our institution, all genetic testing for FLNA is performed on peripheral blood samples, at an off-site UKAS accredited genetic laboratory by use of bi-directional Sanger sequencing. Determination of pathogenicity of variants are carried out at the laboratory using bioinformatics software Alamut v 2.0 (Interactive Biosoftware, Rouen, France).

The clinical presentation, evolution, management and radiological features were recorded and a medical literature review of Medline indexed articles of previously described cases was conducted. The parents of all patients described in this article have provided consent for discussion of their children's cases.

Results:

Over the last 10 years at our institution, there have been 4 genetically confirmed cases of *FLNA* mutation related lung disease. An overview of the genetic abnormalities, key radiographical features and clinical outcomes are provided in **Table 1**. A detailed case review of each patient is outlined below.

Case 1

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A female infant with a normal antenatal course was delivered via elective Caesarean section at 36 + 5 weeks gestation due to breech presentation. The delivery was otherwise unremarkable and the patient was discharged home on her second day of life. Although initially thriving, her weight gain plateaued at 6-8 weeks of age.

At three months of age the patient was admitted to her local hospital with sudden onset difficulty in breathing. Oxygen saturations were low at 80% in air, improving to 100% with 2 litres of oxygen via nasal cannula. A chest radiograph **(Figure 1)** was performed which demonstrated right upper zone consolidation, mediastinal shift to the right and hyperinflation of the left lung with septal thickening. An empirical course of Ceftriaxone was started, however respiratory symptoms deteriorated overnight requiring intubation and high frequency oscillatory ventilation (HFOV).

The patient was transferred to our institution for further medical input. On arrival the patient showed signs of septic shock requiring aggressive fluid resuscitation and inotropic support. Transthoracic echocardiogram revealed pulmonary hypertension, small patent ductus arteriosus (PDA) and patent foramen ovale (PFO). A right pleural effusion was drained on admission and did not culture any micro-organisms. A bronchioalveolar lavage was initially positive for pneumocystis carinii and a three week course of co-trimoxazole was started. Repeat lavage one week later was negative.

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A computed tomography (CT) of the chest **(Figure 2 a to c)** revealed a right sided pneumothorax, extensive bilateral chronic lung changes with left sided abnormal architectural changes and regions of centrilobar emphysema. A tube oesophagram did not reveal a tracheo-oesophagela fistula.

The patient continued to have a difficult clinical course, with failure to extubate, increasing oxygen requirements and recurrent admissions to paediatric intensive care unit. Repeat thoracic CT studies did not reveal any significant change in appearances apart from resolution of a previous right pneumothorax **(Figure 2 d to f)**.

Given the lack of clear diagnosis for the lung changes, molecular genetic testing was sought. There was no evidence for cystic fibrosis, pulmonary surfactant protein B (SFTPB) or C (SFTPC) ABCA3 gene deficiencies or any immunodeficiencies.

At the age of 5 months, she underwent PDA ligation and right lung wedge biopsy (**Figure 3 a**). The biopsy results demonstrated poor alveolar development and immaturity with mild thickening of the small arteries. A post-operative echocardiogram revealed reduction in pulmonary pressures with only a small residual atrial septal defect (ASD) remaining.

The patient was eventually weaned from ventilation and extubated at age 6 months onto bilevel positive airway pressure (BiPAP) support. She was then

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cycled to continuous positive airway pressure (CPAP) for increasing durations and discharged from the intensive care unit to the ward. Shortly after she developed pyrexia and loose stools rapidly escalating to respiratory distress and arrest. She was re-intubated and ventilated and transferred to the paediatric intensive care unit (PICU). After discussion with family and medical teams, the decision was made to form a tracheostomy at age 8 months.

Unfortunately the patient was unable to recover from the event, requiring continued oxygen and ventilator support, and died shortly after aged 9 months. A postmortem examination was interpreted as atypical congenital alveolar dysplasia. There were no areas of normal lung tissue, nor any large cysts or active infection **(Figure 3 b).** Further genetic testing performed post-mortem revealed a mutation in the *FLNA* gene, felt to be contributory to the severe lung changes.

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Case 2

A term female infant with an unremarkable antenatal course was noted to have poor weight gain during the early months of her life. At the age of 7 months, her parents reported one episode of choking during a swimming outing in a chlorinated pool. There was no concern regarding near drowning or requirement for poolside resuscitation however the parents decided to take the infant to her local hospital for review. An admission chest radiograph was performed and demonstratedemphysematous changes within the right lung,

which would not have been consistent with the clinical history. A chest CT was performed during the same admission which confirmed the radiograph findings of hyperinflation of the right upper and middle lobes with areas of bronchial wall thickening and dilatation in the lower lobes **(Figure 4).**

The patient continued to have multiple episodes of intercurrent pulmonary infections necessitating prolonged hospital admission and escalation in ventilatory support with 100% oxygen via high flow nasal cannulae with a maximal FiO2 of 45%.

During her hospital admission, she was discovered to have a PDA which was ligated. Transpulmonary gradient pressures measured at this time were elevated and a course of sildenafil initiated. A transthoracic echocardiogram revealed a hypertrophied right ventricle with preserved function and no significant chamber dilatation.

Given the destructive lung changes, genetic analysis was sought and a mutation of the *FLNA* gene was identified. Due to the known association between *FLNA* mutation and neurological abnormalities, an MRI of the brain was performed demonstrating periventricular nodular heterotopia (PVNH), although the patient was not suffering from any neurological symptoms at this stage.

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During the course of the following months the chest radiograph changes were progressively deteriorating **(Figure 5)** with increasing right upper lobe hyperinflation, left sided mediastinal shift and reduced aeration within the left lung. After careful consideration and discussion at the respiratory multi-disciplinary team (MDT) meeting, a right upper lobectomy was performed at 18 months of age. The histopathology results of the resected lobe demonstrated emphysematous changes but without significant inflammatory lung disease **(Figure 6).**

The patient made a slow, but successful recovery post-lobectomy with improvements in chest symptoms, saturating up to 98% on room air. She has had minor viral illnesses requiring additional supplemental oxygen but no further respiratory support. She is currently 4 years old and progressing well clinically. Her pulmonary vasodilators are slowly being weaned and she no longer requires diuretic medications. The latest echocardiogram does continue to demonstrate some signs of elevated pulmonary arterial pressure and therefore she continues to be monitored in our institution's outpatient clinic for signs of aortic root dilatation or valvulopathy.

Case 3

This female infant was born via vaginal delivery through meconium stained liquor at 40 +4 weeks gestation. At birth she was transferred and intubated on the neonatal intensive care unit (NICU) due to increased work of breathing and low oxygen saturations. The patient continued to have a difficult neonatal

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course requiring high flow oxygen ventilation, nitric oxide and inotropic support.

After multiple unsuccessful attempts at extubation, the patient was transferred to a tertiary specialist children's hospital for further care. A chest CT at this stage, aged 2 months, revealed pan lobular emphysematous changes within both lungs with hyperinflation of the upper lobes and right middle lobe **(Figure 7).** At three months of age she was successfully extubated and placed on non-invasive mechanical ventilation (NIV) support. A bronchoscopy at this time demonstrated left lower lobe bronchomalacia and multiple bronchial stenoses. A transthoracic echocardiography revealed a structurally normal heart without any evidence of pulmonary hypertension.

After a prolonged course of NIV support and difficulty weaning from CPAP, the decision was made to site a tracheostomy at 6 months of age. The patient was slowly established on a portable ventilator via the tracheostomy with improvement in her baseline respiratory rate and work of breathing. She was then transferred to our institution for long term ventilation management. The admission chest radiograph **(Figure 8)** shows bilateral bronchial wall thickening with persistent hyperlucency and inflation of the upper lobes.

During her inpatient stay, she was difficult to wean from ventilation, developing recurrent episodes of wheezing and bronchoconstriction which were responsive to Ipratropium bromide and Salbutamol. Genetic testing was

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sought based on re-review of the external CT imaging. This was negative for cystic fibrosis and myotonin-protein kinase expansion but ~~, which~~ confirmed a novel variant missense mutation within the filamin A gene. Given the uncertainty regarding pathogenicity, the patient's immediate family members were all reviewed and tested for FLNA gene mutation. The patient's mother and younger sister were asymptomatic but found to have the identical missense mutation, and the patient's father did not. After prolonged discussion and genetic consultation, given the absence of other explainable lung disease, the consensus hypothesis was that the lung disease may have been manifested by an invoked unfavourable X inactivation in the patient.

Over the next years, the patient slowly improved with bronchodilator nebulization and satisfactory gas exchange on lower CPAP, tolerating periods on the Swedish nose during the day. She is now 3 years old and has had her tracheostomy removed within the last 6 months. She is continuing to gain weight and only requires the usage of bronchodilator inhalers for a recurrent wheeze.

Case 4

This female infant was born at 38 weeks gestation with a normal antenatal and immediate neonatal course. She was admitted to her local hospital with suspected viral bronchiolitis at 3 months of age and required supplemental oxygen. An echocardiogram at this time revealed a secundum ASD with

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bidirectional flow, mildly dilated and hypertrophied right ventricle and right atrium as well as pulmonary hypertension.

The chest radiograph appearances at this stage included right upper lobe hyperinflation with right middle lobe and left lower lobe atelectasis **(Figure 9).** The subsequent chest CT demonstrated dilatation of the central pulmonary artery with bilateral extensive chronic lung disease with nodular change and bronchial wall thickening. In addition, hyperinflation of the upper lobes bilaterally were seen **(Figure 10).**

The patient was admitted to our institution for a hybrid procedure involving both cardiac catheterisation and cardiac MRI. These studies revealed a PDA, confirmed bidirectional flow through the ASD and significant pulmonary arterial hypertension. The systemic and pulmonary venous connections were normal.

A respiratory consultation was sought and the pulmonary hypertension was presumed to be secondary to the diffuse lung disease. The patient was started on Bosentan in addition to her regular dose of Sildenafil. Investigations for aspiration, gastro-oesophageal reflux, immunodeficiency and cystic fibrosis were all negative.

In view of the severe early onset cystic lung disease, she was screened for a Filamin A gene mutation, which was confirmed. She is currently 6 years old,

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and continues on supplementary oxygen support of 1L at home and pulmonary vasodilators.

Discussion:

Our case series has demonstrated a variable outcome and management course for four patients seen with confirmed *FLNA* gene mutations occurring with interstitial lung disease. One patient required surgical intervention in the form of a right upper lobectomy after which a dramatic clinical improvement was observed, two patients were treated with supplementary oxygen and ventilatory support with pulmonary arterial vasodilators and have demonstrated a steady but slow improvement in respiratory symptoms and one patient died in infancy having been treated with maximal medical therapies and multiple episodes of mechanical ventilatory support.

Compared to the limited number of previously published cases (summarised in Table 2), our case series show similar features with our patients having an early age of onset of respiratory symptoms, presence of cardiac co-morbidities, pulmonary arterial hypertension (seen on echocardiography) and in one patient, the confirmatory diagnosis of periventricular nodular heterotopia on brain MRI. On chest imaging, all our patients demonstrated features both on radiography and CT of predominantly upper lobe hyperinflation and segmental basal atelectasis. This echoes previous cases where imaging appearances have been described as those featuring a

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combination of bronchopulmonary dysplasia, bronchomalacia and congenital lobar emphysema[12][11].

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With regards to the cardiac co-morbidities, two of our patients were found to have a patent ductus arteriosis, which was also seen in 5 previously reported FLNA associated lung disease cases[7][6],[11][10] and was the second commonest associated cardiac abnormality within 17.6% (6 patients) of patients with FLNA associated PVNH (second only to aortic valvular insufficiency)[Error! Bookmark not defined.][13]. This illustrates the importance of echocardiographic findings in this cohort of patients and the need for future extended medical surveillance from a cardiovascular standpoint.

Although one of our four cases was found to have PVNH on brain imaging, none of our cases reported neurological symptoms. This may be explained by the young age group or case mix of our cohort. Lange et al[14] report in their large case series, of 34 patient with detailed clinical history and FLNA associated PVNH, many patients (n=10) had either none or minor neurological symptoms (e.g. headaches) whilst the remainder had seizure onset during adolescence or adulthood (n = 20). Interestingly, only 2 patients in this cohort had any respiratory symptoms described (these are not detailed further, apart from being described as 'obstructive lung disease'). Whilst we acknowledge a strong association between FLNA mutation and PVNH exists, given the young age of our patients, lack of neurological symptoms and increased risk from general anaesthesia in order to perform the MRI studies

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(due to their lung disease), this was not felt to be clinically indicated at the present stage. The patient's parents have all been extensively counseled on associated anomalies in FLNA mutations, and should neurological symptoms arise, further neurological examinations would be arranged.

In terms of treatment, in contrast to 4 other prior cases in the literature[11,10], none of the patients in our series required lung transplantation. Whilst one of our patients died at an early age and may not have been a suitable surgical candidate, transplantation may be an alternative treatment in severe cases where supportive therapies do not appear to work. Additionally, the individual *FLNA* mutations identified within our subgroup are all novel and provide a record by which future cases with *FLNA* mutations can be compared to help guide management and provide information on potential patient prognosis. In one of our cases (case 3), the genetic mutation was a missense variant mutation, highlighting a clinical diagnostic dilemma and necessitating genetic testing of several family members. In the absence of other explainable causes for the patient's lung disease, this was assumed to be contributory and has been included in this case series in the hope that our findings may aid in patient counseling and for comparison to future similar cases to emerge in the medical literature.

Genetic analysis and counseling can also aid family members, particularly in the setting of family planning, to determine options for prenatal genetic testing or preimplantation genetic diagnosis for further pregnancies. However there

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are limitations to this, one being the discovery of missense variant mutations as highlighted and described in case 3 which may pose a diagnostic dilemma. In addition, although our case series highlights the clinical and radiological findings of lung disease in patients with confirmed FLNA mutations, the spectrum of such lung disease is still to be established and there may be 'missed cases' of FLNA mutation in patients with potentially milder forms of the disease, being treated and labeled as having 'abnormalities of lung growth'. This may be an interesting area for further research work.

The pathogenesis of lung disease associated with *FLNA* mutations is unclear but a variety of hypotheses have been proposed. One explanation may be due to abnormal binding properties between Filamin A and beta integrin cell adhesion receptors, crucial in airway development[15]. The association between *FLNA* mutation with connective tissue disorders[16] may also suggest interplay with other gene abnormalities within the same functional pathway. These explanations have also been proposed to account for the associated cardiac anomalies in many observed cases of FLNA associated lung disease.

An alternative mechanism may be from atypical interactions of Filamin A with the cystic fibrosis transmembrane regulator (CFTR) thereby destabilizing chloride channel function in the airways[17]. This is however felt to be a less likely cause for the pulmonary features as florid bronchiectasis and impaired mucociliary fluid clearance has only been described in one patient so far with *FLNA* mutation[12~~11~~]. Furthermore poor immunological function with a super-

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added infective aetiology has been suggested due to the role played by Filamin A in T cell activation and interleukin production[18], however patients with the *FLNA* mutation have not shown susceptibility to any specific organisms despite many of the patients in our case series regularly presenting to hospital in childhood with recurrent pulmonary infections.

Conclusion

We present the clinical radiological, cardiac and histological features of *FLNA* gene mutation ChILd. This diagnosis should be considered for atypical diffuse lung disease with the hallmark feature of marked hyperinflation[19], predominantly in the upper lobe distribution with accompanying pulmonary arterial hypertension in the majority of cases. There may be a potential association with periventricular nodular heterotopia (PNH)[16]. Whilst the treatment options may be predominantly supportive, our case series has shown that some clinical improvements may be achieved and that the prognosis is variable.

Acknowledgements:

None

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Image Legends

Figure 1

Chest radiograph of case 1 at age 3 months demonstrating hyperinflation and lucency within the left lung with mediastinal shift to the right. Fine interstitial thickening and septations are noted in the left lower zone. There is right upper lobe consolidation.

Figure 2 (a-f)

Case 1: Serial axial chest CT images (with lung windows) are displayed demonstrating lung changes with age. The top row (a to c) highlights imaging appearances within the upper, middle and lower zones respectively at 3 months of age. There is a moderate sized right pneumothorax with underlying ground glass change in the lung parenchyma. The left lung is hyperinflated with interlobular septal thickening and areas of air trapping. The bottom row (d to f) demonstrates lung appearances at 5 months of age reflecting on-going left lung hyperinflation and septal thickening. The pneumothorax is no longer present.

Figure 3

Haematoxylin and eosin stained histopathology images of (a) the initial wedge biopsy of lung including the pleura and (b) a representative post-mortem section of the patient's lung from case 1. The wedge biopsy reveals simplification of the alveolar architecture with dilatation of the distal airspaces. There is peribronchial fibrosis and focal haemorrhage. The post-mortem section demonstrates thickened alveolar septa and contain double capillary loops. This appearance was present

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throughout the lung and is the diagnostic feature of atypical congenital alveolar dysplasia. There is marked intra-alveolar oedema. Elsewhere within the lung there was fibrosis and airspace dilatation.

Figure 4

Case 2: Axial chest computed tomography images of the upper (a), middle (b) and lower (c) zones at four months of age. Right upper and middle lobe hyperinflation with patchy ground glass opacification and atelectasis are present within the left lung and right lower lobe.

Figure 5

Serial chest radiographs from case 2 performed at (a) 8 months of age and (b) 15 months of age reveal progressive right sided hyperinflation, mediastinal shift to the left and diffuse ground glass opacification within the left lung.

Figure 6

Histopathology lung biopsy image from case 2 with elastic van Gieson staining. There is marked dilatation of the distal air spaces without thickening of the walls. The included muscular pulmonary arteries are thick walled in keeping with pulmonary arterial hypertension. There is also some artefactual alveolar haemorrhage.

Figure 7

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Case 3: Axial chest CT images of the (a) upper and (b) lower lobes at 2 months of age demonstrate upper lobe hyperinflation with sparse pulmonary vascular markings and bibasal medial atelectatic changes.

Figure 8

A supine chest radiograph of case 3 performed at 10 months of age demonstrates persistent upper lobe hyperinflation, flattening of both hemidiaphragms and perihilar airspace changes. The patient has a tracheostomy in situ at this stage and is fed via a nasogastric tube.

Figure 9

Chest radiograph of patient in case 4 performed at 8 months of age demonstrates right upper lobe hyperinflation and atelectasis within the right midzone and left lower lobe.

Figure 10

Axial chest computed tomography images of case 4 of the (a) upper lobes and (b and c) lung bases reveal marked overinflation of both upper lobes and the right middle lobe with bilateral lower lobe atelectasis

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Table 1.

Summary of patient characteristics, clinical outcomes and investigations with FLNA related lung disease

		Case 1	***Case 2***	***Case 3***	***Case 4***
Genetic sequencing analysis of FLNA exons 2-48		Heterozygous for c.88delG, p.(Ala30fs)	Heterozygous for c.6496dupA, p.(Ile2166fs)	Heterozygous for c.1528G>A, p.(Ala510Thr)	Heterozygous for c.2190_2193delTTAC, p(tyr731fs)
Clinical Presentation	Sex	Female	Female	Female	Female
	Gestational Age	36 + 5 weeks	Term	Term	38 + 0 weeks
	Delivery	Elective Caesarean section	Vaginal delivery	Vaginal delivery	Vaginal delivery
	Presentation	Sudden onset shortness of breath at 3 months	Choking episode at 7 months	Meconium aspiration at birth	Viral bronchiolitis at 3 months
	Clinical Outcome	Died at 9 months age from respiratory arrest.	Now 4 years old. Weaning off pulmonary vasodilators. No longer requires regular diuretics or supplemental 0_2.	Now 3 years old. Gaining weight, tracheostomy decannulated in the last year. Requires bronchodilator inhalers for a recurrent wheeze.	Now 6 years old. Still requiring supplemental O_2 at home, pulmonary vasodilators and bronchodilator nebulisers.
Initial Echocardiography findings		Pulmonary hypertension, small PDA and PFO.	Large PDA, left to right shunting, significant pulmonary hypertension.	Structurally normal heart. Small interatrial communication with left to right flow. No evidence for pulmonary hypertension.	Secundum ASD with bidirectional flow, mildly dilated and hypertrophied right ventricle, right atrium and pulmonary hypertension
Brain imaging		None performed No neurological symptoms	MRI Brain demonstrated periventricular nodular heterotopia. No neurological symptoms	None performed No neurological symptoms	None performed No neurological symptoms

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Pattern of abnormalities on chest radiographs	Left lung hyperinflation. Interstitial thickening in left lower zone. Mediastinal shift to the right. Right lobe consolidation.	Progressive right lung hyperinflation Mediastinal shift to the left.	Bilateral upper lobe hyperinflation Basal atelectasis	Right upper lobe hyperinflation Right middle lobe and left lower lobe atelectasis
Predominant abnormalities seen on chest CTs	Left upper lobe and lower lobe over-inflation Coarse septal thickening Right pneumothorax initially, later resolved.	Right upper and middle lobe over-inflation Coarse septal thickening Lower lobe atelectasis Patchy ground glass changes in lower lobes	Right upper and left upper lobe over-inflation Coarse septal thickening Lower lobe atelectasis	Right upper and middle, left upper lobe over-inflation Coarse septal thickening Lower lobe atelectasis
Lung Histopathology	Right lung wedge biopsy: Poor alveolar development and immaturity with mild thickening of the small arteries Post-mortem examination: Congenital alveolar dysplasia. No pulmonary cysts or evidence for active infection	Right upper lobar resection: Emphysematous changes but without significant inflammatory lung disease	None	None

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Table 2.

Summary of previously published patient characteristics, clinical outcomes and investigations with FLNA related lung disease

		De Wit et al[7] 2011	*Masurel-Paulet et al[6] 2011*	*Singh et al[10] 2013*	*Lord et al[8] 2014*	*Bickel et al[11] 2015*	*Eltahir et al[9] 2016*
City, Country		Rotterdam, Netherlands	Adelaide, Australia	Texas, USA	Montreal, Canada	Florida, USA	Riyadh, Saudi Arabia
Number of Cases		1	1	4	1	1	1
FLNA mutation		Missense mutation (c.220G>P.G74R)	Mosaic nonsense mutation (c.994delG.P.K331X)	3 patients had frameshift mutations, one had missense mutation in FLNA. Mutation location not stated.	Truncating mutation (c.5683G>T,p.G1895)	Mutation seen at (c.988-1 G>C).	Pathogenic variant (c.3153dupC) in exon 21
Clinical Presentation	Sex	Female	Male	4 Female	Female	Female	Female
	Clinical Outcome	Still alive at 3 years of age, with normal cognitive development and delayed motor development. No seizures.	Followed up and still alive at 6 years of age – requires supplementary oxygen whilst sleeping. Mildly developmentally delayed, gastrostomy fed, suffers from mild aortic valve regurgitation.	All patients underwent bilateral lung transplantation at 15, 15, 6 and 5 months of age. One patient underwent a second lung transplantation but died of viral pneumonia aged 3 years old. Remaining 3 patients are thriving and 8	Alive at 22 months of age, no longer requiring supplemental oxygen.	Alive at 9 years of age. Continues to be seen at routine outpatient pulmonology clinic.	The patient required prolonged oxygen support and mechanical ventilation whilst on maximal medical therapy until her death at aged 15 months.

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			years, 1.7 years and 5 months post transplant.			
Surgical Intervention	Right middle lobe lobectomy before the age of 1 years old.	Subtotal left upper lobectomy at 8 months of age	All 4 patients underwent lung transplantation.	None	None	None
Pattern of abnormalities on chest radiographs	Not stated	Bilateral subsegmental atelectasis with hyperlucent areas in midzones.	Not stated	Initial radiographs reveals bilateral perihilar pulmonary infiltrates. Over time until 22 months of age, there is progressive right upper and middle lobe overinflation with right lower lobe atelectasis.	Not stated	Subsegmental atelectasis within the lower lobes and upper lobe air trapping
Predominant abnormalities seen on chest CTs	Lobar emphysema of right middle lobe with displacement of mediastinal structures to the left and compression of left upper lobe	Widespread peribronchial thickening, subsegmental collapse, eventration of the left hemidiaphragm	All studies demonstrated severe pulmonary hyperinflation and hyperlucency simulating emphysema	Patchy ground glass appearances with right upper lobe hyperinflation. Heterogenous areas of atelectasis and interlobular septal thickening, predominantly in the lower lobes .	Ground glass opacification with areas of atelectasis and hyperinflation on initial imaging as infant. Subsequent CT at age 8 years old demonstrated additional broncheictasis	Bibasal lung atelectasis, overinflation of the right middle lobe and left upper lobe with enlargement of the main and proximal branch pulmonary arteries.
Lung Histopathology	Lung emphysema without inflammatory changes	Panpulmonary emphysema with absence of bronchial cartilage	Explanted lung tissue showed severe alveolar simplification and	Mild to moderate chronic lung disease with alveolar simplification and	None	Lung biopsy showed alveolar simplification.

For Peer Review

	and hypertensive pulmonary vascular disease.	moderate pulmonary arteriopathy.	pulmonary hypertension.

For Peer Review

Table 1.

Summary of patient characteristics, clinical outcomes and investigations with FLNA related lung disease

		Case 1	***Case 2***	***Case 3***	***Case 4***
Genetic sequencing analysis of FLNA exons 2-48		Heterozygous for c.88delG, p.(Ala30fs)	Heterozygous for c.6496dupA, p.(Ile2166fs)	Heterozygous for c.1528G>A, p.(Ala510Thr)	Heterozygous for c.2190_2193delTTAC, p(tyr731fs)
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Brain imaging		None performed No neurological symptoms	MRI Brain demonstrated periventricular nodular heterotopia. No neurological symptoms	None performed No neurological symptoms	None performed No neurological symptoms

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Lung Histopathology	Right lung wedge biopsy: Poor alveolar development and immaturity with mild thickening of the small arteries Post-mortem examination: Congenital alveolar dysplasia. No pulmonary cysts or evidence for active infection	Right upper lobar resection: Emphysematous changes but without significant inflammatory lung disease	None	None

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			years, 1.7 years and 5 months post transplant.			
Surgical Intervention	Right middle lobe lobectomy before the age of 1 years old.	Subtotal left upper lobectomy at 8 months of age	All 4 patients underwent lung transplantation.	None	None	None
Pattern of abnormalities on chest radiographs	Not stated	Bilateral subsegmental atelectasis with hyperlucent areas in midzones.	Not stated	Initial radiographs reveals bilateral perihilar pulmonary infiltrates. Over time until 22 months of age, there is progressive right upper and middle lobe overinflation with right lower lobe atelectasis.	Not stated	Subsegmental atelectasis within the lower lobes and upper lobe air trapping
Predominant abnormalities seen on chest CTs	Lobar emphysema of right middle lobe with displacement of mediastinal structures to the left and compression of left upper lobe	Widespread peribronchial thickening, subsegmental collapse, eventration of the left hemidiaphragm	All studies demonstrated severe pulmonary hyperinflation and hyperlucency simulating emphysema	Patchy ground glass appearances with right upper lobe hyperinflation. Heterogenous areas of atelectasis and interlobular septal thickening, predominantly in the lower lobes .	Ground glass opacification with areas of atelectasis and hyperinflation on initial imaging as infant. Subsequent CT at age 8 years old demonstrated additional broncheictasis	Bibasal lung atelectasis, overinflation of the right middle lobe and left upper lobe with enlargement of the main and proximal branch pulmonary arteries.
Lung Histopathology	Lung emphysema without inflammatory changes	Panpulmonary emphysema with absence of bronchial cartilage	Explanted lung tissue showed severe alveolar simplification and	Mild to moderate chronic lung disease with alveolar simplification and	None	Lung biopsy showed alveolar simplification.

For Peer Review

	and hypertensive pulmonary vascular disease.	moderate pulmonary arteriopathy.	pulmonary hypertension.

For Peer Review

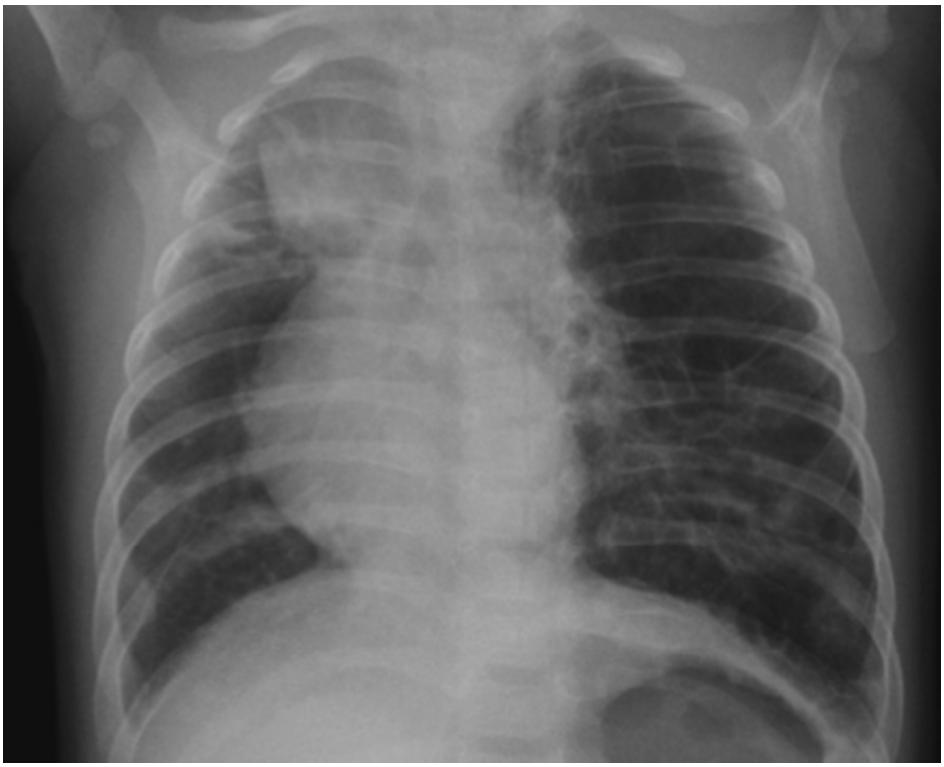

Figure 1. Chest radiograph of case 1 at age 3 months demonstrating hyperinflation and lucency within the left lung with mediastinal shift to the right. Fine interstitial thickening and septations are noted in the left lower zone. There is right upper lobe consolidation.
Figure 1
39x32mm (300 x 300 DPI)

view

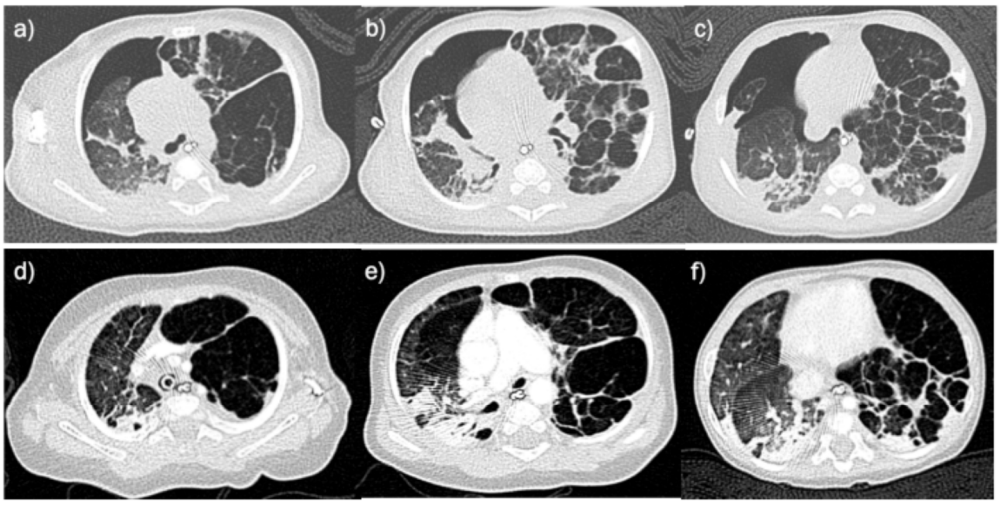

Figure 2. Case 1: Serial axial chest CT images (with lung windows) are displayed demonstrating lung changes with age. The top row (a to c) highlights imaging appearances within the upper, middle and lower zones respectively at 3 months of age. There is a moderate sized right pneumothorax with underlying ground glass change in the lung parenchyma. The left lung is hyperinflated with interlobular septal thickening and areas of air trapping. The bottom row (d to f) demonstrates lung appearances at 5 months of age reflecting on-going left lung hyperinflation and septal thickening. The pneumothorax is no longer present.
Figure 2
250x127mm (300 x 300 DPI)

Review

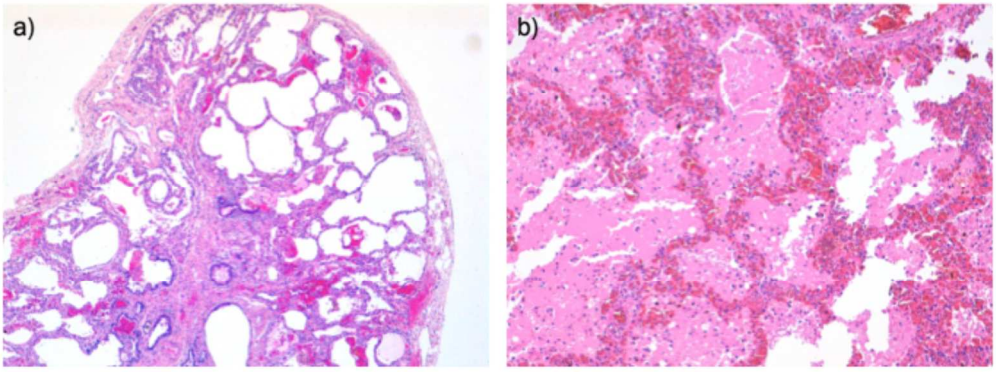

Figure 3. Haematoxylin and eosin stained histopathology images of (a) the initial wedge biopsy of lung including the pleura and (b) a representative post-mortem section of the patient's lung from case 1. The wedge biopsy reveals simplification of the alveolar architecture with dilatation of the distal airspaces. There is peribronchial fibrosis and focal haemorrhage. The post-mortem section demonstrates thickened alveolar septa and contain double capillary loops. This appearance was present throughout the lung and is the diagnostic feature of atypical congenital alveolar dysplasia. There is marked intra-alveolar oedema. Elsewhere within the lung there was fibrosis and airspace dilatation.
Figure 3
241x91mm (300 x 300 DPI)

er Review

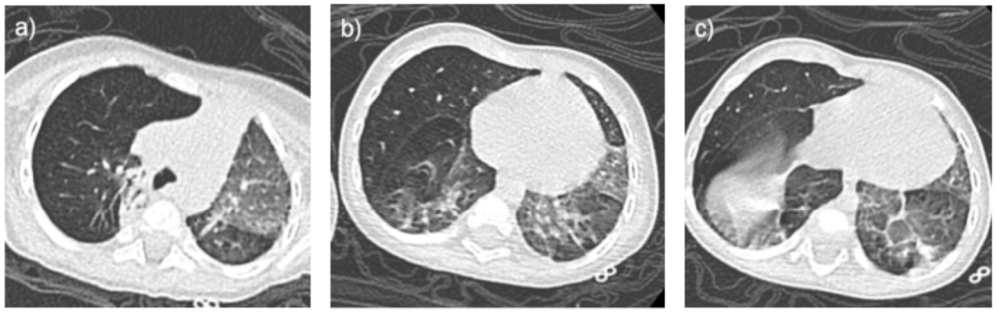

Figure 4. Case 2: Axial chest computed tomography images of the upper (a), middle (b) and lower (c) zones at four months of age. Right upper and middle lobe hyperinflation with patchy ground glass opacification and atelectasis are present within the left lung and right lower lobe.
Figure 4
248x77mm (300 x 300 DPI)

For Peer Review

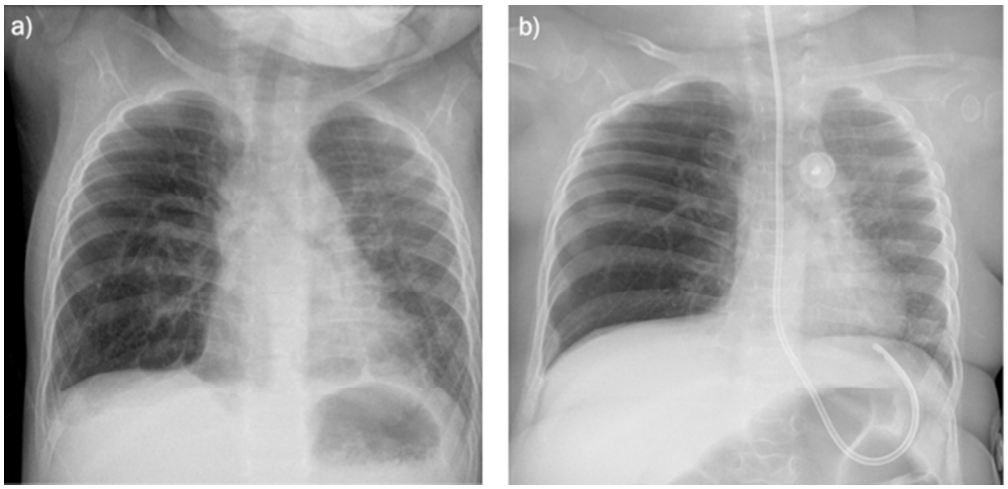

Figure 5. Serial chest radiographs from case 2 performed at (a) 8 months of age and (b) 15 months of age reveal progressive right sided hyperinflation, mediastinal shift to the left and diffuse ground glass opacification within the left lung.
Figure 5
58x27mm (300 x 300 DPI)

For Peer Review

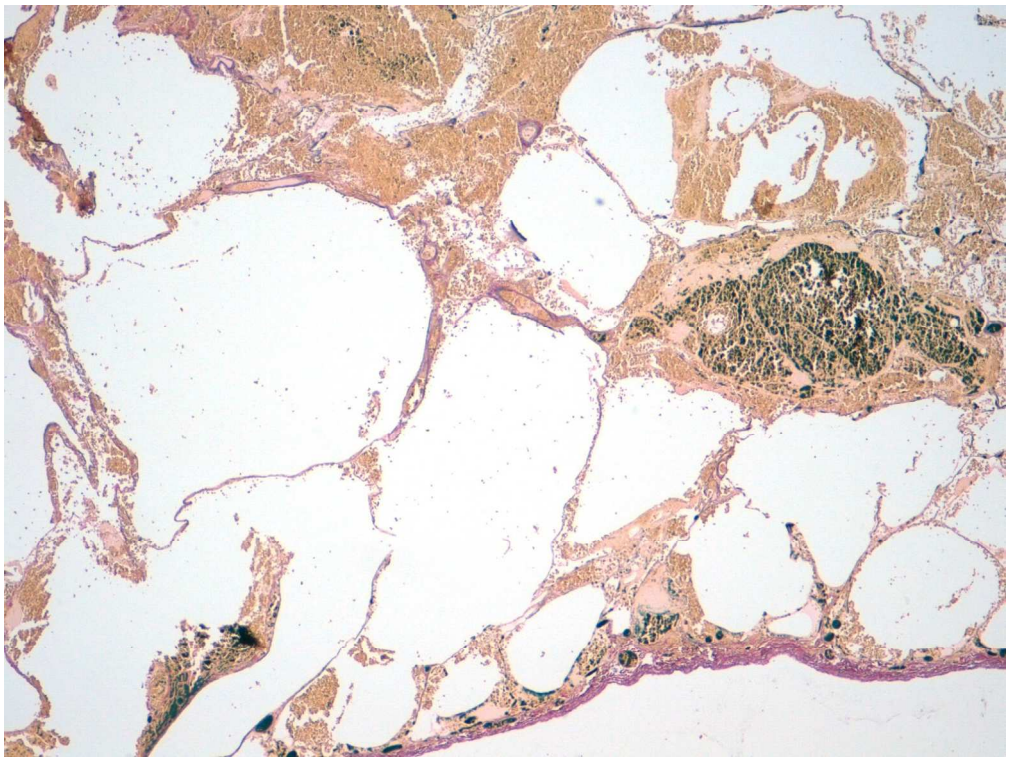

Figure 6. Histopathology lung biopsy image from case 2 with elastic van Gieson staining. There is marked dilatation of the distal air spaces without thickening of the walls. The included muscular pulmonary arteries are thick walled in keeping with pulmonary arterial hypertension. There is also some artefactual alveolar haemorrhage.
Figure 6
173x130mm (300 x 300 DPI)

For Peer Review

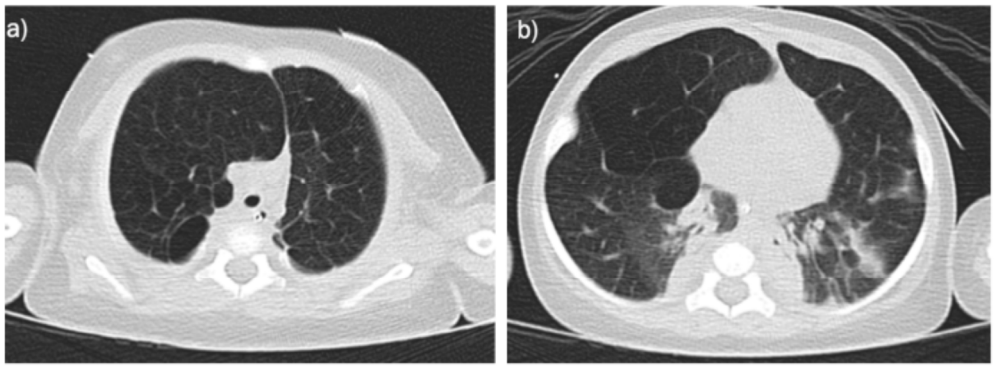

Figure 7. Case 3: Axial chest CT images of the (a) upper and (b) lower lobes at 2 months of age demonstrate upper lobe hyperinflation with sparse pulmonary vascular markings and bibasal medial atelectatic changes.
Figure 7
246x91mm (300 x 300 DPI)

Peer Review

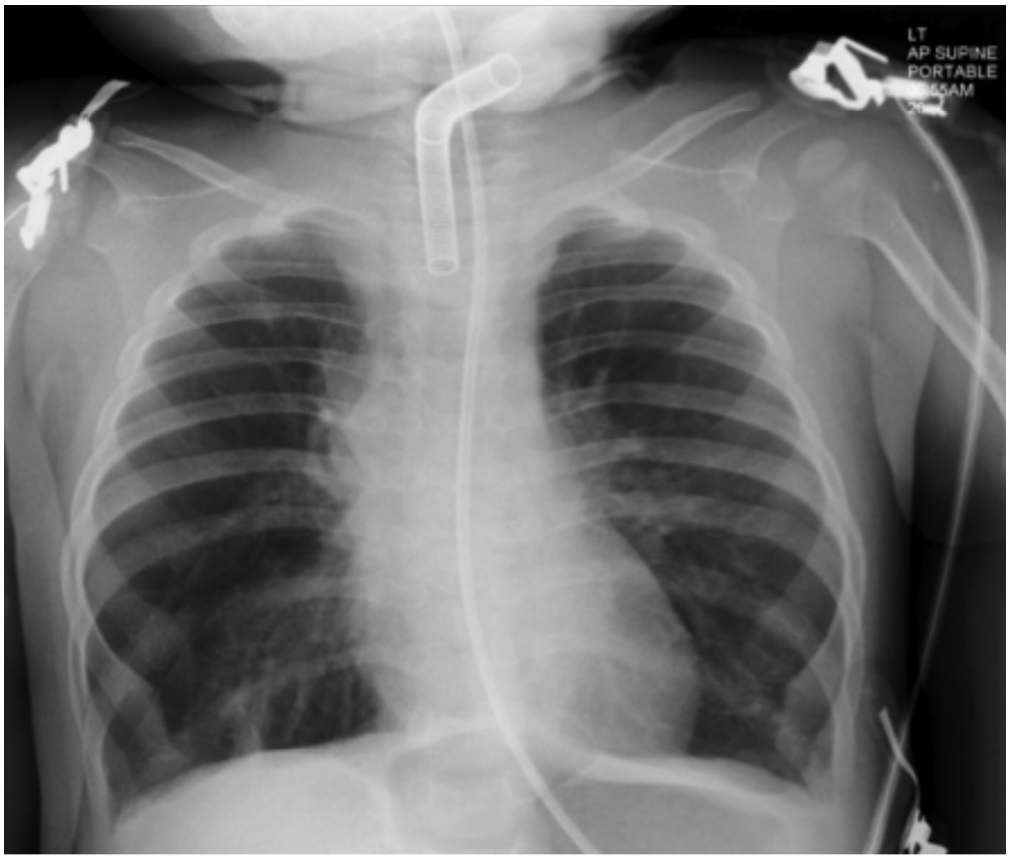

Figure 8. A supine chest radiograph of case 3 performed at 10 months of age demonstrates persistent upper lobe hyperinflation, flattening of both hemidiaphragms and perihilar airspace changes. The patient has a tracheostomy in situ at this stage and is fed via a nasogastric tube.
Figure 8
117x99mm (300 x 300 DPI)

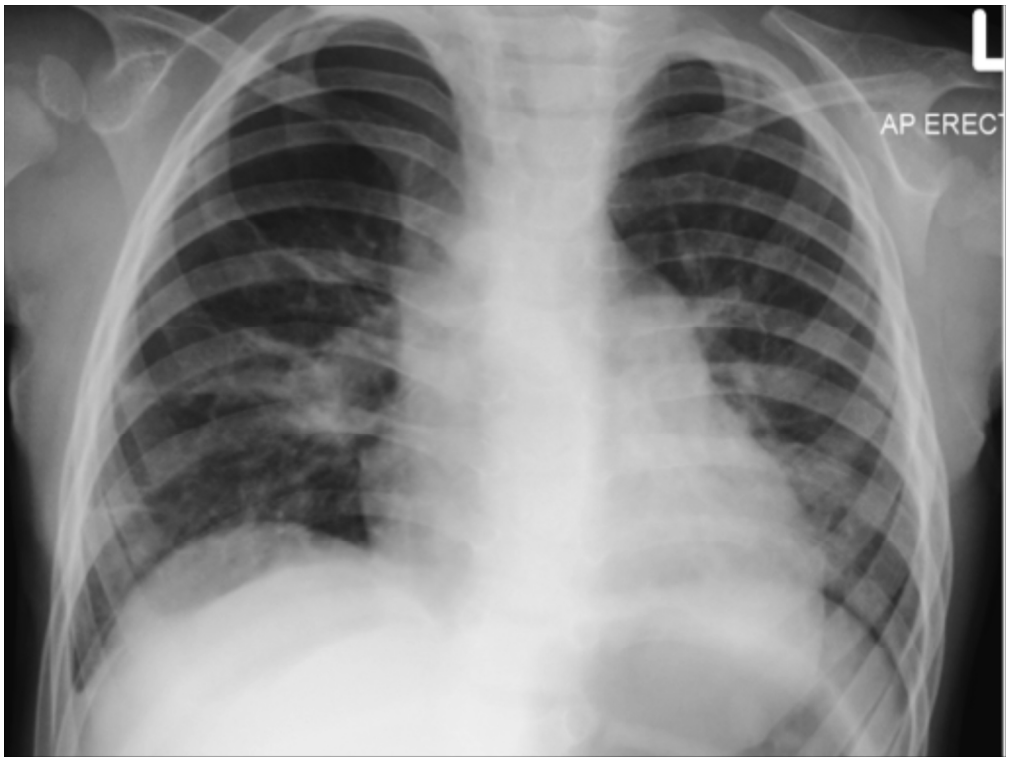

Figure 9. Chest radiograph of patient in case 4 performed at 8 months of age demonstrates right upper lobe hyperinflation and atelectasis within the right midzone and left lower lobe.
Figure 9
135x101mm (300 x 300 DPI)

eview

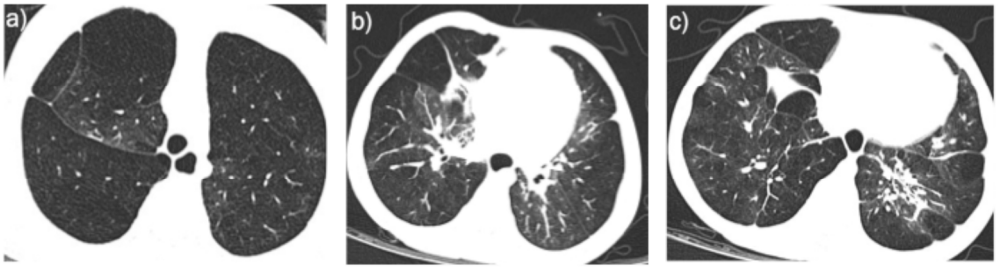

Figure 10. Axial chest computed tomography images of case 4 of the (a) upper lobes and (b and c) lung bases reveal marked overinflation of both upper lobes and the right middle lobe with bilateral lower lobe atelectasis
Figure 10
246x68mm (300 x 300 DPI)

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