Airway remodelling with spatial correlations: Implications for asthma pathogenesis

Christopher D. Pascoe, Francis H.Y. Green, John G. Elliot, Alan L. James, Peter B. Noble, Graham M. Donovan

Abstract

Airway remodelling is a cardinal feature of asthma in which airways undergo structural changes - in particular, increased airway smooth muscle mass and total airway wall area. Remodelling has long been thought to have functional consequences in asthma due to geometric effects that can increase airway narrowing and luminal occlusion. Prior studies have examined the distribution of remodelling between and within patients, but none have yet considered the possibility for spatial correlations in airway remodelling. That is, is remodelling clustered locally, or interrelated along proximal and distal locations of the bronchial tree? In view of recent interest regarding airway remodelling produced by mechanical stimuli, we developed a mathematical model to examine whether spatial correlations in airway remodelling could arise due to cycles of bronchoconstriction and mechanotransduction. Further, we compared modelling predictions to the spatial distribution of airway remodelling in lungs from subjects with and without asthma. Results indicate that spatial correlations in airway remodelling do exist in vivo, and cycles of bronchoconstriction and mechanotransduction are one plausible mechanism for their origin. These findings offer insights into the evolution of airway remodelling in asthma, which may inform strategies for treatment and prevention.

1. Introduction

‘Airway remodelling’ is an important feature of asthma, in which airways undergo structural modification, particularly increased thickness of the airway smooth muscle (ASM) layer and total wall thickness (Redington and Howarth, 1997; Stewart, 1996). While it has long been understood that the extent of airway remodelling contributes to airflow limitation (Bai et al., 1998; James and Wenzel, 2007), what has yet to be established is how remodelling across different airways is organized relative to one another – i.e. the spatial distribution of remodelling is unclear. Are airways with greater remodelling likely to occur near one another (on a local or regional basis), and/or are remodelling processes organized along interconnected branching pathways, or across disconnected airways in close proximity? Distribution of airway remodelling has been shown to be an important determinant of the final organ response, such as total lung resistance and compliance; for example, increased heterogeneity of airway remodelling produces greater functional impairment compared with a more homogenous distribution (Thorpe and Bates, 1997; Gillis and Lutchen, 1999; Pascoe et al., 2017b).

A separate but interrelated question relates to how such correlations in airway structure develop, which is directly relevant to the origin of airway remodelling in disease. One of the most influential studies of airway remodelling in recent years by Grainge et al. (Grainge et al., 2011) demonstrated that bronchoconstriction alone is sufficient to produce altered airway structure. Bronchoconstriction-induced remodelling could theoretically impact at all stages of life: that is, during development where the ASM contracts in utero (Parvez et al., 2006), or during an acute exacerbation experienced by a child or adult who has asthma. Importantly, if bronchoconstriction via mechanotransduction...
(Tschumperlin and Drazen, 2006; Fabry and Fredberg, 2007; Hill et al., 2018; Kılç et al., 2020) produces remodelling, it is reasonable to assume that this may manifest as structure which is spatially correlated.

Through use of a conceptual model (Tschumperlin and Drazen, 2006; Lutchen, 2016; Lutchen et al., 2017), we tested the feasibility of the hypothesis that spatially correlated airway remodelling could be formed by cycles of bronchoconstriction, mechanotransduction, and remodelling; referred to here as the bronchoconstriction-mechanotransduction-remodelling (BMR) cycle. We used a previously developed multi-branch airway tree lung model, adapted to include a simple heuristic remodelling algorithm based solely on the degree of bronchoconstriction occurring in each airway throughout the tree. We then characterized the resulting remodelling distribution using measures of spatial heterogeneity, and compared these predictions with measurements of remodelling in post-mortem lungs from different patient groups.

2. Methods

This study used a combination of theoretical modelling and empirical data on airway wall thickness acquired from subjects with and without asthma. Two different methods of sampling airways from human lungs were followed: planar lung sections assessed correlations in remodelling on short length scales which are not arranged along the airway tree, while similar data sampled along branching pathways assessed correlations between interconnected airways across a broad range of generations (Fig. 1).

2.1. Model overview

The conceptual model is based on the idea that airways undergo cycles of bronchoconstriction, mechanotransduction, and remodelling (BMR cycles). This is made explicit in a mathematical model making use of an existing model of bronchoconstriction combined with a new heuristic model of mechanotransduction and remodelling. A brief conceptual outline is given here, and further details are presented in the following section. We make use of an existing functional model to determine the bronchoconstriction patterns which occur in each cycle (Donovan, 2017; Donovan et al., 2018). This model uses the detailed structure of the airway tree, including airway connectivity, dimensions, and structural properties such as basement membrane perimeter ($P_{bm}$), ASM area, and total wall area (WA) for each airway to compute lung function both in terms of overall flow patterns and respiratory resistance. Importantly this is done in the presence of induced ASM tone and hence bronchoconstriction. The fundamental elements are the airway model of (Lambert et al., 1982), the parenchymal tethering model of (Lai-Fook, 1977), and the airway interdependence ideas of (Anafi and Wilson, 2001). The model shares some common features with the well-known symmetric tree model of (Venegas et al., 2005), but is here extended to apply to anatomically accurate structures and scaled to a whole human lung (Donovan, 2017; Donovan et al., 2018). For more details, the reader is referred to both the following section and earlier studies (Donovan and Kritter, 2015; Donovan, 2017).

For each cycle, remodelling (via mechanotransduction) is determined based on the calculated bronchoconstriction, which is assumed to occur throughout ventilation defects and low ventilation areas (Tgavalekos et al., 2005). Hence the mechanotransduction and remodelling processes also occur throughout these regions. In the absence of specific data, we make the simplest assumption and assume that remodelling, via mechanotransduction, is linearly proportional to the degree of bronchoconstriction. We also perform an extensive sensitivity analysis. For reasons of computational cost, in whole-lung simulations we use a relatively large degree of remodelling per cycle, and hence a relatively small number of cycles; as per the sensitivity analysis, the results are qualitatively unchanged from those assuming a smaller degree of remodelling per cycle, but a larger number of cycles. The important aspect, then, is not the absolute number of cycles, but rather the progression with additional cycles.

2.2. Details of mathematical model

The mathematical model of BMR cycles used in this study is a combination of an existing model of bronchoconstriction, and a new

Fig. 1. Schematic illustration of sampling methods. Left panel: transverse planar sampling, with transverse plane (blue) and core (orange), and in-slide airways marked in red. Note that the core is enlarged for visibility and not shown to scale. Right panel: pathway sampling. In both cases the figure is illustrative of the method rather than indicating specific locations, e.g. for transverse planar sampling, slides are taken from multiple locations, and not just the upper lobe. Similarly, pathway sampling occurs along axial paths in each lobe (see inset).
heuristic model of the mechanotransduction and remodelling portions. Each model is applied sequentially to simulate the evolution of structure and function over repeated BMR cycles.

The bronchoconstriction model uses details of airway tree structure (branching pattern, airway lengths, airway wall parameters including basement membrane perimeter, wall area and ASM area) to predict function and flow patterns during bronchoconstriction (Donovan, 2017; Donovan et al., 2018). The fundamental elements are the airway model of (Lambert et al., 1982), the parenchymal tethering model of (Lai-Fook, 1977), and the airway interdependence ideas of Anaflou and Wilson (Lai-Fook, 1977; Anaflou and Wilson, 2001). With the airway and lung structure determined, we calculate the resulting flow patterns. We use the model of (Donovan, 2017), which is based on the Anaflou-Wilson instability (Anaflou and Wilson, 2001; Venegas et al., 2005; Leary et al., 2014). The interested reader is referred to these references for full details, but briefly the model assumes quasi-steady Poiseuille flow in each airway and describes both airway narrowing due to ASM activation, and the interactions between airways, all joined together as a very large system of coupled ordinary differential equations. Poiseuille flow and pressure balance introduce algebraic constraints, but these can be systematically eliminated (Donovan, 2016). Driving pleural pressures are determined as necessary to maintain target tidal volumes (Donovan and Kritter, 2015). The parameters of this system of equations are determined by the previous remodelling cycles, and the equations are then solved numerically in order to determine the flow patterns within that simulated lung, for a given level of ASM activation.

For the mechanotransduction and remodelling portion, we apply the following heuristic model. Mechanotransduction and remodelling are assumed to occur throughout ventilation defects as follows. Each airway, during each cycle, undergoes scaling of both WA and ASM by a factor of $\Delta$. This is determined, for each airway and cycle, by the dynamics of the simulated agonist challenge (calculated using the model described above). We use a simple piecewise linear function

$$\Delta(\delta) = \begin{cases} 1, & \text{for } \delta \geq 1 \\ 1 + \delta(1 - \delta), & \text{for } \delta < 1 \end{cases}$$

based on the assumption that airways which do not undergo bronchoconstriction do not undergo remodelling, and that for those that do, the remodelling is linearly proportional to the severity of the bronchoconstriction. The concept is based upon (Grainge et al., 2011), though no information is available on the precise dynamics of this process, and as such we assume the simplest plausible form. Here $\delta$ is the parameter which determines the extent of the remodelling during each cycle, while $\delta$ is determined by the bronchoconstriction for that airway, during the challenge, and calculated for each airway as the ratio of the flow through that airway during bronchoconstriction relative to the same flow in the absence of bronchoconstriction. All airways within the simulated left lung are subject to the same remodelling process. Pbm is assumed to remain unchanged. Each simulated lung begins with a fixed branching structure, derived from CT where possible and generated algorithmically for smaller airways (Howatson Tawhai et al., 2000). All airways begin in a fictitious state with homogeneous and relatively little ASM and WA, in order to demonstrate the spatial patterns of remodelling which emerge. A single airway tree geometry is used, based on a healthy subject. Each simulated cycle consists of average tidal breathing over 10 breaths, subject to bronchoconstriction (equal degrees of ASM activation applied everywhere). The precise timescale of a BMR cycle, however, is not explicitly specified; only the degree of remodelling which occurs in response to bronchoconstriction during the cycle.

Model simulations are performed either in a whole left lung (approximately 30,000 conducting airways), or within a restricted subtree consisting of 2133 airways. Using the whole lung is desirable where practicable, however due to the computational cost of simulation the latter is used in applications where much larger numbers of simulations are required, for example in sensitivity analysis. Parameters for the underlying functional model are as given in (Donovan, 2017). Parameter values for the additional BMR components used in the simulations were $\delta = 0.5$ (dimensionless), and the additive noise at each cycle was zero mean and 1% standard deviation. For purposes of sensitivity analysis, $\delta$ was tested over an order of magnitude, the noise standard deviation over $\pm 20\%$, as well as the ASM activation level over $\pm 10\%$ and the airway-airway coupling strength (see (Donovan and Kritter, 2015)) over $\pm 20\%$.

2.3. Human airway sampling – transverse planar

We collected data from post-mortem lung specimens to validate the findings from the mathematical model of BMR cycles. Subject demographics for post-mortem formalin fixed lung sections used for transverse planar sampling can be seen in Table 1. Lungs from subjects with a history of asthma were obtained with written, informed consent through the International Institute for the Advancement of Medicine (IIAM, Edinon, NJ) and with approval from the University of British Columbia and St. Paul’s Hospital ethics committee. Tissues from these lungs have been used in previous studies (Chin et al., 2012; Syngon et al., 2015; Pascoe et al., 2017b, 2017a). All conditions of the ethics approvals were followed. These subjects were used for transverse planar sampling only.

Lungs were prepared for sectioning as previously described (Pascoe et al., 2017b). The method of fixation approximates an inflation pressure of 20 cm H2O. Nevertheless, lung inflation is not a critical factor in measuring airway wall compartment areas; basement membrane perimeter (Pbm), the marker for airway size, is relatively insensitive to the pressure of inflation (James et al., 1988, 2008) and the relationship between Pbm and wall areas remains largely unaffected. Transverse lung sections (5 µm thick) were cut and then, using a sharpened hollow cylinder, cores approximately 15 mm–20 mm in diameter were sampled; the sampling procedure is illustrated schematically in Fig. 1 (left panel). An average of 5 cores per lung were used, with cores randomly sampled throughout the lung. Cores with multiple in-slide airways were used as this allowed pairwise distance measurements to be performed, with an average of 2.5 cross-sectional airways per core (n = 129 airways, n = 61 in-slide airway pairs).

2.4. Human airway sampling – pathway

Subject demographics for formalin fixed lung sections used for pathway sampling can be seen in Table 2. Whole left lungs used for pathway sampling were acquired from the Prairie Provinces Fatal Asthma Study (Tough et al., 1996; Salkie et al., 1998; Hessel et al., 1999; Boser et al., 2005; Green et al., 2010). Ethical approval for the study was obtained from the four major universities in each Canadian province involved in the study: the University of Calgary, University of Edmonton, University of Saskatchewan and the University of Manitoba. Subjects were classified as: Control (n = 31) - no history of asthma, wheeze or other lung disease; non-fatal asthma (n = 32) - death attributed to a non-respiratory cause with a confirmed history of asthma; and fatal asthma (n = 25) - death attributed to asthma with a
confirmed history. Informed consent was obtained from all families of the deceased to use the pathology samples from the autopsy for research purposes, and all conditions of the ethics approvals were followed. These lungs were used for pathway sampling only.

The main bronchus and pulmonary artery were perfused simultaneously with glutaraldehyde (2.5% in 0.05 M phosphate buffer, pH 7.4, 350 mOsm with sucrose) at pressure heads of 20 and 40 cmH₂O respectively, thus maintaining a capillary-alveolar pressure difference of 15–20 cmH₂O. Vascular perfusion was maintained for 2 h and bronchial perfusion overnight. Three segmental bronchi, two from the lower lobe (anterior and posterior basal) and one from the upper lobe (apical), were sampled. Portions of airway (with surrounding parenchyma) were resected from the lungs. In each case these areas are normalized for airway size and sectioned (5 μm), ensuring that the samples were taken horizontal to the axial path. This pulmonary artery was used to identify the correct segment and to enter the pleural surface as markers, and a 1 mm silver probe in the attendant bronchus by the most direct path to the pleural surface for that segment. This was done to ensure that the separation distance between observations (either in linear distance, or branching distance), δ is the bin tolerance, N(h ± δ) is the set of points within that bin, and N(h ± δ) is the number of points in that set. Here the notation $z_i$ and $z_j$ indicates observations from the field. Thus the variogram essentially is calculated as the sum of the squares of the differences at each separation distance, appropriately normalized. Relatively large values of the variogram indicate weaker correlations between samples separated by that distance, and conversely small values of the variogram indicate stronger correlation. The shape of the variogram therein reflects spatial dependence.

A simple illustration of the variogram as a means of quantifying spatial correlation is provided in Fig. 2. The left panel shows a random field which is spatially uncorrelated, while the center panel shows a field with significant spatial correlation/clustering. The values of both are described by the colorbar. The corresponding variograms for each are shown in the right panel. In short, in the absence of spatial correlations, the variogram is approximately constant; otherwise, the shape of the variogram characterizes the spatial correlations. Several observations are worth making: 1) this example is 2D for ease of visualization, but is essentially unchanged in 3D because it is based on the distance and variation between pairs of observations; 2) while the variogram of the data in the left panel is approximately 1, this is not exact, and does vary slightly across separation distances; and 3) the calculated variograms do not extend to zero separation distance but stop at the grid size.

We calculate two variograms in this study: a variogram based on the simple linear distance between airways, and a variogram in which the distance between airways is the tree branching distance (e.g. traced along the pathway of conducting airways connecting any two airways). Note, that because of the sampling techniques, the linear distance variogram can only be computed using the transverse planar sampling data and the branching distance variogram only from the pathway sampling data. In model simulations, both can be computed.

We used a form of analysis for spatial correlations which is well-known in geostatistics; the ‘variogram’. The variogram is more informative than well-known measures of heterogeneity, such as the standard deviation or coefficient of variation, which are insufficient measures of spatially correlated remodelling (because they only capture information about the variation, independent of its spatial arrangement).

The variogram (Cressie and Hawkins, 1980) characterises the spatial dependence of a random field or process, and it does so as a function of the distance between two samples. Thus, the variogram is not a single value (c.f. coefficient of variation), but rather a function of the separation distance. It is calculated as:

$$\gamma(h ± \delta) = \frac{1}{2N(h ± \delta)} \sum_{(i,j) \in N(h±\delta)} |z_i - z_j|^2$$

where $h$ is the separation distance between observations (either in linear distance, or branching distance), $\delta$ is the bin tolerance, $N(h ± \delta)$ is the set of points within that bin, and $N(h ± \delta)$ is the number of points in that set. Here the notation $z_i$ and $z_j$ indicates observations from the field. Thus the variogram essentially is calculated as the sum of the squares of the differences at each separation distance, appropriately normalized. Relatively large values of the variogram indicate weaker correlations between samples separated by that distance, and conversely small values of the variogram indicate stronger correlation. The shape of the variogram therein reflects spatial dependence.

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Variograms are computed for both total WA and ASM as measures of remodelling. In each case these areas are normalized for airway size (and non-dimensionalized) by dividing by the square of basement

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**Table 2**

<table>
<thead>
<tr>
<th>Subject characteristics for pathway sampling.</th>
<th>Control (n = 31)</th>
<th>Nonfatal asthma (n = 32)</th>
<th>Fatal asthma (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, Mean ± SD.</td>
<td>39 ± 10</td>
<td>35 ± 11</td>
<td>33 ± 14</td>
</tr>
<tr>
<td>Gender, male / female</td>
<td>19 / 12</td>
<td>16 / 16</td>
<td>15 / 10</td>
</tr>
<tr>
<td>Corticosteroid use, % Inhaled/Oral, number (%)</td>
<td>–</td>
<td>9 (53)</td>
<td>12* (92)</td>
</tr>
<tr>
<td>Ever smoked, number (%)</td>
<td>8 (57)</td>
<td>11 (52)</td>
<td>9 (64)</td>
</tr>
<tr>
<td>Body mass index Mean ± SD. (range)</td>
<td>27 ± 5* (22-38)</td>
<td>32 ± 8 (16-45)</td>
<td>26 ± 6* (15-41)</td>
</tr>
<tr>
<td>Age at onset of asthma, years median (IQR)</td>
<td>–</td>
<td>17 (10-26)</td>
<td>9 (5-39)</td>
</tr>
<tr>
<td>Duration of asthma, years median (IQR)</td>
<td>–</td>
<td>17 (9-21)</td>
<td>17 (7-22)</td>
</tr>
<tr>
<td>Asthma severity, “Severe” number (%)</td>
<td>–</td>
<td>8 (38)</td>
<td>9 (64)</td>
</tr>
</tbody>
</table>

§ = incomplete data set.  
* = p < 0.05 v Nonfatal asthma.  
# = p = 0.04.  

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**2.5. Morphometry**

Airways were assessed by point counting. Specifically, for transverse planar sampling, tissue sections were stained with hematoxyn and eosin and airways that were determined to be near cross sectional (short to long axis ratio greater than 0.6) were included in the analysis. In-slide airway distance was calculated by measuring the minimum distance between two cross-sectional areas (center to center) on the same section for which morphometric data had been collected. More details on morphometric analysis is provided in the appendix.

Morphometry of airways acquired by pathway sampling was conducted on sections stained with the elastic trichrome technique. The areas of the ASM layer and total WA were measured by point counting. On a contiguous section stained with hematoxyn and eosin, eosinophils and neutrophils were counted within the inner airway wall around the entire airway section, based on their morphology and staining characteristics. The cell densities were calculated by normalization to total wall area. Further detail is provided in the appendix.

**2.6. Spatial correlation analysis: the variogram**

We used a form of analysis for spatial correlations which is well-known in geostatistics; the ‘variogram’. The variogram is more informative than well-known measures of heterogeneity, such as the standard deviation or coefficient of variation, which are insufficient measures of spatially correlated remodelling (because they only capture information about the variation, independent of its spatial arrangement).
perimeter ($P_{b,m}$). Variograms are typically valid only up to a separation distance of approximately half of the maximum separation distance (known as the distance of reliability (Omre, 1984)), and variograms presented adhere to this convention.

For comparison with previous work, we also characterize spatial heterogeneity using a spatial heterogeneity index (SHI) as introduced for this purpose in (Donovan et al., 2018). This is designed to capture information about not just the overall heterogeneity, but also its spatial structure. As such it is constructed to consist of the equally weighted sum of the coefficient of variation, expressing total heterogeneity, and the spatial autocorrelation (capturing the spatial structure, as measured by Moran’s I (Moran, 1950)). However, the SHI does not capture information about how the correlation varies with the distance between samples, and so the variogram is the preferred measure.

3. Results and discussion

3.1. Predictions of spatial remodelling patterns due to bronchoconstriction-mechanotransduction-remodelling (BMR) cycles

Fig. 3 illustrates the effects of BMR cycles in the model, showing the evolution of a typical flow pattern from a single simulation, as well as the emergence of a remodelling pattern after repeated cycles of bronchoconstriction, mechanotransduction, and remodelling. The top row shows the evolution of remodelling (expressed here in terms of normalized wall area (WA)), evolving left to right; the lower row shows the same evolution steps in terms of flow. The bronchoconstriction which occurs in low flow regions leads to mechanotransduction and remodelling, and further remodelling of these airways makes them more prone to future bronchoconstriction in subsequent cycles. Fig. 4 shows the evolution of many such simulations over repeated cycles, in terms of a more simplified analysis, the spatial heterogeneity index (SHI) of normalized WA (left panel), the SHI of flow (center panel), and airway resistance (right panel). Recall that the SHI is an equally-weighted sum of the spatial autocorrelation and the coefficient of variation; it provides a simplified quantification of spatial heterogeneity but does not take into account the spatial scale of that heterogeneity. Clearly evolution of the remodelling process over repeated cycles significantly alters both the resistance and SHI of wall area, though no clear change is evident in the flow SHI, which begins with significant spatial heterogeneity. In comparison there is a rapid increase in SHI of WA from a relatively homogenous initial distribution. The contrast between Figs. 3 and 4 (centre panel) highlights the inadequacy of SHI as a measure of these patterns and supports the use of variograms to assess spatial correlations.

From Figs. 3 & 4 we see that the BMR cycles can alter the structure and function of the simulated lung. The exact character of these changes can be quantified more explicitly in terms of the variograms.

Fig. 2. Illustration of the variogram as a method of characterizing spatial correlations. Left panel: uncorrelated; center panel: correlated; right panel: corresponding variograms. See text for additional details.

Fig. 3. Illustration of BMR cycles in a typical simulation; arrow indicates evolution over BMR cycles (left to right). Top row: WA spatial distribution, normalized to initial. Bottom row: corresponding flow patterns during bronchoconstriction phase, normalized to nominal (Donovan et al., 2018).
Predicted remodelling variograms are given in Fig. 5. Two measures are expressed: the linear distance variogram (e.g. simple linear distance between airway pairs), and the branching distance variogram based on the distance between airways along the conducting airway tree (proximal end to proximal end). Here variograms are shown from 3 independent simulations (grey, solid lines) and after 6, 7, 9, and 11 BMR cycles. Recall that, as per the Methods, in whole-lung simulations we use a relatively large degree of remodelling per cycle, and hence a relatively small number of cycles. The important aspect is not the absolute number of cycles, but rather the progression with additional cycles. The results are qualitatively the same using a larger number of cycles and a smaller amount of remodelling per cycle, but this induces much greater computational cost. The most important aspect is that some number of BMR cycles can produce these spatial patterns.

Fig. 5. Predicted BMR variograms (grey lines); left: ASM; right: WA. Upper: standard linear distance variogram. Lower: distance over branching airway tree variogram. Variograms from experimental data (dashed lines) overlaid for comparison. Transverse planar sampling data showing pairwise variation for normalized ASM depending on pair separation distance h, and variation for normalized WA; (a) corresponding ASM and (b) WA variogram; (c) and (d) branching distance variograms from pathway sampling data, classified as non-asthma (NA, green circles), non-fatal asthma (NFA, red x’s) and fatal asthma (FA, blue triangles). Here variograms are shown after 6, 7, 9, and 11 BMR cycles, from 3 independent simulations. The groups are in monotonically increasing order.

These predicted variograms can then be compared with the human airway data acquired via both pathway sampling and transverse planar sampling. Transverse planar sampling data is used to compute linear distance variograms, up to the distance of reliability, and these are given in the black dashed lines in Fig. 5, panels a and b. Although these data are limited to short length scales (∼ < 10 mm separation), both WA and ASM data suggest a degree of spatial correlation in structure over this length scale. As outlined in Fig. 2, spatially-uncorrelated remodelling would yield a variogram which is constant with respect to the separation distance.

Transverse planar sampling sections (e.g. Fig. 1 left panel) contain airways such that the measured distance between airway pairs is the exact linear distance, allowing direct calculation of the linear distance variogram. Potential bias in airway selection is limited, with the only exclusion criterion being the angle at which the airway is “out of plane” with the slice. However, this methodology is limited to relatively short distances as airway pairs must be contained in the same 15−20 mm diameter core to be assessed. Sample size is also relatively low and is insufficient to differentiate between status and severity of asthma (asthma n = 12, non-asthma n = 9). Nonetheless, analysis of this data in terms of the variogram does suggest a dependent spatial relationship at scales up to ∼ 10 mm. To address limitations described above, longer length scales were assessed using intra-lobe airway pairs acquired through pathway sampling (e.g., Fig. 1 right panel). Variograms for both ASM and WA are...
shown in Fig. 5 panels c and d. Because these data are sampled along the
tree, the corresponding variograms are the branching distance
variograms. Here the larger number of samples allows classification by
group: non-asthma (NA, green circles), non-fatal asthma (NFA, red ‘x’s)
and fatal asthma (FA, blue triangles). Here we have assumed a se-
paration distance of 15 mm per anatomical level, for comparison. There
is a clear distinction between the NA, NFA and FA populations
expressed in this way, as well as an indication of the potential spatial
pattern, in agreement with model predictions. That is, airways which
are closer together (in terms of anatomical levels) appear to have
greater correlation in ASM and WA, while those with greater separation
have decreased correlation. An unbalanced two-way ANOVA analysis of
the intra-lobe airway pairs (e.g. the experimental variograms in Fig. 5)
indicates statistical significance for asthma status (NA, NFA, FA; p < 0.0001),
branching distance (p < 0.0001) and interaction between
asthma status and branching distance (p = 0.02).

Another way of interpreting this is to recall that the variogram
captures how much the structure in two airways differ, averaged across
all airway pairs which are a certain distance apart. If structure was
uniform everywhere, then the variogram would be zero. Hence the
magnitudes of the variogram captures information about heterogeneity.
Moreover it indicates how it is arranged spatially, in that airways which
are near to one another are more similar (smaller variogram) while
airways which are far from each other are less similar (larger vario-
gram).

An alternative approach is to examine spatial associations using
Pearson correlation coefficients between airway pairs separated by
different distances (in terms of anatomical levels), and these data are
given in Table 3. In all cases, airways separated by only one anatomical
level have moderate to strong correlation (> 0.4), with correlations falling
for airways separated by greater distances. For comparison, the
Pearson coefficients for the model simulations are also shown, assuming
a 15 mm branching distance per anatomical level. When comparing
correlations derived from the model and experimental data, it is im-
portant to appreciate that greater numbers of model simulations are
available compared with the experimental data, and that within the
experimental data there are many more pairs separated by fewer ana-
tomical levels.

It is thus clear that BMR cycles are a plausible explanation for the
formation of the observed spatial correlations. Importantly, correlations
are present in the NA population as well as the NFA and FA groups. This
means that the underlying remodelling process must at least respect and
maintain these spatial correlations, if not increase them; if a remodel-
ing process occurred which was completely independent of these
initial spatial correlations, the variograms would be expected to become
independent of the separation distance (e.g. have slope zero).

An alternate approach to this analysis is to normalise WA/Pbm2 and
ASM/Pbm2 to the averages for each subject. Repeating the above anal-
ysis using this approach does not alter the results. An extensive sen-
sitivity analysis of the model was performed with respect to parameters
for degree of ASM activation during challenge, degree of airway-par-
enchymal interdependence, rate of bronchoconstriction-induced re-
modelling, and strength of additive white noise per cycle (see methods).
In all cases the results remain qualitatively consistent with the results
for the parameter set shown.

It is important to appreciate that pathway sampling of the airways
introduces the variable of airway size, or Pbm (basement membrane
length) as is conventionally used in histological studies on airway
tissue. The axial airways (i.e. longest path) at anatomical levels 6–9 are
comprised of large central airways, (defined as having cartilage and
mucous glands in their walls), whilst those at anatomical levels 1–3 are
classified as small airways, and lack these features. Airways in the in-
termediate zone (levels 4–6) exhibit transitional features. Given the
very large number of samples in this analysis it was possible to extract
useful information from the computed variograms. Here it is striking to
notice not only the pattern of increasing variation with separation
distance, but also the clear distinction between NA, NFA and FA groups.

While variograms generated from transverse planar or pathway
sampling have different limitations (and strengths), a clear picture is
emerging: it seems probable that spatial correlations in airway re-
modelling exist, and that the specific spatial correlation patterns are
consistent with those which might be expected if the underlying process
of formation is repeated BMR cycles. Other mechanisms, including
those derived from inflammatory signals, could also be consistent, if
there are local mechanisms to drive such spatial correlations.

Eosinophil and neutrophil counts were available for a subset of the
pathway sampling data (N = 217 airways). Analysis of this subset of
data suggested similar spatial correlations for inflammatory cells.
Although the relative size of this data subset makes more extensive
analysis impossible, these cell count data suggest that inflammatory
processes are also spatially correlated, particularly in more severe
asthma. Eosinophil and neutrophil count, normalised to wall area, were
not significantly correlated with WA/Pbm2 (ρ = 0.12, p = 0.08), but
ASM/Pbm2 showed a modest correlation which did reach signi-
ificance (ρ = 0.23, p < 0.05).

3.2. Clinical implications and testing these hypotheses in patients

The above analyses provide evidence to support correlations in
airway remodelling and also predicts the specific statistical signature
that would be expected if airway remodelling was driven by a BMR-
type process. That is, remodelling produced by functional behaviour
such as bronchoconstriction to an environmental trigger, when re-
peated over many cycles, will yield a variogram dependent on separa-
tion distance. In comparison, remodelling that occurs through a me-
chanism which does not lead to spatial correlations in airway structure
– for example, if remodelling simply occurred at (independent) random
sites – would exhibit variograms without any dependence on the se-
paration distance.

Present findings have potential implications for the treatment of
remodelling in asthma. If remodelling is to be considered a therapeutic
target, understanding the mechanism of formation provides an oppor-
tunity to reverse, or – perhaps of greater significance – prevent disease.
Actively targeting the remodelling process at an early stage might be
most effective in reducing the consequences of remodelling, and this
could have implications for selection of short- or long-acting therapies.
However, there is mounting evidence that airway remodelling is ac-
quired very early in life and lung function is impaired at birth in sub-
jects who go on to develop asthma (Håland et al., 2006), indicating that
the BMR cycles and the resulting spatially correlated remodelling may
occur in utero. If airway remodelling developed in this manner, suc-
cessful intervention would be more difficult and require prenatal ap-
plication.

Spatially correlated airway remodelling provides explanation for
various functional abnormalities that have been documented in patients
with asthma. Lung ventilation defects are detected by hyperpolarized

<table>
<thead>
<tr>
<th>h = 1 level</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>0.06</td>
<td>0.03</td>
<td>0.05</td>
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<td>0.17</td>
<td>0.05</td>
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<td>0.02</td>
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<tr>
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<td>0.16</td>
<td>0.02</td>
<td>-0.07</td>
<td>-0.05</td>
<td>-0.06</td>
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</tr>
<tr>
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<td>0.38</td>
<td>0.37</td>
<td>0.36</td>
<td>0.36</td>
<td>0.38</td>
</tr>
</tbody>
</table>

a = p < 0.01.
b = p < 0.001.
c = p < 0.0001.
Fig. 5 that spatially correlated remodelling patterns associated with the background state via a self-organized process (Venegas et al., 2005) and subjects with or without asthma in the conceptual model, in that all processes were entirely independent of the spatial relationships, the processes which result in progression to NFA and FA. If the remodelling processes were entirely independent of the spatial relationships, the spatial correlations apparent even in the lung must respect and maintain these spatial correlations: a spatially-independent remodelling process would lead to decreases in the apparent spatial correlations with disease progression. Moreover, spatial correlations in airway remodelling are expected to have a significant impact on resulting lung function. Identifying and understanding the evolution of spatial correlations in airway remodelling in asthma should be considered and may be relevant when developing strategies to mitigate disease severity.

3.3. Limitations and future directions

The effect of airway size on study conclusions requires more explicit discussion. Certainly other work has suggested the potential importance of differential remodelling in large vs small airways (Hirota and Martin, 2013; Elliot et al., 2015), and such an outcome would be intrinsically linked with spatial correlations due to their relationships in the structure of the airway tree. We have treated airways of different size equally by normalizing both ASM and WA by the square of Pbm, such that the quantities are dimensionless. Differential responses in airways of different size (e.g. that do not scale in this dimensionless way) would further complicate the analysis of correlated remodelling but would also enable potentially interesting phenomena such as propagation of large airway remodelling toward more distal airways (James et al., 2007, 2009) which is consistent with regions of clustered airway re-modelling toward more distal airways (James et al., 2007)

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points per 1000μm², and the density of the point grid was internally validated as previously described (ref Chiu et al., 2012 supplementary material). Multiplying the ratio of points that fall on the region of interest by the size of the image (in mm²) yielded the area for that layer. The final area values (ASM and total wall area, (WA) were normalized to Pbm² to control for differences in airway size. Pbm was determined by manually tracing the perimeter (ImageScope, Leica Biosystems).

 Morphometry of airways acquired by pathway sampling was conducted on sections stained with the elastic trichrome technique. Point counting was performed using a Nikon light microscope, drawing tube and square lattice grid containing 240 points. The point counts for each feature were converted to area fractions using the formula: Area (mm²) = Z’x (point count value) where Z’ was a constant derived from the distance between the grid lines and the luminal aspect of the basement membrane. The intersection counts were converted to a length (perimeter mm) using the formula: Perimeter = Z’ x number of intersections (Howard and Reed, 2004).

References