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SONOGRAPHIC EVIDENCE OF ABNORMAL TRACHEAL CARTILAGE RING STRUCTURE IN CYSTIC FIBROSIS

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Abstract

Objective—Tracheal cartilage ring structural abnormalities have been reported in cystic fibrosis (CF) mice and pigs. Whether similar findings are present in humans with CF is unknown.

Study Design—Tracheal cartilage ring size and shape were measured in adults with (n=21) and without CF (n=18).

Methods—Ultrasonography was used in human subjects to non-invasively assess tracheal cartilage ring structure in both the sagittal and the transverse planes. Tracheal cartilage ring thickness was also determined from histological sections obtained from newborn non-CF and CF pigs. These values were compared with human data.

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Some of the results of this study have been previously reported in the form of abstracts at the American Thoracic Society Conference, 2013, and the 27th Annual North American Cystic Fibrosis Conference, 2013.

Results—Human CF tracheas had a greater width and were less circular in shape compared to non-CF subjects. CF tracheal cartilage rings had a greater midline cross sectional area and were thicker compared to non-CF rings. Maximal tracheal cartilage ring thickness was also greater in both newborn CF pigs and human adults with CF, compared to non-CF controls.

Conclusion—Our findings demonstrate that structural differences exist in tracheal cartilage rings in adults with CF. Comparison with newborn CF pig data suggests that some of these changes may be congenital in nature.

Keywords

cystic fibrosis; airway; cartilage; ultrasound; trachea; human

INTRODUCTION

Cystic fibrosis (CF) is a multi-system disorder caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR).[1, 2] The leading cause of morbidity and mortality in CF is lung disease. [3–6]

We previously generated pigs with a disrupted *CFTR* gene.[7, 8] Within weeks to months after birth, CF pigs develop upper and lower airway disease like humans with CF.[9–11] We were surprised to find that newborn CF pigs have developmental abnormalities in their upper and lower airway structure, despite no evidence of airway inflammation or mucus accumulation on the day that CF pigs are born.[10] There is sinus hypoplasia and the trachea and more proximal airways have a reduced lumen area.[11–13] Moreover, the tracheal lumen is less circular in shape and its cartilage rings are abnormally shaped and irregularly spaced.[13] Similar cartilage ring structural abnormalities have also been reported in CF mice.[14]

Whether similar airway and cartilage abnormalities exist in humans with CF is unknown, but several pieces of evidence suggest they might. First, tracheal changes of reduced lumen caliber and circularity have been reported in CF infants and young children, similar to the CF pig model.[13] Second, tracheomalacia was identified via bronchoscopy in 15% of young children with CF (median age 16 months), compared to a rate of approximately 0.05% in the general pediatric population.[15] Lastly, in a computed tomographic (CT) study of adults with CF, tracheomalacia was also frequently observed in CF subjects.[16] Therefore, in the current study, we asked if tracheal cartilage ring morphology was different between non-CF and CF adults.

Imaging modalities used to evaluate tracheal structure include CT scanning and magnetic resonance imaging (MRI), but ionizing radiation exposure (CT), cost, image acquisition time (MRI), and frequent need for sedation limit their use. Ultrasonography is an alternative imaging modality that is in widespread use by clinicians due to its favorable safety profile,[17] low cost, portability, ease of use, and accessibility. Ultrasonography has been successfully used to investigate both pleuro-pulmonary and airway structures[18] and has yielded results comparable to CT imaging.[19] Moreover, ultrasonography has also been used to assess vocal cord function and for pre-assessment of endotracheal tube placement.[20, 21] In this study, we hypothesized that tracheal cartilage ring shape and size would be different in people with

CF. To test this hypothesis, we used ultrasound to non-invasively image tracheal cartilage rings in people with and without CF.

MATERIALS AND METHODS

Study subjects

This study was conducted in accordance with the amended Declaration of Helsinki, after approval by the Institutional Review Board of the University of Iowa (IRB #199207428). Written informed consent was obtained from all subjects. We recruited young adult males and females (18–35 years old) with CF, who were receiving care at the University of Iowa Hospitals and Clinics, and healthy non-CF controls. All had stable respiratory health. We also obtained spirometry data and sputum culture results from a recent or same day clinic visit in the CF subjects. The control group consisted of healthy, non-smoking, male and female, young adult (18–35 years old) volunteers with no respiratory disease history.

Ultrasound image acquisition

The ultrasound protocol was adapted from a prior study.[18] Ultrasound images were acquired in a supine position with necks extended. Image acquisition began at the level of the thyroid cartilage and moved in the caudal direction until the sternal notch was reached. Two scans were obtained per person: one in the sagittal plane and another in the transverse plane (Figures 1 A–D). In preliminary studies (data not shown), we determined that we could visualize extra-thoracic, but not intra-thoracic, tracheal cartilage rings and that visualization was optimal at end-expiration. Thus, images of extra-thoracic tracheal cartilage rings were acquired while the subjects performed a breath hold maneuver at end-expiration. Image acquisition required approximately 15 seconds for each view. We used a Philips Sparq ultrasound system (Andover, MA) with a 12–4 MHz broadband linear array transducer. Patient identifier information was removed from scans prior to analysis.

Ultrasound image analysis

We assessed ring structure in both the sagittal (Figures 1A–B), and the transverse planes (Figures 1C–D). From the sonographic images, and blinded to genotype, tracheal cartilage rings were manually segmented with ImageJ analysis software (NIH, Bethesda, MD). In the sagittal plane, we measured the distance from one ring center to the next, which we called inter-ring distance. We also obtained a measure of ring area, thickness, and height. In addition, we report the standard deviation of ring thickness as a measure of intra-subject ring variability.

We acquired a number of measurements from the transverse imaging plane, including ring thickness at the midline, tracheal width, and maximal ring thickness. For maximal ring thickness, we identified the thickest portion of each ring within a subject, and from these identified the thickest ring per individual subject. We report this value as maximal thickness per subject. Our prior studies, of both CF pigs and human neonates with CF, suggest that the CF trachea is less circular in cross-sectional shape than non-CF controls. Because of this finding, we were interested in assessing tracheal shape for the present study. However, with ultrasonography, we were unable to completely visualize the trachea in cross-section,

because tracheal lumen air casts a shadow on posterior image details. Complete cross-sectional visibility is required for conventional tracheal circularity measurement. Thus, for the present study we used a surrogate measure named “roundness error,” which is derived from a best-fit circle. First, we selected a number of points along the anterior aspect of the cartilage ring. Next, we used the least squares method to apply a best-fit circle to those points, and by extension to the cartilage ring itself. We then calculated the Euclidean distance from each selected point to the nearest point along the best fit circle’s circumference. The average of these distances was called the “roundness error.” For example, a highly circular cartilage ring would be closely fitted by a circle and thus would have a low roundness error. Conversely, a less circular ring would have a worse fit, resulting in a relatively greater error.

Animals

Animal studies were reviewed and approved by the University of Iowa Animal Care and Use Committee. Neonatal non-CF ($CFTR^{+/+}$) and CF ($CFTR^{-/-}$) pig littermates were obtained from Exemplar Genetics (Sioux Center, IA). Animals were euthanized (Euthasol; Virbac, Fort Worth, TX) at 6 – 12 hours of age for study.

Histopathology

Newborn CF and non-CF pig tracheas were dissected and placed into 10% neutral buffered formalin for fixation. Tracheas were cross-sectioned, routinely processed, and stained with hematoxylin and eosin for examination. Due to variable trachea sampling between animals, we determined the maximal cartilage ring thickness for each pig trachea using the available histological sections from each animal.[13]

Comparison with CT imaging

We retrospectively reviewed medical records of all subjects with CF who had a tracheal ultrasound performed as part of this study and identified those who had a computed tomography (CT) scan of the chest in the past year. We measured the proximal tracheal width at multiple levels and compared the average in each subject with the average tracheal width as measured with ultrasound.

Statistical Analysis

Statistical analyses were performed with R (R Core Team, 2013). Linear mixed-effects models with a random effect for each subject were used to model correlation of measurements within a subject. A model with a fixed effect for group (CF vs. non-CF) was used to test whether there was a difference in means between groups. A model with a fixed effect for group at each ring was used to test whether there was a significant difference in means at each ring. Data are presented for individual cartilage rings and means for all rings studied. Comparison for any ring with a p value less than 0.05 is reported as statistically significant.

RESULTS

Ultrasonography of tracheal cartilage rings

Patient demographics are outlined in Table 1. The CF group had lower average weight and height as compared to non-CF, but comparable body mass indices. During the end-expiratory breath hold, we were able to image 3–9 rings (median 5) in non-CF and 2–7 rings (median 5) in CF.

Human tracheal cartilage ring analysis

Sagittal plane imaging—In the sagittal view (Figures 1A–B), we obtained cross-sectional images of the tracheal cartilage rings in the midline. Inter-ring distances, for all extra-thoracic cartilage rings studied, were similar between non-CF and CF (Figure 2A) except for the distance between ring 5–6 which was significantly greater in the non-CF group ($p < 0.05$). Compared to tracheal cartilage rings in non-CF adults, rings in CF adults had a greater area and were thicker (Figures 2B and C). The area of rings 3–6 and thickness of rings 2–7 contributed the most to this difference between non-CF and CF. In contrast, no difference was identified in ring height between the two groups (Figure 2D). On a per-person average basis, none of these ultrasound measurements had a significant linear correlation with FEV_1, and no correlation was observed between the subject's height and weight with any of the above parameters (data not shown).

Transverse plane imaging—In the transverse view (Figures 1C–D), we measured the ring thickness in the midline and tracheal width, and calculated the roundness error. Similar to our sagittal view measurements, average ring thickness was greater in CF than non-CF subjects with rings 6 and 7 having the greatest difference (Figure 2E). On a per-person average basis, ring thickness had a significant linear correlation with FEV_1 for people with CF ($R^2 = 0.39$, $p < 0.05$, slope = -0.0004 ± 0.0001 cm/% predicted, y-intercept = 0.22 ± 0.009). The tracheal width was also larger in CF (Figure 2F). In the non-CF group, the tracheal width (cartilage ring size) was largest proximally with narrowing in more distal rings. Interestingly, the rings narrowed initially in the CF group but then showed an increase in tracheal width beyond ring 4, and showed a statistically significant difference for rings 5 and 6. On a per-person average basis, tracheal width had a significant linear correlation with FEV_1 for people with CF ($R^2 = 0.39$, $p < 0.05$, slope = -0.005 ± 0.002 cm/% predicted, y-intercept = 2.34 ± 0.10). The roundness error was larger in CF, suggesting that the tracheal cartilage rings were less circular in shape in adults with CF compared to non-CF (Figure 2G). Ring 3, which was in the narrowest zone of CF tracheal rings (Figure 2F), showed the biggest difference between the two groups (Figure 2G).

Ring thickness variability—Interestingly, we found that the thickness of individual cartilage rings within a subject appeared to be more variable in CF than non-CF (Figures 3A and B). To quantify this variability, we calculated the standard deviation of cartilage ring thickness (sagittal view) for individual subjects and then compared these values between non-CF and CF. Adults with CF had a significantly larger standard deviation from the mean ring thickness when compared to non-CF, suggesting greater variability in ring thickness within individual CF subjects (Figure 3C).

CT scan measurements—We were unable to adequately visualize and measure individual tracheal cartilage rings on images from chest CT scans. However, tracheal width was measured on CT scan images and there was a highly significant correlation with measurements obtained from sonographic images ($R^2 = 0.99$, $p < 0.0001$, slope = 0.77 ± 0.02, y intercept = 4.61 ± 0.45, and x intercept = −5.93). These findings demonstrate the accuracy of the ultrasonographic technique (Figure 4).

Maximal cartilage thickness in newborn CF pigs

Our discovery of cartilage abnormalities in humans with CF led us to re-examine tracheal cartilage in the newborn CF pig. In a prior study, we found that newborn CF pig tracheas were smaller in caliber and less circular in shape than non-CF.[13] We analyzed our previously collected non-CF and CF pig histology and obtained a new measure of tracheal cartilage: maximal ring thickness, which we report on a per-animal basis. We found newborn CF pig maximal cartilage ring thickness to be significantly greater than non-CF (Figure 5A). We then acquired the same measure from our transverse view, human, ultrasound scans. Similar to CF pig tracheal cartilage, we found that on a per-person basis people with CF had a significantly greater maximal cartilage ring thickness, compared to non-CF human subjects (Figure 5B).

DISCUSSION

Tracheal cartilage ring structural abnormalities are already present at birth in both CF pigs[13] and CF mice.[14, 22] Whether these abnormalities are present in humans with CF has not been previously described. Studies in the human newborn period are technically difficult. Using ultrasonography, we found that extra-thoracic tracheal cartilage rings in adults with CF were thicker, had a greater area, were more irregularly shaped, and were more variable in size within a subject.

Trachea structural abnormalities in CF

Upper and lower airway structural abnormalities are a common finding in older people with CF. Over two thirds of adults with CF had tracheomalacia identified on CT scan images,[16] tracheal diameter enlargement was frequently present on chest radiographs from people with CF,[23] and sinus hypoplasia is a common finding in CF subjects.[24] In the current study, we observed a negative correlation of cartilage ring thickness and tracheal width with FEV_1 raising the possibility that chronic inflammation and infection contributed to the reported abnormalities. Despite this, there is increasing evidence of airway structural disease being present within months of birth.[25–29] We previously described less circular and irregularly shaped tracheas on CT scans performed in infants and young children with CF as well as a reduced tracheal caliber size in infants with CF.[13] Moreover, tracheomalacia was diagnosed by bronchoscopy in 15% of young children with CF (median age 16 months), compared to a rate of approximately 0.05% in the general pediatric population.[15] The contribution of our tracheal findings to malacia is unclear. It seems that thickened cartilage rings in CF, through structural reinforcement, would more likely protect against tracheomalacia than contribute to it. In contrast, perhaps the irregular cartilage morphometry creates structurally weak and collapsible tracheal regions, an effect possibly exacerbated by expanded luminal width.[15]

Primary versus secondary

Our findings raise the question of whether human CF trachea abnormalities are secondary to infection, inflammation, and airway wall remodeling or a primary developmental abnormality due to loss of CFTR. The correlation between cartilage ring thickness and tracheal width with FEV_1 are consistent with a secondary consequence. However, the similarities between the abnormal trachea and cartilage ring structural features in newborn CF pigs, which lack airway infection and inflammation, and those found in this study in adults with CF suggest a primary consequence of CFTR disruption. Both newborn CF pigs and adults with CF have an irregularly shaped trachea, thicker cartilage rings, and more variable appearing cartilage ring structure within an individual subject. Moreover, airway developmental abnormalities have been reported in CF mice and recently in the CF rat.[30] In total, these findings suggest a role for CFTR in airway development and that loss of CFTR causes a common defect across species. In contrast to the newborn CF pig, which has a reduced tracheal lumen caliber, we discovered an increased lumen caliber in adults with CF. These data agree with other studies of tracheal size in adults with CF, but may suggest that the larger size of the adult CF trachea is likely a consequence of longstanding inflammation and chronic cough in this adult population.

How might these morphological abnormalities affect airflow? Altered tracheal width could have a dramatic impact on airflow resistance and airflow velocity in the trachea and more distal airways. Abnormal airflow may impact deposition patterns of inhaled particulate and aerosolized drugs. Moreover, these deposition patterns may be further altered if the irregularly shaped cartilage rings contribute to abnormal lumen morphometry.

Advantages and limitations

Our study has both advantages and limitations. Strengths include: (1) Comparisons were made with cartilage ring data from newborn pigs – a model that recapitulates human CF disease in multiple organs. The *in utero* development of the airway tree is similar between pigs and humans,[31] and the similar changes in ring thickness between newborn CF pigs and adults with CF suggest the possibility of a congenital abnormality. (2) Comparisons between CT and sonographic data were highly correlated, supporting the accuracy of the measurements. Limitations include: (1) Our study was done in adults, and we are unable to definitively answer the question of primary versus secondary cartilage changes. Evaluation of the neonate and infant CF trachea will further increase our knowledge regarding primary versus secondary abnormalities related to loss of CFTR function. With ongoing advances in ultrasound imaging technology and newborn screening protocols for CF, studies at earlier time points might be feasible in the future. (2) We only investigated the proximal, extra-thoracic cartilage rings. However, since the most severe congenital abnormalities in the tracheas of CF pigs (unpublished findings) and mice are present in the subglottic/proximal trachea,[14] this area might be the most abnormal in CF humans. (3) We could only assess the anterior portion of the tracheal cartilage rings, since the air in the lumen casts a shadow that did not allow the visualization of the posterior aspect of the trachea. Although we were able to assess several characteristics of the rings as described above, we were unable to assess for the presence of tracheomalacia or expiratory, posterior wall collapse.

CONCLUSION

In summary, adults with CF have abnormal cartilage rings with increased thickness and outer diameter, a reduced circularity, and show greater variability within a subject. Data from assessment of newborn pig tracheal cartilage rings also demonstrate reduced circularity and increased thickness, suggesting that perhaps some of the changes seen in tracheal cartilage rings in adults with CF are congenital in origin.

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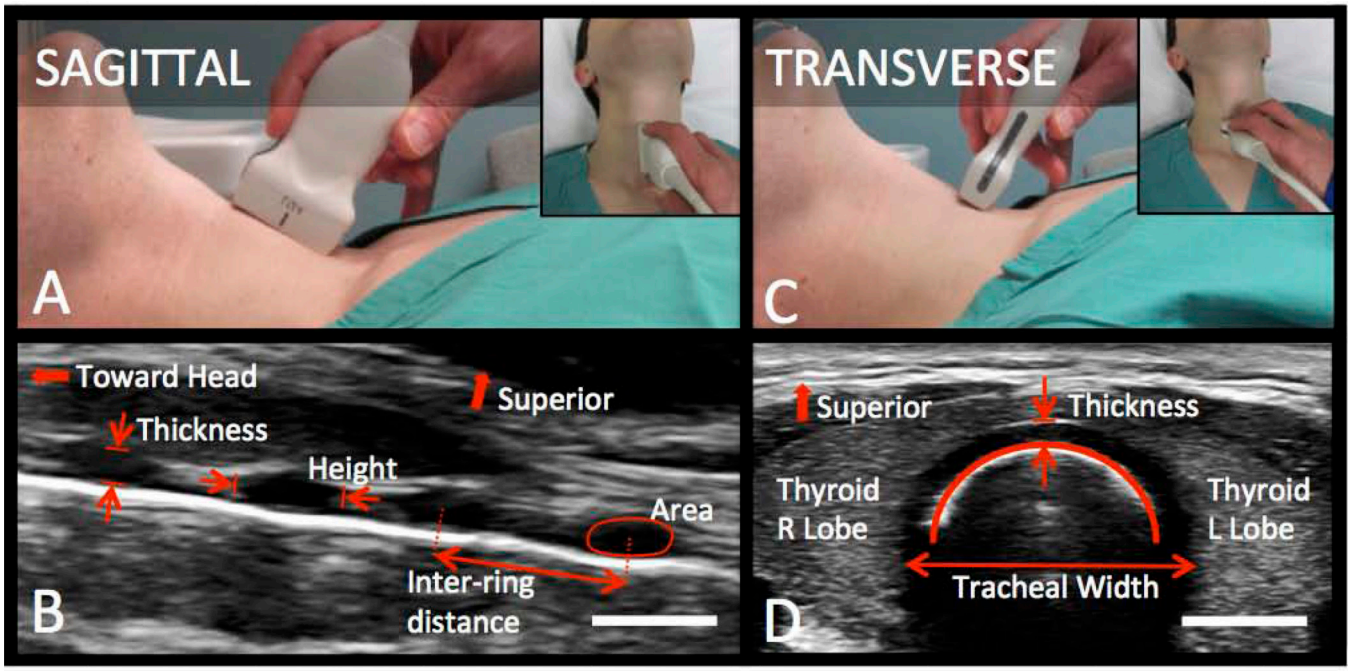

Figure 1. Ultrasound of the tracheal cartilage rings using a linear transducer

(A) Subject is in the supine position with neck extended. The ultrasound probe is positioned longitudinally in the midline of the neck for sagittal plane imaging. (B) Sonogram in the sagittal plane showing multiple tracheal cartilage rings in cross-section. Cartilage ring measurements obtained in the sagittal plane were: ring area, height, thickness and inter-ring distance. (C) The ultrasound probe is positioned perpendicular to the neck for transverse plane imaging. (D) Sonogram in the transverse plane showing a tracheal cartilage ring. Tracheal cartilage rings were seen surrounded by the thyroid gland. Cartilage ring measurements obtained in the transverse plane were: ring thickness, tracheal width, and roundness error.

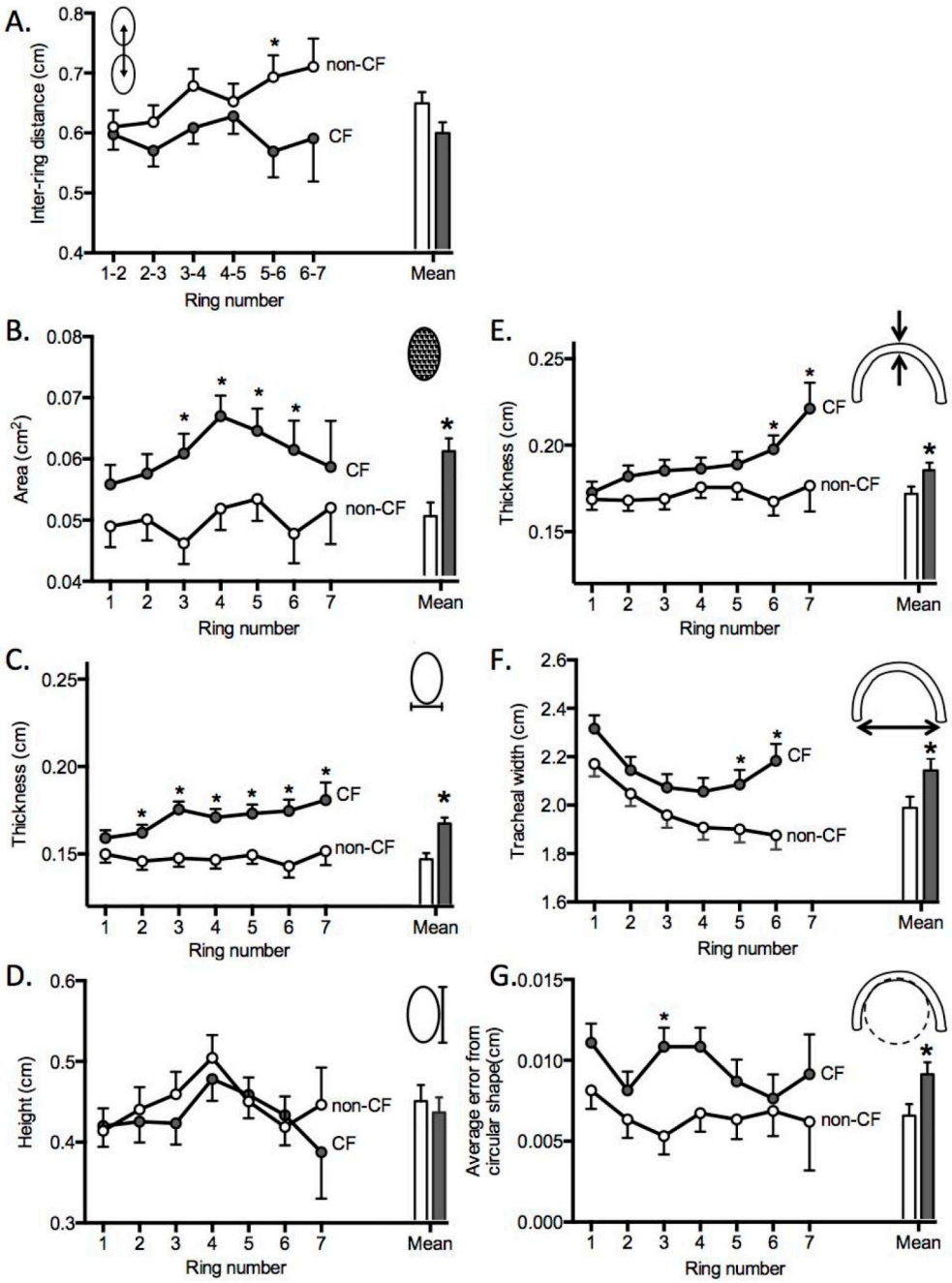

Figure 2. Tracheal cartilage ring measurements

Sagittal view. (A) Inter-ring distance - distance between tracheal cartilage rings. (B) Ring area. (C) Ring thickness. (D) Ring height. *Transverse view*. (E) Ring thickness. (F) Tracheal width. (G) Roundness error. Open symbols/bar denote non-CF and closed symbols/bar denote CF. Data are mean ± SEM. * $p < 0.05$.

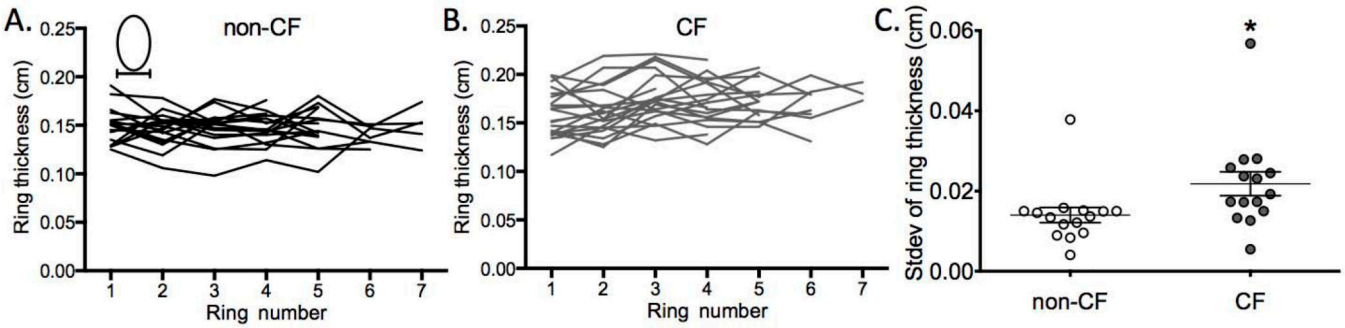

Figure 3. Profile plots for tracheal cartilage ring thickness in non-CF and CF adults

Tracheal cartilage ring thickness in non-CF (A) and CF (B). Each line represents data from an individual subject. (C) Standard deviation of ring thickness for each subject. Each symbol represents the standard deviation for ring thickness calculated from all rings from an individual. Lines represent mean and SEM. $p < 0.05$.

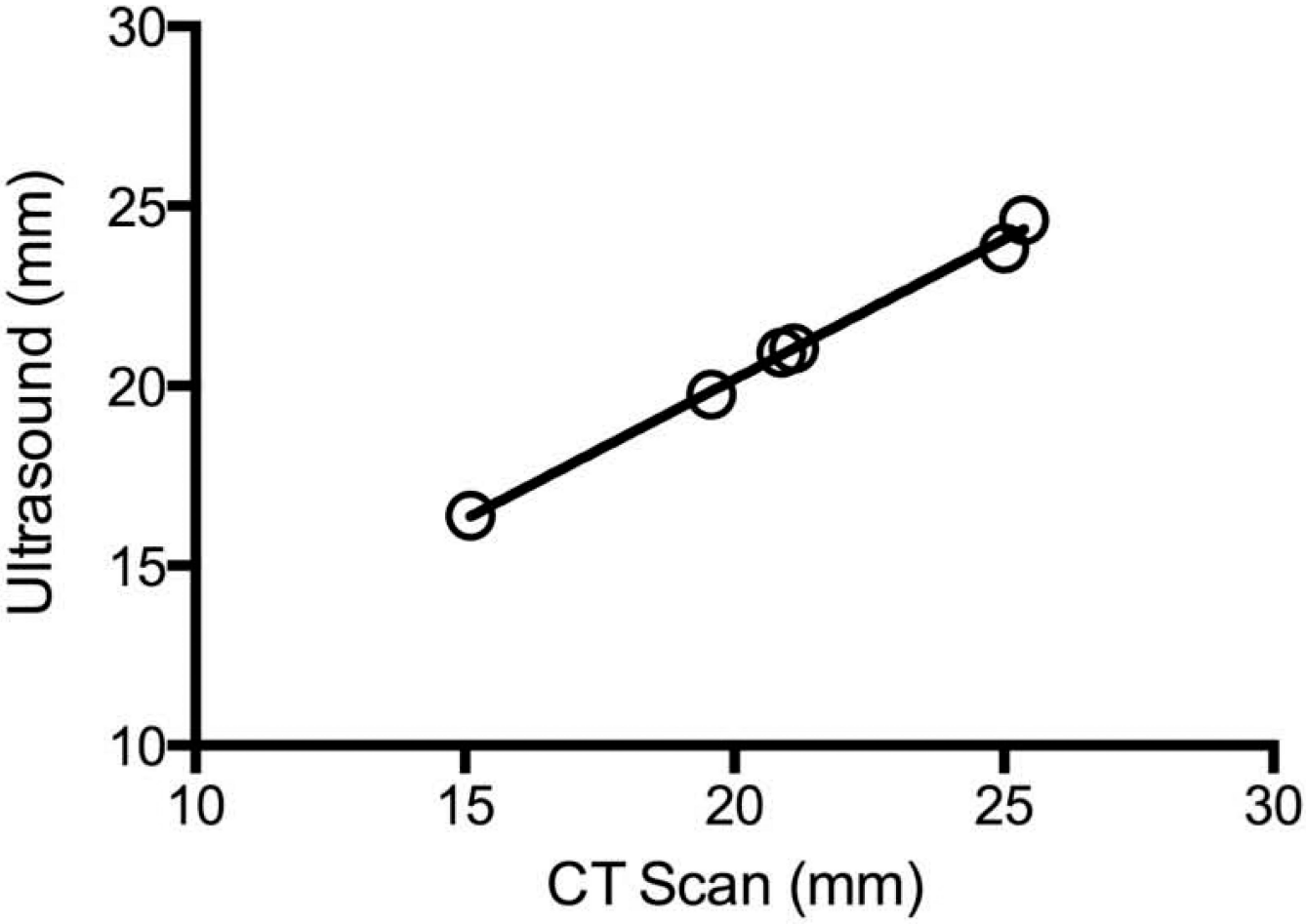

Figure 4. Tracheal width measurements from ultrasonography and chest computed tomography scans

Linear regression of tracheal width from CT scan and ultrasonography data. Each symbol represents data from an individual CF subject. $R^2 = 0.99$, slope = 0.77 ± 0.02, y intercept = 4.61 ± 0.45, x intercept = −5.93.

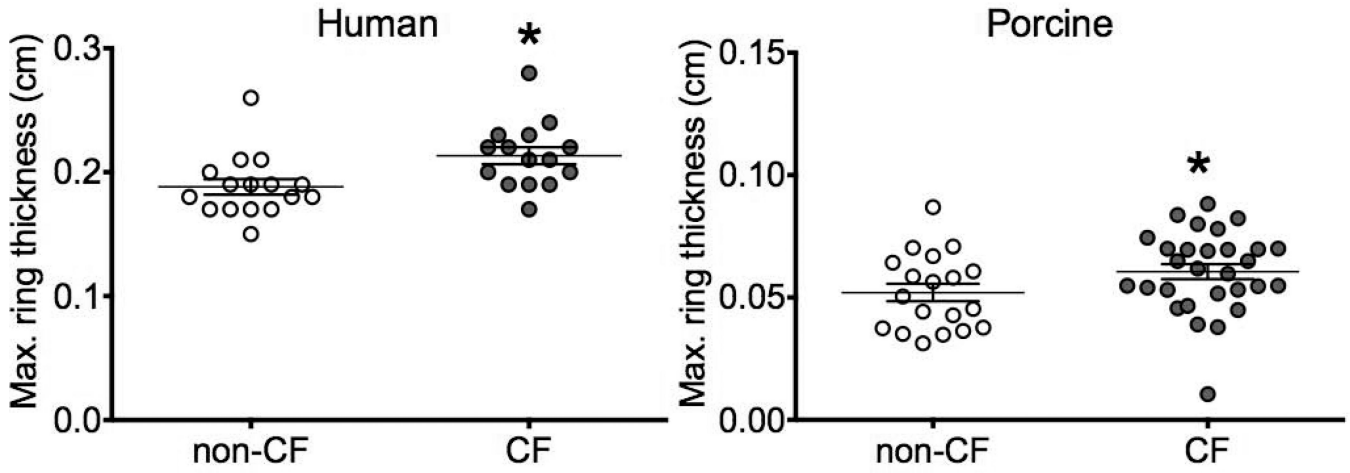

Figure 5. Maximal tracheal cartilage ring thickness

(A) Adult human tracheal cartilage ring maximal thickness obtained from ultrasonographic measurements. (B) Newborn porcine tracheal cartilage ring maximal thickness obtained from histological measurements. Each symbol represents the maximal ring thickness for an individual subject. Data are mean and SEM. $p < 0.05$.

Table 1

Characteristics of study participants

	non-CF (n=18)	CF (n=21)
Age (yrs)	26.2 ± 4.4	25.4 ± 3.5
Male	9 (50%)	8 (38%)
Weight (kg)	68.6 ± 13.6	59.5 ± 10.4*
Height (cm)	171.0 ± 9.3	164.4 ± 6.9*
BMI (kg/m^2)	23.3 ± 3.4	22.0 ± 3.5
FEV_1 (L)		1.77 ± 0.74
FEV_1 % predicted		50.8 ± 24.1
ΔF508 homozygous mutation		16/21 (76.2%)
ΔF508 heterozygous mutation		2/21 (9.5%)
P. aeruginosa colonization		18/21 (85.7%)

* $p < 0.05$