Air Trapping and Airflow Obstruction in Newborn Cystic Fibrosis Piglets

Ryan J. Adam¹, Andrew S. Michalski¹, Christian Bauer², Mahmoud H. Abou Alaiwa¹, Thomas J. Gross¹, Maged S. Awadalla¹, Drake C. Bouzek¹, Nicholas D. Gansemer¹, Peter J. Taft¹, Mark J. Hoegger¹, Amit Diwakar¹, Matthias Ochs^{3,4,5}, Joseph M. Reinhardt⁶, Eric A. Hoffman^{1,6,7}, Reinhard R. Beichel^{1,2}, David K. Meyerholz⁸, and David A. Stoltz^{1,6}

¹Department of Internal Medicine, ²Department of Radiology, and ⁸Department of Pathology, University of Iowa Roy J. and Lucille A. Carver College of Medicine, Iowa City, IA; ³Department of Electrical and Computer Engineering and ⁶Department of Biomedical Engineering, College of Engineering, University of Iowa, Iowa City, IA; ⁴Institute of Functional and Applied Anatomy, Hannover Medical School, Hannover, Germany; ⁵Biomedical Research in Endstage and Obstructive Lung Disease Hannover, German Center for Lung Research, Hannover, Germany; and ⁷Cluster of Excellence REBIRTH (From Regenerative Biology to Reconstructive Therapy), Hannover, Germany

Rationale: Air trapping and airflow obstruction are being increasingly identified in infants with cystic fibrosis. These findings are commonly attributed to airway infection, inflammation, and mucus buildup.

Objectives: To learn if air trapping and airflow obstruction are present before the onset of airway infection and inflammation in cystic fibrosis.

Methods: On the day they are born, piglets with cystic fibrosis lack airway infection and inflammation. Therefore, we used newborn wild-type piglets and piglets with cystic fibrosis to assess air trapping, airway size, and lung volume with inspiratory and expiratory X-ray computed tomography scans. Micro-computed tomography scanning was used to assess more distal airway sizes. Airway resistance was determined with a mechanical ventilator. Mean linear intercept and alveolar surface area were determined using stereologic methods.

Measurements and Main Results: On the day they were born, piglets with cystic fibrosis exhibited air trapping more frequently than wild-type piglets (75% vs. 12.5%, respectively). Moreover, newborn piglets with cystic fibrosis had increased airway resistance that was accompanied by luminal size reduction in the trachea, mainstem bronchi, and proximal airways. In contrast, mean linear intercept length, alveolar surface area, and lung volume were similar between both genotypes.

Conclusions: The presence of air trapping, airflow obstruction, and airway size reduction in newborn piglets with cystic fibrosis before the onset of airway infection, inflammation, and mucus accumulation indicates that cystic fibrosis impacts airway development. Our findings suggest that early airflow obstruction and air trapping in

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Current dogma suggests that the cystic fibrosis lung is structurally normal at birth. However, there is increasing evidence that cystic fibrosis lung disease, including airflow obstruction and air trapping, is present within months after birth. Technical and ethical constraints limit the ability to investigate the human cystic fibrosis lung at birth. Thus, we are unable to distinguish if these abnormalities are secondary to early airway infection, inflammation, wall thickening, and mucus obstruction or represent a primary developmental abnormality in the cystic fibrosis lung.

What This Study Adds to the Field

Pigs with cystic fibrosis spontaneously develop lung disease like humans with cystic fibrosis. We examined elements of lung structure and function in newborn wild-type and cystic fibrosis piglets before the onset of airway infection and inflammation. On the day they were born, piglets with cystic fibrosis, compared with wild-type piglets, exhibited an increased frequency of air trapping and airflow obstruction, which was accompanied by a reduction in proximal airway lumen size. These findings suggest that congenital airway abnormalities in cystic fibrosis might contribute to early disease pathogenesis.

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Correspondence and requests for reprints should be addressed to David A. Stoltz, M.D., Ph.D., Department of Internal Medicine, Carver College of Medicine, 440 EMRB, Iowa City, IA 52242. E-mail: david-stoltz@uiowa.edu

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infants with cystic fibrosis might, in part, be caused by congenital airway abnormalities.

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Obstructive lung disease remains the leading cause of morbidity and mortality in cystic fibrosis (CF) (1–3). With time it has become increasingly clear that CF lung disease begins earlier than previously thought and is present very soon after birth. For example, in the 1990s it was appreciated that by 6 months of age many infants with CF already had airway inflammation when assessed by bronchoalveolar lavage (4–6). More recently, in infants with CF with a median age of approximately 3.5 months, air trapping was identified in up to two-thirds of subjects (7, 8). Although air trapping has typically been attributed to airway infection, inflammation, and mucus accumulation, many of these infants were asymptomatic and lacked evidence of these abnormalities (7, 8). Those findings raise several questions: When does airway disease begin in CF? Is there a component

of airway obstruction that occurs before and independent of airway inflammation, infection, and mucus buildup? Is the CF lung normal at birth? To fully answer these questions, studies need to be performed at birth or shortly thereafter. For technical and ethical reasons, this time period is difficult to study in humans.

To begin to answer some of these questions, we generated pigs with CF with a disrupted CF transmembrane conductance regulator (*CFTR*) gene (9, 10). At birth, piglets with CF have many features similar to newborn babies with CF including exocrine pancreatic destruction, meconium ileus, microgallbladder, vas deferens abnormalities, and focal biliary cirrhosis (10). Within weeks to months after birth, pigs with CF spontaneously develop characteristic features of human CF lung disease including airway infection, inflammation, remodeling, mucus accumulation, and obstruction (11, 12). A persistent question in CF research has been whether airway inflammation and mucus plugging precede infection or vice versa. For example, some studies suggested that airway inflammation or mucus accumulation precede the onset of infection (5, 6, 13), whereas others found that airway infection precedes inflammation (4, 14). In earlier studies, we used newborn piglets with CF to address this question. The presence of airway inflammation, goblet cell hyperplasia, submucosal gland hypertrophy, and luminal mucus was assessed with extensive lung histopathology, bronchoalveolar lavage (inflammatory cell and cytokine measurements), and mRNA microarray analysis of airway samples for inflammatory genes and networks and pathways. We found that on the day they are born, CF piglet airways already have a bacterial host defense defect, yet lack airway inflammation, goblet cell hyperplasia, submucosal gland hypertrophy, or mucus accumulation (10, 12, 15). These studies demonstrated that newborn piglets with CF have pristine airways.

Despite the absence of airway inflammation at birth, we were surprised to find that newborn CF piglet tracheas have a reduced caliber and irregularly shaped structure (15). Similar features have been reported in mice with CF and comparable abnormalities are observed in infants and young children with CF (15–19). Knowing that air trapping is present in humans with CF as early as weeks to months after birth and that loss of *CFTR* function leads to developmental airway abnormalities, we hypothesized that air trapping and airflow obstruction are present at birth and occur before the onset of airway infection, inflammation, and mucus accumulation. To test this hypothesis, we studied newborn piglets with and without CF before they develop airway inflammation and mucus buildup.

Some of the results of these studies have been previously reported in the form of abstracts (20, 21).

METHODS

Animals

We previously reported generation of *CFTR*^{+/−} and *CFTR*^{−/−} pigs. *CFTR*^{+/−} animals were mated and progeny were studied (9, 10, 12). *CFTR*^{−/−} newborn piglet airways lack normal *CFTR* transcripts and *CFTR* protein, and exhibit defective chloride and bicarbonate transport (10, 22, 23). All animal experiments were approved by the University of Iowa Institutional Animal Care and Use Committee.

In Vivo Multi-Detector Row Computed Tomography Scanning and Air Trapping

Air trapping, airway size (trachea and mainstem bronchi), and lung volume measurements were assessed from multi-detector row computed tomography (MDCT) scans. Most air trapping studies in very young infants with CF have been performed in intubated subjects (25 cm H₂O for the inspiratory scan and 0 cm H₂O airway pressure for the

expiratory scan) (7, 8, 24–28). Because these studies evaluated the greatest number of infants with CF identified by newborn screening and at the earliest time points (closest to the newborn period), we adopted a similar air trapping protocol to allow for comparisons with these human studies. Therefore, air trapping was assessed from paired 25 cm H₂O (inspiratory) and 0 cm H₂O (expiratory) chest MDCT scans in intubated newborn *CFTR*^{+/+} and *CFTR*^{−/−} piglets. Air trapping was quantified with a semiquantitative scoring system that we developed and adapted from previously published scoring systems (7, 25, 29, 30). The right and left lungs were each divided into three zones: the cephalad, middle, and caudal (total of six zones per animal). Easily identifiable anatomic landmarks were used to define the zones. The cephalad zone was demarcated from the middle zone by the carina, and the middle zone from the caudal zone by the inferior margin of the heart. Three experienced pulmonologists, masked to genotype, independently scored the scans for evidence of air trapping. All scans were reviewed with a standardized Hounsfield unit window and at the same computer workstation. Air trapping was defined as parenchymal areas that failed to increase in attenuation and reduce in volume on expiratory, compared with inspiratory, MDCT scans. The scoring system was binary. Reviewers visually graded each lung zone for the presence of air trapping (0 = no air trapping; 1 = air trapping). The median air trapping score for each lung zone was calculated from the individual scores of the three reviewers. The total air trapping score, determined by summing the individual lung zone scores, was calculated (score range, 0–6).

Cranial Lobe Micro-CT Scanning

Limitations in MDCT scanner resolution coupled with the small size of the newborn pig lung restricted our ability to measure airway size distal to the mainstem bronchi with the MDCT protocol. Therefore, we used micro-CT, which has comparatively superior spatial resolution. However, the newborn piglet lung is larger than the micro-CT scanner's field of view. Thus, we focused our airway measurement studies on an appropriately sized lobe, the right cranial lobe, the porcine equivalent of the human right upper lobe. Newborn pig right cranial lobes were excised, cannulated, and micro-CT scanned as previously described (31).

Additional details for the methods and materials are provided in the online supplement.

RESULTS

Piglets with CF Have Air Trapping at Birth

To test the hypothesis that newborn *CFTR*^{−/−} piglets (hereafter piglets with CF) have a predisposition to air trapping before the onset of airway infection and inflammation, we obtained MDCT scans of newborn piglet lungs at 25 and 0 cm H₂O pressure and examined them for air trapping (Figure 1). Only one of eight *CFTR*^{+/+} (hereafter piglets without CF) piglets had air trapping, whereas six of eight piglets with CF had air trapping (Figure 2A). In piglets with CF, air trapping was observed in all lung regions (Figure 2B). These data demonstrate that air trapping occurs in the newborn CF piglet lung before the onset of airway infection, inflammation, or mucus obstruction (10, 12, 15).

Piglets with CF Have Airflow Obstruction at Birth

Given the reduced tracheal lumen size in the newborn piglet with CF (15), and reports of abnormal pulmonary function tests in infants with CF, we hypothesized that piglets with CF would have airflow obstruction at birth, before the onset of infection and inflammation. Using a flexiVent system (SCIREQ, Montreal, QC, Canada), we measured airway resistance in newborn piglets with and without CF (Figure 3). The resistance of the non-CF airways was 0.025 ± 0.004 cm H₂O/s/ml and for CF 0.054 ± 0.007 cm H₂O/s/ml (*P* < 0.05). These findings demonstrate that the reduced caliber of the CF trachea causes, at least in part, an elevated airway resistance in newborn CF piglet lungs. However, from these data, we are unable to conclude if

Figure 1. Air trapping in newborn piglets with cystic fibrosis (CF). Shown are representative multi-detector row computed tomography images from newborn piglets with and without CF. For each genotype, the transverse, coronal, and sagittal plane images came from the same animal. Identical Hounsfield unit window settings were applied to all images. Air trapping is identified by arrows.

the reduced CF tracheal lumen size is sufficient to cause the increased airway resistance in piglets with CF.

Therefore, we calculated the contribution of the trachea (Figures 4A and 4B) to total airway resistance with Poiseuille's equation. The calculated tracheal resistance was 0.001 ± 0.001 cm H₂O/s/ml in newborn piglets without CF and 0.005 ± 0.001 cm H₂O/s/ml in piglets with CF ($n = 8$ for both groups; $P < 0.0001$). Comparison of the flexiVent measured airway resistance and the calculated tracheal resistance suggests two conclusions. First, tracheal resistance represents only a fraction of the total measured airway resistance in the newborn pig. This is in agreement with other data showing that, at least in humans, the greatest resistance to airflow is in the medium-sized bronchi (32). Second, by itself, the reduction in the CF tracheal size fails to account for the observed differences in total airway resistance between non-CF and CF. These findings suggest that the airway size reduction in newborn piglets with CF is not restricted to the trachea, but likely extends further down the airway tree.

Newborn CF Piglet Mainstem Bronchi Have Reduced Caliber

To characterize the extent and patterning of newborn CF piglet airway size reduction, we measured the mainstem bronchi cross-sectional area from MDCT datasets of newborn non-CF and CF piglet lungs. Measurements were obtained perpendicular to the airway centerline on a segment by segment basis with Pulmonary Workstation 2.0 (VIDA Diagnostics, Coralville, IA). An airway segment was defined as the portion of an airway that extends

from one branch point to the next. Compared with non-CF, the CF mainstem bronchi sizes were significantly reduced at every measured segment (Figures 4C and 4D).

Airway Size Reduction in Newborn Piglets with CF Persists in Proximal Airways

Limitations in MDCT scanner resolution coupled with the small size of the newborn pig lung restricted our ability to measure airway size further down the airway tree with the MDCT protocol. Therefore, to more fully investigate the airway size reduction phenotype in the newborn piglet with CF we used an alternative imaging modality: micro-CT, which has comparatively superior spatial resolution. We developed a high-resolution, *ex vivo*, micro-CT-based imaging protocol to evaluate airway size (31). The newborn piglet lung, however, is larger than the micro-CT scanner's field of view. Thus, we focused our airway measurement studies on an appropriately sized lobe, the right cranial lobe, the porcine equivalent of the human right upper lobe. Freshly excised right cranial lobes were scanned with micro-CT; the airways segmented with a purpose built, highly automated computer algorithm; and then measured.

Micro-CT measurement validation. Pulmonary Workstation 2.0 has been validated for use with traditional CT scans of human subjects, but not for micro-CT scan datasets of newborn piglet lungs. Therefore, we validated its use in this study by obtaining two independent size measurements of the same airway: one with Pulmonary Workstation 2.0 and the other by airway microdissection (Figure 5A). The measured airways ranged in size from 0.1 to 2.48 mm² in cross-sectional area (corresponding to a radius of

Figure 2. Quantitative assessment of air trapping in newborn piglets with and without cystic fibrosis (CF). (A) The lungs were divided into six zones (three zones per lung): the cephalad, middle, and caudal; for both the right and left lungs. Each zone was scored for the presence of air trapping in a binary manner (0 = no air trapping; 1 = air trapping). Scores range from 0 to 6 for each animal. Each data point represents an individual animal. Horizontal lines represent mean $\pm$ SEM. $P < 0.05$, Mann-Whitney test. (B) Percentage of piglets with and without CF with air trapping in each lung zone.

Figure 3. Airway resistance in newborn piglets with and without cystic fibrosis (CF). Airway resistance measurements were performed in newborn piglets with the flexiVent system. $n = 5$ for non-CF and 8 for CF. Data represent mean $\pm$ SEM. * P less than 0.05.

0.18 and 0.89 mm, respectively) (Figure 5B). The measurement correlation was strong throughout (linear R^2 , 0.99), demonstrating that we can accurately measure micro-CT imaged airways in this size range with Pulmonary Workstation 2.0.

Airway size reduction in the newborn CF piglet lung. We measured six major airways of the right cranial lobe including the caudal branch (denoted as T.TB.Ca; henceforth, the “T.TB” designation is omitted; see online supplement for further description of airway tree nomenclature system), and the first five branches from the caudal airway (Ca.B1, Ca.B2, Ca.B3, Ca.B4, and Ca.B5) (Figures 6A and 6B; see Table E1 in the online supplement).

The greatest percentage of size reduction in the CF right cranial lobe airways was observed in the largest airways (Figure 6C). In the first segment of the caudal branch (Ca.1) the cross-sectional area in CF was reduced by approximately 50%, similar to the size reduction observed in the newborn CF piglet trachea and mainstem bronchi. In the first segment of airway Ca.B1, there again was a significant size reduction in CF. However, continuing distally down this branch, the caliber difference between genotypes tapered until ultimately reaching size concordance. This similarly held for Ca.B2 and Ca.B3. Branches Ca.B4 and Ca.B5 showed little difference between genotypes for segments of any number. A similar pattern of airway size reduction was observed when the lobes were scanned at an airway pressure of 0 cm H₂O (see Figure E1). Micro-CT-based airway measurements of the right cranial lobe indicate that the airway size reduction observed in the trachea and mainstem bronchi extends into the airway tree, but is restricted to the large, conducting airways (Figure 7).

Lung Volume and Alveolar Surface Area Are Normal in the Newborn Piglet with CF

The micro-CT study demonstrated airway size reduction in the large CF airways, but differences dissipated in more distal airways. Based on these findings, we hypothesized that airspace size, alveolar surface area, and lung volume would be similar between newborn pigs with and without CF.

To assess whether distal airspace development was dependent on CFTR expression, we used tissue samples obtained from the right cranial lobe to quantify mean linear intercept, a measure of the acinar volume to surface ratio, and alveolar surface area. The mean linear intercept and lobar alveolar surface area were similar in piglets with and without CF (Figures 8A and 8B). Additionally, we performed controlled ventilation MDCT scanning to determine total lung volumes at 0 and 25 cm H₂O. Lung volumes were similar in piglets with and without CF at both pressures studied (Figure 8C). Collectively, these data suggest that loss of CFTR function affects the development of proximal,

Figure 4. Multi-detector row computed tomography-based airway size measurements in newborn piglets with and without cystic fibrosis (CF). (A) Multi-detector row computed tomography-based reconstruction of the newborn porcine airway tree. Airway lumen cross-sectional area was determined in the trachea (B), right mainstem bronchus (RMB) (C), and left mainstem bronchus (LMB) (D). The airway trees were reconstructed and analyzed with Pulmonary Workstation 2.0. $n = 8$ for non-CF and 5 for CF. Data are mean $\pm$ SEM. * P less than 0.05. RMB and LMB were statistically different between non-CF and CF at all segments measured ($P < 0.05$). TB = tracheal bronchus. Scale bar is approximate.

larger airways in the piglet lung and is associated with air trapping before the onset of infection and inflammation.

DISCUSSION

We previously reported that the trachea of newborn piglets with CF has a reduced caliber and lumen circularity, irregularly shaped cartilage rings, and abnormally appearing airway smooth muscle bundles (15). Our data now demonstrate that air trapping, one of the earliest radiographic findings in humans with CF (7), and airflow obstruction, a clinical feature of CF lung disease (2, 33), are present on the day that pigs with CF are born. Moreover, proximal airway development, but not distal airway or alveolar development, depends on CFTR function. Because these findings were present before the onset of airway infection, inflammation, and mucus obstruction our data suggest a developmental origin for some aspects of CF airway disease.

Several factors likely contribute to CF lung disease including reduced bacterial killing by CF airway surface liquid (23), impaired mucociliary transport (34), abnormal mucus (35, 36), and others (2, 37). Our findings raise the possibility that developmental abnormalities might also contribute to CF lung disease. However, our data indicate that the changes in CF airways are not as severe as those in other congenital lung diseases, such as congenital lobar emphysema, in which approximately 50% of cases are

Figure 5. Validation of Pulmonary Workstation 2.0 micro-computed tomography (micro-CT) airway measurements. (A) The right cranial lobe in four forms relevant to method validation including freshly excised (*upper left*), installation fixed (*lower left*), a computer-generated airway tree reconstruction (*upper right*), and an airway microdissection (*lower right*). Newborn piglet right cranial lobes were instillation fixed, scanned with micro-CT, and select airways were measured. Then, airways from these right cranial lobes were microdissected, sectioned, imaged with a standard light microscope, and measured. Thus, two measurements of the same airway were obtained. Bar = 3 mm. (B) Cross-comparison of microdissection-based airway lumen measurements and Pulmonary Workstation 2.0 (PW2) micro-CT measurements of the same airway segments (linear R^2 , 0.99). Each point represents a different airway segment.

caused by a ball-valve-type defect, airway lumen obstruction, or external airway compression causing airspace enlargement (38, 39). The remaining cases have no identifiable cause, suggesting additional etiologies. Nor are these airway changes in the pig with CF as severe as the tracheobronchomegaly of Mounier-Kuhn syndrome (40). Nevertheless, these findings will have important implications for how clinicians and scientists think about the pathogenesis of early CF lung disease and may impact future diagnostic and therapeutic approaches in early CF.

Air Trapping Occurs in the Absence of Infection or Inflammation in Newborn Piglets with CF

Air trapping is one of the earliest observed radiographic abnormalities on chest CT scans in infants and young children with CF (7, 8). Moreover, the existence of air trapping in infants with CF

is predictive of future pulmonary exacerbations (41) and is a risk factor for bronchiectasis (28). In newborn piglets with CF, air trapping cannot be attributed to mucus plugging, infection, inflammation, or their secondary consequences, because these changes are not present (10, 12, 15). This finding has consistency with two recent studies of air trapping in infants with CF; both studies noted air trapping despite lack of measurable infection in some subjects. Hall and coworkers (8) found that 49% of infants with CF had air trapping, yet only 12% were classified as infected. In infants with CF, Sly and coworkers (7) found that two-thirds had air trapping (a similar proportion to our newborn piglets with CF), and that air trapping was not associated with infection.

Airway Size Reduction and Potential Mechanisms

How does lack of functional CFTR affect airway growth and development? The mechanisms remain unknown, but the

Figure 6. Micro-computed tomography (micro-CT)-based measurements of the right cranial lobe airway size. (A) Micro-CT based three-dimensional reconstruction of right cranial lobe airways from a piglet with and without cystic fibrosis (CF). Scale bar is approximate. (B) A schematic of the right cranial lobe airways with pertinent branches labeled. (C) Airway lumen cross-sectional area measurements from micro-CT scans. Freshly excised right cranial lobes from newborn piglets with and without CF were micro-CT scanned at a pressure of 20 cm H_2O . Shown are measurements of six major airway branches within the right cranial lobe. Airway measurements were obtained from eight non-CF and six CF newborn piglets. Data are mean $\pm$ SEM. See Table E1 for statistical comparison.

Figure 7. Airways with size reduction in the newborn piglet with cystic fibrosis right cranial lobe. The measured airways (dotted overlay) within the shaded triangular region had size reduction in the newborn piglet with cystic fibrosis and no size reduction outside of the shaded region (approximate). See Figure 6 for airway labels.

patterning of the congenital airway caliber reduction might provide some clues.

First, lung growth and development are widely considered to be dependent on fetal lung liquid and chloride secretion. Fetal lamb studies have demonstrated that tying off the trachea leads to lung enlargement, whereas lung liquid drainage retards lung growth and development (42). Because the newborn porcine CF lung lacks morphologic evidence of reduced lung growth, it seems unlikely that reduced fetal lung liquid secretion is directly or solely responsible for the reduced caliber of proximal CF airways.

Second, our findings suggest that the distribution of airway developmental abnormalities could be related to the temporal order of airway development. The more proximal and larger airways develop early in gestation (43). With time, airway branching continues, and the more distal, smaller airways and alveoli develop. The distribution of airway size reduction in the CF pig airway tree indicates that the airways that develop earlier in gestation are predominantly affected.

Third, the pattern of airway size reduction might be related to the distribution and timing of *CFTR* expression during lung development. *CFTR* mRNA is expressed in human airway epithelium during the first trimester of gestation (44, 45). In the sheep lung, *CFTR* mRNA is more highly expressed earlier in gestation than later (46), and in humans the transcript abundance in airway epithelium is less in adults than in the fetus (44). Airway development and growth may be more dependent on *CFTR* during earlier gestational time points, when the proximal airways form, than later time points, when the smaller and more distal airways and airspaces develop.

Finally, the distribution of airway narrowing raises the possibility that in addition to airway epithelial cells, other cell types might be responsible.

Chondrocytes. During gestation in humans, cartilage precursors first appear in the trachea during the fourth week of gestation (47). Whether lack of *CFTR* in chondrocytes leads to defects in airway growth, or the cartilage ring defects represent a secondary consequence of smaller-sized airways, remains to be determined. Perhaps lack of functional *CFTR* alters the cross-talk at the epithelial-mesenchymal junction leading to abnormal cartilage development thereby causing airway size reduction (48).

Neural cells. Pulmonary innervation begins very early in gestation (47) and baseline airway caliber is in part regulated by neural control (49). Innervation abnormalities have been reported in the airways of a CF mouse model (50), and we recently reported that pigs with CF have nervous system abnormalities at birth (51). Might altered nervous system function and/or innervation contribute to the airway phenotype?

Airway smooth muscle cells. Fetal airways exhibit peristaltic contractile activity that is dependent on phasic airway smooth muscle contractions. These distending forces are transmitted to the developing airway and may be important in airway and lung growth (52). *CFTR* is present in human airway smooth muscle cells (53), and abnormal smooth muscle has been reported in people with CF (53, 54). Whether altered airway smooth muscle function is responsible for the proximal airway size reduction we observed in the newborn piglet with CF is unknown, but remains a possibility.

Advantages and Limitations of this Study

Our study has advantages and limitations. Strengths include: (1) Studies were conducted on newborn piglets with CF, allowing us to remove secondary consequences of infection and inflammation (10, 12, 15). (2) Comparisons were made between CF and non-CF littermate control animals. Studies in human infants with CF are often limited by lack of non-CF control subjects. (3) The *in utero* development of the airway tree is similar between pigs and humans (43, 55). (4) We were able to take advantage of the micro-CT's superior imaging resolution and advanced airway segmentation analysis tools to obtain a continuous and more complete visualization of the lung and

Figure 8. Distal airspace and lung volume measurements in newborn piglets with and without cystic fibrosis (CF). (A) Mean linear intercept ($n = 25$ animals for non-CF and 15 for CF). (B) Alveolar surface area of the instillation fixed right cranial lobe ($n = 24$ animals for non-CF and 15 for CF). (C) Multi-detector row computed tomography derived measures of lung volume ($n = 8$ animals for non-CF and 8 for CF). Data are mean $\pm$ SEM.

airway tree. Limitations include: (1) Because of size constraints of the newborn pig lung, some of our measurements were performed only on the right cranial lobe. Based on our MDCT studies of the mainstem bronchi, lung volumes, and air trapping data, we expect that the pattern of airway narrowing in the right cranial lobe is representative of the airway structure in the remainder of the CF lung and not unique to this lobe. (2) Our conclusions on the pattern of airway size reduction are based only on the airways that we measured. (3) The pigs with CF used in this study were *CFTR*^{-/-}, but most mutations in human *CFTR* have some residual *CFTR* function (i.e., $\Delta F508$). We previously reported similar, but slightly less severe, tracheal structural abnormalities in newborn *CFTR* ^{$\Delta F508/\Delta F508$} piglets suggesting that similar features might be present in humans with CF (11).

Implications for Humans with CF

Newborn screening has facilitated studies of infants with CF, demonstrating that CF airway disease, including air trapping and airflow obstruction, is present within months after birth (7, 8, 17, 25, 26, 33, 56). These findings are generally interpreted to mean that airway disease develops soon after birth. An additional explanation is that some airway abnormalities are present at birth in humans with CF and these structural defects contribute to the early airway changes observed in infants with CF.

The severity of these changes might be dependent on residual *CFTR* function, specific *CFTR* mutations, modifier genes, and/or additional factors present *in utero* during lung development. Studies designed to address these questions in newborns with CF or at earlier time points are important to conduct, but may be difficult for technical and ethical reasons.

Results from our study also raise questions about where in the airway tree CF disease starts and whether structural abnormalities in the large airways contribute to the CF disease phenotype. The “small airways” are commonly implicated as the site of origin of CF airway disease. Yet, the data supporting this idea are limited. Could structural abnormalities in the larger airways contribute to early disease pathogenesis? Potential mechanisms could include disrupting mucociliary transport, increasing airway resistance or wall shear stress, or altering microbe deposition on the airway surface. CF animal models might allow us to answer some of these questions.

In summary, findings from this study demonstrate that newborn piglets with CF have air trapping and airflow obstruction before the onset of airway infection, inflammation, or mucus accumulation. With the development of *CFTR*-specific therapies, and initiation of treatments at earlier time points in people with CF, it will be important to better understand the role of these congenital airway abnormalities in disease onset and progression.

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