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Journal

Annals of Biomedical Engineering, 39(6)

ISSN

0090-6964

Authors

Lee, DongYoub

Willits, Neil

Wexler, Anthony S

Publication Date

2011-06-01

DOI

10.1007/s10439-011-0279-4

Annals of Biomedical Engineering, Vol. 39, No. 6, June 2011 *(© 2011)* pp. 1805–1814
DOI: 10.1007/s10439-011-0279-4

Detecting Alterations in Pulmonary Airway Development with Airway-by-Airway Comparison

DONGYOUB LEE,[1] NEIL WILLITS,[2] and ANTHONY S. WEXLER[1,3,4,5]

[1]Department of Mechanical and Aerospace Engineering, University of California, One Shields Ave., Davis, CA 95616, USA; [2]Department of Statistics, University of California, Davis, CA 95616, USA; [3]Air Quality Research Center, University of California, Davis, CA 95616, USA; [4]Department of Civil and Environmental Engineering, University of California, Davis, CA 95616, USA; and [5]Department of Land, Air and Water Resources, University of California, Davis, CA 95616, USA

(Received 6 December 2010; accepted 12 February 2011; published online 23 February 2011)

Associate Editor Kenneth R. Lutchen oversaw the review of this article.

Abstract—Neonatal and postnatal exposures to air pollutants have adverse effects on lung development resulting in airway structure changes. Usually, generation-averaged analysis of airway geometric parameters is employed to differentiate between pulmonary airway trees. However, this method is limited, especially for monopodial branching trees such as in rat airways, because both quite proximal and less proximal airways that have very different structure and function may be in the same generation. To avoid limitations inherent in generation averaging, we developed a method that compares two trees airway-by-airway using micro CT image data from rat lungs. This computerized technique (1) identifies the geometry and architecture of the conducting airways from CT images, (2) extracts the main tree, (3) associates paired airways from the two different trees, and (4) develops summary statistics on the degree of similarity between populations of animals. By comparing the trees airway-by-airway, we found that the variance in airway length of the group exposed to diffusion flame particles (DFP) is significantly larger than the group raised in filtered air (FA). This method also found that rotation angle of the DFP group is significantly larger than FA, which is not as certain in the generation-based analysis. We suggest that airway-by-airway analysis complements generation-based averaging for detecting airway alterations.

Keywords—Development disruption, Pulmonary airways, CT image, Rat lungs, Airway-by-airway comparison, Diffusion flame particles.

INTRODUCTION

Air pollutants, such as particulate matter (PM) and ozone, have been shown to adversely affect human health. Epidemiologic studies suggest that childhood chronic exposure to air pollution is associated with reduced lung function growth.[1,2,5,6,8,18]

Also there are a growing number of mechanistic studies using animal models, showing that chronic exposure to air pollutants results in various lung structure changes in both conducting and alveolar regions. The lungs of mice chronically exposed perinatally to traffic-related PM are less alveolarized than control.[14] Postnatal exposure of rats to ultra-fine particles with a high fraction of organic compounds reduce conducting airway size at adulthood.[13] Rats exposed to sidestream smoke in the postnatal period were found to have accelerated airway epithelial differentiation.[10] A study of perinatal exposure to sidestream tobacco smoke in the rhesus monkey found decreased alveolar number and size and an increase in respiratory bronchial volume.[1] Airway size changes have been observed in distal airways of Rhesus monkeys following postnatal exposure to ozone.[4] Ozone exposures over 18 months in young monkeys resulted in significant increases in the volume proportion of the respiratory bronchioles.[23]

To differentiate airway structure in the conducting region between different pulmonary airway trees, airways were observed only near terminal bronchioles[4] or along the main pathway.[15] Recently Lee *et al.*[13] found changes in airway geometry due to particle exposures. These observations were based on detailed airway architecture of the entire conducting airway of multiple rats using a generation-average analysis, where the airway size and branching angle were averaged as a function of generation to quantify airway structure changes after chronic particle exposure. Although generation-based analysis is widely used, since it is a very simple and efficient way to present overall airway

Address correspondence to DongYoub Lee, Department of Mechanical and Aerospace Engineering, University of California, One Shields Ave., Davis, CA 95616, USA. Electronic mail: dolee@ucdavis.edu

architecture, it does not properly categorize airways especially for highly monopodial airways of small animal lungs such as rats or mice. In addition, averaging over many airways in a generation may obscure severe geometry changes that occur in a small fraction of a generation's airways.

In this study, we suggest an alternative way to detect airway structure changes by comparing pulmonary airway trees on an airway-by-airway basis. We applied this method to rat lungs exposed to flame generated particles during a three-week postnatal development period and compared them to lungs from rats raised in filtered air. The airway-by-airway analysis has potential to detect structural differences between two groups that are hidden or hard to detect in the generation-average analysis.

METHODS

Animal Exposure and Preparation of the Lungs

Particles were generated by an annular tubular burner that can be run in different modes to generate a variety of environmentally relevant particle types. We selected diffusion flame particles (DFP) among various air pollutants because the majority of particles emitted from combustion sources contain elemental carbon. Particle concentration and size were controlled by varying ethylene fuel flow rate between 0.22 and 0.25 L/min with a surrounding co-flow of 30 L/min of clean air. The flame was surrounded by a nitrogen jacket flowing at 10 L/min to prevent oxidation of the particles. All flame products from diffusion flames were mixed with HEPA/CBR (Chemical Bacteriological Radiological)-filtered air to obtain the desired dilution ratio.

Particle size distribution and number concentration were measured in the exposure chamber with a TSI 3071 SMPS (Scanning Mobility Particle Sizer) or TSI 3775 CPC (Condensation Particle Counter). The diffusion flame produced a concentration of 2.4×10^4 particles/cm^3 in the exposure chamber with a number mean aerodynamic particle diameter (NMAD) of 230 nm with a geometric standard deviation of 1.89 and a mass concentration of 72 μg/m^3; we refer to these as DFP. To analyze elemental (EC) and organic (OC) carbon content, particles were sampled simultaneously from exposure and filtered air chambers twice weekly onto Pallflex Tissuquartz filters. DFP was sampled for 180 min per filter. The filters were analyzed by a Sunset Labs OC/EC instrument. OC for FA and DFP was 3.2 and 8.7 μg/m^3, respectively, and EC for FA and DFP was 0.3 and 57.5 μg/m^3, respectively. Organic carbon content in the FA exposures is likely due to dander and food particles in the chamber. More details were reported previously.[13]

Litters of Sprague-Dawley rat pups with a lactating mother were delivered from the vendor (Harlan Laboratories) and were housed in filtered air chambers in AAALAC approved facilities. All procedures were part of an IACUC approved animal protocol. Male pups housed with lactating mothers were placed in filtered air chambers at age 1 day. Neonatal rats were exposed to particles for 6 h a day, 5 days a week for 19 days starting when the pups were 7 days old and ended at 25 days old. At 21 days of age, the rat pups were weaned and transferred into an open wire mesh rodent inhalation cage module with two animals per cage. An age-matched set of pups was exposed to filtered air (FA) using the same protocol. At 28 days old, the animals were transferred from the exposure chambers to HEPA filtered enclosures where they matured until 80–81 days of age when lung casting for airway architecture analysis were conducted. Food (Purina rodent chow) and micro-filtered deionized water are provided *ad libitum*.

All animals were euthanized through intraperitoneal injection of pentobarbital (150 mg/kg) of body weight. Lungs were fixed in chest via tracheal cannula with Karnovsky's fixative at 30 cm H_2O pressure for 1 h, then removed from the chest and stored in fixative. Fixed lungs were placed in buffered saline prior to casting. Silicone RTV was introduced to the lung through the trachea under a slight negative pressure (-80 mmHg) until it reached the distal airways. The silicone RTV was allowed to cure for 48 h, after which the airway tissue was removed with bleach. Full details about animal exposure and lung preparation can be found in our previous studies.[11,13]

Architecture Extraction from CT Data

Lung casts were imaged using a commercially available microCT scanner, MicroCAT II (Siemens, Knoxville, TN) in high resolution mode. The image was reconstructed using the Feldkamp reconstruction algorithm with corresponding voxel size of 53 μm $\times$ 53 μm $\times$ 53 μm. Image resolution was 51 μm (Fig. 1).

Custom software was employed to extract the branching patterns of conducting airways, including airway diameter, length, branching angle, rotation angle as well as connectivity between airways from the CT images.[11,12] This was accomplished using a flexible bifurcation model. The software searched for a parameter set that minimized the distance between the airway CT image and the bifurcation model at each bifurcation, thereby characterizing airway architecture. The software reported average diameter when the airway diameter changed along its length. Branching angle is defined as the angle between parent branch and its daughter branch. Rotation angle is defined as

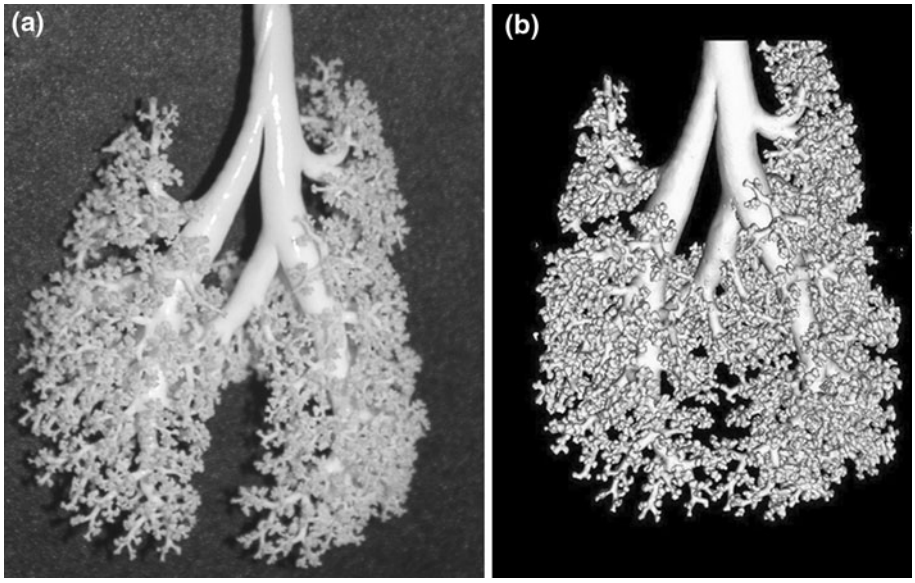

FIGURE 1. Example of (a) lung airway cast and (b) 3D reconstruction of CT image from the cast.

the angle between successive bifurcation planes, where the bifurcation plane is defined by a parent branch and its two daughters. The vast majority of bifurcations were in-plane or nearly so. Using computerized analysis of lung cast CT images, most conducting airways were measured but the alveolar region was not considered in this study.

Generation-Average Analysis

Lung airways have a tree branching structure. Starting from trachea the airway successively bifurcates into many branches. In rats, some airways terminate after relatively few rounds of branching, whereas others terminate after many more. Normally the size and branching angle of two daughter branches from the same parent are not same. These asymmetries in structure and branching are different between species. Trees with high asymmetry are termed monopodial. Airway diameter and length are important because they are directly related with air flow resistance and branching, and rotation angles are also important geometry parameters that could be altered by airway remodeling and changes in smooth muscle orientation.

The most common way to describe airway tree architecture is to plot average values of various geometry parameters such as airway size and branching angle as a function of generation.[25] Despite the fact that generation-based analysis has substantial limitations for describing monopodial branching trees, it is a simple and useful way to show differences between airway trees.[13]

Airway-by-Airway Comparison

While proximal airways, such as the trachea and lobe branches, have regular branching patterns, in distal regions it is more difficult to distinguish branches since the diameters of the two daughters are similar and inter-subject variability increases in smaller airways.[11,16] As a result, we limited the application of airway-by-airway analysis to the main tree. The airway-by-airway comparison algorithm is outlined below.

Step 1: Extracting the Main Tree from All the Conducting Airways

The number of sub-airways attached to a branch (airways that are distal to a selected airway) is used as a criterion to determine whether the branch is part of the main tree or not. Considering that conducting airways are pathways to the alveolar region, a branch with many sub-airways will perfuse more alveoli, so those with many sub-airways are considered part of the main tree. In this study, we identified a branch with the main tree if the number of airways attached to it were equal to or larger than 14. Note that a dichotomous tree with three generations has 14 daughter branches.

Step 2: Associate Paired Airways from the Two Different Airway Trees

To compare airways to each other, we must determine which airways correspond to each other in the tree. These two comparable airways are called pairs. This is the most challenging part of the algorithm since simple changes, such as insertion or deletion of a

branch is difficult for a computerized algorithm to unravel. Six characteristic parameters of each branch are employed to associate pairs: (1) airway diameter, (2) number of sub-airways, (3) parent branch id, (4) cumulative airway length to the branch, (5) coordinates of the branch, and (6) if its sibling is part of the main tree. The following equation quantifies how we use these parameters to determine an objective function. Smaller values of the objective function indicate airways that are more likely to be paired.

$$\text{Objective} = 1.5 \cdot \frac{d_1}{d_2} + 0.2 \cdot \frac{n_1}{n_2} + 0.5 \cdot \frac{\sum l_1}{\sum l_2} + 0.2 \cdot \sqrt{\sum_{i=1}^{3} (x_{1i} - x_{2i})^2} + k + j \quad (1)$$

In Eq. (1), d is airway diameter, n is number of sub-airways, l is length, and x_i is coordinate of the branch, $i = 1, 2, 3$ indicates x, y, z coordinate, respectively. k is equal to 0 if parents of the two comparing branches are also pairs and equal to 1 otherwise, and j is equal to 0 if the branch's sibling is also main tree and equal to 1 otherwise. In the first three terms, the larger of the two comparing values is placed in the numerator.

The coefficients were adjusted by trial and error based on the tests with artificial airway trees and real rat airway casts. The number of sub-airways in Eq. (1) was weighed less because this value was highly dependent on the lung airway casting condition and CT image resolution. High variability in this value was inevitable at this stage. Diameter was weighed more because observation of real airway trees showed that paired airways had similar size. The weighting of the coordinate term was adjusted so that its value was comparable in magnitude to other terms, therefore this coefficient value should be smaller for larger species such as human. The objective was calculated for all possible combinations and the two branches with smallest objective were considered a pair. To reduce the effects of potential wrong pair associations, a branch was considered not paired if the objective function value in Eq. (1) was larger than 15. The value 15 was determined experimentally based on the objective function values from comparisons of rat airway trees. This criterion value should be selected carefully because the algorithm could mis-associate pairs if it was too large whereas the algorithm might not associate correct pairs if it was too strict.

Step 3: Compare Main Trees from Two Animals to Each Other

To measure differences between two trees, the difference between each pair normalized by their mean value was calculated for airway diameter and length and the difference between each pair was calculated for branching angle and rotation angle, using the Eq. (2). In Eq. (2), x_A and x_B were geometric parameters such as airway diameter for different trees A and B, respectively, and N was the total number of pairs associated between the two main trees (for example, Fig. 2a). Comparing the differences for each pair could detect differences between trees even when average features of the two trees are same. For example, tree A had a mother branch with diameter 5 and two daughters with diameter 4 and 1, while tree B had a mother branch with diameter 5 and two daughters with diameter 4.2 and 0.8. Although the two trees were different in daughter diameters, generation-average analysis would consider them identical because the average diameter of daughter generation was equal to 2.5 for both trees. On the other hand, diameter difference of the two trees calculated using Eq. (2a) was not zero. This simple example demonstrates how the airway-by-airway analysis complements generation-average analysis.

$$\text{Difference} = \frac{\sum_{i=1}^{N} \left| \frac{x_A - x_B}{(x_A + x_B)/2} \right|_i}{N} \quad \text{for airway size} \quad (2a)$$

$$\text{Difference} = \frac{\sum_{i=1}^{N} |x_A - x_B|_i}{N} \quad \text{for angles} \quad (2b)$$

We performed extensive tests using artificial trees as well as real airway trees from the rat casts to validate this pair-association algorithm, part of which is presented in the "Results" section.

Step 4: Statistics

The difference in Eq. (2) was obtained for all combinations of subjects from both FA and DFP groups. To compare the magnitudes of geometric parameters between DFP and FA, we calculated the signed difference in Eq. (2) for all combinations of DFP and FA subjects. In this case, x_A indicates a geometric parameter for a DFP subject and x_B indicates one for a FA subject. If the average difference from all combinations had a positive sign, the parameter for DFP was larger than FA. For inter-subject variance of a geometric parameter for each group, absolute values were used in Eq. (2). The average difference from all combinations between subjects in each group indicated the inter-subject variability of the geometric parameter for each group. If the average value from the DFP subjects was larger than that from FA subjects, inter-subject variability of the geometric parameter for DFP was larger than that for FA.

To test the significance of the difference between DFP and FA, permutation tests with null distribution were performed on 50,000 permuted samples,

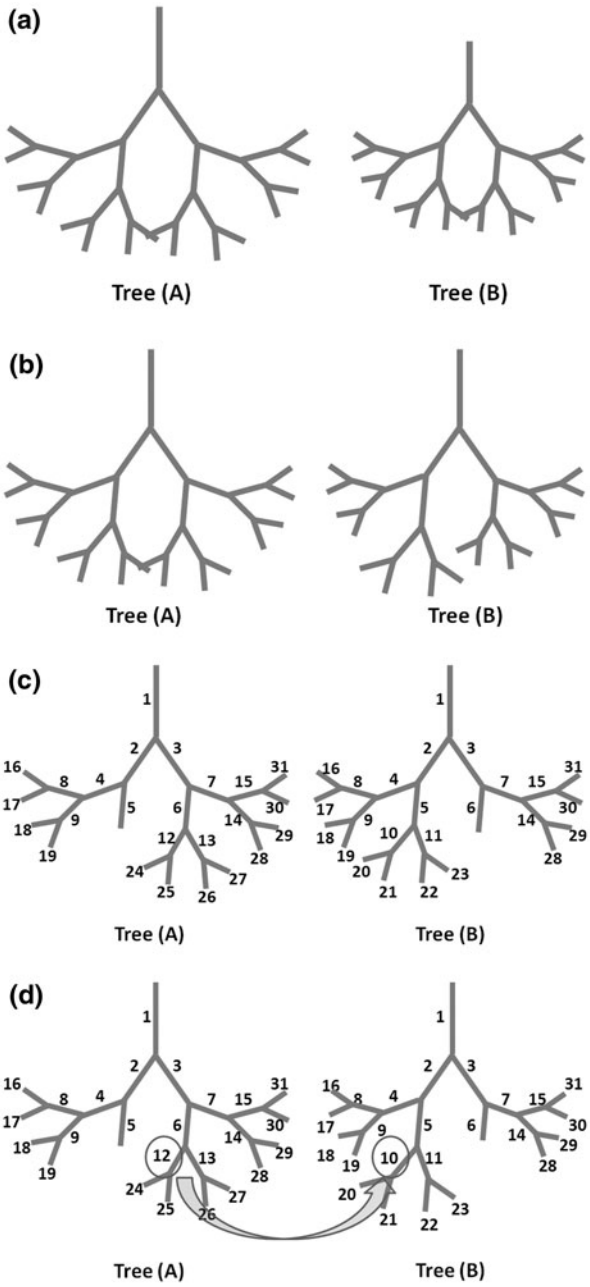

FIGURE 2. Artificial trees to test our pairs searching algorithm. (a) Two trees are different in airway size with constant ratio for all branches. (b) Two trees are different in airway size at random for each branch. (c) Two trees are different in topology. (d) Two trees are different both in airway size and topology.

summarized as follows. In this study we had an n by n matrix of differences calculated by Eq. (2), where n is the total number of subjects in the combined DFP and FA groups. We could then calculate any summary statistic from that matrix, such as the average paired difference between DFP and FA subjects. To determine whether the test statistic was surprising or not, a null distribution for the test statistic was generated. (1) The subjects were shuffled without respect to their group membership, so that both the rows and the columns of the matrix were shuffled in the same way. (2) The test statistic was recalculated based on the shuffled data. (3) Finally, this process was repeated a large number of times, computing the test statistic each time and giving a null distribution for that statistic. If the test statistic based on the original (unshuffled) data was sufficiently out in the tails of the null distribution, then the null hypothesis was rejected, with a p value equal to the proportion of trials when an even more extreme test statistic value was obtained. In this study, we investigated airway diameter, length, branching angle, and rotation angle.

RESULTS

Tests of Tree Comparison Algorithm

To test the pair-searching algorithm, artificial trees were generated. We performed extensive tests and several typical results are presented in this section. Figure 2a shows two airway trees with 4 generations. In tree A, daughter to parent diameter ratio and length to diameter ratio was 0.8 and 2.5, respectively, for all bifurcations and branching angle was 35°. Airway diameter and length of tree B were smaller than those of tree A by a constant size ratio for all airways. For a size ratio between 1 and 0.4, the algorithm was successful in associating pairs. When the size ratio was very small such as 0.3, the algorithm sometimes fails, however, such dramatic differences in airway geometric parameters have not been observed in actual airway trees. Figure 2b shows the case where airway diameter and length of tree B were different from tree A at random, i.e., $d_A = R_1 \times d_B$ and $L_A = R_2 \times L_B$ where d and L were airway diameter and length and R was a random number. Even for a wide range of R between 0.5 and 2, the algorithm successfully associated pairs. In Fig. 2c, branch 5 in tree A was a terminal airway while branch 6 in tree B was terminal one. This example demonstrated a topological difference between trees. In our algorithm, branches 12, 13, 24, 25, 26, 27 of tree A do not have corresponding pairs in tree B and branches 10, 11, 20, 21, 22, 23 of tree B do not have corresponding pairs in tree A. The algorithm was also successful when the two trees are different both in airway size and topology.

The algorithm could mis-associate pairs when the structural differences between trees were large. Figure 2d shows an example where the airway sizes were different as in Fig. 2b and the topology was different as in Fig. 2c. In this case, the algorithm wrongly associated branches 12, 13, 24, 25 of tree A with branches 10, 11, 22, 23 of tree B, respectively.

However, their objective function value (Eq. 1) was larger than 15, indicating those pair associations were wrong. Since the algorithm could mis-associate pairs and the chance of this occurring would increase in distal regions, the algorithm could only be applied to the main tree; the criterion for determining mis-associated pairs should be selected carefully. We also performed tests using real airway trees from the rat casts to validate the pair-searching algorithm (results are not presented).

Generation-Based Analysis

Body weight and lung weight for FA and DFP groups are presented in Table 1. Average number of airways analyzed was 4636 and 4864 for FA and DFP groups, respectively. There was no significant difference between DFP and FA both in body and lung weight.

TABLE 1. Body and lung weights in the different groups at age 80–81 days.

Study group	Body weight (g)	Lung weight (g)	Number of subjects
FA	365 ± 29	17.9 ± 1.7	33
DFP	362 ± 32	18.2 ± 2.1	9

No significant difference between exposed and FA groups in body and lung weight.

Figures 3a and 3b show airway diameter and airway length as a function of generation, respectively. Both airway diameter and airway length were not significantly different between FA and DFP for all generations. Branching angle for DFP was significantly larger ($p < 0.05$) than FA in 2 generations (Fig. 3c). Rotation angles for DFP are significantly larger than FA in 4 generations (Fig. 3d). However, there were no significant changes in both branching and rotation angle after Bonferroni correction.

Airway-by-Airway Analysis

Figure 4 shows signed differences in geometric parameters between DFP and FA groups calculated by Eq. (2). Airway-by-airway analysis indicated that there was no significant difference in the magnitudes of diameter, length, and branching angle, which agrees with generation-based analysis. However, rotation angle differences between DFP and FA were more

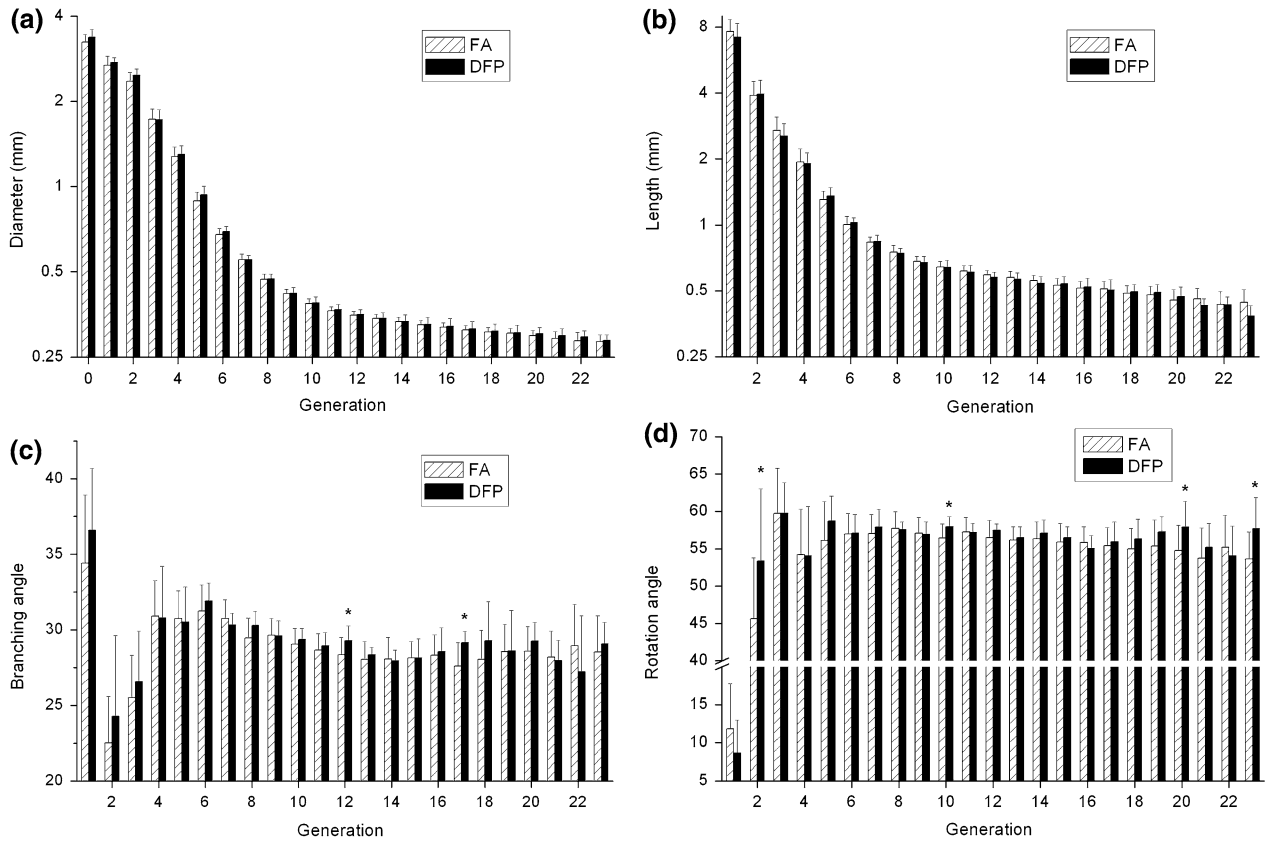

FIGURE 3. (a) Airway diameter (means ± S.D.) as a function of generation, (b) airway length as a function of generation, (c) branching angle as a function of generation, and (d) rotation angle as a function of generation in the FA and DFP group. *p value less than 0.05 from Student t test.

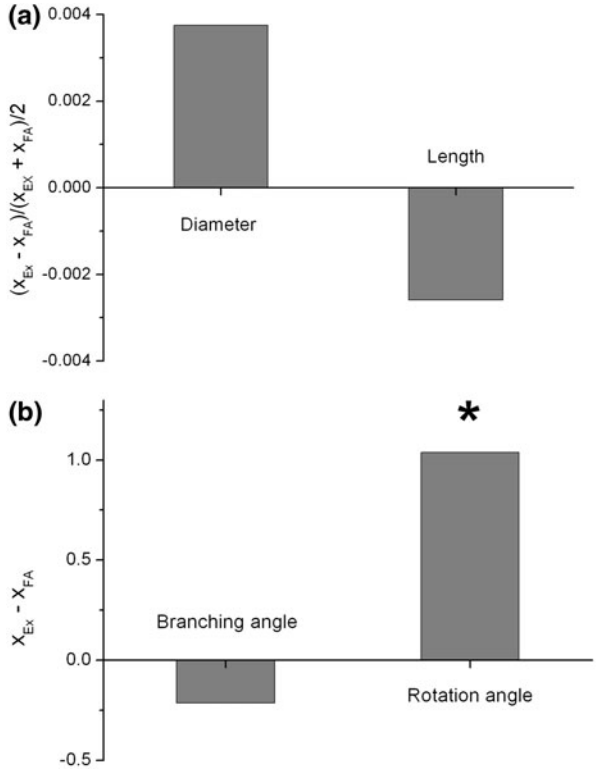

FIGURE 4. (a) Average normalized difference in airway diameter and length between DFP and FA and (b) average difference in branching angle and rotation angle between DFP and FA. Positive value indicates that the corresponding geometric parameter of DFP is larger than FA. *p value less than 0.05 from permutation test.

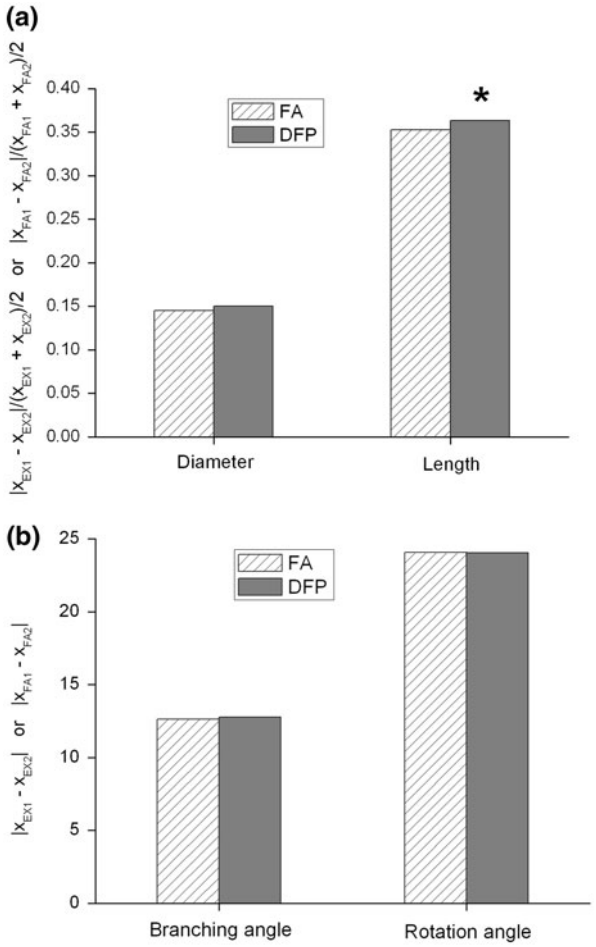

FIGURE 5. (a) Average normalized variability in airway diameter and length for DFP and FA and (b) average variability in branching angle and rotation angle between DFP and FA. *p value less than 0.05 from permutation test. EX1, EX2 and FA1, FA2 represent arbitrary subjects from DFP and FA groups, the value is from the all combinations of trees for each group not just two trees.

significant ($p = 0.020$) (Fig. 4b). Although generation-based analysis also showed that rotation angle of DFP was larger than FA in 4 generations (Fig. 3d), it was hard to say whether the changes in 4 among 23 generations were statistically meaningful or not. Therefore the permutation test in the airway-by-airway analysis was a good complement to the generation-based analysis.

Figure 5 shows inter-subject variances of the geometric parameters calculated by Eq. (2). The larger variance in length for DFP compared to FA indicated that lung airways for DFP have more variability in length than FA group. We also conducted this analysis for each lobe. Rotation angle for DFP was larger than FA for each lobe except for right cardiac (Table 2). Inter-subject variability in length for DFP was larger than FA for all lobes consistently (Table 3).

TABLE 2. Signed difference in rotation angles between DFP and FA for each lobe.

Lobe	Average difference	p value
RA	2.0	0.062
RC	−1.9	0.097
RD	1.3	0.16
RI	0.56	0.37
L	2.0	0.015

RA, RC, RD, RI, and L indicate right apical, right cardiac, right diaphragmatic, right intermediate, and left lobe, respectively.

DISCUSSION

Before the computerized methods for pulmonary airway analysis[10,11,19,20] were developed, manual measurements of sampled airways were used to observe airway size changes.[3] Some studies[3,15] suggested that the entire pulmonary arterial tree or airway tree can be fully characterized by measuring only the main pathway and its sub-branches as the pulmonary tree has highly self-similar structure at all scales. If airway structure information of the whole airways are

TABLE 3. Inter-subject variability in airway length for DFP and FA for each lobe.

Lobe	Variability for FA	Variability for DFP	*p* value
RA	0.34	0.35	0.36
RC	0.35	0.38	0.018
RD	0.37	0.38	0.35
RI	0.34	0.35	0.31
L	0.35	0.36	0.28

available either by manual or computerized methods,[11,12,17,19,20,22] a common way to describe the overall airway tree architecture is to present averaged geometric parameters as a function of Weibel generation. Although this method is a simple and useful way to show differences between airway trees,[13] averaging poses the risk of missing local changes especially for monopodial branching trees such as rat airways because both quite proximal and less proximal airways could be categorized in the same generation. Horsfield's scheme based on airway order numbering from terminal bronchioles to trachea is less affected by this problem.[9] However, our software cannot reliably identify terminal bronchioles so Horsfield's scheme cannot be applied. Note that both Weibel's and Horsfield's schemes compare averages of geometric parameter between trees whereas the airway-by-airway analysis presented here compares the associated pairs to judge the degree of difference between trees.

In this study, we suggest a method for comparing pulmonary airway trees based on airway-by-airway analysis and applied the method for comparing airway structure between DFP exposed subjects and FA subjects. Airway-by-airway analysis shows that rotation angle for DFP is significantly larger than FA ($p = 0.02$). Although the rotation angles for DFP are significantly larger than FA in 4 generations using a generation-averaged analysis, it is difficult to judge if the overall difference is significant or not because the rotation angles for DFP are smaller than the FA in many other generations. In fact, after applying a Bonferroni correction for multiple comparisons, rotation angle differences were not significant in any of the generations. This result demonstrates a merit of airway-by-airway analysis. In addition, airway-by-airway analysis enables us to employ statistics that test if inter-subject variability of geometric parameters is different between groups (Fig. 5). Inter-subject variances can be calculated for each generation in the generation-averaged analysis as well, however, there is no way to test the statistical significance of the difference between groups. Actually inter-subject variances of airway length for DFP are larger than FA in 14 generations and smaller in 9 generations (Fig. 6), so it is difficult to judge if the variance for DFP is significantly larger

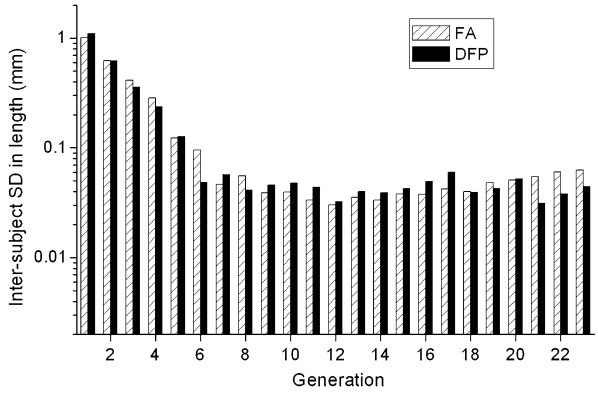

FIGURE 6. Inter-subject variability (standard deviation) in airway length for each generation.

than FA. Of course, the permutation test *p* value in the airway-by-airway analysis is not a universal ruler to judge the difference between groups but it is a useful quantitative measure.

Although airway-by-airway analysis suggests there are changes in rotation angle and variance of airway length between DFP and FA, overall the differences in airway structure seem minor. As discussed in earlier studies, [11,24] lung damage due to small particles may be caused by organic carbon mass rather than elemental carbon. More studies are necessary to explore the full range of air pollutants that disrupt normal lung development. Also, further study with other air pollutants is warranted to investigate if the airway-by-airway analysis can detect lung alterations that are hidden in the generation-based analysis.

Regional particle deposition changes depend on airway structure, such as airway size and alveolar volume attached to the airway, breathing condition, and particle size. These could disrupt airway development in only a specific range of airway sizes or locations. We grouped the main tree into three different airway sizes (diameter larger than 1.5 mm, between 1 and 1.5 mm, and smaller than 1 mm) and did the comparison for each group. However, we did not find any appreciable difference between airway size groups. That would be because the airway structure changes in DFP are not very significant.

Generation-based analysis[13,25] or analysis along a principal pathway[3,15] are certainly very efficient ways for differentiating between normal and abnormal pulmonary airways, especially when the differences in architecture between groups are appreciable.[13] Additional methods such as the airway-by-airway analysis would help to detect slight or local changes in lung airway structure. Substantial changes in a subpopulation at a generation may not be significant when averaged with other airways in a generation that may not have changed. But the changes in this subpopulation

may indicate substantial airway development disruption in a lobe or a portion of a lobe. For example, in this study we used average difference of all the airway pairs to measure the overall difference between trees, but if we investigate the difference from each pair one by one then airway-by-airway analysis could be used for detecting local alterations in the airways. As well, there are huge number of studies on the measures of dissimilarity (metrics) for comparing trees in the quantitative analysis of evolutionary trees (reviewed well by Steel and Penny[21]). Applying these tree comparison methods to our problem, in addition to the analysis done in this study, could provide more comprehensive information especially on the topological differences between pulmonary trees. Airway-by-airway analysis would be applicable to other studies such as understanding the relative contribution of genetics and randomness to airway structure in small animals similar to a recent study by Glenny *et al.*[7]

ACKNOWLEDGMENTS

Although the research described in the article has been funded in part by the United States Environmental Protection Agency through Grant RD-83241401-0 to the University of California, Davis, it has not been subject to the Agency's required peer and policy review and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

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REFERENCES

[1]Avdalovic, M., L. Putney, N. Tyler, W. Finkbeiner, K. Pinkerton, and D. Hyde. In utero and postnatal exposure to environmental tobacco smoke (ETS) alters alveolar and respiratory bronchiole (RB) growth and development in infant monkeys. *Toxicol. Pathol.* 37:256–263, 2009.

[2]Avol, E. L., W. J. Gauderman, S. M. Tan, S. J. London, and J. M. Peters. Respiratory effects of relocating to areas of differing air pollution levels. *Am. J. Respir. Crit. Care Med.* 164:2067–2072, 2001.

[3]Einstein, D. R., B. Neradilak, N. Pollisar, K. R. Minard, C. Wallis, M. Fanucchi, J. P. Carson, A. P. Kuprat, S. Kabilan, R. E. Jacob, and R. A. Corley. An automated self-similarity analysis of the pulmonary tree of the Sprague-Dawley rat. *Anat. Rec.* 291:1628–1648, 2008.

[4]Fanucchi, M. V., C. G. Plopper, M. J. Evans, D. M. Hyde, L. S. Van Winkle, L. J. Gershwin, and E. S. Schelegle. Cyclic exposure to ozone alters distal airway development in infant rhesus monkeys. *Am. J. Physiol. Lung Cell Mol. Physiol.* 291:L644–L650, 2006.

[5]Frischer, T., M. Studnicka, C. Gartner, E. Tauber, F. Horak, A. Veiter, J. Spengler, J. Kühr, and R. Urbanek. Lung function growth and ambient ozone: a three-year population study in school children. *Am. J. Respir. Crit. Care Med.* 160:390–396, 1999.

[6]Gauderman, W. J., E. Avol, F. Gilliland, H. Vora, D. Thomas, K. Berhane, R. McConnell, N. Kuenzli, F. Lurmann, E. Rappaport, H. Margolis, D. Bates, and J. Peters. The effect of air pollution on lung development from 10 to 18 years of age. *New Engl. J. Med.* 351:1057–1067, 2004.

[7]Glenney, R., S. Bernard, B. Neradilek, and N. Polissar. Quantifying the genetic influence on mammalian vascular tree structure. *PNAS* 104:6858–6863, 2007.

[8]Horak, Jr., F., M. Studnicka, C. Gartner, J. D. Spengler, E. Tauber, and R. Urbanek. Particulate matter and lung function growth in children: a 3-yr follow-up study in Austrian schoolchildren. *Eur. Respir. J.* 19:838–845, 2002.

[9]Horsfield, K., G. Dart, D. E. Olsen, G. F. Filley, and G. Cumming. Models of the human bronchial tree. *J. Appl. Physiol.* 31:207–217, 1971.

[10]Ji, C. M., C. G. Plopper, H. P. Witschi, and K. E. Pinkerton. Exposure to sidestream cigarette smoke alters bronchiolar epithelial cell differentiation in the postnatal rat lung. *Am. J. Respir. Cell Mol. Biol.* 11:312–320, 1994.

[11]Lee, D., M. V. Fanucchi, C. G. Plopper, J. Fung, and A. S. Wexler. Pulmonary architecture in the conducting regions of six rats. *Anat. Rec.* 291:916–926, 2008.

[12]Lee, D., S. S. Park, G. A. Ban-Weiss, M. V. Fanucchi, C. G. Plopper, and A. S. Wexler. Bifurcation model for characterization of pulmonary architecture. *Anat. Rec.* 291:379–389, 2008.

[13]Lee, D., C. Wallis, A. S. Wexler, E. S. Schelegle, L. S. Van Winkle, C. G. Plopper, M. V. Fanucchi, B. Kumfer, I. Kennedy, and J. Chan. Inhaled particles disrupt postnatal airway development. *J. Appl. Physiol.* 109:1115–1124, 2010.

[14]Mauad, T., D. H. Rivero, R. C. de Oliveira, A. J. Lichtenfels, E. T. Guimarães, P. A. de Andre, D. I. Kasahara, H. M. Bueno, and P. H. Saldiva. Chronic exposure to ambient levels of urban particles affects mouse lung development. *Am. J. Respir. Crit. Care Med.* 178:721–728, 2008.

[15]Molthen, R. C., K. L. Karau, and C. A. Dawson. Quantitative models of the rat pulmonary arterial tree morphometry applied to hypoxia-induced arterial remodeling. *J. Appl. Physiol.* 97:2372–2384, 2004.

[16]Phillips, C. G., and S. R. Kaye. Diameter-based analysis of the bronchial geometry of four mammalian bronchial trees. *Respir. Physiol.* 102:303–316, 1995.

[17]Raabe, O. G., H. C. Yeh, G. M. Schum, and R. F. Phalen. Tracheobronchial Geometry: Human, Dog, Rat, Hamster. LF-53. Albuquerque, NM: Lovelace Foundation for Medical Education and Research, 1976.

[18]Rojas-Martinez, R., R. Perez-Padilla, G. Olaiz-Fernandez, L. Mendoza-Alvarado, H. Moreno-Macias, T. Fortoul, W. McDonnell, D. Loomis, and I. Romieu. Lung function growth in children with long-term exposure to air pollutants in Mexico City. *Am. J. Respir. Crit. Care Med.* 176:377–384, 2007.

[19]Sauret, V., R. M. Halson, I. W. Brown, J. S. Fleming, and A. G. Bailey. Study of the three-dimensional geometry of the central conducting airways in man using computed tomographic (CT) images. *J. Anat*. 200:123–134, 2002.

[20]Sera, T., H. Fujioka, H. Yokota, A. Makinouchi, R. Himeno, R. C. Schroter, and K. Tanishita. Three-dimensional visualization and morphometry of small airways from microfocal X-ray computed tomography. *J. Biomech*. 36:1587–1594, 2003.

[21]Steel, M. A., and D. Penny. Distribution of the comparison metrics-some new results. *Syst. Biol*. 42:126–141, 1993.

[22]Tawhai, M. H., P. Hunter, J. Tschirren, J. Reinhardt, G. McLennan, and E. A. Hoffman. CT-based geometry analysis and finite element models of the human and ovine bronchial tree. *J. Appl. Physiol*. 97:2310–2321, 2004.

[23]Tyler, W. S., N. K. Tyler, J. A. Last, M. J. Gillespie, and T. J. Barstow. Comparison of daily and seasonal exposures of young monkeys to ozone. *Toxicology* 50:131–144, 1988.

[24]Warheit, D. B., T. R. Webb, V. L. Colvin, K. L. Reed, and C. M. Sayes. Pulmonary bioassay studies with nanoscale and fine-quartz particles in rats: toxicity is not dependent upon particle size but on surface characteristics. *Toxicol. Sci*. 95:270–280, 2007.

[25]Weibel, E. R. Morphometry of the Human Lung. New York: Academic, 1963.