Sestrin2 Was Involved in Airway Remodeling in COPD: A Multi-level Research

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Research article

Keywords: Sestrin2, Airway remodeling, COPD, Matrix metalloproteinase 9, Quantitative computed tomography

Posted Date: August 25th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-54810/v1

Version of Record: A version of this preprint was published on November 16th, 2020. See the published version at https://doi.org/10.1186/s12890-020-01329-x.

Abstract

Background. Airway remodeling is a major pathological characteristic of chronic obstructive pulmonary disease (COPD), which is greatly associated with oxidative stress. Sestrin2 has recently drawn attention as a representative antioxidant protein. However, the underlying correlation between sestrin2 and airway remodeling in COPD has not yet been clarified.

Methods. A total of 124 subjects were enrolled in this study, including 62 control subjects and 62 COPD patients. The pathological changes in airway tissues were showed by different staining methods. The expression of sestrin2 and matrix metalloproteinase 9 (MMP9) in airway tissues was assessed by Immunohistochemistry staining. Enzyme-linked immunosorbent assay (ELISA) was used to detect the serum concentrations of sestrin2 and MMP9. The airway parameters on computed tomography (CT) of all participants were measured for the evaluation of airway remodeling. Serum sestrin2 concentrations' relationship with MMP9 and airway parameters on chest CT was further analyzed.

Results. In patients with COPD, staining results of airway structure showed noticeable pathological changes of remodeling, including cilia cluttered, subepithelial fibrosis, and reticular basement membrane (Rbm) fragmentation. Compared with control subjects, the expression of sestrin2 and MMP9 was significantly increased in both human airway tissues and serum. Typical imaging characteristics of airway remodeling and increased airway parameters were found on chest CT. Additionally, the serum sestrin2 concentration was positively correlated with serum MMP9 concentration and airway parameters on chest CT.

Conclusion. Increased expression of sestrin2 is related to airway remodeling in COPD at three levels including histology, biomarkers and imaging. It is demonstrated for the first time that sestrin2 may be a novel biomarker for airway remodeling in COPD.

Background

Chronic obstructive pulmonary disease is the most common chronic airway inflammatory disease, which is characterized by persistent respiratory symptoms and irreversible airflow limitation. The parenchymal destruction and airway remodeling in pathological changes can greatly contribute to the development of COPD(1). Airway remodeling refers to changes in size, mass, and the quantity of airway structural components(2).Functionally, airway remodeling produces airway obstruction, airway hyperactivity and reduces lung compliance(2), leading to poor clinical outcomes(3). Therefore, it is necessary to find the possible biomarker involved in airway remodeling in COPD.

Patients with COPD suffer an excessive oxidant burden due to tobacco, air pollution, and other harmful particles(1, 4). Excessive oxidative stress contributes to enhance airway remodeling and inversely correlates with lung function in patients with COPD(5, 6).Sestrins, a family of highly conserved proteins, are induced by oxidative and persistent hypoxia and expressed in the lung, brain, liver, heart and many other organs(7, 8). Sestrin2, a leading member of sestrins, is the main component of the systemic

antioxidant defense mechanism. Recent studies show sestrin2 is involved in many oxidative stress-related respiratory diseases(9–11), including COPD. It was found that sestrin2 is upregulated and can suppress the progression of pulmonary emphysema in mouse models of COPD(12).However, whether there is a potential relationship between sestrin2 and airway remodeling in COPD remains unknown.

The main evaluation methods for airway remodeling involve three levels, histology, biomarkers and imaging technology. Major pathological features of airway remodeling in COPD include epithelial-mesenchymal transition (EMT), enhanced collage deposition, extracellular matrix degradation and repair, cilia dysfunction, and inflammatory infiltration(13, 14). There is considerable literature showing that matrix metalloproteinases (MMPs), a family of zinc-dependent proteolytic enzymes, involved in respiratory tract remodeling, particularly MMP9 (15, 16).With the rapid development of imaging technology in recent years, CT is useful in assessing the airway dimensions and provide a valuable tool for the study of airway disease(17). The previous study found that quantitative CT-assessed parameters of the airway were correlated with pathological changes in airway remodeling(18, 19). At present, there is still a lack of multi-level research on the relationship between sestrin2 and airway remodeling. The goal of this research was to investigate the expression characteristics of sestrin2 in patients with COPD and analyze the correlation between sestrins2 and airway remodeling in levels of histology, biomarkers and imaging.

Methods

1.Subjects

Airway tissues were obtained from lobectomy or segmentectomy in 4 patients with lung cancer in situ and 4 COPD patients with comorbidity of lung cancer in situ at the First Affiliated Hospital of Anhui Medical University. Serum were obtained from a total of 124 participants (62 COPD patients and 62 controls) recruited from March 2018 to September 2019. The control group was collected from the physical examination center of our hospital in the same period. All patients with COPD met the diagnostic criteria of the 2018 GOLD guideline. The exclusion criteria include patients with severe cardiovascular, hepatic and renal dysfunction, hematological system diseases, diabetes, obesity and malignant tumor; patients with mental illness; patients with other lung diseases (such as asthma, acute exacerbation of COPD, pneumonia, cystic fibrosis, active pulmonary tuberculosis, interstitial lung disease, etc.); patients with a history of taking corticosteroids or immunosuppressants regularly; patients with a history of participating in any health care activities before attending this study.

2. Pulmonary function tests

Pulmonary function test was performed on each subject at 15 minutes after inhaling salbutamol 400 μg (Ventolin, GlaxoSmithKline, London, UK) by a dry spirometer device (Erich Jaeger GmbH, Hoechberg, Germany). Pulmonary function results were reported by experienced respiratory and critical care physicians. The following parameters were recorded: forced vital capacity (FVC), forced expiratory

volume in one second (FEV1), FEV1% predicted, and the FEV1/FVC ratio. FEV1/FVC□0.7 was classified as COPD.

3.Chest computed tomography scan and analysis

The GE LightSpeed VCT, GE Discovery C1750, and Toshiba Aquilion 16-slice CT were used for scanning. All parameters were obtained by CT plain image reconstruction and were reconstructed by the standard algorithm. The lung window (window width 1500 HU, window level − 500 HU) and mediastinum window (window width 400 HU, window level 40 HU) were observed. Scanning parameters: GE LightSpeed VCT and GE Discovery CTI SO CT machines adopt tube voltage 120 kV, tube current automatic regulation, layer thickness 5.0 mm, reconstruction layer thickness 0.625 mm, pitch 1.375; Toshiba Aquilion 16-layer CT machine adopts tube voltage 120 kV, tube current 150 mA, layer thickness 5.0 mm, reconstruction layer thickness 1.0 mm, pitch 0.980. Airway quantitative measurement was performed using the thoracic VCAR software supplied by GE in the apical segment of the right upper lobe (RB1). The following parameters can be automatically measured using the thoracic VCAR software: square root of the wall area at an internal airway area of 8 mm^2 (Ai8), the percentage of bronchial wall area (WA %), total airway area(A_0) and relative wall thickness (RWT) (expressed as the ratio of wall thickness and external diameter)(20).

4.Measurement of serum sestrin2 and MMP9 concentrations

The blood samples were collected from all subjects by venipuncture with a tube without anticoagulants. The serum was collected after centrifuged for 20 min at 1000 × g and stored at − 80 °C. The serum sestrin2 and MMP9 concentration were detected using human enzyme-linked immunosorbent assay kits following the manufacturer's instruction. The ELISA kits were against sestrin2(Cloud-Clone Crop, Wuhan, China) and MMP9 (CUSABIO, Wuhan, China).

5. Histological staining of airway tissues

Human lung tissues were fixed in 4% paraformaldehyde and then embedded in paraffin.5-μm-thick sections were prepared before staining. HE staining, PASM staining and Masson staining kits (Solarbio, Beijing, China) were employed to test structure changes of the human airway. The experimental methods followed the manufacturer’s instructions. Immunohistochemical staining was applied to detect the expressions of sestrin2 and MMP9 of the sections. Antigens were repaired according to primary antibody specifications. The endogenous peroxidase blocker was added and incubated for 30 minutes at 37℃. The slides were incubated with rabbit anti-sestrin2 antibody (ProteinTech#10795-1-AP, Chicago, USA, diluted with 1:200) and mouse anti-MMP9 antibody (Abcam #ab58803, Cambridge, UK, diluted with 1:100) at 4℃ overnights. Then, the slides were washed with PBS, followed by incubated with biotinylated goat anti-rabbit IgG and then incubated with streptavidin-peroxidase. Diaminobenzidine (DAB) solution (ZSGB-Bio, Beijing, China) was used for staining. Finally, all slides were counterstained with hematoxylin. Staining was imaged under a light microscope (Leica ICC50 W, Wetzlar, Germany).

6.Statistical analysis

GraphPad Prism 5 (San Diego, California, USA) and SPSS 22.0 (IBM, Armonk, NY, USA) software was used to analyze the data. Categorical variables were expressed as count (%). The chi-square test examined differences between sex ratios and smoking status. Normally distributed data were compared using an unpaired t-test and expressed as mean (standard deviation). All non-normally distributed were compared by the Mann-Whitney U test and expressed as median (interquartile range). The correlation between serum sestrin2 concentration and other measurement indexes in the COPD group was analyzed by Pearson's correlation test. A P-value of less than 0.05 was regarded as statistically significant.

Results

1. Demographic characteristics of all subjects

All subjects recruited in this study were divided into two groups: the COPD group(n = 62) and control group(n = 62). There were no significant differences in age, sex, BMI and smoking index between the two groups. However, the pulmonary function indicators (FEV1, FEV1%, and FEV1/FVC) were significantly decreased in the COPD group ($P < 0.001$). The demographic characteristics of all subjects were listed in Table 1.

Table 1
Demographic characteristics of the control group and COPD group

Variables	Control group(n = 62)	COPD group(n = 62)	P value
Age, years	67.37 (6.87)	68.73 (8.47)	0.330[a]
Mal sex, n (%)	34(54.84%)	31(50.00%)	0.590[c]
BMI, kg/m^2	22.90(3.32)	22.17 (4.29)	0.826[b]
Smoking status			0.234[c]
Never smoked, n (%)	50(80.65%)	45(72.58%)	
Current smoker, n (%)	8(12.90%)	7(11.29%)	
Ex-smoker, n (%)	4(8.06%)	10(16.13%)	
Smoking, pack-years*	29.38 (15.16)	31.94 (17.87)	0.689[a]
Pulmonary function			
FEV1, L	2.84 (0.66)	1.06 (0.55)	0.000[a]
FEV1/FVC, %	82.95(7.19)	59.58 (7.76)	0.000[b]
FEV1, % of predicted	104.30 (12.58)	38.00(21.20)	0.000[b]

Notes: Data are presented as number (%) or means (standard deviation) or median (interquartile range). *(number of cigarettes per day × number of years of smoking) /20.

Abbreviations: COPD, chronic obstructive pulmonary disease; BMI, body mass index; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; [a]t-test; [b]Mann-Whitney U test; [c]$\chi 2$ test.

2. Histological staining of airway tissues

To characterize airway remodeling at the pathological level, airway tissues were isolated and various staining methods were carried. HE staining demonstrated that the airway epithelial structure and ciliated cells were intact in the control group, while the ciliated cells were detached and lodging in different degrees in the COPD group (Fig. 1A). Masson staining showed that collagen deposition in the bronchial epithelial area of the COPD group was significantly increased compared with the control group (Fig. 1B). Continuous Rbm was observed clearly in the control group, but the reticular basement membrane was fragmentary in the COPD group through PASM staining (Fig. 1C).

3.Expression of sestrin2 and MMP9 in airway tissues

Compared with the control group, immunohistochemical staining for sestrin2 demonstrated that the expression of sestrin2 in airway tissues was significantly increased in the COPD group, especially in bronchial epithelial cells (Fig. 2C). Meanwhile, the bronchial epithelial cells with sestrin2 positive

expression also highly expressed MMP9, which is known to be a biomarker of airway remodeling (Fig. 2D).

4. Serum concentrations of sestrin2 and MMP9

Compared with the control group,the serum sestrin2 concentration was significantly increased in patients with COPD($P < 0.001$) (Table 2, Fig. 2A). Similarly, the expression of MMP9 in serum was significantly higher in patients with COPD than in controls($P < 0.001$) (Table 2, Fig. 2B).

Table 2
Serum sestrin2 and MMP9 concentrations and quantitative CT measurements in the total subjects

Test index	Control group(n = 62)	COPD group(n = 62)	P value
Serum assay			
Sestrin2(ng/ml)	5.00(3.93)	8.61 (2.89)	0.000[b]
MMP9(ng/ml)	993.02 (421.30)	2125.65 (840.81)	0.000[a]
Airway parameters on chest CT			
Ai8(mm)	4.45 (0.28)	5.51 (0.64)	0.000[a]
A_0 (mm^2)	28.05 (2.50)	38.72 (7.41)	0.000[a]
WA% (%)	70.53 (3.01)	78.31 (3.73)	0.000[a]
RWT	0.23 (0.11)	0.27 (0.02)	0.000[a]

Notes: Data are presented as means (standard deviation) or median (interquartile range). P-values were calculated by analysis of variance.

Abbreviations: CT, computed tomography; MMP9, matrix metalloproteinases 9; Ai8, Square root of the wall area at an internal airway area of 8 mm^2; A_0, total airway area; WA%, wall area percentage; RWT, the ratio of airway wall thickness to overall diameter; [a]t-test;[b]Mann-Whitney U test.

5.Airway parameters on chest CT in subjects

Compared with the control group, the chest CT imaging shows that the bronchial wall was thickening, and the bronchial lumen was rough and curved in patients with COPD(Fig. 3). We demonstrated that the values of airway parameters on chest CT(Ai8, A_0, WA% and RWT) were all significantly increased in the COPD group(P□0.001) (Table 2, Fig. 4).

6.Relationship between sestrin2 and airway remodeling in patients with COPD

To elucidate the relationship between sestrin2 and airway remodeling in patients with COPD, the correlation between serum sestrin2 concentration and MMP9, as well as airway parameters on chest CT, were analyzed in patients with COPD. The result showed that serum sestrin2 concentration was positively correlated with serum MMP9 concentration (r = 0.264;P = 0.038) (Fig. 5), Ai8 (r = 0.287;P = 0.024) (Fig. 6A), A_0(r = 0.273;P = 0.032) (Fig. 6B),WA% (r = 0.294;P = 0.020) (Fig. 6C) and RWT (r = 0.304;P = 0.016) (Fig. 6D).The detailed results of Pearson rank correlation analysis were showed in Table 3.

Table 3
Association between serum sestrin2 concentrations and other measurement indexes in COPD group

Test index	**r**	**P value**
Serum assay		
MMP9(ng/ml)	0.264	0.038
Airway parameters on chest CT		
Ai8(mm)	0.287	0.024
A_0 (mm^2)	0.273	0.032
WA% (%)	0.294	0.020
RWT	0.304	0.016
Notes: Correlations were determined by Pearson rank correlation analysis.		
Abbreviations: CT, computed tomography; MMP9, matrix metalloproteinases 9; Ai8, Square root of the wall area at an internal airway area of 8 mm^2; Ao, total airway area; WA%, wall area percentage; RWT, ratio of airway wall thickness to overall diameter; r, Pearson rank correlation coefficient;		

Discussion

The goal of this study was to find out the possible relationship between sestrin2 and airway remodeling in COPD. At the histological level, immunohistochemical staining of human airway tissues demonstrated that sestrin2 and MMP9 were expressed much higher in the COPD group than that in the control group. At the biomarker level, the serum concentrations of sestrin2 and MMP9 were increased in patients with COPD. At the imaging level, the airway structure showed visible imaging characteristics of the airway remodeling and quantitative airway parameters on chest CT were significantly increased in patients with COPD. We further demonstrated that serum sestrin2 level correlated well with serum MMP9 level and quantitative airway parameters on chest CT. Thus, this study demonstrated a new idea that sestrin2 may participate in the airway remodeling in COPD.

Airway remodeling is a recognized characteristic in the course of COPD(21), which is a complex structural change caused by multiple factors(15, 22, 23). Our study demonstrated that there are typical pathological characteristics of airway remodeling in the airway tissues of COPD, including airway subepithelial

fibrosis, reticular basement membrane fragmentation and cilia clutter. MMP9, a member of matrix metalloproteinases, was often described as an indicator of airway remodeling in COPD(24). In our study, immunohistochemical staining showed that there was a significantly higher expression of MMP9 in the airway in the COPD group. These findings showed that significant airway remodeling was evident in patients with COPD at the histological level.

Our study demonstrated that the concentration of sestrin2 in the COPD group was significantly increased in human serum compared with the control group. Sestrin2, a critical antioxidant protein, plays a protective role in antioxidant defense and reducing intracellular reactive oxygen species (ROS) (25–28). Emerging evidence have shown that sestrin2 plays a vital role in the formation and development of fibrosis in many organs, including heart, liver and renal(8, 29–31). Consistent with previous reports(19, 24, 32), the serum concentration of MMP9 was significantly increased in the COPD group in our study. It has been proven that MMP9 was associated with pulmonary function and indicators of small airway disease in COPD(33, 34).To further explore whether sestrin2 is related to airway remodeling in COPD, immunohistochemical staining in the airway was done and showed that there was a significantly higher expression of sestrin2 in the airway in the COPD group, especially in bronchial epithelial cells full of MMP9. Furthermore, we also found that the serum sestrin2 concentrations were positively correlated with serum MMP9 concentrations. Therefore, these findings highlighted the possible utility of sestrin2 as a blood biomarker for airway remodeling in COPD.

Given that airway parameters on chest CT have been regarded as radiological biomarkers for COPD and associated with airflow obstruction in all GOLD stages(35–37), airway structure and airway parameters on chest CT were obtained automatically to assess airway remodeling in our study. We demonstrated that the COPD group showed apparent changes in the airway structure, characterized by the thickened bronchial wall and rough and curved bronchial lumen. The four quantitative airway parameters on chest CT (Ai8, A_0, WA% and RWT) were all statistically increased, which demonstrate airway remodeling in COPD at the imaging level. The previous study of our team found that airway parameters on chest CT were related to the CAT score and the mMRC classification of COPD(38). It was reported that there was also a significant correlation of sputum elastase with the thickness of bronchial wall measured by chest CT(39). In this study, our study demonstrated that there was a positive relationship between serum sestrin2 concentration and quantitative airway parameters on chest CT, further suggesting that the levels of sestrin2 may reflect the airway remodeling in COPD at the imaging level.

In our study, we demonstrated that sestrin2 is closely related to airway remodeling in COPD. Early airway disease can be undetected before impairments of lung function are observed in COPD(13). However, these early airway diseases gradually lead to thickening of the airway wall and narrowing of the lumen in later life, known as airway remodeling, which accelerated decline in lung function over time(2).Therefore, sestrin2 may be used as a potential therapeutic target for delaying the progression of COPD, and it is meaningful to further study the particular mechanism of sestrin2 regulating airway remodeling. The present study also had certain limitations. First, due to most patients with mild COPD are unaware of their condition or never receive regular lung function tests, our subjects were most moderate to severe patients.

Furthermore, sestrin2 levels have been reported to decline after treatment in Parkinson's, asthma and obstructive sleep apnea (OSA)(9, 11, 40). We did not follow up on the changes in serum sestrin2 and MMP9 concentration and quantitative airway parameters on chest CT in patients with COPD after regular treatment.

Conclusions

In summary, the airway of patients with COPD showed significant airway remodeling from levels of histology, biomarker and imaging. The expression of sestrin2 was significantly increased in patients with COPD. A positive correlation was found in serum sestrin2 concentration and MMP9 concentration as well as quantitative airway parameters on chest CT as markers of airway remodeling. Sestrin2 may serve as a potential clinical biomarker for airway remodeling in COPD.

List Of Abbreviations

Chronic obstructive pulmonary disease, COPD; Matrix metalloproteinase 9, MMP9; Enzyme-linked immunosorbent assay, ELISA; Computed tomography, CT; Reticular basement membrane, Rbm; Epithelial-mesenchymal transition, EMT; Matrix metalloproteinases, MMPs; Forced vital capacity, FVC; Forced expiratory volume in one second, FEV1;Square root of the wall area at an internal airway area of 8 mm2, Ai8;The percentage of bronchial wall area, WA %;Total airway area, AO; Relative wall thickness, RWT; Diaminobenzidine, DAB; Reactive oxygen species, ROS; Obstructive sleep apnea, OSA.

Declarations

Ethics approval and consent to participate. All subjects signed the informed consent and the study protocol was reviewed and approved by the ethics committee of Anhui Medical University.NO. 20180388..

Consent for publication. Not applicable.

Availability of data and materials. All data generated or analyzed during this study are included in this published article.

Competing interests. The authors reported no conflicts of interest in this work.

Funding. This work was funded by the Natural Science Foundation of China (NSFC grant 81870036) and the Key Technology Research and Development Program of Anhui Province (1804h08020237). The funder designed the study and had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Authors' contributions. GHF, DWZ and YYW designed the study and had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. GHF, DWZ and YYW drafted the paper. GHF, DWZ, YYW and SJ did the analysis, and all authors critically

revised the manuscript for important intellectual content and gave final approval for the version to be published. DWZ, SJ and YYW collected the data. All authors were expected to have made substantial contributions to the conception. All authors read and approved the final manuscript.

Acknowledgements. The authors would like to sincerely thank the Pulmonary Department, Medical laboratory Department, Medical Imaging Department, Physical Examination Center, First Affiliated Hospital of Anhui Medical University and the Center for Scientific Research of Anhui Medical University for valuable help in our study.

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Figures

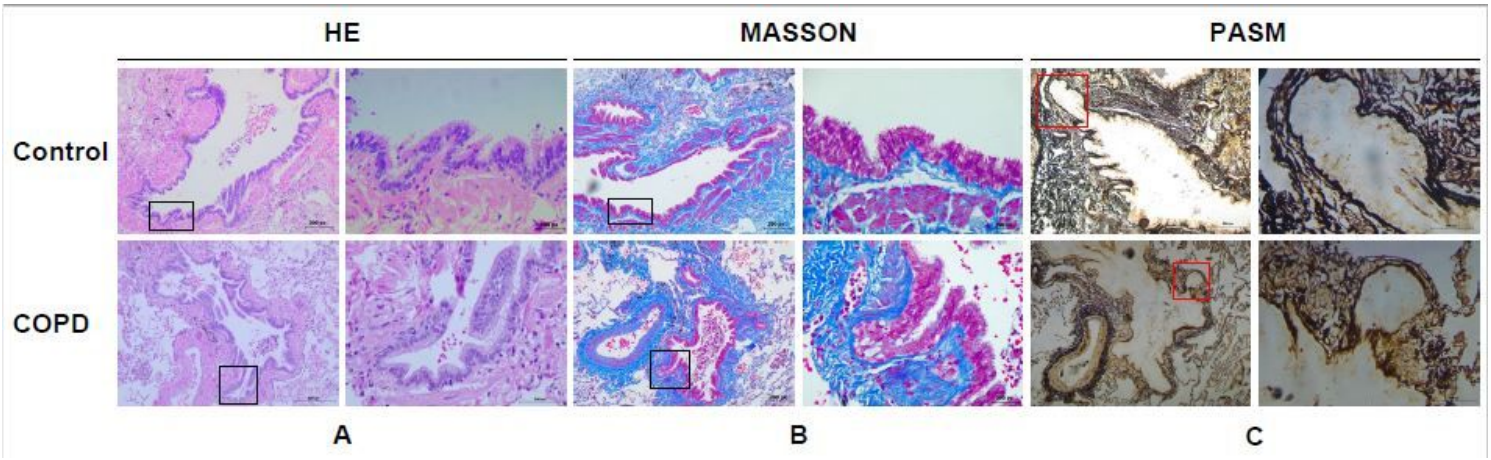

Figure 1

Staining of airway tissues in control and COPD group. Notes: (A) H&E stains in control and COPD group; (B) Subepithelial fibrosis by Masson staining in control and COPD group; (C) Change of reticular basement membrane thickness by PASM staining in control and COPD group.

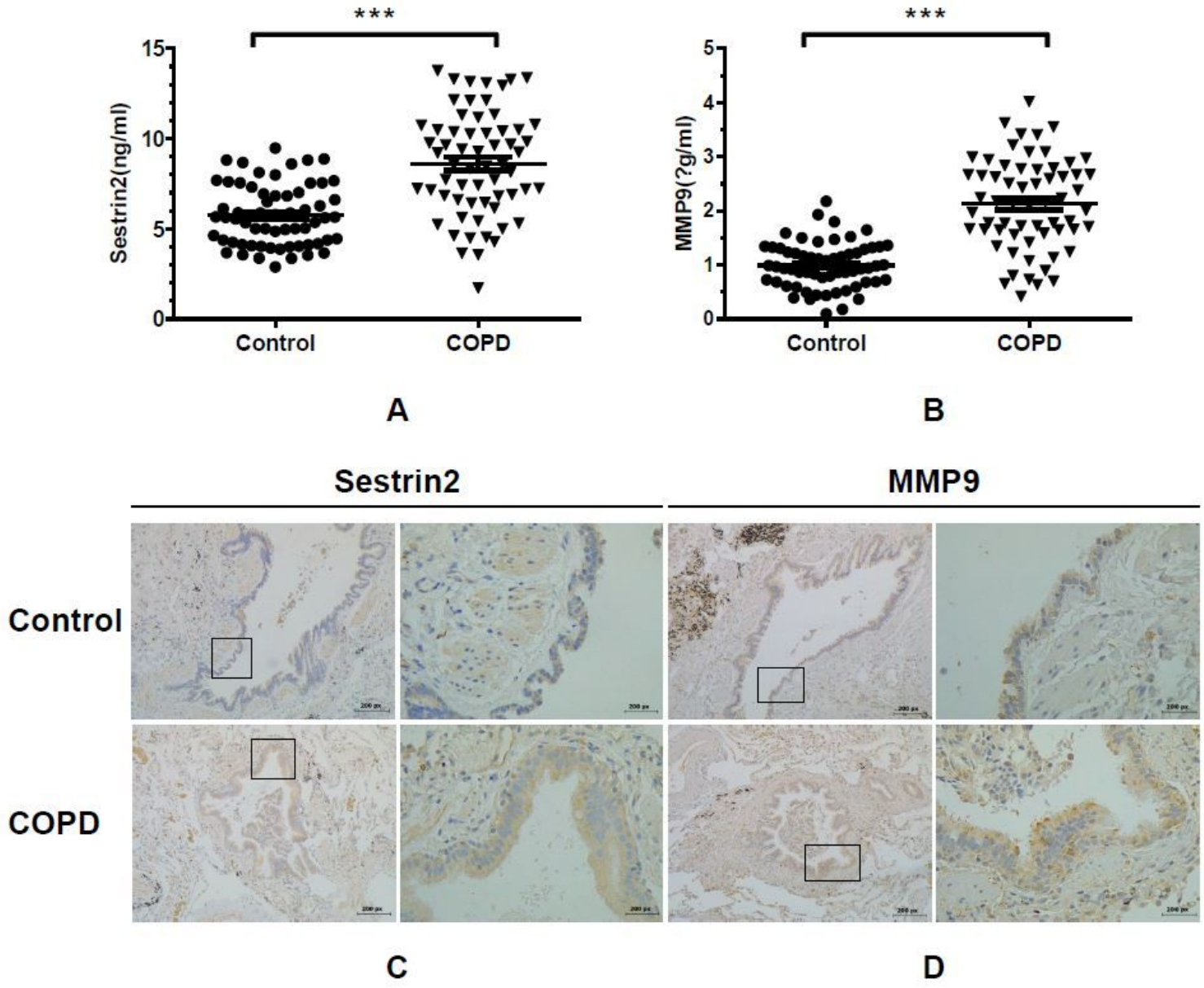

Figure 2

Sestrin2 and MMP9 expression in serum and airway tissue in control and COPD group. Notes: (A)Serum sestrin2 concentrations in control and COPD groups; (B) Serum MMP9 concentrations in control and COPD groups; (C)Expression of sestrin2 by IHC in control and patients with COPD; (D)Expression of matrix metalloproteinases 9 by IHC in control and patients with COPD. *P□0.05, **P□0.01, ***P□0.001 versus control group.

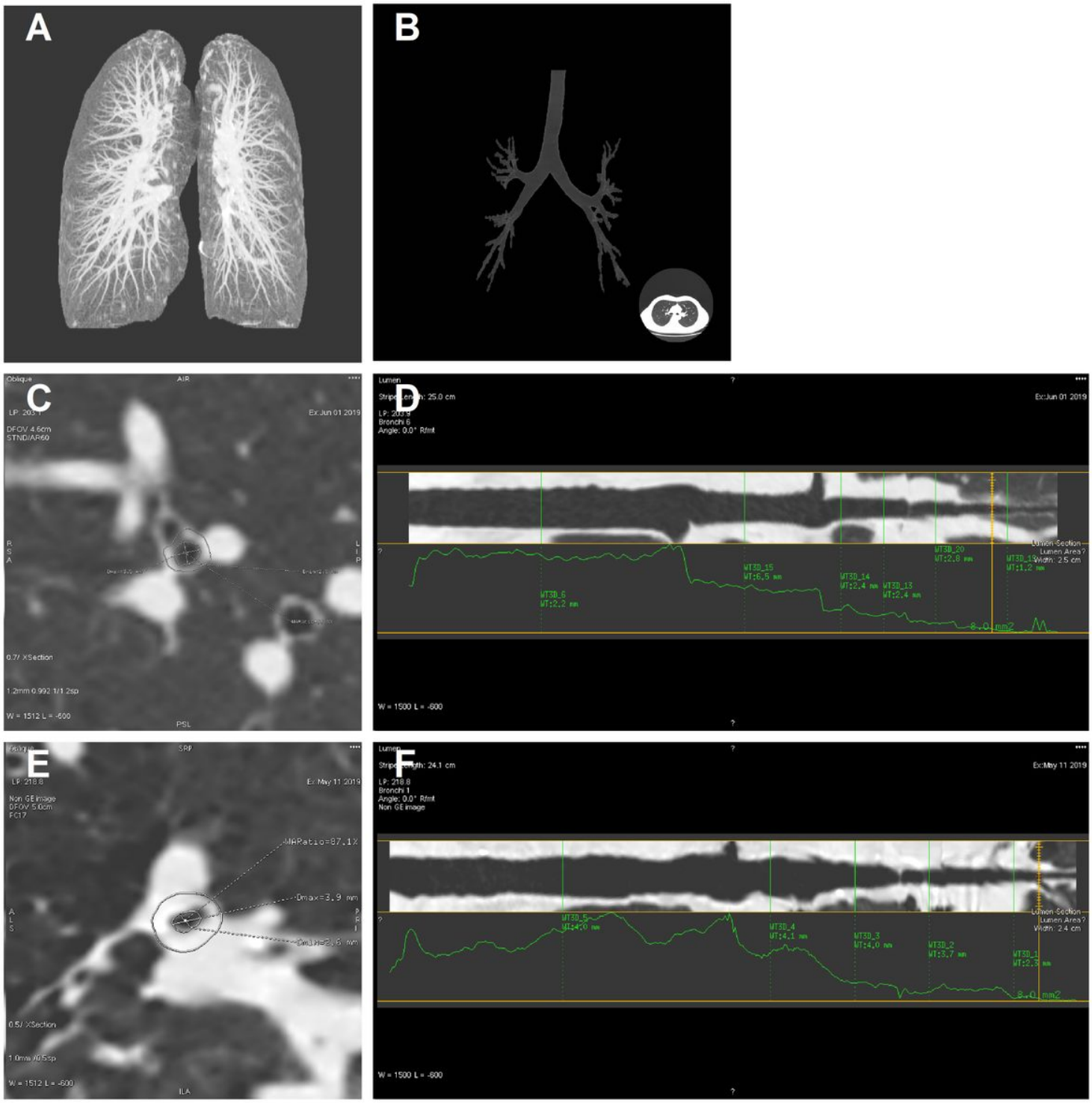

Figure 3

Chest computed tomography image in control and COPD group. Notes:(A) Lung tissue extracted automatically by software by Thoracic VCAR software;(B) Airway tree extracted automatically by software by Thoracic VCAR software;(C)–(E) Cross-section of an exact airway with lumen area 8mm2, shows delineation of outer and inner bronchial wall, permitting calculation of airway measurements in control and COPD groups;(D)–(F) Curved planar reformation of the bronchial pathway in control and COPD group.

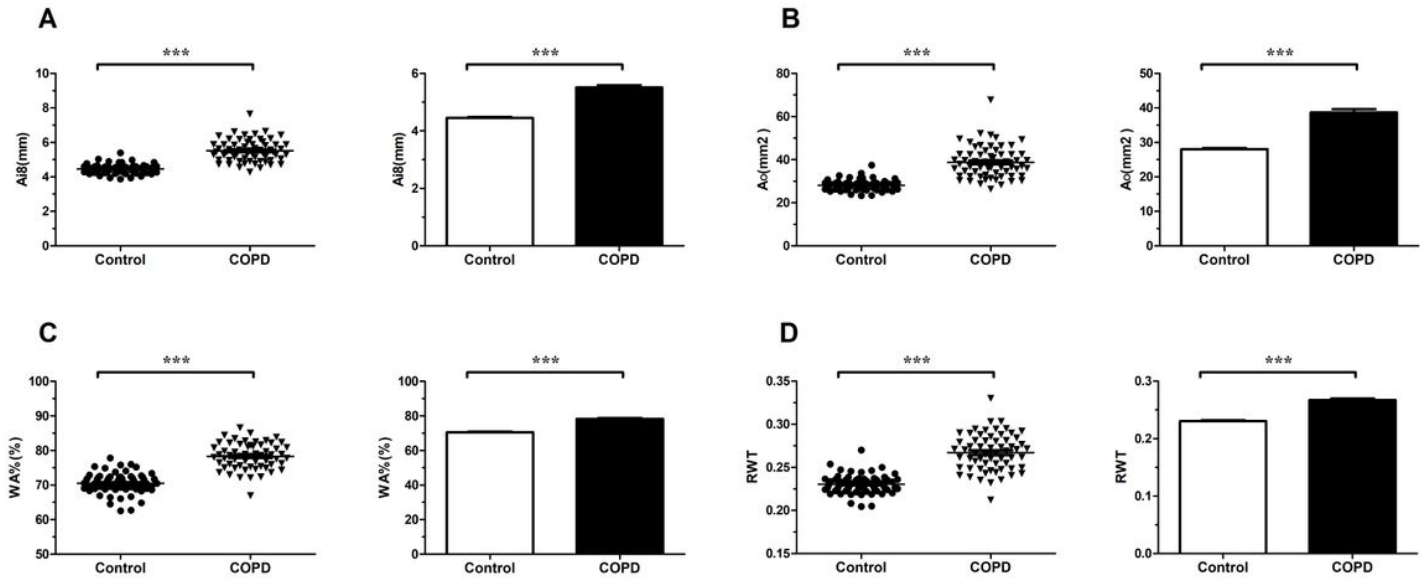

Figure 4

Airway parameters on chest CT in control and COPD group. Notes: (A)Ai8 in control and COPD group;(B) AO in control and COPD group;(C) WA% in control and COPD group;(D) RWT in control and COPD group. *P□0.05, **P□0.01, ***P□0.001 versus control group.

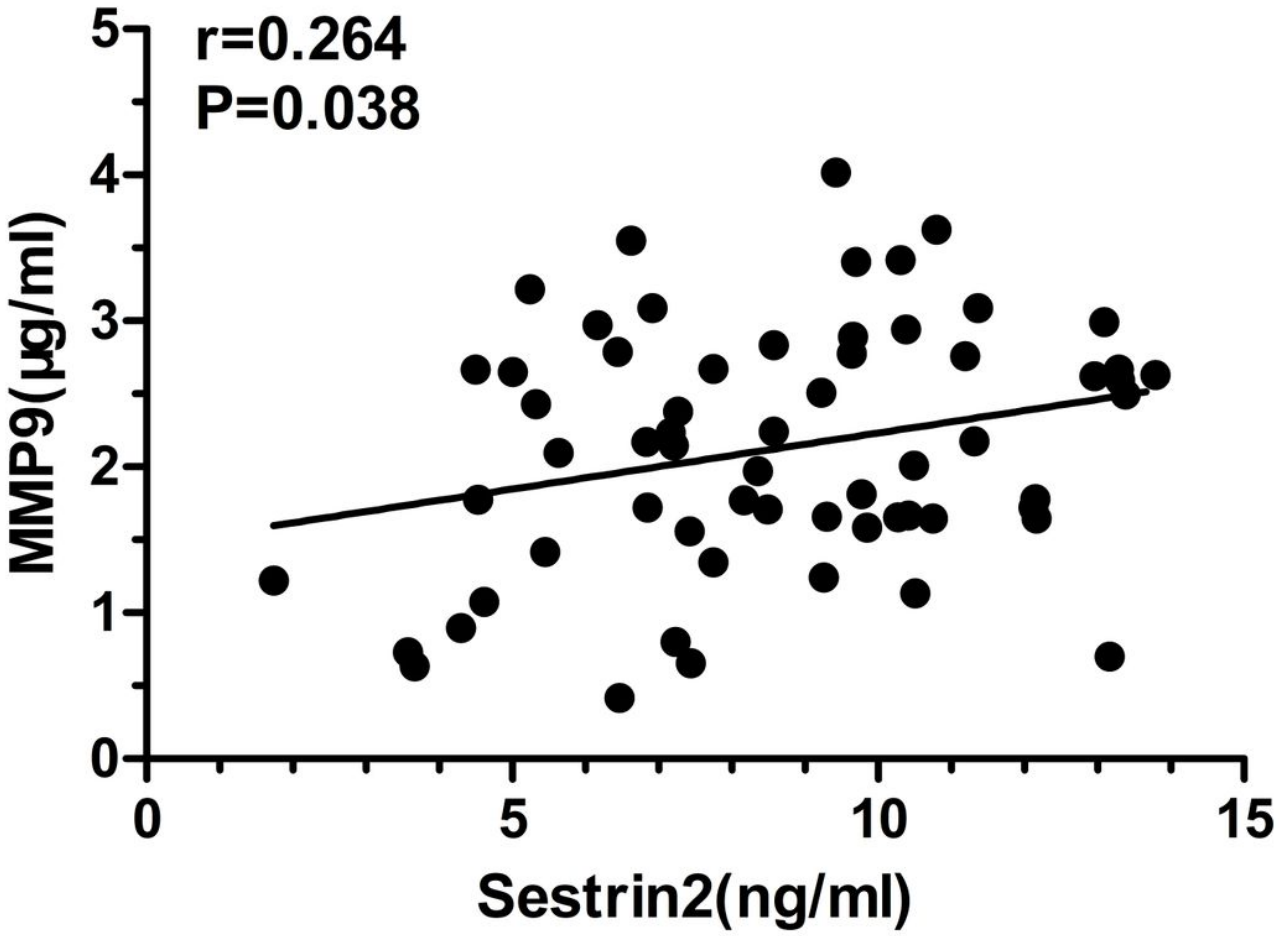

Figure 5

Correlation between serum sestrin2 concentrations and serum MMP9 concentrations. Notes: Correlations between serum sestrin2 concentrations and serum MMP9 concentrations. Correlations were determined by Pearson rank correlation analysis.

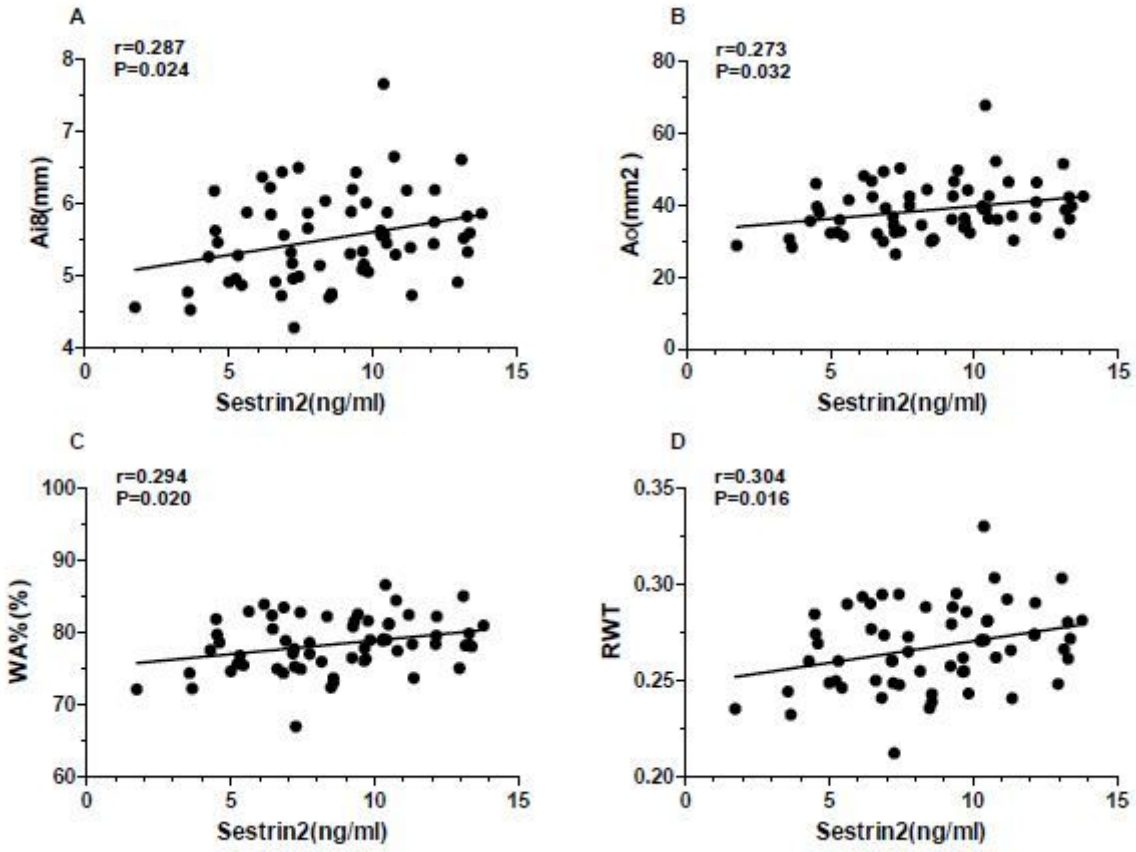

Figure 6

Correlation between serum sestrin2 concentrations and airway parameters on chest CT. Notes: Correlations between serum sestrin2 concentrations and Ai8(A), AO(B), WA%(C), and RWT(D). Correlations were determined by Pearson rank correlation analysis.