Airway remodeling and bronchodilator responses in asthma assessed by endobronchial optical coherence tomography

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Abstract

Background Understanding asthmatic airway structural changes and the bronchodilator responses may help unravel targets for intervention. However, structural abnormalities of asthmatic airways with different disease severity and the major anatomical site of bronchodilator responses have not been well elucidated. We aim to evaluate the airway remodeling characteristics and the bronchodilator responses in medium-sized and small airways of asthma. Methods We recruited 104 asthmatic patients and 31 non-smoking control subjects to compare the airway inner area (Ai) and airway wall area percentage (Aw%) with endobronchial optical coherence tomography. We also enrolled 32 patients with moderate-to-severe asthma to dynamically assess the airway morphological changes after salbutamol inhalation. Results More prominent airway structural abnormalities correlated with greater asthma severity, evidenced by the decreased Ai and greater Aw% in medium-sized and small airways. Patients with mild asthma yielded comparable Ai but greater Aw% than control subjects. Salbutamol inhalation led to a rapid dilatation of both medium-sized and small airways, the lung function improvement correlated significantly with the increase in Ai of the medium-sized, but not small, airways at 15 min. Conclusion Luminal narrowing and airway wall thickening of the medium-sized and small airways are present in mild asthma and reflect asthma severity, lending support to the use of anti-imflammatory intervention in mild asthma. The medium-sized airways are the crucial site of the bronchodilator responses, providing the scientific rationale for future development of more effective delivery of inhaled medications for asthma.

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Running title: OCT assessed bronchodilation and remodeling in asthma

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Abstract

Background Understanding asthmatic airway structural changes and the bronchodilator responses may help unravel targets for intervention. However, structural abnormalities of asthmatic airways with different disease severity and the major anatomical site of bronchodilator responses have not been well elucidated. We aim to evaluate the airway remodeling characteristics and the bronchodilator responses in medium-sized and small airways of asthma.

Methods We recruited 104 asthmatic patients and 31 non-smoking control subjects to compare the airway inner area (Ai) and airway wall area percentage (Aw%) with endobronchial optical coherence tomography. We also enrolled 32 patients with moderate-to-severe asthma to dynamically assess the airway morphological changes after salbutamol inhalation.

Results More prominent airway structural abnormalities correlated with greater asthma severity, evidenced by the decreased Ai and greater Aw% in medium-sized and small airways. Patients with mild asthma yielded comparable Ai but greater Aw% than control subjects. Salbutamol inhalation led to a rapid dilatation of both medium-sized and small airways, the lung function improvement correlated significantly with the increase in Ai of the medium-sized, but not small, airways at 15 min.

Conclusion Luminal narrowing and airway wall thickening of the medium-sized and small airways are present in mild asthma and reflect asthma severity, lending support to the use of anti-imflammatory intervention in mild asthma. The medium-sized airways are the crucial site of the bronchodilator responses, providing the scientific rationale for future development of more effective delivery of inhaled medications for asthma.

Keywords: Asthma; airway remodeling; bronchodilation; optical coherence tomography

Abbreviations list

Ai = airway inner area;

Ao = total airway wall area;

Aw% = airway wall area percentage;

EB-OCT = endobronchial optical coherence tomography;

Ai3-6 = the mean airway inner area from the third to sixth generation of bronchi;

Ai7-9 = the mean airway inner area from the seventh to ninth generation of bronchi;

Aw%3-6 = the mean airway wall area percentage from the third to sixth generation of bronchi;

Aw%7-9 = the mean airway wall area percentage from the seventh to ninth generation of bronchi;

BSA = body surface area;

RB8 = anterior basal segment of the right lower lobe;

RB9 = lateral basal segment of the right lower lobe;

FEV_1 = forced expiratory volume in one second;

FVC = forced vital capacity;

MMEF = maximal mid-expiratory flow;

MEF_{25} = Maximal expiratory volume when 25% of the lung volume has been expired;

MEF_{50} = Maximal expiratory volume when 50% of the lung volume has been expired;

Fres = resonant frequency;

R_5 = airway resistance at 5 Hz;

R_{20} = airway resistance at 20 Hz;

R_5-R_{20} = peripheral airway resistance;

X_5 = pulmonary reactance at 5 Hz;

AX = area of reactance;

Z_5 = respiratory impedance at 5 Hz.

Introduction

Asthma is a chronic airway inflammatory disease characterized by recurrent episodes of wheezing which have been associated with bronchoconstriction[1, 2]. Chronic airway inflammation has been a critical driver of airway remodeling, characterized by luminal narrowing and airway wall thickening that are mainly responsible for the significantly increased airway resistance and airflow limitation[3-5]. Importantly, airway remodeling was thought to mainly occur in large and middle-sized airways in asthma[6, 7]. Few studies, however, have systematically compared the structural abnormalities from medium-sized to small airways in asthmatic patients with different disease severity. Clinically, inhalation of bronchodilators rapidly improves lung function and

ameliorates respiratory symptoms (particularly wheezing) in asthma, but how and where the bronchodilator effects take place have not been thoroughly elucidated with an objective measurement. Exploration of the structural abnormalities of asthmatic airways and bronchodilator responses might help unravel the pathophysiology of, and highlight therapeutic targets for, asthma.

Both computed tomography (CT) and histology have limited ability to measure small airway morphology because of the technical constraints (e.g. low resolution with a limited value in detecting small airways beyond the 6^{th} generation bronchi, and unavailability of obtaining biopsy specimens). Endobronchial optical coherence tomography (EB-OCT), accessible to distal airways up to the 10^{th} generation of bronchi, is a validated real-time imaging technique that generates high-resolution transverse and longitudinal images of the airway architecture *in vivo* , which have a high concordance with CT and histologic measurements[8, 9]. By using EB-OCT, we have recently revealed that small airways, refer to the airways with an inner diameter of less than 2 mm, are mainly located at the 7^{th}generation or more distal bronchi[10], and that more advanced stages of COPD were characterized by a greater magnitude of abnormality in airway architecture (i.e. tapering airway caliber and greater airway wall thickness). More importantly, EB-OCT has confirmed the presence of airway remodeling in early-stage COPD[11].

We hypothesized that more prominent airway structural abnormalities correlated with greater asthma severity, and that changes in medium-sized airways caliber correlated with the improvement in lung function. Based on EB-OCT measurement, we sought to investigate airway remodeling in asthmatic patients with different disease severity, and elucidate the bronchodilator responses of different airway generations after inhalation of salbutamol.

Methods

Study participants

We recruited 167 study participants, aged 18 years or greater, from out-patient clinics of The First Affiliated Hospital of Guangzhou Medical University between April 2014 and December 2019. First, we studied 104 asthmatic patients and 31 control subjects to compare the airway structural characteristics by using EB-OCT (Study 1). Thereafter, 32 patients with moderate-to-severe asthma participated in Study 2 to assess the bronchodilator responses with EB-OCT. The diagnosis of asthma was made by respiratory physician based on the history of respiratory symptoms and variable expiratory airflow limitation. Asthma severity was graded according to *Global Initiative for Asthma* [12]. Overall, 30.8% (32/104) patients in Study 1 and 31.2% (10/32) in Study 2 were well controlled, while 69.2% in Study 1 and 68.8% in Study 2 remained partly controlled. All asthmatic patients were non-smokers or had smoked for less than 5 pack-years. We excluded patients with asthma attacks or acute respiratory tract infection within 4 weeks and cardiopulmonary diseases. Control subjects, who underwent bronchoscopy for upper lobe peripheral nodules, were otherwise never-smokers with normal lung function and free from respiratory diseases with symptoms.

This study was approved by the Ethics Committee of The First Affiliated Hospital of Guangzhou Medical University (Medical Ethics [Year 2014] No. 51 and [Year 2019] No. K09). All subjects gave written informed consent.

Procedures

Study 1 : In this case-control study, 135 eligible participants attended a single clinical visit in which they were directly inquired the clinical history, induced sputum, peripheral blood test and EB-OCT measurements of the right anterior basal segment (RB8) and right lateral basal segment (RB9). We performed IOS, and subsequently, spirometry (Jaeger, Hoechberg, Germany) according to the international guidelines[13]. Forced vital capacity (FVC), forced expiratory volume in one second (FEV_1) and maximal mid-expiratory flow (MMEF) were measured in asthmatic patients before and at 10 min after 400μg salbutamol (GlaxoSmithKline Co. Ltd., UK) inhalation via a pressurised metered dose inhalers (pMDI) with a spacer (DHN200, Koka Co. Ltd., China).

Study 2 : We performed EB-OCT and spirometry in 32 asthmatic patients before and after salbutamol

inhalation to assess bronchodilator response within the same clinical visit. Spirometry was performed at baseline before EB-OCT assessment. To secure the comparability of EB-OCT measurement within the same segment, we advanced an OCT catheter to the RB9 segment immediately (within 1 min) after inhaling 400 μg salbutamol (denoted as baseline EB-OCT measurement) with the guidence of bronchial navigation system. Next, the catheter was maintained within the bronchi for conducting EB-OCT scanning at 5-min intervals (up to 15 min) to directly determine the dynamic airway morphological changes. Spirometry was performed at 60 min after salbutamol inhalation (post-bronchodilation test). For all asthmatic patients, short- and long-acting bronchodilators were withheld for at least 8 and 24 hours respectively prior to spirometry or EB-OCT measurement.

EB-OCT imaging

EB-OCT scans was performed by using Lightlabs C7XR OCT system (St. Jude Medical, St. Paul, MN). The OCT catheter (0.9 mm in diameter) was advanced to the right lower bronchus under flexible bronchoscopy (B260F, Olympus, Japan) and via the DirectPath navigation system (Olympus Co. Ltd., Japan)[9]. The 3rd to 9th generation of bronchi were detected upon breath hold at the end of full inspiration. At least three reproducible EB-OCT measurements were performed for each segment.

The generation of bronchi was determined based on the airway branch-points in longitudinal section of EB-OCT images[14]. The EB-OCT imagings were analyzed with the Lightlabs C7XR software system workstation, which automatically measured and calculated the airway inner area (Ai) and total airway wall area (Ao) based upon the luminal perimeter and the inner perimeter of the cartilage[8, 9, 15]. The airway wall area percentage (Aw%) was calculated as (Ao–Ai) /Ao×100%. The Ai was further corrected for the body surface area (BSA). The ΔAi/BSA was defined as the difference of Ai/BSA before and after inhalation of salbutamol. Given that small airways refers to the airways with an inner diameter less than 2 mm, we defined the small airways as the 7th generation and more distal bronchi based on our latest publication[10, 11]. Accordingly, the Ai/BSA and Aw% detected from the 3rd to 6th, and from the 7th to 9th generation of bronchi were averaged respectively to reflect the morphology of the medium-sized and small airways.

Statistical Analysis

In Study 1, assuming a two-sided α of 0.05 and β of 0.20, we estimated at least 21 cases per each group, based on the difference of 2.12 mm^2 and a standard deviation of 2.39 mm^2 of Ai between asthmatic patients and control subjects[16]. In Study 2, based on the difference in Ai before and after inhalation of bronchodilator[17], assuming a difference of 54.9% and a standard deviation of 22.5% for airway volume, with a two-sided significance level of 5% and a power of 80%, a total sample size of 8 cases would be required.

Statistical analysis was performed using SPSS version 22.0 (SPSS Inc., Chicago, USA) and GraphPad Prism 5.0 (GraphPad Inc., USA). Data were expressed as mean ± standard deviation. In Study 1, the differences in Ai/BSA and Aw% between the RB8 and RB9 segment were determined by paired t-tests. Paired t-test was applied to compare the spirometric and EB-OCT data before and after salbutamol inhalation. Differences among groups were analyzed using one-way analysis-of-variance or Kruskal-Wallis test where appropriate. Bonferroni correction was applied to adjust for multiple comparisons. The association between EB-OCT and spirometric parameters, and sputum cell counts were determined with Pearson's or Spearman's correlation model when appropriate. $P<0.05$ was deemed statistically significant unless otherwise stated.

Results

Demographic and clinical characteristics

Overall, 21 patients with mild asthma, 36 with moderate asthma, 47 with severe asthma and 31 control subjects were included in Study 1. The gender distribution, age, body-mass index and BSA were comparable (all$P$ >0.05, **Table 1**). The difference in atopic status, sputum and peripheral blood eosinophil counts was unremarkable among asthmatic patients with varying severity (all$P$ >0.05). Patients with severe asthma had a longer disease duration than those with mild asthma. Furthermore, compared with patients with mild asthma and control subjects, patients with moderate-to-severe asthma had markedly lower percentage

predicted value of forced expiratory volume in one second (FEV_1) and the ratio between FEV_1 and forced vital capacity (FVC). The percentage predicted of FEV_1 and maximal mid-expiratory flow (MMEF) was significantly lower in patients with mild asthma than in control subjects (all P <0.05). The magnitude of abnormality of IOS parameters was greater in patients with asthma than in control subjects (**Table E1**). Patients with severe asthma had significantly higher R_5, R_5-R_{20}, X_5 and Z_5 compared with those with moderate asthma (all$P$ <0.05). The magnitudes of abnormality of Fres, R_5, R_5-R_{20}, X_5, AX and Z_5 were markedly higher in patients with severe asthma than those with mild asthma (all$P$ <0.05).

In Study 2, we recruited 16 patients with moderate asthma and 16 with severe asthma. The baseline clinical characteristics of patients with moderate-to-severe asthma in Study 1 did not differ from those in Study 2 (**Table E2**) .

Airway structural abnormalities were more prominent in patients with greater asthma severity

In Study 1, the Ai/BSA and Aw% from the 3^{rd} to 9^{th} generation of bronchi did not differ significantly from those in the RB8 to RB9 segment (all P >0.05,**Table E3**). In medium-sized airways (the 3^{rd}-6^{th} generation), both patients with moderate and severe asthma had significantly lower Ai/BSA than control subjects and patients with mild asthma (both$P$ <0.05, **Figure 1**); however, the Ai/BSA in control subjects and patients with mild asthma were comparable (both$P$ >0.05). Compared with those with moderate asthma, patients with severe asthma yielded markedly smaller caliber from the 5^{th} to 6^{th} generation of bronchi (P =0.018). Furthermore, significantly greater Aw% from the 3^{rd} to 6^{th} generation of bronchi was seen in both patients with moderate and severe asthma than in control subjects and patients with mild asthma (all P <0.05). Severe asthma was associated with greater Aw% from the 5^{th} to 6^{th} generation of bronchi compared with moderate asthma (all P <0.001). Aw% from the 3^{rd} to 6^{th} generation of bronchi was notably greater in patients with mild asthma than in control subjects (P <0.05).

In small airways (the 7^{th}-9^{th}generation), both patients with moderate and severe asthma presented with significantly smaller Ai/BSA and greater Aw% compared with patients with mild asthma and control subjects (all$P$ <0.05, **Figure 1**). Severe asthma yielded markedly lower Ai/BSA and greater Aw% from the 7^{th} to 9^{th} generation of bronchi compared with moderate asthma (all P <0.05). Despite comparable Ai/BSA, patients with mild asthma yielded greater Aw% from the 7^{th} to 9^{th} generation compared with control subjects. Collectively, patients with mild asthma had developed airway remodeling (wall thickening) from the 3^{rd} to 9^{th} generation, even in the absence of airway luminal narrowing. More prominent structural abnormalities were associated with greater asthma severity, as evidenced by decreased airway caliber and greater wall thickness (**Figure 2**). The airway inner area of medium-sized airways (Ai/BSA3-6) correlated negatively, and the airway wall area of medium-sized airways (Aw%3-6) correlated positively with the duration of asthmatic symptoms (r=-0.306, P =0.002; r=0.282,P =0.004, respectively). However, the sputum eosinophil count did not correlate with EB-OCT parameters (all P >0.05,**Table E4 and Table E5**).

Dynamic changes in airway caliber after bronchodilation

In Study 2, compared with baseline levels, salbutamol inhalation led to a significant improvement not only in FEV_1, FVC and FEV_1/FVC, but also the small-airway spirometric parameters such as MMEF in 32 patients with moderate-to-severe asthma (all P <0.05, **Table E6)** . Both the Ai/BSA in medium-sized (the 3^{rd}-6^{th}generation) and small airways (the 7^{th}-9^{th} generation) increased substantially after salbutamol inhalation in 30 (93.8%) patients (**Figure 3** and **Figure 4**). The Ai/BSA3-6 progressively increased by 14.3%, 20.1%, and 22.0% at 5 min, 10 min and 15 min post-bronchodilation compared with baseline (**Figure 5**). Meanwhile, Ai/BSA7-9 increased by 11.0%, 22.0% and 38.0% at 5 min, 10 min and 15 min post-bronchodilation compared with baseline. The Aw% of medium-sized and small airways did not change significantly at 15 min after bronchodilation. The EB-OCT assessement of dynamic changes in the medium-sized and small airways caliber within 15 min after salbutamol inhalation was shown in **Video 1** .

The improvement of spirometric parameters and the airway structure in moderate asthma did not differ significantly from those of severe asthma (all P> 0.05, **Table E6** and **Table E7**). Importantly, the magnitude of increase in Ai/BSA3-6, but not Ai/BSA7-9, Aw%3-6 or Aw%7-9, correlated positively with the

improvement in FEV_1 (r=0.636, P =0.001), FVC (r=0.506, P =0.003) and MMEF (r=0.391, P =0.027) at 15 min after salbutamol inhalation (**Table 2**).

Discussion

This is the first study that employed EB-OCT measurement to unravel the objective evidence of luminal narrowing and airway wall thickening in medium-sized and small airways that correlate with asthma severity. Airway remodeling in mild asthma was characterized by airway wall thickening from the 3^{rd} to 9^{th}generation, without notable luminal narrowing. The duration of symptoms, but not sputum eosinophil count, correlated with airway remodeling. After salbutamol inhalation, medium-sized and small airways elicited the most prominent changes in moderate-to-severe asthma, and the increase of medium-sized airway inner area correlated significantly with that of FEV_1 and FVC.

Remodeling of the asthmatic airways has been attributable to mucus gland hypertrophy, smooth muscle hyperplasia, increased fibroblasts and collagen deposition[18-25]. High-resolution CT showed that asthmatic patients had significantly greater Aw and smaller airway Ai compared with control subjects[26]. Our study has extended these findings with EB-OCT measurement which provided high-resolution images and had a high consistency to histological findings [8, 9]. Furthermore, EB-OCT could measure airway architectures as distal as to the 9^{th}generation of bronchi. Consistent with CT studies which probed the airways up to the 6^{th} generation bronchi[27], both Ai and Aw% of the medium-sized and small airways correlated significantly with asthma severity. The Ai tapered and Aw% increased more considerably in patients with greater asthma severity (particularly the 5^{th}-9^{th} generation). The concomitant decrease in Ai/BSA and increase in Aw% indicated the myriad of smooth muscle and mucus gland hypertrophy, increased collagen deposition and mucus plugging in moderate-to-severe asthma[28-30]. Importantly, patients with mild asthma have already developed airway remodeling, evidenced by the significantly greater Aw% in the 3^{rd}-9^{th} generation of bronchi compared with control subjects, which was in accordance with the results of previous studies that airways of patients with mild asthma yielded greater collagen and matrix production from fibroblasts compared with control subjects[31-33]. However, thickened airway wall with a relative nomal inner airway luminal area in mild asthma indicated different remodeling patterns as compared with that of early-stage COPD, characterized by airway narrowing with airway wall thickening in medium-sized and small airways (see **Table E8** in Online Supplment)[34]. Endobronchial biopsies from COPD patients with features of asthma and the asthmatic patients who have smoked showed an overlap of the histological features of inflammatory cell infiltration and thickening of the basement membrane[35]. Findings of the present study coupled with the published evidence suggested that OCT measurements would provide a complementary evidence of the airway wall structural characteristics, particularly in small airways. This will help illuminate the discrepancies between asthma and COPD, or asthma-COPD overlap. However, asthma remained suboptimally controlled in a considerable proportion of patients with mild asthma. Notably, the airway structural changes in patients with mild asthma may be explained by the chronic inflammatory that predisposed to airway wall thickening and remodeling. Our results have offered indirect support to the findings of the recent clinical trials – as-needed inhaled corticosteroids effectively improved syptom control and prevented from excebations in patients with mild asthma[36, 37]. Hence, the airway remodeling of the medium-sized and small airways might the rationale for targeted anti-inflammatory therapies for asthma.

The morphological changes in response to bronchodilators has been related to the variable airflow limitation in asthma. Multiple-breath nitrogen washout technique revealed that both conductive airways and acinar airways were partly reversible after salbutamol inhalation in asthma[38]. Furthermore, xenon-enhanced CT showed that the changes in Ai correlated significantly with improvements of FEV_1[39]. Here, we have further extended the morphological assessment directly with EB-OCT to small airways in a real-time fashion. Notably, the airway caliber significantly increased in both medium-sized and small airways after bronchodilator inhalation. Furthermore, the improvement in FEV_1 correlated with the increase in Ai/BSA of medium-sized, but not small, airways. FEV_1 primarily reflects the airflow in central airways and reportedly correlated with wheezing in asthma[40-42]. Because bronchoconstriction in medium-sized airways mainly contributed to the increase of airway resistance[41], the dilatation of medium-sized airways might lead to re-opening of the oc-

cluded small airways and improvement in ventilatory heterogeneity[43]. A study with technetium-99m-labeled albuterol aerosols showed that large particles had a more proximal deposition, and achieved greater short-term lung function improvement than smaller particles[44, 45]. The proximal deposition might be responsible for the dilatation of medium-sized airways, which significantly correlated with the improvement of FEV_1. Hence, proximal (the 3^{rd}-6^{th} generation) airways responded most prominently to salbutamol inhalation. Our observations also indicated the modes of action of bronchial thermoplasty, the effects of which were confined to a small number of large and medium-sized airways (the 3^{rd}-5^{th}generation) that contributed to symptom control in severe refractory asthma[46]. From morphological and physiological perspectives, these findings added to our understanding of the mechanisms on how and when bronchodilators improve lung function and alleviate asthmatic symptoms.

Persistent chronic inflammation is related to airway remodeling (e.g. inflammatory cells infiltration, collagen deposition, connective tissue and smooth muscle proliferation) in asthma.[28-30]Patients with severe asthma and airflow limitation usually had a longer disease duration[47], which significantly correlated with more prominent airway wall thickening[28, 48]. Consistently, Ai and Aw% of medium-sized bronchus (the 3^{rd}-6^{th} generation), but not small airways (the 7^{th}-9^{th} generation), markedly correlated with the disease duration. Hence, prolonged courses of asthma might aggravate medium-sized airways remodeling and smooth muscle contraction, and lung function decline. However, we did not observe a significant correlation between sputum or blood eosinophil count and airway remodeling.

EB-OCT measurement has its unique advantage over CT assessment, including the avoidance of radiation exposure, the ability to continuously measure the airway morphology *in vivo* , and the ability to detect dynamic airway morphologic changes in terms of bronchodilator responses. Nonetheless, some caveats should be considered. This study is limited by the lack of direct bronchoscopic biopsies from the asthmatic airways to compare the OCT images and histological findings. However, the consistency between OCT and histological measurements had been well acknowledged [9, 15]. We therefore believed that OCT measurements might better reflect the asthmatic airway morphological changes (particularly the caliber)*in vivo* . Second, our study was limited by its cross-sectional design. A longitudinal study with a longer follow-up duration (i.e. 12 months) or different medication categories (i.e. long-acting beta-receptor agonists or muscarinic receptor antagonists) would be needed. To secure the comparability of EB-OCT measurement in an identical bronchial segment during scanning, we have only detected the bronchodilator responses at a single segment (RB9), because it would not be ethical and practical to detect the whole airway structure before and after salbutamol inhalation.

In conclusion, we have unraveled airway remodeling (luminal narrowing and airway wall thickening) that correlated with asthma severity. Patients with mild asthma have already developed airway remodeling, lending support to the use of anti-imflammatory intervention in mild asthma. The understanding of the principal anatomical site of bronchodilator responses might shed light on the development of inhaled bronchodilators to more effectively improve lung function and ameliorate symptoms in asthma.

Author contributions: Z. Q. S., Z. Q. Z. and W. J. G. performed the literature search and drafted the manuscript; Z. Q. S., Z. Q. Z., Y. C., X. B. C., C. H. Z. and C. L. T. were responsible for subject recruitment; Z. Q. S., Z. Q. Z., and W. J. G. contributed to the data collection, data analysis and data interpretation; Z. Q. S., Z. Q. Z., W. J. G., S. Y. L. and N. S. Z. contributed to study conception; S. Y. L. and N. S. Z. provided critical review of the manuscript and approved the final submission.

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Table 1: Demographics and clinical characteristics of study participants (Study 1)

	Control	**Mild asthma**	**Moderate asthma**	**Severe asthma**
Case (n)	31	21	36	47
Sex (M/F)	12/15	9/12	18/18	26/19
Age (y)	53.0±8.4	45.5±14.9	49.0±11.8	50.6±10.2
Body-mass index (kg/m^2)	23.3±2.8	23.7±4.0	24.4±3.3	23.5±3.2
Body surface area (m^2)	1.62±0.16	1.62±0.17	1.63±0.34	1.66±0.20
Atopy (n, %)	-	8 (38.1%)	27 (75.0%)	36 (76.6%)
Disease duration (yrs)	-	5.6±9.3§	12.8±10.2	13.2±11.7*
FEV$_1$ (L)	2.60±0.61++SS	2.19±0.72§	1.92±0.84*	1.65±0.67*+
FVC (L)	3.29±0.80	3.04±1.04	3.02±0.93	2.85±0.77
FEV$_1$% predicted	99.6±10.4 +++SS	82.1±14.6 *++SS	68.8±20.3 *+	60.6±19.2 *+
FEV$_1$/FVC ratio	79.3±5.5 ++SS	72.1±8.7 ++SS	62.7±14.0 *+	57.5±13.7 *+
MMEF % predicted	68.9±17.3 +++SS	41.8±19.6 *	36.1±27.1 *	27.3±23.6 *
Blood eosinophils ×10^9 (cells/L)	-	0.48±0.66	0.40±0.42	0.37±0.37

	Control	Mild asthma	Moderate asthma	Severe asthma
Sputum eosinophils (%)	-	23.7±23.0	26.9±27.0	25.0±24.8

* Compared with control, P<0.05;

+ Compared with mild asthma, P<0.05;

++ Compared with moderate asthma, P<0.05;

SS Compared with severe asthma, P<0.05.

Multiple-group comparisons were made by using one-way ANOVA, Bonferroni correction was applied to adjust for multiple comparisons.

Abbreviation: FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; MMEF: maximal mid-expiratory flow; ICS: inhaled corticosteroid.

Table 2: Correlation between the increase in Ai/BSA and lung function parameters after salbutamol inhalation in patients with moderate-to-severe asthma (n=32)

	ΔAι/BΣA οφ 3ρδ-6τη γενερατιον	**ΔAι/BΣA οφ 3ρδ-6τη γενερατιον**	**ΔAι/BΣA οφ 7τ**
	r	P	**r**
$\Delta FE''_1$	**0.636**	**0.001**	0.111
$\Delta F''$	**0.506**	**0.003**	0.192
$\Delta FE''_1/F''$	0.297	0.099	0.086
ΔMMEΦ	**0.391**	**0.027**	0.014
$\Delta ME\Phi_{25}$	0.306	0.089	0.010
$\Delta ME\Phi_{50}$	0.03	0.101	0.079

Δ Spirometric parameters (ΔFEV_1, ΔFVC, ΔFEV_1/FVC, ΔMMEF, ΔMEF_{25}, ΔMEF_{50}) denoted the difference before and at 60 min after salbutamol inhalation.

ΔAi/BSA denoted the difference of Ai/BSA before and at 15 min after inhaling salbutamol.

Data in bold indicated the correlation coefficient with statistical significance.

Abbreviation: FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; MMEF: maximum midexpiratory flow; MEF_{25}: Maximal expiratory volume when 25% of the lung volume has been expired; MEF_{50}: Maximal expiratory volume when 50% of the lung volume has been expired.

Figure Legends

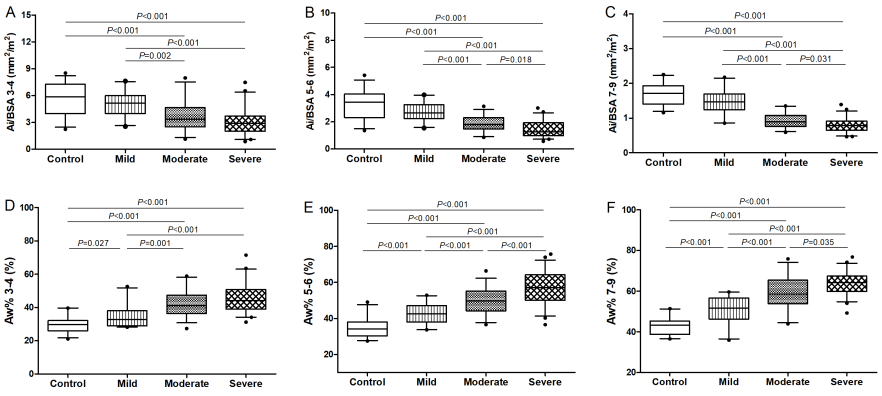

Figure 1: Airway inner area from the 3rd to 9th generation of bronchi among control subjects and asthmatic patients with different severity

(A and D) Ai/BSA and Aw% from the 3rd to 4th generation of bronchi. (B and E) Ai/BSA and Aw% from the 5th to 6th generation of bronchi. (C and F) Ai/BSA and Aw% from the 7th to 9th generation of bronchi.

Abbreviation: Ai: airway inner area; BSA: body surface area; Aw%: airway wall area percentage; 3-4: 3rd to 4th generation of bronchi; 5-6: 5thto 6th generation of bronchi; 7-9: 7th to 9th generation of bronchi.

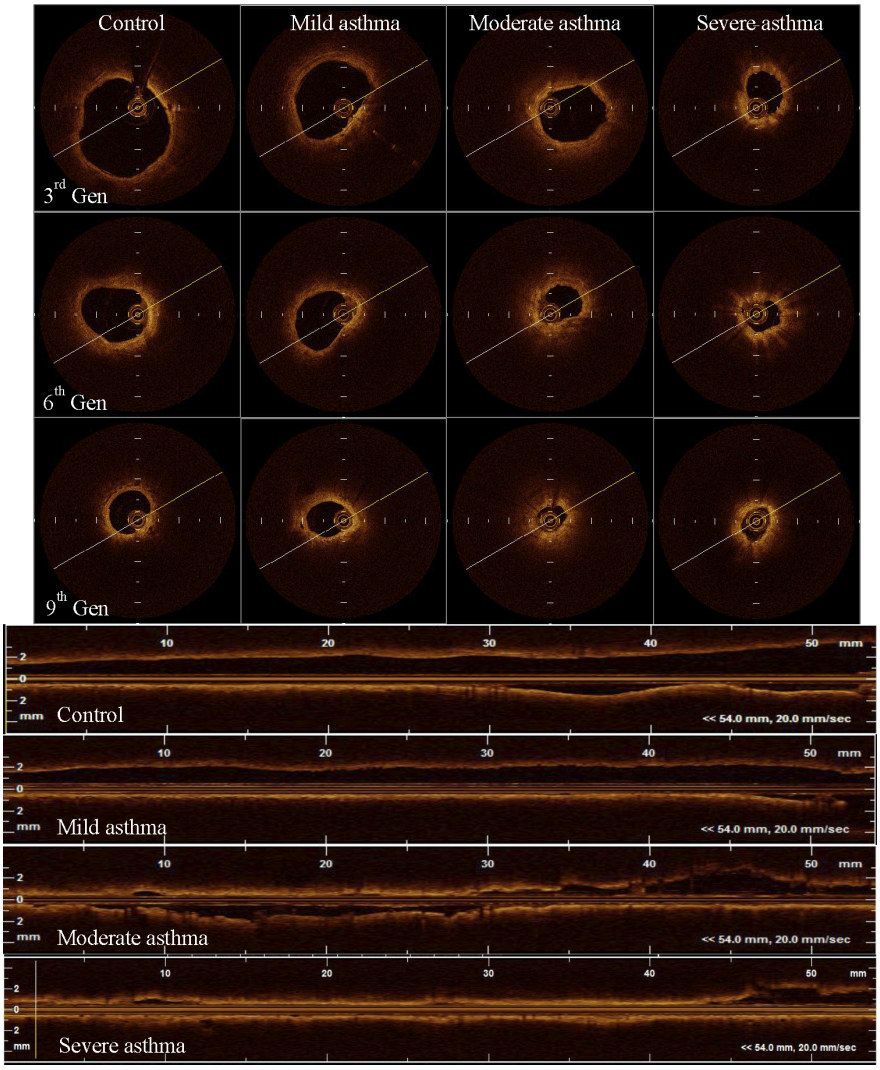

Figure 2: Cross- and longitudinal-sectional endobronchial optical coherence tomography images from the 3rd to 9th generation of bronchi

Compared with control subjects, patients with asthma were characterized by significant airway wall thickening and luminal narrowing. More prominent structural abnormalities were associated with greater severity of asthma, as evidenced by the decreased airway caliber and greater airway wall area.

Abbreviation: gen: generation of bronchi; 3rd: the 3rd generation of bronchi; 6th: the 6th generation of bronchi; 9th: the 9th generation of bronchi.

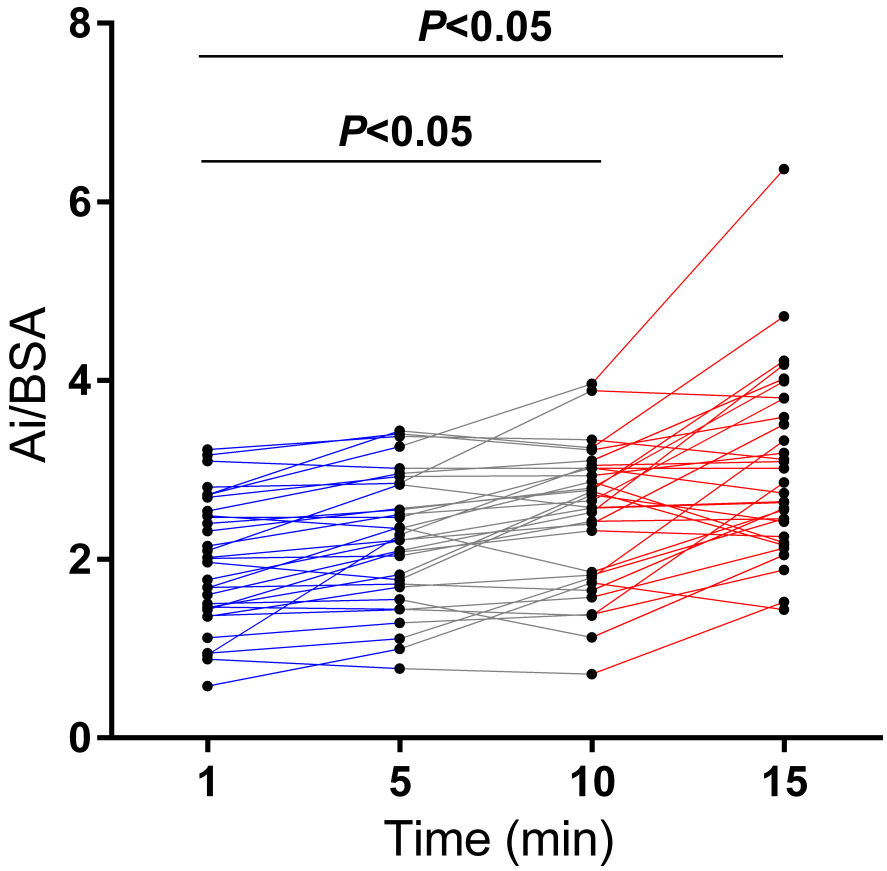

Figure 3: Changes of Ai/BSA from the 3rd to 9th generation of bronchi in response to salbutamol inhalation in each individual (n=32)

The average Ai/BSA at 10th and 15thmin after bronchodilation were significantly increased, compared with the Ai/BSA obtained at 1st min (all$P$ <0.05).

Abbreviation: Ai: airway inner area; BSA: body surface area.

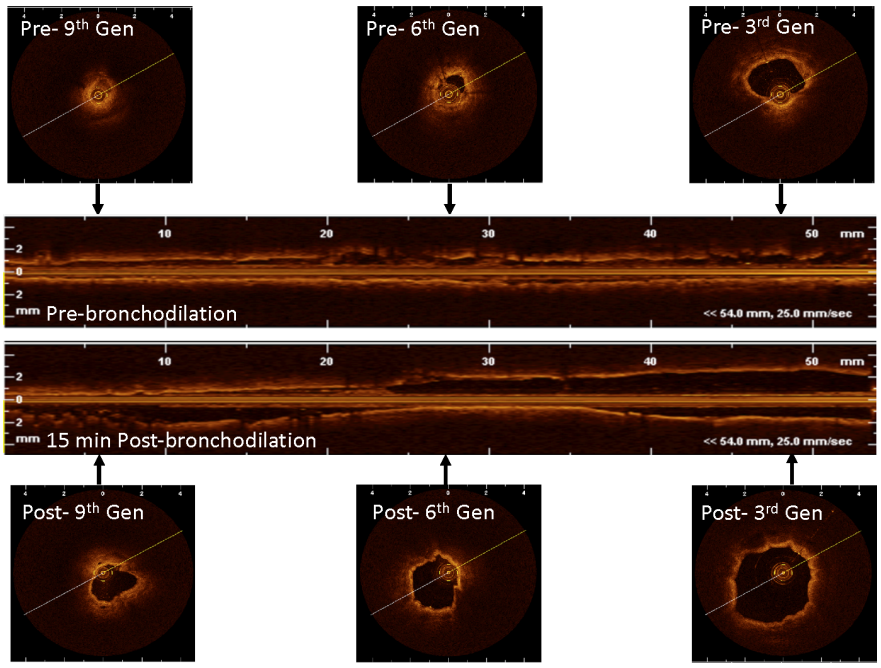

Figure 4: Changes of the airway structures assessed with EB-OCT before and at 15 min after salbutamol inhalation

Shown in the figures are the EB-OCT images from a 60-year-old man with moderate asthma. The average increment of Ai/BSA was 32.6%, 48.9% and 176.6% respectively in the 3rd, 6thand 9th generation of bronchi at 15 min after salbutamol inhalation.

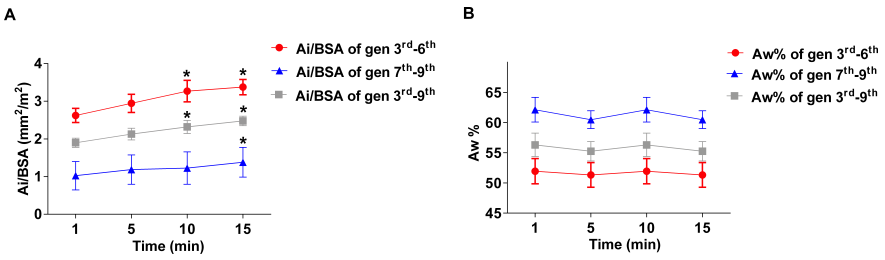

Figure 5: Dynamic changes of airway inner area and airway wall thickness percentage after salbutamol inhalation.

(A) The average changes of Ai from the 3rd to 9th generation of bronchi. (B) The average changes of Aw% of medium and small airways.

* Compared with baseline, P<0.05.

Abbreviation: Ai: airway inner area; BSA: body surface area; Aw%: airway wall area percentage; gen: generation of bronchi.