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Managing chronic obstruction pulmonary disease: From translational research to public health practice

Edited by
Shu-Chuan Ho, Chia-Li Han, Kin-fai Ho, Hsiao-Chi Chuang and Ting-Yu Lin

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Managing chronic obstruction pulmonary disease: From translational research to public health practice

Topic editors

Shu-Chuan Ho — Taipei Medical University, Taiwan
Chia-Li Han — Taipei Medical University, Taiwan
Kin-fai Ho — The Chinese University of Hong Kong, China
Hsiao-Chi Chuang — Taipei Medical University, Taiwan
Ting-Yu Lin — Linkou Chang Gung Memorial Hospital, Taiwan

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Alys Clark,
University of Auckland, New Zealand

*CORRESPONDENCE
Hsiao-Chi Chuang
chuanghc@tmu.edu.tw

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Editorial: Managing chronic obstruction pulmonary disease: From translational research to public health practice

Chia-Li Han[1], Kin-Fai Ho[2], Shu-Chuan Ho[3] and Hsiao-Chi Chuang[3,4,5,6]*

[1]Master Program in Clinical Genomics and Proteomics, College of Pharmacy, Taipei Medical University, Taipei City, Taiwan, [2]JC School of Public Health and Primary Care, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China, [3]School of Respiratory Therapy, College of Medicine, Taipei Medical University, Taipei City, Taiwan, [4]Division of Pulmonary Medicine, Department of Internal Medicine, Shuang Ho Hospital, Taipei Medical University, Taipei City, Taiwan, [5]Graduate Institute of Clinical Medicine, College of Medicine, Taipei Medical University, Taipei City, Taiwan, [6]Cell Physiology and Molecular Image Research Center, Wan Fang Hospital, Taipei Medical University, Taipei City, Taiwan

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Editorial on the Research Topic
Managing chronic obstruction pulmonary disease: From translational research to public health practice

Chronic obstructive pulmonary disease (COPD) is an important public health issue, which is the fourth leading cause of death in the world (1). Approximately 6% of all deaths (more than 3 million people) occurred as a result of COPD (2). Because of continuous exposure of COPD risk factors and aging of the population, the incidence of COPD is projected to increase in coming decades (3). Exposure to particles from cigarette smoke, occupational hazards, and air pollution are recognized as risk factors in the development and progression of COPD (2). It is worth noting that no effective treatment has been found that can fundamentally modify the disease and decrease the mortality of COPD currently, and health care of the disease often causes high medical costs.

Cigarette smoking is an important public health problem which has a direct effect on the respiratory system. Previous studies have demonstrated the harmful effect of smoking on the pulmonary function. Smoking accelerates decline in lung function, and often leads to COPD. Therefore, it is important to understand the possible ongoing impairment in lung function in smokers. In this special issue, Tian et al. reported that the annual decline rate of current male smokers with high smoking intensity (≥30 cigarettes per day) was 13.80 and 14.17 times greater than that of never-smokers in FEV1 and FVC. Moreover, a recent study indicated that lung function decline occurred in former smokers and low-intensity current smokers compared with never-smokers (4). All levels of smoking habit are probably linked with lung impairment and smoking cessation is the most effective way for risk reduction in COPD.

Emphysema, usually associated with cigarette smoking, is a phenotype of COPD in which alveoli become damaged and destroyed. But many people diagnosed with COPD

have never smoked. Tung et al. investigated the relationship of various air pollutants with emphysema measured through high-resolution CT (HR-CT) lung scans and lung function testing. The results indicated that particulate matter $<2.5\,\mu m$ in aerodynamic diameter ($PM_{2.5}$), nitrogen dioxide (NO_2), and ozone (O_3) were associated with an increased degree of upper lobe emphysema and lower lobe emphysema. It is important to explore factors that contribute to emphysema, particularly in a large, multi-ethnic group of adults. Moreover, the combined health effect of multiple air pollutants—$PM_{2.5}$, NO_2, and O_3 can be addressed which can aid in our understanding and control of emphysema in COPD in the future.

Increasing reports showed the advantages of CT on quantification of COPD severity. Cao et al. identified that expiratory CT scans provided a more accurate assessment of COPD than the inspiratory CT scans. Also, the results of the quantitative parameter intrapulmonary vascular volume (IPVV) was significantly associated with FEV1%, emphysema degree and airway disease. Based on the powerful approaches with different advanced quantitative models, CT would provide more information regarding COPD severity for clinical diagnosis and treatment strategy.

Since the pathogenesis of COPD is unclear, there is no cure but pharmacological therapies to slow the progression of COPD. A multicenter prospective longitudinal study in China was conducted to evaluate the effectiveness of inhaled combination LABA/LAMA treatment and triple (ICS/LABA/LAMA) therapy in a total of 695 symptomatic COPD patients *via* assessing the minimum clinical important difference (MCID) defined by attaining a COPD assessment test decrease ≥ 2 (Cheng et al.). Nearly 50% of patients attained MCID, especially the female patients. Among these, patients treated with LABA/LAMA or ICS/LABA/LAMA were more likely to attain MCID than patients treated with LAMA monotherapy. A higher incidence of severe exacerbations was observed in patients treated with LABA/LAMA than those with ICS/LABA/LAMA. Apart from the approved inhalation therapy, Chen et al. reported the usage of human umbilical cord-derived mesenchymal stem cells (MSCs) in treating the mouse model of cigarette smoke-induced COPD emphysema. A number of inflammatory molecules were found to be decreased not only locally in the lung tissues but also systematically in serum after MSC administration. Significant reduction in emphysema severity was also observed, suggesting the immunoregulation and repair potential of MSCs in treating COPD. In addition, a novel long non-coding RNA, Nqo1 antisense transcript 1 (Nqo1-AS1), was reported by Zhang et al. to attenuate the cigarette smoke-induced oxidative stress by increasing the Serpina mRNA expression as well as the protein level of Nqo1 through stabilizing its mRNA.

COPD is a chronic inflammatory disease of the lung associated with the structural remodeling of airways and irreversible airflow obstruction caused by various factors. With the advanced CT with quantification models, precision medicine for diagnosis of COPD with emphysema could be conducted for evaluating disease stability and severity. In addition to traditional strategies for COPD management, an increasing development of molecular drugs and stem cell therapy provides a bright future for patient welfare and quality of life. Taken together, this Research Topic has pushed forward our understanding of COPD in terms of risk factors, pathophysiology, diagnosis, and treatment.

Author contributions

C-LH, K-FH, S-CH, and H-CC drafted and revised this editorial. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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CASE REPORT
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Case Report: Ketogenic Diet Is Associated With Improvements in Chronic Obstructive Pulmonary Disease

Nicholas G. Norwitz[1]*, *Russell Winwood*[2], *Brianna J. Stubbs*[3], *Dominic P. D'Agostino*[4,5] *and Peter J. Barnes*[6]

[1] Department of Nutrition, Harvard Medical School, Boston, MA, United States, [2] Respiratory Network, Ministry of Health Agency for Clinical Innovation, St Leonards, NSW, Australia, [3] Buck Institute for Research on Aging, Novato, CA, United States, [4] Morsani College of Medicine, University of South Florida, Tampa, FL, United States, [5] Institute for Human and Machine Cognition, Pensacola, FL, United States, [6] National Heart and Lung Institute, Imperial College, London, United Kingdom

Chronic Obstructive Pulmonary Disease (COPD) is a debilitating inflammatory respiratory condition that presents with worsening breathing difficulties and it is assumed to be progressive and incurable. As an inflammatory disease, COPD is associated with recruitment of immune cells to lung tissue and increased levels of pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, IL-8, and GM-CSF. Low-carbohydrate ketogenic diets have anti-inflammatory properties that could, in theory, improve COPD symptoms and progression. Herein, we report on a 54-year-old patient (C.A.) with COPD who adopted a ketogenic diet (70% calories from fat). Subsequently, C.A. experienced a reduction in inflammatory markers in association with a meaningful improvement in lung function. His inflammatory markers decreased into the normal range and his forced expiratory volume increased by 37.5% relative to its pre-ketogenic diet value. Future research should explore nutritional ketosis and ketogenic diets as possible therapeutic options for individuals with COPD.

Keywords: chronic objective pulmonary disease, ketogenic diet, forced expiratory volume 1, inflammation, case report

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Hsiao-Chi Chuang, Taipei Medical University, Taiwan

Reviewed by:
Mark Wewers, The Ohio State University, United States
Eleni Papakonstantinou, Aristotle University of Thessaloniki, Greece

***Correspondence:**
Nicholas G. Norwitz
nicholas_norwitz@hms.harvard.edu

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INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) affects over 300 million patients worldwide and is currently the third ranked cause of death globally (1). COPD is characterized by slowly progressive airflow limitation as a result of peripheral airway obstruction (chronic bronchiolitis) and lung parenchymal destruction (emphysema), which lead to increasing shortness of breath on exertion (2). COPD is further associated with inflammation of the lung, including the recruitment of macrophages, neutrophils and lymphocytes and the secretion of multiple inflammatory mediators, including cytokines such as TNF-α, IL-1β, IL-6, IL-8, and GM-CSF (3). This pulmonary inflammation is not reduced by the mainstay of current therapy, inhaled long-acting bronchodilators, and is also largely resistant to corticosteroids (4). Targeting of individual cytokines has been unsuccessful, reflecting the fact that many mediators are involved in pathogenesis of the disease (5). Importantly, there are no current therapies that significantly improve disease progression. Therefore, there is a pressing need to find broader spectrum anti-inflammatory treatments for COPD that will improve symptoms, disease progression, and patient quality of life (6).

Ketogenic diets (KD) have a near century long history of being used to treat pediatric epilepsy (7), and newer research is beginning to explore their therapeutic potential in other chronic disease, such as type II diabetes (8, 9), polycystic ovarian syndrome (10), Alzheimer's disease (11), Parkinson's disease (12), cardiovascular risk (13), metabolic syndrome (14), and various mental illnesses (15). Many of these disease have an inflammatory component and, correspondingly, well-formulated ketogenic diets have been shown to improve a broad spectrum of inflammatory markers (16).

The anti-inflammatory effects of KD may be mediated, in part, by the ability of the ketone molecule, β-hydroxybutyrate (βHB), to inhibit the NLRP3 inflammasome (17, 18). NLRP3 is a protein complex that positively regulates the inflammatory response, and inhibition of NLRP3 is a mechanism whereby βHB is thought to mitigate inflammatory conditions such as gout (19). NLRP3 is also elevated in active COPD, as measured by circulating and local levels of NLRP3, Asc, and caspase-1 mRNAs (20). These mechanistic data, along with the clinical data mentioned above, suggest that a KD could have a beneficial effect in COPD patients, possibly by inhibiting NLRP3.

With respect to pulmonary diseases, KD improve symptoms in asthma (21) and trials are ongoing to determine whether KD may protect against severe COVID-19 disease, including lung disease (22). Importantly, a 3-week controlled trial including 60 COPD patients demonstrated a small but significant improvement in forced expiratory volume in 1 s (FEV_1) with a lower carbohydrate group (47% calories from carbohydrates) compared to a higher carbohydrate group (65% calories from carbohydrates) (23). In this study 10% of calories in the lower carbohydrate group were obtained from medium chain triglycerides to induce mild ketosis.

While COPD is an inflammatory disease and KD are known to be anti-inflammatory, there are no reports of KD being used to treat COPD existing in the medical literature. Herein we report on such a case in which an individual with COPD adopted a KD and subsequently observed improvements in inflammatory markers and lung function.

CASE DESCRIPTION

The subject of this study (C.A.) is a 54-year-old male in whom COPD was diagnosed in 2011, at age 45 years. Early in childhood, at age 10, C.A. was diagnosed with asthma. He also smoked cigarettes from age 17 and to 37 (∼15 pack-years) and his grandfather died of lung cancer at age 67. C.A.'s only co-morbidity was and remains asthma, and he is free of other common comorbidities of COPD, including diabetes, pre-diabetes, cardiovascular disease, osteoporosis, sleep or mood disorders, metabolic syndrome, or obesity.

Abbreviations: COPD, Chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 second; GM-CSF, granulocyte-macrophage colony stimulating factor; KD, ketogenic diet; IL-1, interleukin; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; TNF-α, tumor necrosis factor-α; βHB, β-hydroxybutyrate.

In year prior to diagnosis, C.A.'s chief complaints were worsening shortness of breath and more frequent chest infections. In 2011, spirometry showed an FEV_1 of 0.79L (22% predicted normal) with an FEV_1/FVC ratio of 40%. There was an increase in lung volume measured by body plethysmography, with an increase in air trapping, but there was no reduction in gas transfer. There was no significant bronchodilator response to inhaled salbutamol, nor any response to a trial of oral corticosteroids (prednisolone 40 mg od for 4 weeks). A computerized tomography scan showed no evidence of emphysema, indicating that the COPD is due to small airway disease. C.A. was diagnosed with very severe COPD (GOLD stage 4) and was treated with inhaled tiotropium bromide once daily and budesonide-formoterol combination twice daily, which he has continued. He has also taken zinc and vitamin C supplements daily since his diagnosis in 2011.

Over the ensuring 4 years C.A. undertook an aerobic exercise program consisting of daily running, cycling or swimming and ate a "balanced diet" consisting of fats, proteins, and carbohydrates, including fruits, vegetables and whole grains. In association with his daily activity and diet, his BMI decreased (from 29.4 kg/m^2 at time of diagnosis in 2011 to 23.8 kg/m^2 in 2017), his exercise maximal peak capacity increased from 165 to a peak of 193 watts, as measured by cycle ergometer, and his FEV_1 improved only marginally to 0.91L (25% predicted normal).

In 2017, he began a low-carbohydrate, high-fat diet KD. The macronutrient composition of the diet comprised calories from 70% fat, 20% protein, and 10% carbohydrates and C.A. confirmed nutritional ketosis of >0.5 mmol/L daily by fingerstick using an Abbott Optium Neo device that measured blood D-βHB. While on the KD, he lost no more weight, remaining at a BMI of 23.8 kg/m^2 at the time of the 2019 and 2020 measurements listed below.

Prior to starting the KD, baseline plasma TNF-α, IL-1β, IL-6, IL-8, and GM-CSF, concentrations were 16, 4.9, 13.9, 499, and 193.5 pg/mL, respectively, and CRP was 7 mg/L. All measurements were above the upper threshold of normal. In 2019, 2 years into his KD, blood concentrations decreased to 3.4 (TNF-α), 0.6 (IL-1β), 4.2 (IL-6), 2.7 (IL-8), and 21.6 pg/mL (GM-CSF), and CRP was 1 – 2 mg/L (**Figure 1**). These measurements were repeated in 2020, with maintenance of the KD, and results were similar. On both occasions all post-KD inflammatory markers were within the normal range with the singular exception of the 2020 IL-8 (62 pg/mL), although this measure still represented an 8-fold decrease from its pre-KD level. All three cytokine panels were ordered direct to consumer (NutriPath) by C.A. The 2017 baseline test was ordered for interest following his self-study on COPD. At this time, there was no thought of a case report; however, C.A.'s response to dietary change was—in his perspective (below)—so remarkable that, in 2019, he decided he wanted to collect follow-up data to correlate with his improved symptoms and quality of life out of interest. He also ordered the 2020 tests as a replicate.

In conjunction with the improvement in inflammatory blood biomarkers (**Figure 1**), C.A.'s FEV_1 improved to 1.24 (35% predicted) and 1.25L in 2019 and in 2020, respectively, representing an improvement in FEV_1 of 38% relative to

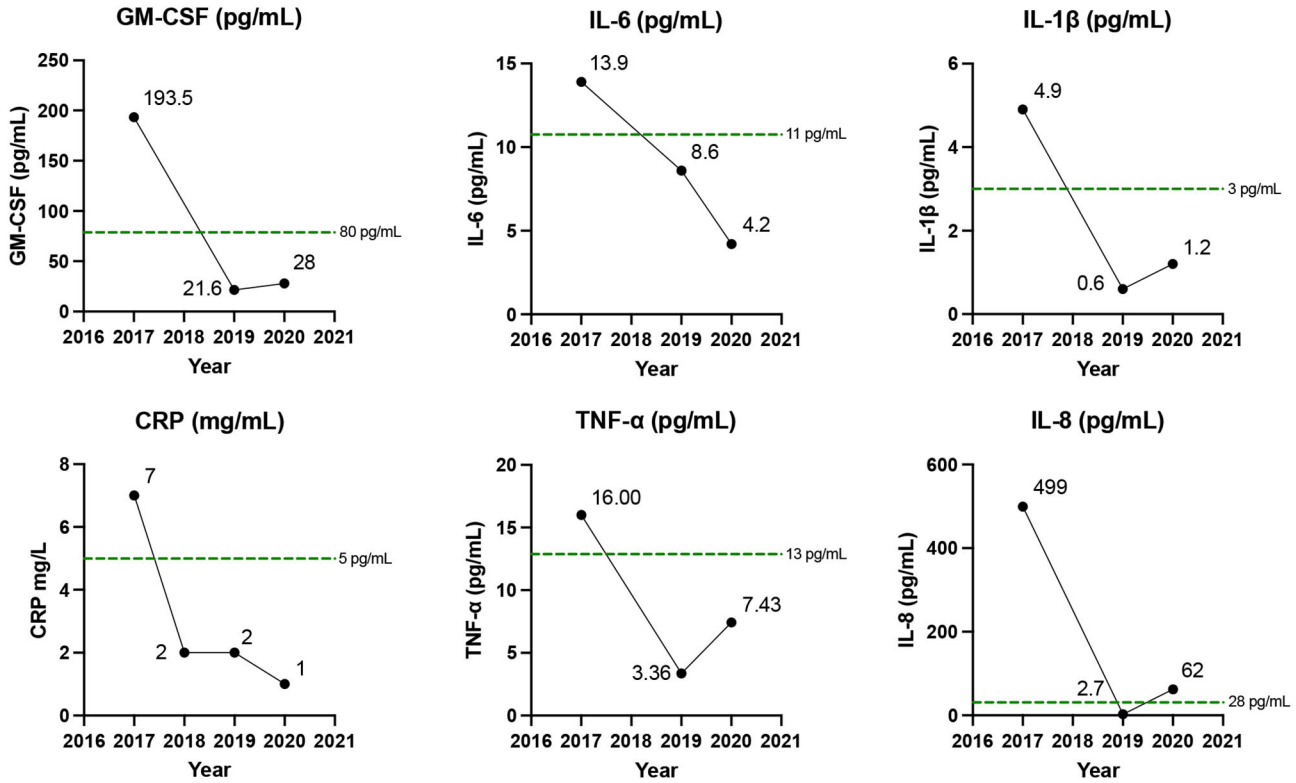

FIGURE 1 | Inflammatory biomarkers. Data represent C.A.'s inflammatory biomarkers prior to starting a ketogenic diet and at two time points, 2019 and 2020, after commencing the diet. Green dotted line represent the threshold of normal range for each biomarker. All markers improved on a ketogenic diet.

pre-KD measurements (**Figure 2**). All lung function tests, including the baseline test, were administered 24 h after stopping bronchodilators. Prior to the diet he suffered from 1 to 2 acute exacerbations/year but reported no exacerbations since taking the KD. He also reported improvement in symptoms and quality of life, with reduced use of rescue salbutamol inhaler from 3 to 4 puffs daily before the KD to only one or less puffs on the diet. His exercise tolerance also improved markedly to the extent that he was able to complete marathons.

DISCUSSION

C.A. suffers from severe COPD, predominantly due to small airway disease and showed only modest improvement in symptoms and lung function with maximal inhaler therapy (inhaled corticosteroid, long-acting muscarinic antagonist and long-acting β_2-agonist), as recommended by current management strategies (24). After switching to a KD, he had reduced symptoms and improved exercise tolerance, used less rescue inhaler and had no further acute exacerbations. His FEV_1 improved by over 35%, relative to baseline, whereas there had previously been no significant improvement with a bronchodilator or a systemic corticosteroid. Furthermore, between 2011 and 2017, his self-prescribed exercise program increased his FEV_1 from 0.79L (22% predicted normal) to FEV_1 from 0.91L (25% predicted normal), in conjunction with a BMI decrease from 29.4 to 23.8 kg/m^2. By comparison, the addition of a KD was associated with a much larger improvement in FEV_1 to 1.24 and 1.25, in 2019 and 2020, respectively (∼35% predicted), without any change in weight. As the KD improved C.A.'s exercise tolerance, it's possible that there was a synergy between the KD and exercise, i.e., that the KD acted as a therapeutic adjunct. It is also possible, and perhaps more likely, that the KD had an independent effect.

The clinical improvements C.A. experienced after adopting the KD were accompanied by a marked fall in plasma concentrations of several inflammatory cytokines known to be increased in COPD (TNF-α, IL-1β, IL6, IL-8, GM-CSF, and CRP) that were elevated prior to the diet. While the data on hand do not permit us to draw a causal conclusion that the KD improved C.A.'s COPD, the coincident drop in serum cytokines and improvements in FEV_1, along with prior clinical and non-clinical literature demonstrating anti-inflammatory and therapeutic beneficial effects of a KD in inflammatory disorders, suggest that a KD could have potential for the treatment of COPD. This is an area that deserves more structured investigation.

Several dietary interventions have been evaluated in COPD, mainly with a view to increasing skeletal muscle mass as well as addition of nutraceuticals, such as antioxidant vitamins and vitamin D, but so far there is no convincing evidence for the efficacy of these diets, and they are not recommended in the routine management of COPD patients (25). Since diagnosis in 2011, C.A. only ever reported consuming a zinc and vitamin

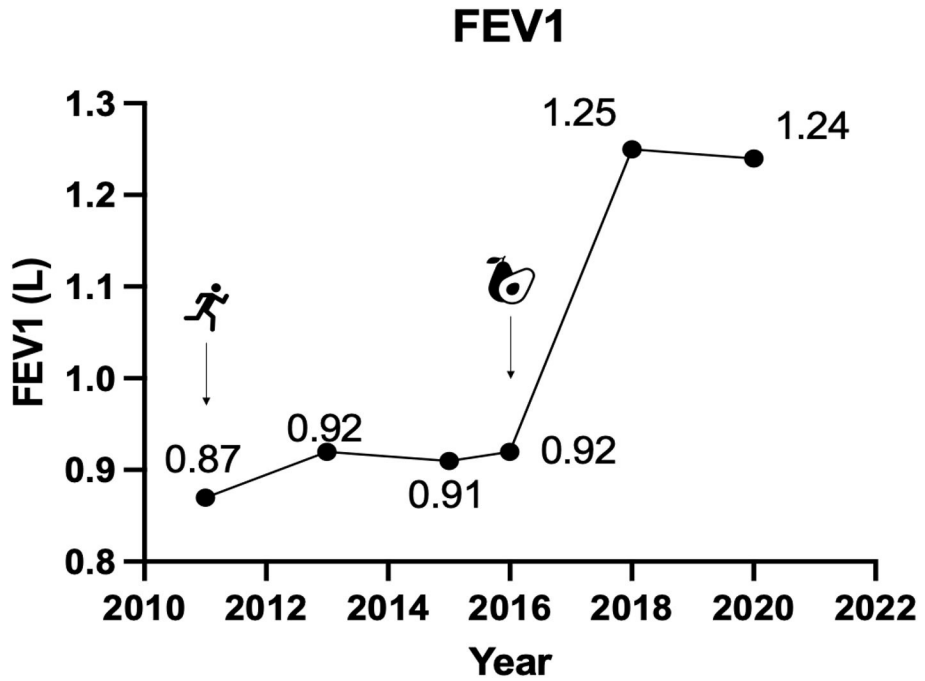

FIGURE 2 | Forced expiratory volume in 1 s. Data represent C.A.'s FEV1 at four time points prior to starting a ketogenic diet and at two time points, 2019 and 2020, after commencing the diet.

C supplement and their dosing did not change upon starting a KD. Dietary interventions may theoretically benefit the lung disease, but also the comorbidities that are commonly seen in COPD patients (26), including diabetes, pre-diabetes, obesity, ad metabolic syndrome, although C.A. presented with none of these conditions nor did the KD cause C.A. to lose weight. Furthermore, dietary interventions may be more acceptable to patients than long-term drug therapies, which have poor adherence in COPD (27). Conversely, despite the common conception that KD are not sustainable for patients, trials show that patients given adequate education and support adhere to and enjoy KD as much as other diets and standard of care (8, 28).

Although a KD has been shown to improve asthma, there are no previous reports of its use in COPD patients. Given the parallel decrease in cytokines and improvement in FEV_1, it is possible, if not likely, that the direct anti-inflammatory effect of the KD mediate part of the therapeutic benefit of the lifestyle in this case. βHB is known to inhibit the NLRP3 inflammasome (17, 18), which plays a pathological role in COPD (20). Ketogenic diets have also been shown to lower a wide range of inflammatory cytokines and have been shown to outperform isocaloric low-fat diets in their ability to lower TNF-α, IL-6, and IL-8 (16) and reverse insulin resistance and metabolic syndrome (14), which are common inflammatory comorbidities associated with COPD (29, 30).

Another possible mechanism for the clinical improvement in COPD may be a change in the gut or lung microbiome. Lung microbiome is abnormal in COPD, and patients with severe COPD commonly have colonization of the lower respiratory tract with bacteria such as *Haemphilus influenzae, Streptococcus pneumoniae*, and *Moraxella cattarhalis* (31, 32). These colonizing bacteria may be important in inducing a chronic inflammatory response, with increases in cytokines TNF-α, IL-1β, IL-6, and IL-8. Correspondingly, mouse data suggest that changes in the microbiome mediate some of the therapeutic effects of ketogenic diet against epilepsy (33) and that KDs can alter the microbiome to protect against activation of Th17 cells and other inflammatory mediators (34).

An obvious limitation of this case study is that the data do not allow us to draw conclusions about the mechanism of action by which a KD improved inflammation and respiratory function in the patient, C.A. We can only note the striking association among the onset and maintenance of KD and concomitant improvements in all measured inflammatory markers and FEV_1. Nevertheless, C.A.'s improvements make mechanistic sense in the context of the broader literature on COPD as an inflammatory disorder and KD as an anti-inflammatory intervention. Future animal model research should focus on exploring the mechanisms of action of KD on COPD and related respiratory conditions with a mind toward providing patients with a lifestyle option to treat disease and improve quality of life.

PATIENT PERSPECTIVE

After my COPD diagnosis, I made a commitment to myself that I would do anything I could to improve my health so took up running, swimming, and cycling. Even though, I wasn't very good, I especially loved distance running. I started listening to podcasts and reading about how nutrition could improve my

breathing and endurance running performance. The information I gathered suggested a low-carb ketogenic diet could help burn fat as fuel more efficiently, I thought I'd give it a go. I was surprised to find my breathlessness diminished as soon as I was in ketosis and, 4 months later, I ran my personal best marathon time of 5½ h, which for me was astonishing. I started to notice when I adhered to my diet my breathing was less labored. Encouraged, I started to read more including a paper that showed ketones inhibit the NLRP3 inflammasome. I had also read about the NLRP3 and its role in COPD. I asked a respiratory professional whom I met at a conference, "what if we had a medication to inhibit NLRP3?" He said, "that could represent a remarkable step forward in the treatment and patient care." That sealed it for me. I've been on a ketogenic diet ever since and, combined with my exercise, my respiration appears to be ever improving. It's unlikely I'll ever have completely normal breathing, but I'm so grateful to being trending upward, not downward. I hope my experience will encourage others to try a lifestyle that significantly improved my quality of life.

LIMITATIONS

As with any retrospective $n = 1$ patient case, this report contains limitations that must be acknowledged. (i) First, the duration of time between the initiation of the KD and subsequent FEV_1 and cytokine tests was ∼2 years. It is plausible that other changes in the patient's lifestyle during this time contributed to his symptomatic and inflammatory improvements. While the patient attests that "I was surprised to find my breathlessness diminished as soon as I was in ketosis," and that "when I adhered to my diet my breathing was less labored," and that he ran his personal best marathon time 4 months after the initiation of his diet, these data are self-reported and/or subjective and should be taken as such. (ii) Second, and building on the above, there is the possibility of a placebo interaction. As the patient was encouraged about the possibility that a ketogenic diet could improve his athletic performance and symptoms, it is feasible that this optimism changed his exercise confidence, or other aspects of lifestyle, contributing to his improvements. It is also possible that his enthusiasm and optimism led him to order the direct-to-consumer cytokine panels at symptomatically favorable moments when inflammation might have been in a trough. Although he denies this was the case, and it seems unlikely that a placebo interaction could explain the full effect and its consistency (assuming the effect is genuine and due to the diet), it is an important caveat, nonetheless. (iii) Third, the patient represents a highly particular case of COPD, as most patients are not marathon runners and do not engage in intensive physical activities. It would therefore be premature to generalize the findings of this report to a wider COPD population. (iv) Finally, it would have been ideal to not only have more data timepoints but also more functional measures of lung function. Unfortunately, as this is a retrospective case, the data are limited to that which the patients and treating physician had available to us.

SUMMARY

We report on a case in which initiation and continuation of a ketogenic diet was associated with improvements in the lung function and inflammatory markers of a patient with COPD. As ketogenic diets have the potential to be anti-inflammatory diets, COPD is an inflammatory disorder, and ketogenic diets are being explored for an increasing array of inflammatory conditions, this case suggests ketogenic diets could have therapeutic potential in COPD and that more research is needed.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Written, informed consent was obtained from the participant for the publication of this case report and any potentially-identifying information/images.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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LncRNA Nqo1-AS1 Attenuates Cigarette Smoke-Induced Oxidative Stress by Upregulating its Natural Antisense Transcript Nqo1

Haiyun Zhang[1,2†], Ruijuan Guan[2†], Zili Zhang[2†], Defu Li[2], Jingyi Xu[2], Yuxin Gong[1], Xin Chen[1] and Wenju Lu[2*]*

[1]Department of Pulmonary and Critical Care Medicine, Zhujiang Hosptial, Southern Medical University, Guangzhou, China, [2]State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China

Evidence of the involvement of long noncoding RNAs (lncRNAs) in the pathogenesis of chronic obstructive pulmonary disease (COPD) is growing but still largely unknown. This study aims to explore the expression, functions and molecular mechanisms of Fantom3_F830212L20, a lncRNA that transcribes in an antisense orientation to Nqo1.We name this lncRNA as Nqo1 antisense transcript 1 (Nqo1-AS1). The distribution, expression level and protein coding potential of Nqo1-AS1 were determined. The effects of Nqo1-AS1 on cigarette smoke (CS)-induced oxidative stress were also evaluated. The results showed that Nqo1-AS1 were mainly located in the cytoplasm of mouse alveolar epithelium and had a very low protein coding potential. Nqo1-AS1 (or its human homologue) was increased with the increase of CS exposure. Nqo1-AS1 overexpression enhanced the mRNA and protein levels of Nqo1 and Serpina1 mRNA expression, and attenuated CS-induced oxidative stress, whereas knockdown of Nqo1-AS1 significantly decreased Nqo1 and Serpina1 mRNA expressions, and aggravated CS-induced oxidative stress. Nqo1-AS1 increased Nqo1 mRNA stability and upregulated Nqo1 expression through antisense pairing with Nqo1 3′UTR. In conclusion, these results suggest that Nqo1-AS1 attenuates CS-induced oxidative stress by increasing Nqo1 mRNA stability and upregulating Nqo1 expression, which might serve as a novel approach for the treatment of COPD.

Keywords: COPD, lncRNA, Nqo1 antisense transcript 1 (Nqo1-AS1), oxidative stress, cigarette smoke

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Gang Hou,
China-Japan Friendship Hospital, China
Takayuki Shiomi,
International University of Health and Welfare, Narita, Japan

****Correspondence:***
Xin Chen
chen_xin1020@163.com
Wenju Lu
wlu92@yahoo.com

[†]These authors have contributed equally to this work

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a lung disease that is usually progressive degenerative and characterized by persistent respiratory symptoms and incompletely reversible expiratory airflow limitation (Duffy and Criner, 2019; Gu et al., 2021), which is a leading cause of death and disability worldwide (Wang et al., 2018; Riley and Sciurba, 2019). Cigarette smoke (CS)-induced oxidative stress is one of the most important pathogenetic mechanisms involved in pulmonary emphysema and COPD (Lu et al., 2018; Barnes, 2020; Guan et al., 2020). NAD(P)H quinone oxidoreductase 1 (Nqo1), one of the most critical quinone reductases, has been well-documented to play crucial roles in antioxidant protection and tumor-killing (Zhang et al., 2018a; Li et al., 2019). It has been well established that Nqo1 is closely related to CS, CS-induced oxidative

stress or obstructive bronchitis. It is demonstrated that NQO1 expression is the activation of aryl hydrocarbon receptor (AhR) pathway by propolis, which promotes lung repair in a mouse emphysema model caused by CS exposure (Barroso et al., 2017).Also, studies have proved that NQO1 P187S polymorphisms is determined as risk genotype in children with obstructive bronchitis, whose mother smoke actively during their pregnancies. Previous studies have reported that Nqo1 expression in lung tissue was upregulated by CS exposure (Adair-Kirk et al., 2008; Shahdoust et al., 2013).Recently, a study showed that overexpression of Nqo1 was able to increase scavenging of superoxide in Chinese hamster ovary cells, suggesting that Nqo1 plays a critical role in antioxidant protection (Ross and Siegel, 2017). However, the role of Nqo1 in COPD is still unknown.

Long non-coding RNAs (lncRNAs) are a class of transcripts with length of more than 200 nucleotides but without protein-coding capacity. Through epigenetic modification, control of transcription, RNA processing and translation, lncRNAs have been shown to play crucial roles in various biological processes such as cell growth, metabolism, differentiation and apoptosis (Liao et al., 2018). Some lncRNAs have been shown to be correlated with the occurrence and development of COPD. For example, COPDA1 promotes the proliferation of smooth muscle cells through upregulating the expression of MS4A1 in COPD (Zheng et al., 2019). LINC00987 modulates LPS-induced cell apoptosis, oxidative stress, inflammation and autophagy through sponging let-7b-5p in COPD (Wang et al., 2020, Chen, Chen, Liu, Dong, Ji, Hu, Zhang). MEG3 targets miR-218 thereby regulating cigarette smoke extract (CSE)-inhibited proliferation and CSE-induced apoptosis in COPD (Song et al., 2020). MALAT1 exhibits clinical implications in acute exacerbation risk prediction and management of COPD (Liu et al., 2020). However, the expressions and functions of lncRNAs in COPD progression are largely unknown.

In previous study, we reported that lncRNA Fantom3_F830212L20 and Nqo1 were co-expressed lncRNA and protein-coding gene, and both of two were significantly up-regulated in lung tissues of chronic CS-induced COPD mouse model, 16HBE cells and A549 cells exposed to CSE treatment when compared to their controls (Zhang et al., 2018b). In the present study, we identified the characterization of Fantom3_F830212L20, a lncRNA that transcribed in an antisense orientation to Nqo1 and had a very low protein coding potential, which were mainly located in the cytoplasm of alveolar epithelial cells of mouse lung tissues. We named this lncRNA as Nqo1 antisense transcript 1 (Nqo1-AS1). We further proved that Nqo1-AS1 was upregulated in lung tissues of mice exposed to CS and the mle-12 cells treated with CSE, and its human homologue expression was upregulated in peripheral blood mononuclear cells (PBMCs) of patients with COPD when compared to those of the control group. Nqo1-AS1 was able to inhibit CS-induced oxidative stress as indicated by increased levels of malondialdehyde (MDA), glutathione disulfide (GSSG) and reactive oxygen specicies (ROS). Mechanistically, Nqo1-AS1 upregulated Nqo1 expression through binding Nqo1 3′UTR and increasing Nqo1 mRNA stability thereby attenuating CS-induced oxidative stress. This study provides new insights into the therapy strategy for the treatment of COPD.

MATERIALS AND METHODS

Animal Experiments

C57BL/6J male mice (6–8 week old) were purchased from Guangdong Medical Laboratory Animal Center (Guangzhou, China). Mice were randomly divided into two groups. One group was exposed to CS generated from 9 filter-tipped cigarettes in a 342 L fume chamber (60 cm × 57 cm × 100 cm) each time, twice a day, 6 days per week. Each CS exposure was lasted for over 2 h per time with the interval between two CS exposures more than 4 h. To better demonstrate the effect of CS on the expression levels of Nqo1 and Nqo1-AS1 in lung tissues of mice, mice in the CS group were exposed to CS for 1 week, 1 month and 3 months. Moreover, chronic CS-induced COPD mouse model was constructed as we did before in order to elucidate the distribution of Nqo1-AS1 in lung tissues of mice (Zhang et al., 2018b). The Red Roses cigarettes (manufactured from Guangdong Cigarette Factory) emitting 13 mg tar and 1.3 mg nicotine per cigarette were used in this experiment. Mice in the control group were housed in a smoke-free environment. All experimental procedures were approved by the Animal Care and Use Committee of The First Affiliated Hospital of Guangzhou Medical University.

Human Samples

A total of seven patients with COPD and seven healthy individuals were recruited between March and May at 2018 in The First Affiliated Hospital of Guangzhou Medical University. Clinical data, including age, smoking information and lung function were collected. Blood samples were obtained with written informed consents from all participants. Whole blood samples were drawn and centrifuged at 1,000×*g* for 15 min. Plasma supernatants were collected and stored at − 80° C until analysis. PBMCs were isolated using lymphocyte separation medium according to the method described previously. This study was approved by the Ethics Committee of The First Affiliated Hospital of Guangzhou Medical University (Ethic Ref No.GZMC 2009-08-1336) and adhered to the Declaration of Helsinki as described previously.

Protein-Coding Potential Analysis

To verify whether Nqo1-AS1 was able to encode protein, bioinformatics analysis and *in vitro* translation assay were performed as described previously (Liu et al., 2020). Briefly, the RNA sequences of Nqo1-AS1, Nqo1 and Hotair were put into the Coding Potential Calculator http://cpc.cbi.pku.edu.cn/ and Coding-Potential Assessment Tool http://lilab.research.bcm.edu/cpat/index.php. Next, the open reading frame (ORF) sequence of Nqo1-AS1, Nqo1 or Hotair was predicted using the ORF finder database (https://www.ncbi.nlm.nih.gov/orffinder), respectively. Then the predicted ORF sequence of Nqo1-AS1, Nqo1 or Hotair was synthesized and subcloned into the BsrGI and XhoI

sites of pcDNA3.1-EGFP vector (Invitrogen). Next, the recombinant plasmid pcDNA3.1-EGFP- Nqo1-AS1 (pc-EGFP-Nqo1-AS1), pcDNA3.1-EGFP-Nqo1 (pc-EGFP-Nqo1) or pcDNA3.1-EGFP- Hotair (pc-EGFP- Hotair) was transfected into the mle-12 cells using Lipofectamine 3000 (Thermo) according to the manufacturer's instructions, respectively. After transfection for 72 h, the nuclei were stained with DAPI (Beyotime), and the immunofluorescence of cells was observed using a fluorescence microscope. Pc-EGFP-Nqo1 was used as a positive control. Pc-EGFP- Hotair was used as a negative control.

RNA ISH

Nqo1-AS1 expression was checked by *in situ* hybridization in lung tissues of mouse. The RNA ISH probe mixture of Nqo1-AS1, Gapdh or U6 RNA was synthesized and labeled with digoxigenin from Biosense Bioscience Co. Ltd. (Guangzhou, China). The probe sequences for RNA ISH were as follows: Nqo1-AS1 antisense probe: 5′- TATTTAGGTGTGTATGCATACGTG AGCCATGGCGCGCCCTGTGGA-3′; Nqo1-AS1 sense probe: 5′- TCCACAGGGCGCGCCATGGCTCACGTATGCATACAC ACCTAAATA -3′; Gapdh: 5′-TAAGCAGTTGGTGGTGCA GGATGCATTGCTGACAATCTTGAGTGAGTTGTCATATTT CTC GTGGTTCACACCCATCA -3′. The Gapdh or U6 RNA probe was used as a positive control. The Nqo1-AS1 sense probe was used as a negative control. RNA ISH was performed as previously described (Mehta-Mujoo et al., 2019). Briefly, the mouse lung tissues were first fixed and embedded with paraffin. Embedded specimens were sectioned at 4 μm thickness. Then sample sections were incubated in graded alcohols, 3% hydrogen peroxide for 30 min and pre-hybridization solution for 2 h. After that, digoxigenin-labeled probes were added in the hybridization solution and incubated with the sections at 37°C overnight in the dark. Next, the sections were incubated with anti-DIG and horseradish peroxidase and observed. The staining scores were assessed based on both immunostaining intensity and the proportion of positive staining cells. The immunostaining intensity was scored 0–3 as follows: 0 (negative staining), 1 (weak staining), 2 (moderate staining) and 3 (dark staining).The proportion of positive staining cells was evaluated as follows: 0 (no positive cells), 1 (< 10%), 2 (10–50%) and 3 (> 50%). Expression of Nqo1-AS1 was evaluated by the final score that was multiplication of the immunostaining intensity and the proportion of positive staining cells. The final scores were divided into two levels: low expression (≤4) and high expression (> 4).

Nuclear and Cytoplasmic RNA Fractionation Analysis

Nuclear and cytoplasmic RNA isolation in the mle-12 cells was performed using the Cytoplasmic and Nuclear RNA Purification Kit (Norgen, Belmont, CA, United States) according to the manufacturer's instruction.

Cell Line and Cell Culture

The mouse alveolar epithelium mle-12 cells were obtained from Shanghai fuxiang biotechnology co., LTD., (Shanghai, China).The mle-12 cells were cultured in DMEM/F12 supplemented with 2% fetal bovine serum (Gibco,United States), 100 IU/mL peniciliin and 100 μg/ml streptomycin, and maintained in a humidified atmosphere of 5% CO_2 at 37°C.

Preparation of Cigarette Smoke Extract

CSE was prepared according to the method described previously (Zhang et al., 2018b). Briefly, two cigarettes (Red Roses, China Tobacco Guangdong Zhongyan Industry CO. Ltd., tar, 13 mg; nicotine, 1.3 mg) without filter were burned and then the smoke was collected and finally bubbled through 10 ml serum-free DMEM medium with the use of a vacuum-pump. The resulting solution was filtered through a 0.22 μm filter to remove particles and bacteria and the pH was adjusted to 7.4. The obtained solution was represented 100% CSE and applied to mle-12 cells within 30 min of preparation.

Plasmid Construction and Transfection

The full-length (FL) Nqo1-AS1, Nqo1-overlapping region (OL) of Nqo1-AS1, Nqo1-non-overlapping region (NOL) of Nqo1-AS1, FL-Nqo1 mRNA were PCR amplified using the SuperScript® III First-Strand Synthesis System (Invitrogen) and subcloned into the ApaI and NotI , NheI and NotI, NheI and XbaI, Nhel and NotI sites of pcDNA3.1 vector (Invitrogen), named pc-Nqo1-AS1, pc-Nqo1-AS1- OL, pc-Nqo1-AS1-NOL or pc-Nqo1, respectively. The primers used were as follows: pc- Nqo1-AS1: 5′-ATAAGAATGCGGCCGCGTTTCTTTGCTTTAGCC-3′ (forward), 5′-TTGCGGGCCCGATAGTTCTGCCATAACAAC-3' (reverse); pc- Nqo1-AS1-OL: 5′-CTAGCTAGCGATGTGTGA TGTATTCATTTATTTCG-3′ (forward), 5′-ATAAGAATG CGGCCGCGATAGTTCTGCCATAAC-3′ (reverse); pc- Nqo1-AS1-NOL: 5′-GTTTCTTTGCTTTAGCCTGGCT-3′ (forward), 5′-AGATGGTGGAGCATGCCTTTAA-3′ (reverse); pc-Nqo1: 5′- CTAGCTAGCAGGCTCAGCTCTTACTAGCCTAG-3′ (forward), 5′-ATAAGAATGCGGCCGCGATGTGTGATGTAT TC-3′ (reverse). The 3′UTR of Nqo1 was PCR amplified using the SuperScript® III First-Strand Synthesis System (Invitrogen) and subcloned into the Xhol and XbaI sites of Dual-Luciferase reporter plasmid pmirGLO vector (Promega), named pmirGLO-Nqo1 3′UTR.The primers used were as follows: 5′-CCGCTCGAGGGATTTTTTTCCTAACATATAGTTAGAC-3′ (forward), 5′-GCTCTAGAGATGTGTGATGTATTCATTTAT TTCG-3′ (reverse). The pcDNA3.1 empty vector was used as a control. A total of 5×10^5 mle-12 cells were seeded in 6-well plates, and cultured for 18–24 h, and 80–90% cells were used for transfection. Then transfection was performed on cells using Lipofectamine 3000 (Invitrogen) according to the manufacturer's instructions. After transfection for 24 h, cells were treated with 0 and 0.5% CSE for 24 h and then harvested for analysis.

Small Interfering RNA Transfection

siRNA specially targeting Nqo1-AS1 (Nqo1-AS1 siRNA) and scrambled negative control siRNA (siRNA CTL) were synthesized by GenePharma (Shanghai, China).The Nqo1-AS1 siRNA sequence was 5′-GCAUGUUGCUGUGUGCCUATT-3′

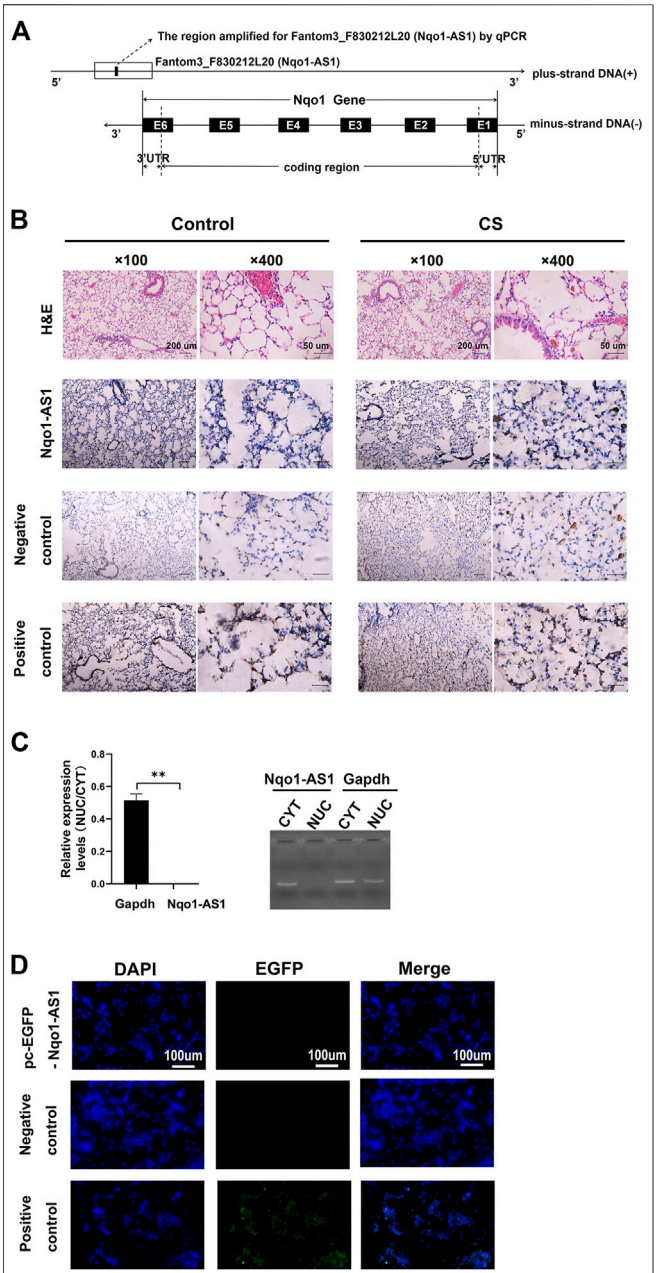

FIGURE 1 | Characterization of lncRNA Fantom3_F830212L20 (Nqo1-AS1) about gene location, distribution and protein coding potential. **(A)** Fantom3_F830212L20 (Nqo1-AS1) located next to the Nqo1 gene on mouse chromosome 8. Nqo1-AS1 was encoded by the (+) DNA strand, while Nqo1was encoded by the (-) DNA strand. Nqo1-AS1 and Nqo1 formed a "tail to tail" pairing pattern with 460 bp full complementarity. **(B)** Representative images of H&E and RNA ISH staining for Nqo1-AS1 in lung tissues of chronic CS-induced COPD mouse model and those of control animals. The expression of Nqo1-AS1 was stronger in lung tissues of chronic CS-induced COPD mouse model than those of control animals. The Nqo1-AS1 sense probe was used as a negative control. The Gapdh or U6 RNA probe was used as a positive control. (Claybank, positive staining). **(C)** Nqo1-AS1 expression in purified nuclear or cytoplasmic RNAs was detected using qPCR. Nqo1-AS1 was enriched in cytoplasm of mle-12 cells. GAPDH served as a control. **(D)** Nqo1-AS1 was verified to have a very low protein coding potential. The recombinant plasmid pcDNA3.1-EGFP- Nqo1-AS1 (pc-EGFP- Nqo1-AS1), (*Continued*)

FIGURE 1 | pcDNA3.1-EGFP- Hotair (pc-EGFP- Hotair) or pcDNA3.1-EGFP-Nqo1 (pc-EGFP-Nqo1) was transfected into the mle-12 cells for 72 h. Then the immunofluorescence of cells was observed using a fluorescence microscope. Pc-EGFP-Nqo1 was used as a positive control. Pc-EGFP- Hotair was used as a negative control.*$p < 0.05$ and **$p < 0.01$. Data represented the mean ± SEM from three independent experiments.

and the siRNA CTL sequence was 5′-UUCUCCGAACGUGUC ACGUTT-3′. A 20 μM siRNA solution was transfected into the mle-12 cells using HiPerFect Transfection (Qiagen) according to the manufacturer's instructions. After 24 h, more than 95% of the mle-12 cells were still viable. Cells were then treated with 0 and 0.5% CSE for 24 h prior to being collected, and analyzed.

ROS Assay

Intracellular ROS was measured using 2, 7-dichlorodihydrofluorescein diacetate (DCFH-DA, Beyotime, Shanghai, China) according to the manufacturer's recommendation. Briefly, the mle-12 cells were seeded into 96-well plates and incubated with 10 mM DCFH-DA for 20 min at 37°C. Cells were washed with 1 × PBS and resuspended in DMEM. Then, DCF fluorescence was detected using fluorescence spectrophotometer (Thermo, MA, United States). The cells treated with Rosup (50 mg/ml) for 30 min were used as positive controls.

Measurement of MDA

Concentrations of MDA in the mle-12 cells, the mouse lung tissues and serums from patients with COPD and healthy individuals were measured using MDA Assay kit (Beyotime, Shanghai, China) according to the manufacturer's instructions. Briefly, the mle-12 cells and the homogenate of mouse lung tissues were lysed in lysis buffers for 30 min on ice, respectively. The lysates were then centrifuged at 10,000×*g* for 10 min at 4°C, and the supernatants were collected. The serums from patients with COPD and healthy individuals were collected as well. After being treated with thiobarbituric acid (TBA) working solution, the supernatants and the serums were heated at 100°C for 15 min and then cooled down and centrifuged at 1,000×*g* for 10 min, respectively. The absorbance was measured spectrophotometrically at 532 nm.

Measurements of Glutathione and GSSG

The levels of GSH and GSSG in the mle-12 cells, the mouse lung tissues and serums from patients with COPD and healthy controls were measured using GSH and GSSG Assay Kit (Beyotime, Shanghai, China) according to the protocols of manufacturer. Briefly, the mle-12 cells, the mouse lung tissues and the serums from patients with COPD and healthy individuals were treated with protein removal reagent M solution at 4°C for 10 min and then centrifuged at 10,000×*g* for 10 min, respectively. The supernatants were collected. Then the GSH test solution and 0.5 mg/ml NADPH solution were added to a 96-well plate containing a standard solution of GSH or the mentioned supernatants. The absorbance was measured spectrophotometrically at 412 nm. Then the content of

TABLE 1 | Prediction of protein coding potential for Nqo1-AS1 (Fantom3_F830212L20).

ID	Peptide length	Fickett score	Isoelectric point (pI)	ORF integrity	Coding probability	label
Nqo1-AS1	87	0.32762	5.12103271484	1	0.171312	noncoding
Hotair	48	0.34113	11.539855957	−1	0.184882	noncoding
Nqo1 (NM_008,706.5)	275	0.43184	8.74053955078	1	0.999935	coding

The RNA sequence of Nqo1-AS1, HOTAIR or Nqo1 was put into the Coding Potential Calculator (CPC) algorithm version 2. CPC2 was available freely at http://cpc2.cbi.pku.edu.cn. Hotair was used as a negative control. Nqo1 was used as a positive control.

TABLE 2 | Prediction of the open reading frame (ORF) sequence of Nqo1-AS1.

ID	Predicted ORF length (bp)	Predicted ORF nucleotide sequence
Nqo1-AS1	261	ATGTCAAGTTGTTTTTCTTTGGTAGAAGGCTACCGGTTTTCATTGTGGCACTTAGGATTATTTTTATATGTACACCGCTTT ATTATTTATTTATTTATATCTACTTATTTATTTATTTATTTATTTATATTTACTTATCTATGCATGCAGTGGCTGCAGGAGCCA GAAGAGGGCACTGGATCCCTCGGAACTGGGGTTAGAAAAGAGGTTGGCCTTTCTGAAGGTTTTCTGCAAGAGCCA ACAAGTGCACTTGGCTGCTGA
Hotair	294	ATGGAAGGGTTTTACAAGTCTGCAGGGGAGTCAGGGAGTAAAGAAATCGTGCCCAGATTTAGAGACAATGGTGAAAGA TACAGAAGACAGAAGAGATGGGGGCCGCCCCAGCTGGCAGGGAGTGGAGCCAGAGGCAGAAAAGGAGAGAAAAGT TTCCTGCCATCTTCATTAGTTGACTTCCCAGTCCACAGCCACAGCTTCCCGGGGGCTGCAGAATTCACTCTCAATAAAG AAAGGAGGCTTAAAAAAAAAAAAAAAAAGTCCTGTGTTTACAAGACCAGAAATGCCAGCGCTAA
Nqo1 (NM_008706.5)	825	ATGGCGGCGAGAAGAGCCCTGATTGTACTGGCCCATTCAGAGAAGACATCATTCAACTACGCCATGAAGGAGGCT GCTGTAGAGGCTCTGAAGAAGAGAGGATGGGAGGTACTCGAATCTGACCTCTATGCTATGAACTTCAACCCCATCATT TCCAGAAATGACATCACAGGTGAGCTGAAGGACTCGAAGAACTTTCAGTATCCTTCCGAGTCATCTCTAGCATATAAG GAAGGACGCCTGAGCCCAGATATTGTGGCCGAACACAAGAAGCTGGAAGCTGCAGACCTGGTGATATTTCAGTTC CCATTGCAGTGGTTTGGGGTGCCAGCCATTCTGAAAGGCTGGTTTGAGAGAGTGCTCGTAGCAGGATTTGCCTACACA TATGCTGCCATGTACGACAACGGTCCTTTCCAGAATAAGAAGACCTTGCTTTCTATCACCACTGGGGGTAGCGGCTCC ATGTACTCTCTTCAGGGTGTCCACGGGGACATGAACGTCATTCTCTGGCCGATTCAGAGTGGCATCCTGCGTTTCTGT GGCTTCCAGGTCTTAGAACCTCAACTGGTTTACAGCATTGGCCACACTCCACCAGATGCCCGCATGCAGATCCTGGAA GGATGGAAGAAACGTCTGGAAACCGTCTGGGAGGAGACCCCACTCTATTTTGCTCCAAGCAGCCTGTTTGACCTAAAC TTTCAGGCAGGATTCTTAATGAAAAAGGAAGTTCAAGAGGAGCAGAAGAAGAACAAGTTTGGCCTCTCTGTGGGCCAT CACCTGGGCAAGTCCATTCCAGCTGACAACCAGATCAAAGCTAGAAAATAA

The predicted ORF sequence of Nqo1-AS1, Hotair or Nqo1 was obtained from the ORF finder database (https://www.ncbi.nlm.nih.gov/orffinder), respectively. Hotair was used as a negative control. Nqo1 was used as a positive control.

reduced GSH or GSSG and the ration of reduced GSH/GSSG were calculated.

Quantitative Real-Time PCR

The total RNA was extracted from mouse lung tissues, cultured cells or PBMCs from patients with COPD and healthy individuals using Trizol reagent (Invitrogen) and reversely transcribed to cDNA using PrimeScript™ RT reagent Kit (TaKaRa, China). QRT-PCR expression analysis was performed on CFX96–C1000 system (Bio-Rad, CA) using SsoFast™ EvaGreen® supermix kit (Bio-Rad). Primers used for qRT-PCR were as follows: mouse Nqo1-AS1 non-overlapping region (Nqo1-AS1-NOL): 5′-TTG GAATGCTGAGACCCTGT-3′ (forward), 5′-GGAGTGAAA ACACGTGGCTT-3′ (reverse); mouse Nqo1-AS1 overlapping region (Nqo1-AS1-OL): 5′-TCGGGCTAGTCCCAGTTAGA-3′ (forward), 5′-AAGTTAGTCCCTCGGCCATT-3′ (reverse); mouse Nqo1 non-overlapping region (Nqo1-NOL): 5′-GGA AGCTGCAGACCTGGTGA-3′ (forward), 5′-CCTTTCAGA ATGGCTGGCA-3′ (reverse); mouse Nqo1 overlapping region (Nqo1-OL): 5′-TCGGGCTAGTCCCAGTTAGA (forward), 5′-AAGTTAGTCCCTCGGCCATT-3′ (reverse); mouse Gapdh: 5′-AGGTCGGTGTGAACGGATTTG-3′ (forward), 5′-GGGGTC GTTGATGGCAACA-3′ (reverse); Nqo1-AS1 human homologue: 5′-TATGGCAGAAGGGAATTGCT (forward), 5′-GCTTTGTAATTGAAAGCAAGAAA (reverse); human NQO1: 5′-GAAGAGCACTGATCGTACTGGC-3′ (forward), 5′-GGA TACTGAAAGTTCGCAGGG-3′ (reverse); human GAPDH: 5′-ACAACTTTGGTATCGTGGAAGG-3′ (forward), 5′-GCC ATCACGCCACAGTTTC-3′ (reverse).A primer sequence for mouse Nqo1-NOL has been previously described (Amara et al., 2012). Primer sequences of mouse Gapdh, human NQO1 and human GAPDH were retrieved from PrimerBank Database (http://pga.mgh.harvard.edu/primerbank/). The relative expression of each gene was normalized to Gapdh expression and calculated using the $2^{-\triangle\triangle Ct}$ method.

RNase Protection Assay and the Infection of Mle-12 Cells With α-amanitin

To detect whether Nqo1-AS1 was associated with Nqo1 mRNA, RNase protection assay was performed as previously described (Xia et al., 2021). Briefly, pc-Nqo1-AS1-OL, pc-Nqo1-AS1-NOL

TABLE 3 | General characteristics of patients with COPD and healthy controls.

	Patients with COPD (n = 7)	Healthy controls (n = 7)	p value
Sex	Male	Male	
Age (year)	64.71 ± 3.06	71.14 ± 4.77	0.28
Smoke (pack-years)	28.36 ± 5.70	0.00	0.00
Height (cm)	162.86 ± 1.10	167.29 ± 1.97	0.07
Weight (kg)	62.86 ± 3.84	70.43 ± 3.27	0.16
BMI (kg/m^2)	23.66 ± 1.32	25.15 ± 1.05	0.39
FEV_1 (L)	2.15 ± 0.33	3.31 ± 0.13	0.01
FVC (L)	3.42 ± 0.35	4.08 ± 0.12	0.12
FEV_1/ FVC (%)	60.28 ± 4.43	82.52 ± 2.28	0.00
FEV_1%Pred (%)	68.56 ± 8.99	97.26 ± 2.88	0.02

Variables are expressed as mean ± standard error of the mean (SEM). Italicized p values resulted from Student t *test for parametric variables between the two groups are statistically significant, ie.,* p < *0.05.*
BMI, body mass index; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s; %Pred, percent predicted.

or pcDNA3.1 was cotransfected with pc-Nqo1 into mle-12 cells. After transfection for 48 h, the total RNA was extracted from the cells. RNA sample was digested by DNaseI (Invitrogen) and followed by RNAse A + T cocktail (AM2286, Thermo Fisher Scientific) treatment at 37°C for 30 min. Then, the RNA sample was extracted using RNeasy kits (QIAGEN) and was reversely transcribed to cDNA as described above. Nqo1-AS1 overlapping region of Nqo1 (Nqo1-OL), Nqo1-AS1 non-overlapping region of Nqo1 (Nqo1-NOL) and mouse Gapdh mRNA were amplified by PCR and analyzed by agarose gel electrophoresis. Gapdh PCR product was used as a control. Next, to detect whether Nqo1-AS1 increased Nqo1 mRNA stability, mle-12 cells were transfected with pc-Nqo1-AS1-OL, pc-Nqo1-AS1-NOL or pcDNA3.1 for 24 h, then further exposed to 10 μg/ml α-amanitin (MedChemExpress) for 0 h, 6 h, 12 h, 18 and 24 h. Finally, the cells were harvested and RNA was extracted and analyzed by qRT-PCR.

Luciferase Reporter Assay

To detect whether Nqo1-AS1 increased the stability of Nqo1 mRNA by binding to its 3′UTR, luciferase reporter assay was performed. Briefly, pmirGLO-Nqo1-3′UTR was cotransfected with Nqo1-AS1 siRNA or siRNA CTL, pc- Nqo1-AS1-OL, pc-Nqo1-AS1-NOL or pcDNA3.1 into mle-12 cells. After transfection for 48 h, cells were harvested and were lysed with lysis buffer. Firefly and Renilla luciferase activities were measured using dual-luciferase reporter assay kit (Promega) according to the manufacturer's instructions.

Western Blot

The mle-12 cells and the homogenate of mouse lung tissues were lysed in RIPA lysis buffer with PMSF for 30 min on ice. Total protein concentration was measured using BCA protein assay (Beyotime Biotechnology, China). Protein samples were separated by SDS-PAGE and then transferred to PVDF membranes. The PVDF membranes were blocked with 5% skim milk and then incubated with the primary antibodies NQO1 (1:20,000, Abcam Biotechnology, Cambridge, MA, United States) and β-actin (1:5,000, Abcam Biotechnology, Cambridge, MA, United States) overnight at 4°C. After being washed with TBST, the membranes were incubated with the secondary antibody at room temperature for 2 h. Protein bands were detected with ECL reagents (CoWin Biosciences, China) and then visualized using Tanon 5200 chemiluminescence imaging system (Tanon, Shanghai, China).Scanned images were quantified with Image-Pro 6 software.

Statistical Analysis

Data were presented as mean ± standard error of the mean (SEM). Normality of the variables was evaluated using a Kolmogorov–Smirnov test. Analysis of parametric variables were performed using the Student t test or one-way ANOVA followed by Bonferroni correction for multiple comparisons, while the analysis of non-parametric variables were performed using the chi-square test. All statistical analyses were performed using SPSS version 13.0 software. The correlation between Nqo1-AS1 human homologues expression and the smoking amount of patients with COPD or healthy individuals was evaluated by Pearson's correlation. A p-value less than 0.05 was regarded as statistically significant.

RESULTS

Characterization of the Nqo1 Antisense Transcript 1 About Gene Location, Distribution and Protein Coding Potential

In previous study, we reported that lncRNA Fantom3_F830212L20 and Nqo1 were co-expressed lncRNA and protein-coding gene (Zhang et al., 2018b). To investigate the association between genome loci of Fantom3_F830212L20 and Nqo1, bioinformatics analysis was performed. The results showed that Fantom3_F830212L20 oriented in antisense direction with respect to Nqo1, which formed a "tail to tail" pairing pattern with 460 bp full complementarity between each other. We named this lncRNA as Nqo1 antisense transcript 1 (Nqo1-AS1) (**Figure 1A**).RNA ISH revealed that the majority of Nqo1-AS1 expression existed in alveolar epithelial cells of mouse with chronic CS exposure, whereas the positive staining was occasionally observed in mouse without CS exposure (**Figure 1B**). Moreover, subcellular fractionation assay shown that Nqo1-AS1 mainly located in the cytoplasm of mouse alveolar epithelium (**Figure 1C**). To verify whether Nqo1-AS1 had a coding potential, bioinformatics analysis and an *in vitro* translation assay were performed as described previously (Zhang et al., 2017). The Coding Potential Caculator computational algorithm predicted that Nqo1-AS1 had a very low protein coding potential, similar to Hotair, a well-known lncRNA, whereas Nqo1 was predicted to code for protein (**Table 1**).Then the recombinant plasmid pc-EGFP-Nqo1-AS1 with the predicted ORF sequence of Nqo1-AS1 was overexpressed in the mle-12 cells (**Table 2**). Pc-EGFP-Nqo1 was used as a positive control, and pc-EGFP- Hotair

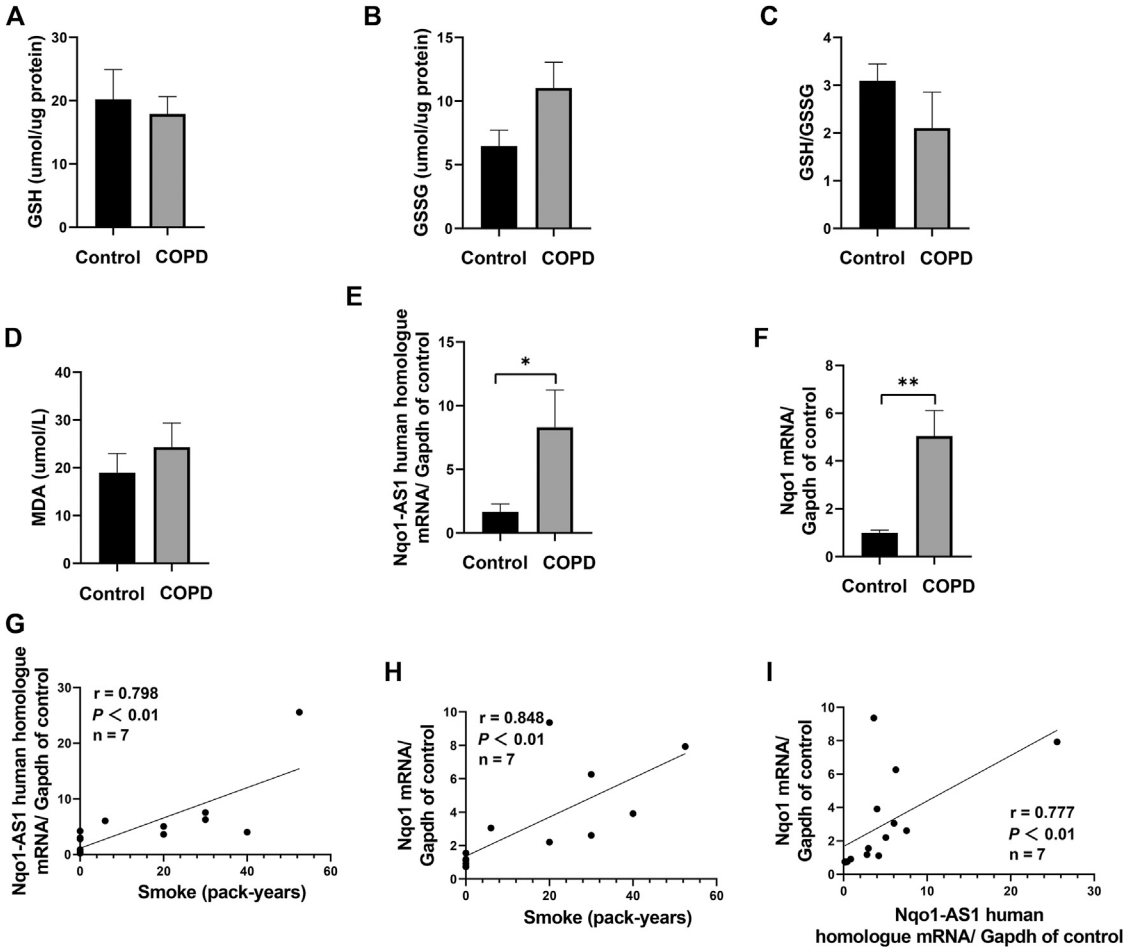

FIGURE 2 | Nqo1-AS1 human homologue is both positively correlated with smoking amount and Nqo1 mRNA expression in patients with COPD. Levels of reduced glutathione (GSH) **(A)**, Glutathione disulfide (GSSG) **(B)**, GSH/GSSG ratio **(C)** and MDA **(D)** were assessed in serums from patients with COPD and healthy controls. Expressions of Nqo1-AS1 human homologue **(E)** and Nqo1 mRNA **(F)** were examined in PBMCs from patients with COPD and healthy controls (*$p < 0.05$; **$p < 0.01$). Both Nqo1-AS1 human homologue **(G)** and Nqo1 mRNA **(H)** expressions were positively correlated with smoking amount of patients with COPD. **(I)** The expression levels of Nqo1-AS1 human homologue and Nqo1 mRNA in PBMCs from patients with COPD and healthy controls were positively correlated with each other ($n = 7$/group; $p < 0.01$; r represents spearman correlation coefficient). Nqo1-AS1 is positively correlated with Nqo1 mRNA expression in lung tissue of mice exposed to CS.

was used as a negative control. Immunofluorescence staining displayed that EGFP was hardly detected in cells transfected with pc-EGFP- Nqo1-AS1 or pc-EGFP- Hotair, whereas it was easily detectable in cells transfected with Pc-EGFP-Nqo1. These results suggest that Nqo1-AS1 mainly locates in the cytoplasm of mouse alveolar epithelium and has a very low protein coding potential.

The RNA sequence of Nqo1-AS1, HOTAIR or Nqo1 was put into the Coding Potential Calculator (CPC) algorithm version 2. CPC2 was available freely at http://cpc2.cbi.pku.edu.cn. Hotair was used as a negative control. Nqo1 was used as a positive control.

The predicted ORF sequence of Nqo1-AS1, Hotair or Nqo1 was obtained from the ORF finder database (https://www.ncbi.nlm.nih.gov/orffinder), respectively. Hotair was used as a negative control. Nqo1 was used as a positive control.

Nqo1-AS1 human homologue is both positively correlated with smoking amount and Nqo1 mRNA expression in PBMCs of patients with COPD or healthy controls

To assess whether Nqo1-AS1 human homologue expression was associated with smoking amounts and Nqo1 mRNA expression, the expression levels of Nqo1-AS1 human homologue and Nqo1 mRNA in PBMCs of patients with COPD or healthy controls were examined, and the correlation between these two gene expressions and smoking amounts of patients with COPD or healthy controls were analyzed. A total of seven patients with COPD and seven healthy controls were enrolled in this study. The general characteristics of study participants were summarized in **Table 3**. As compared to the control group, the GSH concentration and the GSH/GSSG ratio in serum of patients with COPD were lower, whereas concentrations of GSSG and MDA were higher (**Figures**

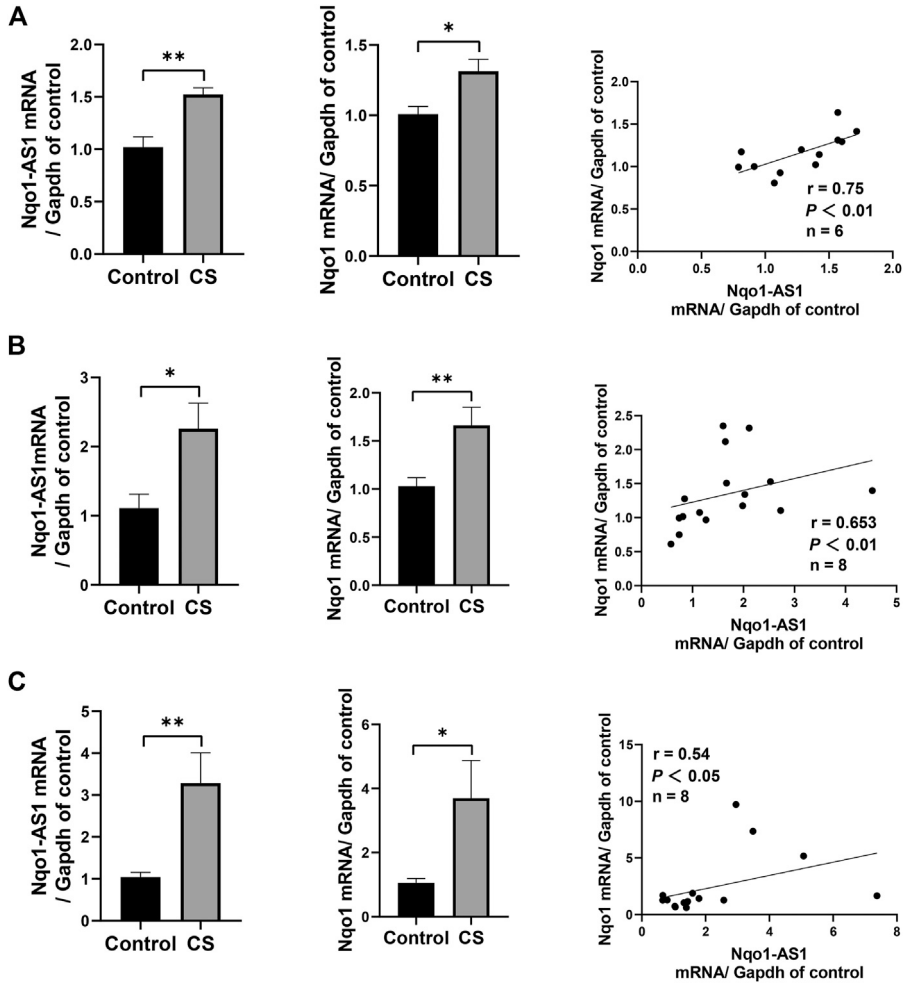

FIGURE 3 | Nqo1-AS1 and Nqo1 mRNA expressions in lung tissues of mice exposed to cigarette smoke (CS) for 1 week **(A)**, 1 month **(B)** and 3 months **(C)**. The expression levels of Nqo1-AS1 and Nqo1 mRNA in lung tissues of mice exposed to CS and those of control animals were measured by qRT-PCR. Expression level of Nqo1-AS1 was positively correlated with Nqo1 mRNA expression in lung tissues of mice with and without CS exposure. n = 6/group for **(A)**. n = 7/group for **(B)** and **(C)** (*$p < 0.05$; **$p < 0.01$; r represents spearman correlation coefficient).

2A–D). The expression levels of Nqo1-AS1 human homologue and Nqo1 mRNA in PBMCs from patients with COPD were significantly upregualted than those of the control group (**Figures 2E,F**). Correlation analysis shown that Nqo1-AS1 human homologue expression was both positively associated with smoking amounts and Nqo1 mRNA expression (**Figures 2G–I**). These results indicate that Nqo1-AS1 human homologue is both positively associated with Nqo1 mRNA expression and smoking amounts of patients with COPD.

Nqo1 Antisense Transcript 1 is Positively Correlated With Nqo1 mRNA Expression in Lung Tissue of Mice Exposed to Cigarette Smoke

Given that Nqo1-AS1 human homologue is positively correlated with Nqo1 mRNA expression in PBMCs of patients with COPD, we speculated that Nqo1-AS1 might also be positively associated with Nqo1 mRNA expression in lung tissue of mice exposed to cigarette smoke. QRT-PCR revealed that both Nqo1-AS1 and Nqo1 mRNA expressions were upregulated in lung tissue of mice exposed to CS for 1 week, 1 month and 3 months in comparison with control animals. Correlation analysis shown that Nqo1-AS1 expression was positively associated with Nqo1 mRNA expression in lung tissues of mice (**Figures 3A–C**). These results suggest that the expression levels of Nqo1-AS1 and Nqo1 mRNA are elevated in lung tissue of mice exposed to CS, and there is a positive correlation between Nqo1-AS1 and Nqo1 mRNA expression.

Both Nqo1 Antisense Transcript 1 and Nqo1 mRNA Expressions Are Associated With Cigarette Smoke Extract Concentration and Duration in Mle-12 Cells

Given that CS exposure was closely associated with the expression levels of Nqo1-AS1 (or its human homologue) and Nqo1 mRNA

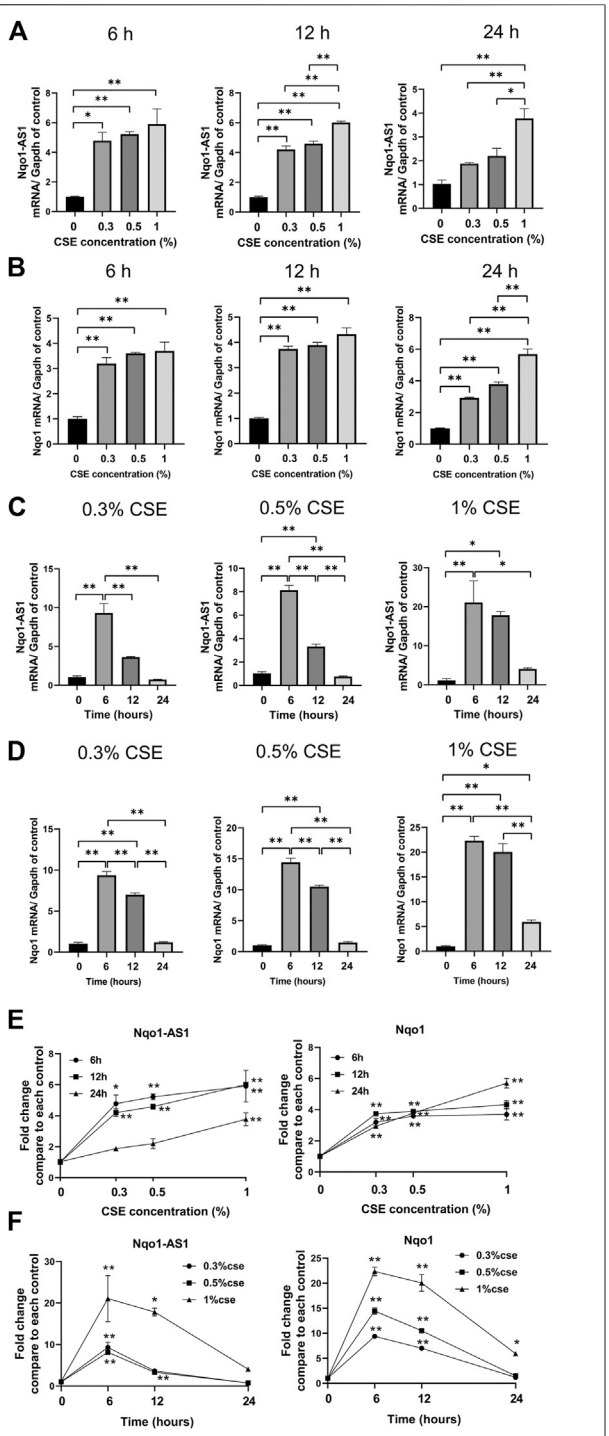

FIGURE 4 | Expression levels of Nqo1-AS1 and Nqo1 mRNA in the mle-12 cells treated with cigarette smoke extract (CSE) at different concentrations for varied time points. Relative mRNA levels of Nqo1-AS1 and Nqo1 were assessed by qRT-PCR. The relative expression of each gene was normalized to Gapdh expression. Relative mRNA levels of Nqo1-AS1 **(A)** and Nqo1 **(B)** were elevated along with the increase of CSE concentration (0–1%) for 6 h, 12 and 24 h. Relative mRNA levels of Nqo1-AS1 **(C)** and Nqo1 **(D)** were elevated predominantly at CSE exposure (0.3–1%) for 6 and 12 h when (*Continued*)

FIGURE 4 | compared to cells exposed to 0% CSE. **(E)** Line chart shown the trends of Nqo1-AS1 and Nqo1 mRNA expressions in cells exposed to CSE (0–1%) for 6 h, 12 and 24 h exposures (derived from **Figures 3A,B**). $^{*}p < 0.05$ and $^{**}p < 0.01$ vs the cell group treated with 0% CSE. **(F)** Line chart shown the relative mRNA levels of Nqo1-AS1 and Nqo1 were elevated predominantly at CSE exposure (0.3–1%) for 6 and 12 h when compared to cells exposed to CSE (0.3–1%) for 0 h (derived from **Figures 3C,D**). $^{*}p < 0.05$ and $^{**}p < 0.01$ vs the cell group treated with the same CSE concentration for 0 h. Data represented the mean ± SEM from three independent experiments. Statistical significance were indicated ($^{*}p < 0.05$;$^{*}p < 0.01$) One-way ANOVA and Bonferroni correction for multiple comparisons.

in lung tissues of mouse or PBMCs from patients with COPD, we speculated that CSE might have effects on the expression levels of Nqo1-AS1 and Nqo1 *in vitro*. The murine alveolar mle-12 cells were treated with CSE at different concentrations for varied time points, and the expression levels of Nqo1-AS1 and Nqo1 mRNA were detected. Compared to the control group, cells treated with 0% CSE, Nqo1-AS1 and Nqo1 mRNA expressions in cells exposed to CSE (0.3–1%) for 6 h, 12 and 24 h were significantly upregulated. Furthermore, Nqo1-AS1 and Nqo1 mRNA expressions were increased after being exposed to CSE in a concentration dependent manner (**Figures 4A,B**).Additionally, Nqo1-AS1 and Nqo1 mRNA expressions were significantly increased after being exposed to CSE for 6 h, yet both these two gene expressions were gradually decreased in response to the increasing CSE duration (**Figures 4C,D**). Line chart depicted the trends of Nqo1-AS1 and Nqo1 mRNA expressions in cells exposed to CSE, which elevated with the increase of CSE concentration whereas decreased with the increase of the CSE exposure duration (**Figures 4E,F**). These results indicate that Nqo1-AS1 and Nqo1 mRNA expressions are closely correlated with CSE concentration, and both of them were elevated mainly in the early stage of exposure of cells to CSE.

Nqo1 Protein Expression Correlates With Cigarette Smoke Extract Concentration and Duration in Mle-12 Cells

Compared to the control group, cells treated with 0% CSE, Nqo1 protein level in mle-12 cells exposed to CSE (0.3–1%) for 6 h, 12 and 24 h were significantly enhanced (**Figure 5A**). Similarly, compared to cells treated with CSE (0.3–1%) for 0 h, Nqo1 protein level in cells was significantly increased after being exposed to CSE (0.3–1%) for 24 h (**Figure 5B**). Line chart displayed the trends of Nqo1 protein level in cells exposed to CSE, which was enhanced with the increase of CSE concentration and exposure duration (**Figure 5C**). These results indicate that CSE enhances Nqo1 protein level of mle-12 in a dose-and time-dependent manner.

Nqo1-Antisense Transcript 1 Attenuates Cigarette Smoke Extract-Induced Oxidative Stress *in vitro*

Accumulating evidences suggest that CS induced oxidative stress plays a critical role in the pathological mechanism of COPD (Kim

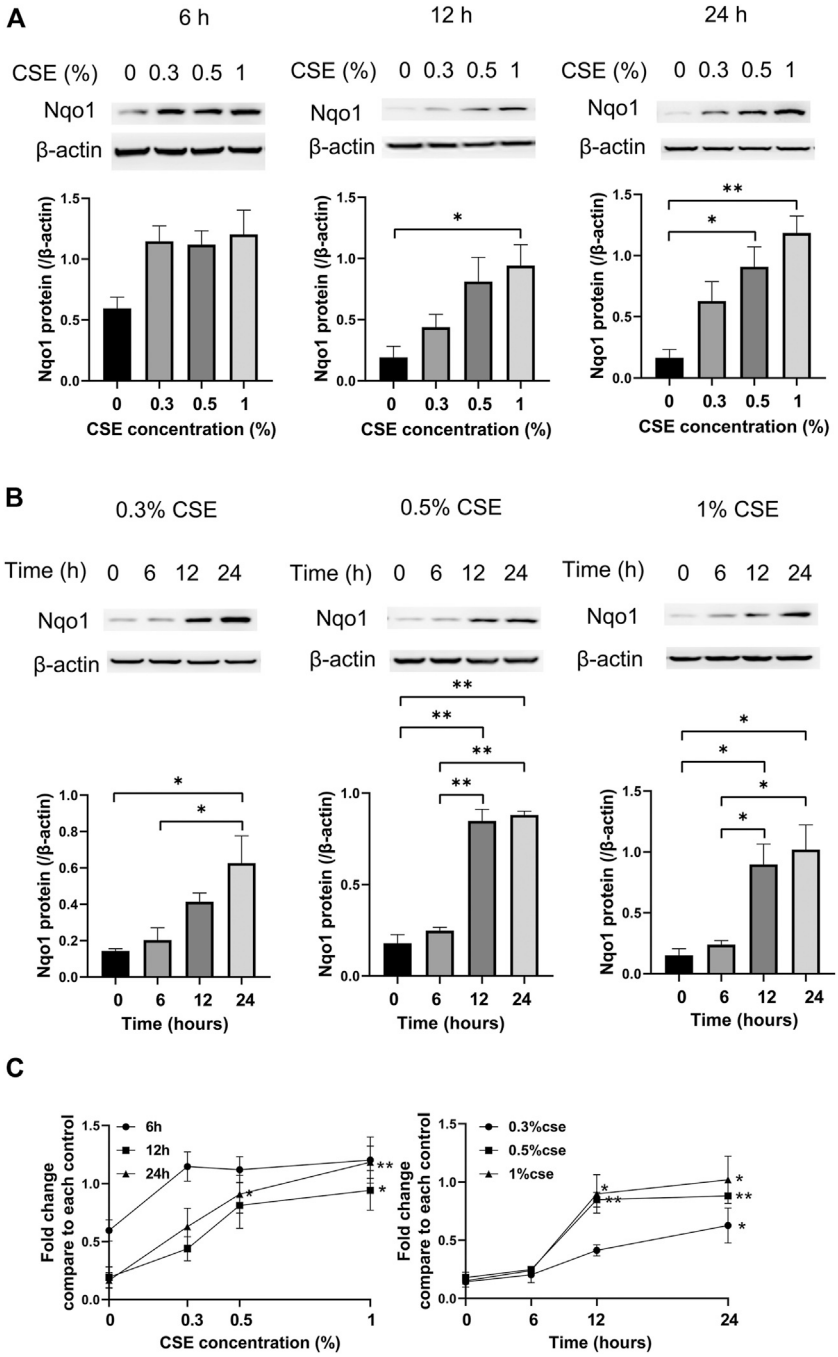

FIGURE 5 | Western blot analysis of Nqo1 expression in the mle-12 cells treated with CSE at different concentrations for varied time points. **(A)** The expression level of Nqo1 protein was elevated along with the increase of CSE concentration (0–1%) for 6 h, 12 and 24 h. **(B)** The expression level of Nqo1 protein was elevated along with the increase of CSE exposure duration. The mel-12 cells were treated with 0.3, 0.5 and 1% CSE respectively. **(C)** Line chart depicted the Nqo1 protein level was increased with the increase of CSE concentration and exposure duration. *$p < 0.05$ and **$p < 0.01$. Data represented the mean ± SEM from three independent experiments. One-way ANOVA and Bonferroni correction for multiple comparisons.

et al., 2019). To elucidate the effect of Nqo1-AS1 against CSE-induced oxidative stress, mle-12 cells were transfected with Nqo1-AS1 siRNA or siRNA CTL prior to being treated with 0% CSE or 0.5% CSE for 24 h. Then oxidative stress parameters such as GSH, GSSG, MDA and ROS in cells were measured. The cells treated with Rosup (50 mg/ml) were used as positive controls. Compared to cells transfected with siRNA CTL and followed by 0% CSE treatment for 24 h, the GSH content and [GSH/GSSG] ratios in cells transfected with siRNA CTL and followed by 0.5% CSE treatment were significantly reduced,

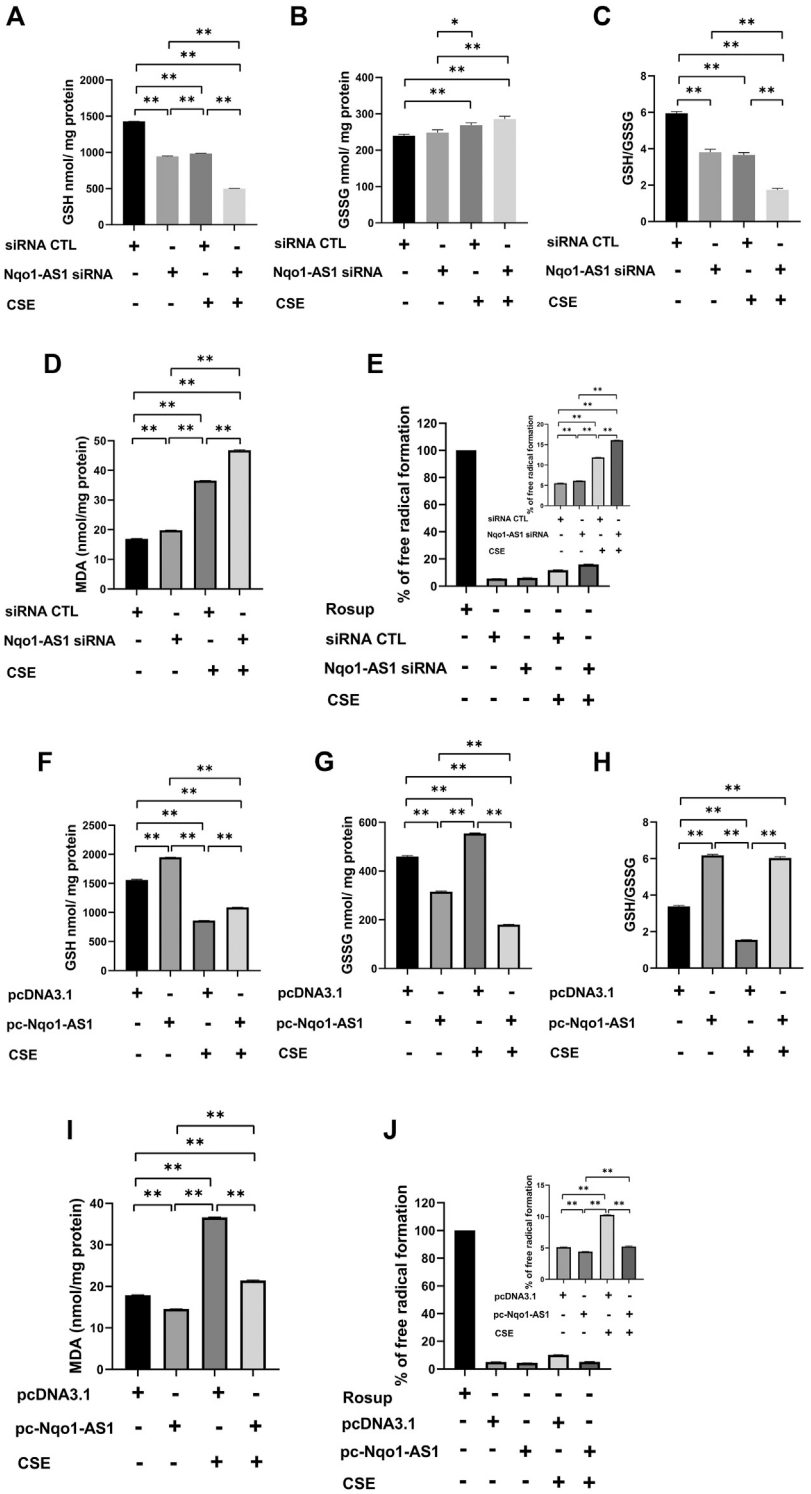

FIGURE 6 | Effect of Nqo1-AS1 on CSE-induced oxidative stress in the mle-12 cells. **(A–E)** Nqo1-AS1 siRNA or siRNA CTL was transfected into the mle-12 cells for 24 h, and followed by 0.5% CSE treatment or medium for 24 h. Then, levels of reduced glutathione (GSH) **(A)**, Glutathione disulfide (GSSG) **(B)**, GSH/GSSG ratio **(C)**, MDA **(D)** and ROS **(E)** were examined in cells with or without CSE treatment. **(F–J)** The pcDNA3.1-Nqo1-AS1 (pc-Nqo1-AS1) or pcDNA3.1 was transfected into the mle-12 cells for 24 h, and followed by 0.5% CSE treatment or medium for 24 h. Oxidative stress indexs such as reduced GSH **(F)**, GSSG **(G)**, GSH/GSSG ratio **(H)**, MDA **(I)** and ROS **(J)** were examined in cells with or without CSE treatment. The cells treated with Rosup (50 mg/ml) for 30 min were used as positive controls. $^{*}p < 0.05$ and $^{**}p < 0.01$. Data represented the mean ± SEM from three independent experiments. One-way ANOVA and Bonferroni correction for multiple comparisons.

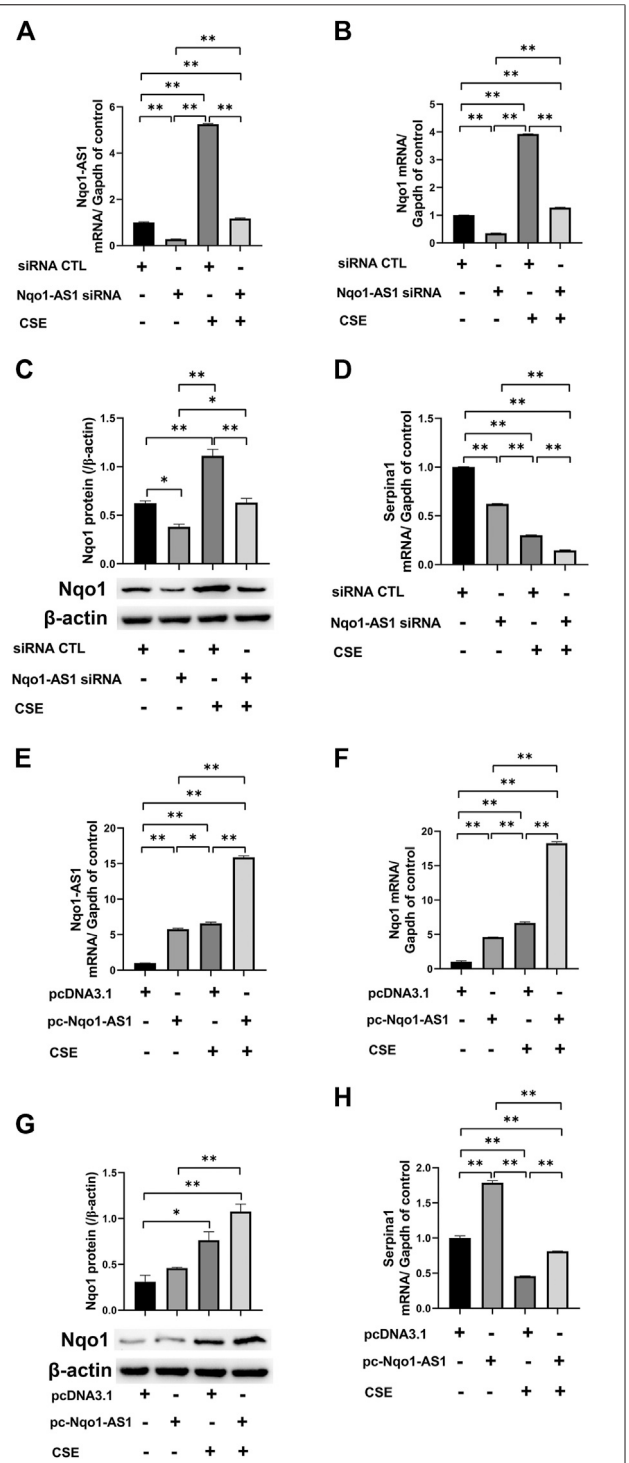

FIGURE 7 | Nqo1-AS1 regulates CSE-induced Nqo1 and Serpina1 expressions in mle-12 cells. **(A)** Nqo1-AS1 siRNA or siRNA control (siRNA CTL) was transfected into the mle-12 cells for 24 h, and followed by 0.5% CSE treatment or medium for 24 h. Next, the interference efficiency of Nqo1-AS1 siRNA was assessed by qRT-PCR. **(B, C)** Nqo1 mRNA **(B)** and protein **(C)** expression levels in cells with or without CSE treatment were examined after knockdown of Nqo1-AS1. The mRNA or protein level of Nqo1 was detected by qRT-PCR or western blotting, respectively. **(D)** Serpina1 mRNA (*Continued*)

FIGURE 7 | level in cells with or without CSE treatment was measured after knockdown of Nqo1-AS1. **(E)** The pc-Nqo1-AS1 or pcDNA3.1 (control) was transfected into the mle-12 cells for 24 h, and followed by 0.5% CSE treatment or medium for 24 h. Next, the transfection efficiency of pc-Nqo1-AS1 was examined by qRT-PCR. **(F, G)** Nqo1 mRNA **(F)** and protein **(G)** expression levels in cells with or without CSE treatment were examined after overexpression of Nqo1-AS1.The mRNA or protein level of Nqo1 was detected by qRT-PCR or western blotting, respectively. **(H)** Serpina1 mRNA level in cells with or without CSE treatment was measured after overexpression of Nqo1-AS1.*$p < 0.05$ and **$p < 0.01$. Data represented the mean ± SEM from three independent experiments. One-way ANOVA and Bonferroni correction for multiple comparisons.

whereas the levels of GSSG, MDA and ROS were significantly enhanced. Knockdown of Nqo1-AS1 worsen the decrease of GSH content and [GSH/GSSG] ratios in cells due to CSE exposure, whereas increasing the levels of GSSG, MDA and ROS (**Figures 6A–E**). On the contrary, the overexpression of Nqo1-AS1 was able to rescue the decrease of GSH content and [GSH/GSSG] ratios due to CSE, and to alleviate the CSE-increased MDA and ROS in cells (**Figures 6F–J**). These results lend strong support to our hypothesis that Nqo1-AS1 has a protective effect on CSE-induced oxidative damage to mle-12 cells *in vitro*.

It has been demonstrated that Nqo1 functions as a crucial antioxidant enzyme and is able to bind to Serpina1 mRNA thereby having effect on COPD progression (Di Francesco et al., 2016). We then determined whether Nqo1-AS1 regulated the expressions of Nqo1 and Serpina1 expression. The Nqo1-AS1 siRNA was transfecting into mle-12 cells and the interference efficiency was detected (**Figure 7A**). QRT-PCR and western blotting shown that knockdown of Nqo1-AS1 expression significantly decreased Nqo1 at mRNA and protein levels. Moreover, down regulation of Nqo1-AS1 inhibited the CSE-induced upregulation of Nqo1 (**Figures 7B,C**). Interestingly, we also observed that silencing Nqo1-AS1 down-regulated the Serpina1 mRNA expression, and even aggravated the decrease of Serpina1 mRNA expression due to CSE (**Figure 7D**). To better evaluate the effect of Nqo1-AS1 on the CSE-induced Nqo1 and Serpina1 expressions, we further upregulated the Nqo1-AS1 expression through transfecting pc-Nqo1-AS1 plasmids into the mle-12 cells. The transfection efficiency of pc-Nqo1-AS1 was detected (**Figure 7E**). As expected, the overexpression of Nqo1-AS1 increased Nqo1 at mRNA and protein levels in cells with or without CSE treatment (**Figures 7F,G**). In addition, overexpressing Nqo1-AS1 not only enhanced Serpina1 mRNA expression but also rescued CSE-induced downregulation of Serpina1 mRNA (**Figure 7H**). These results suggest that Nqo1-AS1 is able to regulate CSE-induced Nqo1 and Serpina1 expressions.

Nqo1-Antisense Transcript1 Increased Nqo1 mRNA Stability and Expression

Since bioinformatics analysis indicated that Nqo1-AS1 was able to form RNA-RNA hybrid with Nqo1 mRNA (**Table 4**), and Nqo1-AS1 and Nqo1 formed a "tail to tail" pairing pattern with 460 bp full complementarity between each other, we then

TABLE 4 | Prediction of the potential interaction between Nqo1-AS1 and Nqo1 mRNA.

Query	Target	dG	ndG	Start Position Query	End Position Query	Start Position target	End Position target
Nqo1-AS1	Nqo1 (NM_008706.5)	−500.43	−250.2150	3,113	3,572	1	460

The RNA sequences of Nqo1-AS1 and Nqo1 were put into the lncRNATargets (http://www.cuilab.cn/lnctar) to analyze the potential interaction between each other.

determined whether Nqo1-AS1 was physically associated with Nqo1 mRNA. RNase protection assay shown that Nqo1-AS1-OL, but not Nqo1-AS1-NOL, protected the overlapping part of Nqo1 (Nqo1-OL) mRNA from RNase digestion by forming the RNA duplexes between Nqo1-AS1 and Nqo1 mRNA, whereas the non-overlapping part of Nqo1 (Nqo1-NOL) mRNA and Gapdh mRNA was totally digested (**Figures 8A–C**). Gapdh PCR product was used as a control. It was the RNA duplexes formation between the overlapping part of Nqo1-AS1 (Nqo1-AS1-OL) and the overlapping part of Nqo1 (Nqo1-OL) mRNA that protected both of them from RNase digestion (**Figure 8D**).To further determine whether Nqo1-AS1 regulated Nqo1 mRNA stability, we silenced the Nqo1-AS1 expression by transiently transfecting Nqo1-AS1 siRNA and followed by a-amanitin treatment to block new RNA synthesis in the mle-12 cells. QRT-PCR analysis revealed that knockdown of Nqo1-AS1 decreased Nqo1 mRNA stability (**Figure 8E**).On the contrary, overexpression of Nqo1-AS1 by transfecting pc-Nqo1-AS1 plasmids into the mle-12 cells increased the stability of Nqo1 mRNA (**Figure 8F**). As the overlapping region of Nqo1-AS1 antisense paired with Nqo1 3′UTR, we constructed a luciferase reporter plasmid that carried the Nqo1 3′UTR, named pmirGLO-Nqo1-3′UTR.A 594-bp fragment of the Nqo1-3′UTR, which contained the entire overlapping region with Nqo1-AS1, was inserted downstream of the luciferase reporter gene in the pmirGLO-Nqo1-3′UTR vector. We examined the effects of Nqo1-AS1 knockdown or overexpression on pmirGLO-Nqo1-3′UTR activity. The results shown that knockdown of Nqo1-AS1 reduced the luciferase activity of pmirGLO-Nqo1-3′UTR, whereas Overexpressing Nqo1-AS1-OL, but not Nqo1-AS1-NOL, increased the luciferase activity of pmirGLO-Nqo1-3′UTR (**Figures 8G,H**).Taken together, these results demonstrate that Nqo1-AS1 increases the Nqo1 mRNA stability and promotes the expression of Nqo1 through forming RNA-RNA hybrid with Nqo1 mRNA.

The RNA sequences of Nqo1-AS1 and Nqo1 were put into the lncRNATargets (http://www.cuilab.cn/lnctar) to analyze the potential interaction between each other.

DISCUSSION

Increasing evidence suggests that lncRNAs play crucial roles in respiratory diseases, including COPD (Poulet et al., 2020). In a previous study, we reported that lncRNA Fantom3_F830212L20 and Nqo1 were co-expressed lncRNA and protein-coding genes, and both of two were significantly up-regulated in lung tissues of chronic CS-induced COPD mouse model, 16HBE cells and A549 cells exposed to CSE when compared to their controls (Zhang et al., 2018b). In this paper, we identified the characterization of Fantom3_F830212L20 about gene location, distribution and protein coding potential, and assessed whether Fantom3_F830212L20 inhibited CS-induced oxidative stress through regulating Nqo1 expression.

To better understand the characterization of fantom3_F830212L20, we evaluated the genomic locations between fantom3_F830212L20 and Nqo1.Interestingly, fantom3_F830212L20 oriented in antisense direction with respect to Nqo1 and formed a “tail to tail” antisense pairing with Nqo1. So we named fantom3_F830212L20 as Nqo1 antisense transcript I (Nqo1-AS1). Recently, quite a few of lncRNAs have been reported to serve as natural antisense transcripts (NATs), which transcribe from the opposite strands of their cognate sense genes and play important roles in various diseases (Latgé et al., 2018). It is the subcellular localization of NATs that are closely related to their different mechanisms of biological functions (Bergalet et al., 2020). Normally, nuclear NATs are mainly involved in transcriptional interference, epigenetic modifications, RNA pocessing and alternative splicing, whereas cytoplasmic NATs are mainly involved in RNA stability and/or mRNA translatability (Xu et al., 2019; Ma et al., 2020; Xu et al., 2020). Therefore we examined the subcellular localization and the protein coding potential of Nqo1-AS1. We observed that Nqo1-AS1 were mainly expressed in cytoplasm of alveolar epithelial cells, and had a very low coding potential. These findings suggest that Nqo1-AS1 serves as a NAT of Nqo1, which has low ability to encode for proteins.

Recently, increasing studies have documented oxidative stress to be a major driving mechanism in the pathogenesis of COPD (Magallón et al., 2019). In this study, we first measured the levels of GSH, GSSG and MDA in the serum of patients with COPD and healthy controls, which were important biomarkers of the oxidative and antioxidant balance system. Notably, we observed that the GSH concentration and the GSH/GSSG ratio were lower in serum of patients with COPD, whereas concentrations of GSSG and MDA were higher, which was consistent with the previous studies about COPD (Leelarungrayub et al., 2018). Moreover, we further found that both Nqo1-AS1 human homologue and Nqo1 mRNA were up-regulated in PBMCs of patients with COPD compared to the healthy controls, and both Nqo1-AS1 human homologue and Nqo1 were not only positively correlated with smoking amount of patients with COPD, but also positively correlated with each other. Since the expression levels of Nqo1-AS1 human homologue and Nqo1 mRNA were intimately associated with the CS exposure duration, we examined the mRNA expression levels of Nqo1-AS1 and Nqo1 in lung tissues of mice as well as in the mle-12 cells, which were exposed to CS or CSE for different

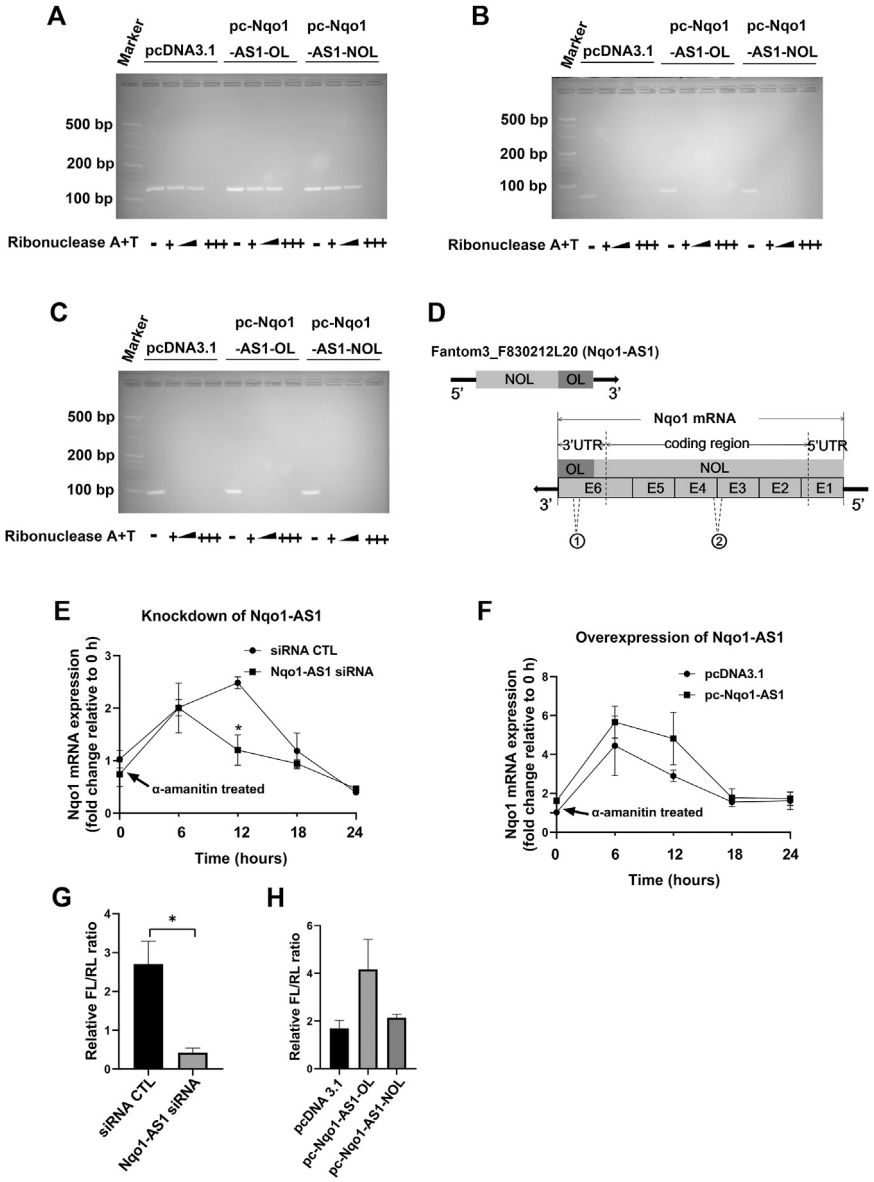

FIGURE 8 | Nqo1-AS1 increases Nqo1 mRNA stability and expression. **(A–C)** RNase protection assay was performed to examine the RNA duplexes formation between Nqo1-AS1 and Nqo1 mRNA. PcDNA3.1-Nqo1-AS1 overlapping region (pc-Nqo1-AS1-OL), PcDNA3.1-Nqo1-AS1 non-overlapping region (pc-Nqo1-AS1-NOL) or pcDNA3.1 (control) vector was cotransfected with pcDNA3.1- Nqo1 (pc-Nqo1) into mle-12 cells. After transfection for 48 h, the total RNA was extracted from the cells. RNA sample was digested with increasing amounts of RNAse A + T cocktail (represented as the black wedge and multiple "+++") in various samples.Then the remaining double-stranded RNA was reversely transcribed to cDNA and amplified the overlapping part of Nqo1 (Nqo1-OL) mRNA **(A)** and the non-overlapping part of Nqo1 (Nqo1-NOL) mRNA **(B)** by PCR. Gapdh PCR product **(C)** was used as a control. **(D)** Schematic diagram displayed the RNA duplexes formation between the overlapping part of Nqo1-AS1 (Nqo1-AS1-OL) and the overlapping part of Nqo1 (Nqo1-OL) mRNA, which protected both of them from RNase digestion. "E" followed by number represented exon. The sites of primers used in RNase protection assay were indicated as follows:1 Nqo1-OL PCR primer; 2Nqo1-NOL PCR primer. **(E–F)** Line chart shown the stability of Nqo1 mRNA over time relative to time 0 after blocking new RNA synthesis with a-amanitin treatment (10 μg/ml). 18S rRNA was used as an internal control, which was a product of RNA polymerase I and was unchanged after a-amanitin treatment. **(E)** Nqo1-AS1 siRNA or siRNA CTL was transfected into the mle-12 cells for 24 h, and followed by a-amanitin treatment for 0 h, 6 h, 12 h, 18 and 24 h. Then the Nqo1 mRNA expression level was measured by qRT-PCR. **(F)** The pc-Nqo1-AS1 or pcDNA3.1 vector was transfected into the mle-12 cells for 24 h and then treated with a-amanitin for 0 h, 6 h, 12 h, 18 and 24 h. Subsequently, the Nqo1 mRNA expression level was detected by qRT-PCR. **(G)** The luciferase activity of pmirGLO-Nqo1 3′UTR was markedly decreased in the mle-12 cells transfected with Nqo1-AS1 siRNA compared to cells transfected with siRNA CTL. **(H)** The luciferase activity of pmirGLO-Nqo1 3′UTR was increased significantly in the mle-12 cells that overexpressing the Nqo1-OL of Nqo1-AS1, but not the Nqo1-NOL of Nqo1-AS1. *$p < 0.05$. Data represented the mean ± SEM from three independent experiments.

durations. We observed that both Nqo1-AS1 and Nqo1 mRNA levels were remarkably upregulated in lung tissues of mice exposed to CS for 1week, 1 month and 3 months, and the fold changes of these two genes between mice exposed to CS and control animals were gradually increased along with the prolongation of CS exposure. Similarly, Nqo1-AS1 and Nqo1 mRNA expressions were also enhanced along with the increase of CSE concentration whereas decreased with the decrease of the CSE exposure duration. Furthermore, the Nqo1 protein level was also enhanced along with the increase of CSE concentration and the CSE exposure duration. Together with these findings, our data imply that Nqo1-AS1 (or its human homologue) and Nqo1 mRNA expression levels are increased with the increase of CS exposure, and both Nqo1-AS1 and Nqo1 mRNA expression levels are positively correlated with each other under CS exposure.

Nqo1 has been reported to be a multifunctional antioxidant enzyme, which plays critical roles in protecting cells from oxidative damage through proteasomal degradation, xenobiotic detoxification, regulation of p53, superoxide scavenging and the maintenance of endogenous antioxidants (Zhu et al., 2020). Furthermore, Nqo1 promotes Serpina1 mRNA translation, whose absence is involved in the pathogenesis of COPD (Di Francesco et al., 2016). Thus we speculated that Nqo1-AS1 might exert an effect on the CS-induced oxidative stress through regulating the expressions of Nqo1 and Serpina1. As expected, we found that Nqo1-AS1 overexpression enhanced the mRNA and protein levels of Nqo1 and Serpina1 mRNA expression in mle-12 cells, and attenuated CSE-induced oxidative stress (GSH, MDA and ROS). On the contrary, knockdown of Nqo1-AS1 significantly decreased Nqo1 at mRNA and protein levels as well as Serpina1 mRNA expression, and aggravated CSE-induced oxidative stress (GSH, MDA and ROS). Therefore, we concluded that Nqo1-AS1 is able to attenuate CS-induced oxidative stress through regulating the expression of Nqo1 in me-12 cells. Taken together, these findings clearly suggest that Nqo1-AS1 might exert its antioxidant effect by regulating Nqo1 expression.

Since NATs are capable of binding to their corresponding sense transcripts thereby regulating the expression of the latter (Jadaliha et al., 2018), we are interested in whether Nqo1-AS1 regulates the expression of Nqo1 through antisense pairing with Nqo1 mRNA. Interestingly, we observed that Nqo1-AS1 upregulated Nqo1 expression through binding to Nqo1 3′UTR and increasing Nqo1 mRNA stability.

The strengths of our study include determining the characterization of Nqo1-AS1, investigating the expression patterns of Nqo1-AS1 (or its human homologue) and Nqo1 in lung tissues of mice exposed to CS, mle-12 cells treated with CSE and PBMCs from patients with COPD, and examining the role of Nqo1-AS1 in the regulation of CS-induced oxidative stress. However, there are still some limitations in our study. An important limitation of our study is that we detected the expression levels of Nqo-AS1 and Nqo1 in PBMCs from patients with COPD and healthy donors, rather than those in lung tissues from patients with COPD and healthy donors. Undoubtedly, it would be better to detect the expressions of Nqo-AS1 and Nqo1 in lung tissues from patients with COPD and healthy donors, and analyze the correlations between smoking history and the expressions of Nqo-AS1 and Nqo1. However, as we all know, it is very difficult to obtain lung tissues from patients with COPD or healthy donors. In fact, PBMCs from patients with COPD and healthy donors are widely used in studies about CS-induced COPD. For example, Shen, W. et al. detected the mRNA expression levels of MBD2, miR-301a-5p, CXCL12 and CXCR4 in PBMCs from healthy controls and patients with stable COPD or with an acute exacerbation of COPD, and found that the MBD2/miR-301a-5p/CXCL12/CXCR4 pathway was shown to affect the migration of lung fibroblasts and monocyte-derived macrophages, which may play an important role during COPD exacerbations (Shen et al., 2020). Zhong, S. et al. analyzed hsa-miR-664a-3p and FHL1 mRNA expressions both in lung tissues from smokers with COPD and normal smokers from the GEO dataset GSE38974 and PBMCs from smokers with COPD and normal smokers, and found that the expression trends of hsa-miR-664a-3p and FHL1 in PBMCs from smokers with COPD were both consistent with those in lung tissues of smokers with COPD from the GEO dataset GSE38974, which demonstrating that the axis of hsa-miR-664a-3p and FHL1 might play a key role in CS-induced COPD (Zhong et al., 2019). Recently, the impacts of CS on both innate and adaptive immunity cells such as T lymphocytes, B lymphocytes, monocytes and macrophages have been widely discussed, which are the main components of PBMCs (Qiu et al., 2017). Thus, it is reasonable to speculate that the aberrant expression patterns of Nqo-AS1 and Nqo1 mRNA in PBMCs from patients with COPD might represent the CS-induced oxidative damage to innate and adaptive immunity cells of patients with COPD to some extent. Simultaneously, it is convincing that the smoking history of patients with COPD are correlated with the expression level of Nqo-AS1 or Nqo1 mRNA in their PBMCs.

In summary, our work demonstrated that Nqo1-AS1 (fantom3_F830212L20) oriented in antisense direction with respect to Nqo1, which is mainly located in the cytoplasm of mouse alveolar epithelium and had a very low protein coding potential. Nqo1-AS1 and Nqo1 mRNA expressions were increased with the increase of CS exposure. Nqo1-AS1 alleviated CS-induced oxidative stress by upregulating Nqo1 expression through antisense pairing withNqo1 3′UTR and increasing Nqo1 mRNA stability. Thus, our findings demonstrate that Nqo1-AS1 might play a critical role in inhibiting CS-induced oxidative stress, and may serve as a pivotal therapeutic target for COPD.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The First Affiliated Hospital of Guangzhou Medical University (Ethic Ref No.GZMC 2009-08-1336). The

patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by the Animal Care and Use Committee of The First Affiliated Hospital of Guangzhou Medical University.

AUTHOR CONTRIBUTIONS

Conceived the project and designed the experiment: WL and XC. Performed the experiment: HZ, RG, DL, JX. Analyzed data: HZ, ZZ, YG. Provided reagents and materials: XC, WL. Wrote the paper: HZ and WL. All authors have read and agreed to the published version of the manuscript

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Real-World Effectiveness of Inhalation Therapy Among Patients With Symptomatic COPD in China: A Multicenter Prospective Study

*Wei Cheng[1], Jiaxi Duan[1], Aiyuan Zhou[2], Yiyang Zhao[1], Rong Yi[3], Yi Liu[3], Dingding Deng[4], Xin Li[5], Yuqin Zeng[1], Yating Peng[1], Qing Song[1], Ling Lin[1], Min Yang[1] and Ping Chen[1]**

[1]Department of Pulmonary and Critical Care Medicine, Research Unit of Respiratory Disease, Diagnosis and Treatment Center of Respiratory Disease, The Second Xiangya Hospital, Central South University, Changsha, China, [2]Department of Pulmonary and Critical Care Medicine, Xiangya Hospital, Central South University, Changsha, China, [3]Department of Pulmonary and Critical Care Medicine, Zhuzhou Central Hospital, Zhuzhou, China, [4]Department of Respiratory Medicine, The First Affiliated People's Hospital, Shaoyang College, Shaoyang, China, [5]Division 4 of Occupational Diseases, Hunan Prevention and Treatment Institute for Occupational Diseases, Changsha, China

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***Correspondence:**
Ping Chen
pingchen0731@csu.edu.cn

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Purpose: This real-world study evaluated the effectiveness of different inhalation therapies in patients with symptomatic chronic obstructive pulmonary disease (COPD) in China and also explored the relevant factors that influence the effectiveness of inhalation therapy.

Patients and Methods: We conducted a multicenter prospective longitudinal study that was carried out in 12 hospitals in China from December 2016 to June 2021. A face-to-face interview was conducted to collect data. Baseline data were collected at the first visit. Minimum clinically important difference (MCID) was defined as attaining a COPD assessment test (CAT) decrease ≥2. We mainly assessed the MCID and the incidence of exacerbations at the 6 months follow-up.

Results: In 695 patients, the mean age was 62.5 ± 8.2 years, with a mean CAT score of 15.1 ± 6.0. Overall, 341 (49.1%) patients attained the MCID of CAT and the incidence of exacerbation during follow-up was 22.3%. Females were significantly more likely to attain MCID than male in COPD patients (adjusted odd ratio (aOR) = 1.93, adjusted 95% confidence interval (a95%CI) = 1.09–3.42, $p = 0.024$). Patients treated with LABA/LAMA or ICS/LABA/LAMA (ICS, inhaled corticosteroid; LABA, long-acting β2-agonist; LAMA, long-acting muscarinic antagonist) were more likely to attain MCID than patients treated with LAMA (aOR = 3.97, a95%CI = 2.48–6.35, $p < 0.001$; aOR = 3.17, a95%CI = 2.09–4.80, $p < 0.001$, respectively). Patients treated with LABA/LAMA had a higher incidence of severe exacerbation than patients treated with ICS/LABA/LAMA (aOR = 1.95, a95%CI = 1.04–3.66, $p = 0.038$).

Conclusion: The incidence of MCID in symptomatic COPD patients treated with inhalation therapy was nearly 50%. Patients treated with LABA/LAMA or ICS/LABA/LAMA were more likely to attain MCID than patients treated with LAMA. Patients treated with LABA/LAMA had a higher incidence of severe exacerbations than with ICS/LABA/LAMA.

Keywords: COPD-chronic obstructive pulmonary disease, symptomatic, inhalation therapy, real-world, exacerbation, MCID (minimal clinically important differences), COPD assessment test (CAT)

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a chronic respiratory disease with persistent airflow limitation caused by toxic particles or gases (Vogelmeier et al., 2017). Globally, 174.5 million (2.4%) people suffer from COPD (GBD 2015 Disease and Injury Incidence and Prevalence Collaborators, 2016), and the prevalence in patients over 40 years of age in China is 13.7% (Wang et al., 2018). COPD is now one of the top three causes of death worldwide (Lozano et al., 2012).

With the progression of COPD, the burden of symptoms increases and quality of life declines. Symptomatic patients with COPD (group B and D) account for the vast majority in China (Duan et al., 2020). Furthermore, compared with patients with well-controlled symptoms, more symptomatic patients have a higher risk of acute exacerbations and poorer disease prognosis (Roche et al., 2013; Miravitlles and Ribera, 2017). Thus, we need to pay more attention to this group so as to further optimize the management of patients with symptomatic COPD.

At present, the effectiveness of different inhaled bronchodilators (long-acting muscarinic antagonist (LAMA); inhaled corticosteroids (ICS)/long-acting β_2-agonists (LABA); as well as the combinations LABA/LAMA and ICS/LABA/LAMA) in the treatment of COPD patients is still controversial (Wedzicha et al., 2016; Lipson et al., 2018; Maltais et al., 2019; Suissa et al., 2019; Lipson et al., 2020; Rabe et al., 2020). These therapies have been tested in randomized controlled trials (RCTs) with strict inclusion and exclusion criteria. The effectiveness of treatment evaluated in real-world studies can complement traditional RCTs by providing a comprehensive overview of treatments in routine clinical practice. Previous real-world studies usually selected one or two types of bronchodilators in mono, dual combination or triple combinations for analysis (Kalhan et al., 2021; Sansbury et al., 2021; Xu et al., 2021), and some studies have compared the effect between open triple and closed triple therapy (Ferguson et al., 2020; Huang et al., 2021). However, there is a lack of real-world data on the effects of the inhalation therapies including mono, dual combination and triple combination therapies among patients with symptomatic COPD in China.

Therefore, the purpose of this real-world study was to compare the effectiveness of different inhalation therapies for symptomatic COPD patients in China and to explore the relevant factors that influence the effectiveness of inhalation therapy.

MATERIALS AND METHODS

Study Participants and Procedures

We conducted a multicenter prospective longitudinal cohort study that was carried out in 12 comprehensive hospitals (**Supplementary Table S1**) in China from December 2016 to June 2021. We collected data by conducting face-to-face interviews with patients. All study participants provided signed informed consent. The baseline data of all participants were collected at the first visit. At the first visit of 695 patients at these centers, 624 (89.8%) patients received inhalation treatment for the first time, and 71 (10.2%) patients received adjusted treatment including 26 patients adjusted from LAMA to LABA/LAMA, two patients adjusted from LAMA to ICS/LABA/LAMA, 16 patients adjusted from ICS/LABA to LABA/LAMA, two patients adjusted ICS/LABA to ICS/LABA/LAMA, and 25 patients adjusted from ICS/LABA/LAMA to LABA/LAMA.

We confirmed that this research was conducted in accordance with the Declaration of Helsinki and has been registered in the Chinese Clinical Trial Registry (ChiCTR-POC-17010431). The study protocol was approved by the local Ethics Committee of the Second Xiangya Hospital of Central South University.

The inclusion criteria for patients in this study were that they: 1) met the diagnosis criterion of COPD defined by the 2017 Global Initiative for Chronic Obstructive Lung Disease (GOLD) recommendations [spirometry with a ratio of the forced expiratory volume in 1 s to the forced vital capacity (FEV1/FVC) lower than 0.70 after bronchodilator administration] (Vogelmeier et al., 2017); 2) were over 40 years of age; 3) a score on the COPD Assessment Test (CAT) ≥10 and or mMRC ≥2. Exclusion criteria were: 1) patients with acute exacerbation of COPD (AECOPD, an acute worsening of respiratory symptoms that results in additional therapy in patients with COPD (Vogelmeier et al., 2017); 2) patients with other chronic respiratory diseases, such as bronchiectasis (based on high-resolution computed tomography), asthma (clinically diagnosed and reversibility >12%), interstitial lung disease, or concurrent malignancy (including lung cancer); 3) patients with severe heart, liver, or kidney diseases (based on actual diagnoses from case records).

Baseline Demographics and Clinical Characteristics

Baseline characteristics included age at index date, sex, body height (BH), body weight (BW), body mass index (BMI), and smoking status. A smoker was defined as continuous smoking exposure of more than 10 pack-years. Patients who had abstained for more than 6 months were classified as former smokers (Liu et al., 2020). Never smokers were defined as those with a lifetime exposure of <1/20 pack-year (Tan et al., 2015). Clinical characteristics of interest were pulmonary function tests, CAT score, Modified Medical Research Council Dyspnea Scale (mMRC) score, number of previous exacerbations at baseline, severity of exacerbation (moderate or severe), smoking history, occupational exposure or biofuel exposure history, the presence of comorbidities ever recorded, and inhalation therapy drugs.

COPD disease severity was classified using the GOLD guidelines and was divided into four stages: mild (FEV1 ≥80% predicted), moderate (FEV1 50–80% predicted), severe (FEV1 30–50% predicted), or very severe (FEV1 <30% predicted). Dyspnea was measured by using the mMRC. The COPD assessment test (CAT) consists of eight items, including cough, expectoration, dyspnea, chest tightness, confidence, limitation of daily activities, quality of sleep, and levels of energy with a total scores ranging from 0 to 40. Our study only investigated moderate and severe exacerbations in the previous year and during the follow-up. Moderate exacerbations were defined as those requiring a prescription for an oral corticosteroid and/or an antibiotic on the same date, and severe exacerbation required an emergency department attendance or a hospital admission (Vogelmeier et al., 2017). The GOLD BD groups (symptomatic COPD) were defined according to the patient's symptoms and the history of exacerbations in the past 1 year as follows: Group B: 0–1 exacerbations per year, no hospitalization, mMRC ≥2 and or CAT ≥10; Group D: ≥2 exacerbations per year, ≥1 exacerbation with hospitalization, mMRC ≥2 and or CAT ≥10.

Treatment Assessment

We evaluated the effectiveness of inhalation therapy based on the response rate of the minimum clinically important difference (MCID) of CAT during the 6 months follow-up. MCID, defined as attaining minimum clinically important difference of CAT (decrease ≥2) (Kon et al., 2014), was assessed at 6 months follow-up. Response rates were calculated based on the proportion of individual patients with a ≥2-unit improvement in CAT score from baseline. We also assessed the incidence of moderate/severe acute exacerbations (AEs) and prescription outcome during the 6 months follow-up.

Adherence was calculated using the medication possession ratio (MPR). MPR was calculated by summing the days of medication supply provided and dividing by the total time treated (Covvey et al., 2014). Patients with poor adherence (MPR <80% or MPR >120%) were not included in the evaluation of effectiveness during the 6 months follow-up. Five mutually exclusive prescription outcomes were defined: continuous use (no modification), discontinuation (permanent [≥91 days with no restart] or temporary [≥91 days with subsequent restart]), switch, and augmentation (Meeraus et al., 2019). Participants who received escalation long-acting bronchodilator therapy or augmented long-acting bronchodilator therapy before the 6 months follow-up, regardless of whether they met the above requirements, were classified as non-MCID.

Sample Size Estimation

The sample size was calculated by using PASS 15.0 in the part of confidence intervals for one proportion. We used the MCID incidence rate (44.9%) obtained from the pre-experiment as the assumed sample proportion, set the interval type as two-sided, and entered the confidence level (1-alpha) as 0.95 and dropout rate as 10%. Finally, the sample we acquired was 679.

Statistical Analysis

Categorical variables are described as counts and percentages. Continuous variables are expressed as mean ± standard deviation or median with interquartile range (IQR) according to normally or non-normally distributed. The chi-squared or Fisher's test was used for categorical variables, and Student's t-test, Mann-Whitney U test, and Kruskal-Wallis H test were used for continuous variables. Risk factors for MCID of CAT and severe exacerbation during follow-up were identified, and their crude odds ratios (cORs), adjusted odds ratios (aORs), and 95% confidence intervals were estimated using logistic regression analyses. All tests of significance were two sided, and a p value < 0.05 was considered to be statistically significant. Multiple comparisons of differences between groups were Bonferroni adjusted. All analyses were performed using IBM SPSS Statistics version 25.0 for Windows (IBM Corp, Armonk, NY, United States).

RESULTS

Baseline Characteristics

A total of 696 patients completed the center 6 months follow-up. One patient discontinued long-acting bronchodilators with poor compliance (MPR<80%). Finally, we included 695 patients for analysis (**Figure 1**).

Of the 695 patients in the baseline, 90.6% were male, with a mean age of 62.5 ± 8.2 years, a mean CAT score of 15.1 ± 6.0, a median FEV1 percentage predicted of 48.3 ± 25.5% and a median FEV1 of 1.21 ± 0.54. These COPD patients included 344 patients in group B and 351 patients in group D. The distribution of inhalation therapy was as follows: LAMA (24.3%), LAMA/LABA (21.4%), ICS/LABA (10.4%), ICS/LABA/LAMA (35.3%), Others (8.6%) including ICS/LAMA and short-acting bronchodilators. Baseline demographics and clinical characteristics are summarized in **Table 1**.

As shown in **Supplementary Table S2**, the proportion of patient with a history of exacerbation in the past year was higher in COPD patients treated with LAMA (111/169 = 65.7%) than in patients treated with LABA/LAMA (78/149 = 52.3%). Furthermore, the proportion of patients with a history of severe exacerbations in the past year was higher in COPD patients treated with LABA/LAMA (60/169 = 40.3%) and ICS/LABA/LAMA (93/245 = 38.0%) than in patients treated with ICS/LABA (16/72 = 22.2%).

Effectiveness of Different Inhalation Therapies

As exhibited in **Table 2**, 341 (49.1%) patients attaining MCID of CAT (decrease ≥2) assessed at the 6 months follow-up. There were 275 (39.6%) patients attaining an mMRC decrease ≥1 assessed at the 6 months follow-up. In all participants, the inhalation treatment of COPD patients with LAMA/LABA (98/149 = 65.8%) or ICS/LABA/LAMA (150/245 = 61.2%) had a higher response rate regarding MCID than LAMA (54/169 = 32.0%) or ICS/LABA (23/72 = 31.9%). Regardless of group B or D, the inhalation therapy of COPD patients with LAMA/LABA or ICS/LABA/LAMA (triple therapy) had a higher response rate

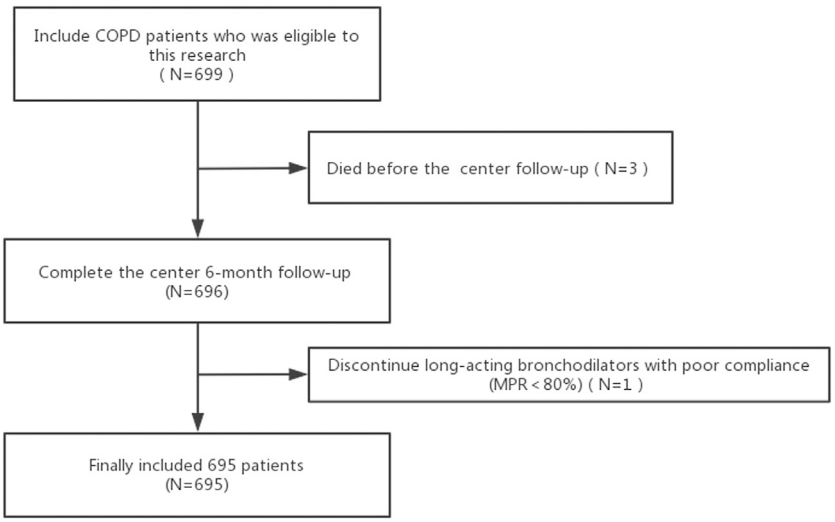

FIGURE 1 | Flow diagram of the inclusion of studies. Abbreviations: COPD, chronic obstructive pulmonary disease; MPR, medication possession ratio.

regarding MCID than therapy with LAMA or ICS/LABA (**Figure 2**).

Overall, the incidence of exacerbations during follow-up was 22.25%. The incidence of exacerbations during the 6 months follow-up with different inhalation therapies was as follows: LAMA (21.9%), LAMA/LABA (20.1%), ICS/LABA (19.4%), ICS/LABA/LAMA (23.7%), others (23.3%); however, we found no difference in the rate of exacerbations between these inhalation treatments. We found that there were significant differences in the incidence of severe exacerbations among patients on different inhalation therapies during follow-up (p = 0.011) (**Table 2**). Further subgroup analysis showed that, with different inhalation therapies, patients who had a history of exacerbation in the past year exhibited a variable incidence of severe exacerbations in follow-up (p = 0.009), while patients without a history of exacerbation had a similar prognosis (p = 0.752).

Factors Correlated With the MCID Response Rate

In **Table 3**, female (66.2%) COPD patients had a higher MCID response rate than males with COPD (47.3%). As shown in **Figure 2**, there were significant differences in the MCID response rate between different inhalation therapies (p < 0.01). We found no significant differences in the MCID response rate during the 6 months follow-up according to different treatment status at baseline, while patients in the LABA/LAMA subgroup had similar results (p = 0.158). After adjusting for sex, age, smoking status, treatment status at baseline, exacerbation history in the past year, GOLD stage, group B/D, and inhalation therapy, the logistic regression model showed that females were significantly more likely to attain MCID than male COPD patients (aOR = 1.93, a95%CI = 1.09–3.42, p = 0.024). We also found that patients treated with LABA/LAMA or ICS/LABA/LAMA were more likely to attain MCID than patients treated with LAMA (aOR = 3.97, a95%CI = 2.48–6.35, p < 0.001; aOR = 3.17, a95%CI = 2.09–4.80, p < 0.001, respectively) (**Table 4**).

Factors Correlated With the Incidence of Severe Exacerbations

The incidence of severe exacerbations in patients was significantly related to the CAT score and the mMRC score (9.7 vs. 3.4%, p = 0.029; 4.5 vs. 10.3%, p = 0.015, respectively). Inhalation treatment of COPD patients with LAMA (8/169 = 4.7%), ICS/LABA (4/72 = 5.6%), and ICS/LABA/LAMA (21/245 = 8.6%) had a lower incidence of severe exacerbations than LABA/LAMA (23/149 = 15.4%) during the 6 months follow-up (**Table 2** and **Figure 3**). After adjusting for sex, age, treatment status at baseline, exacerbation in the past year, severe exacerbation in the past year, CAT score, mMRC score, GOLD stage, group B/D, and inhalation therapy, the logistic regression model showed that patients treated with LABA/LAMA had a higher incidence of severe exacerbations than patients treated with ICS/LABA/LAMA (aOR = 1.95, a95%CI = 1.04–3.66, p = 0.038) (**Table 5**).

DISCUSSION

To the best of our knowledge, this is the first real-world study to assess the effectiveness of inhalation therapies including mono, dual combination and triple combination therapies for symptomatic COPD patients in China.

Our results show that the MCID response rate (CAT improved ≥2) in symptomatic COPD patients treated with inhalation therapy was nearly 50% and the inhalation treatment of COPD patients with LAMA/LABA or triple therapy had a higher MCID response rate than LAMA or ICS/LABA. The total MCID response rate is consistent with previous studies showing that

TABLE 1 | Baseline demographics and clinical characteristics.

Baseline characteristics	Total group (*N* = 695)
Age[a] (year)	62.5 (8.2)
BMI[a] (kg/m^2)	22.3 (3.1)
FEV_1[c] (liter)	1.21 (0.54)
FEV_1% predicted[c] (%)	48.3 (25.5)
CAT[a]	15.1 (6.0)
mMRC[c]	2.0 (2.0)
Male[b]	630 (90.6)
Current smoker[b]	287 (41.3)
Occupational exposure[b]	242 (34.8)
Biofuel exposure[b]	219 (31.5)
Exacerbation in the past 1 year[b]	
0	283 (40.7)
≥1	412 (59.3)
COPD severity[b]	
Mild	40 (5.8)
Moderate	282 (40.6)
Severe	288 (41.4)
Very severe	85 (12.2)
Group B/D[b]	
B	344 (49.5)
D	351 (50.5)
Inhalation[b]	
LAMA	169 (24.3)
LAMA/LABA	149 (21.4)
ICS/LABA	72 (10.4)
ICS/LABA/LAMA	245 (35.3)
Others	60 (8.6)

[a]*Mean (SD).*
[b]*Counts with percentage are indicated.*
[c]*Median (IQR).*
Abbreviations: *BMI, body mass index; FEV1%, forced expiratory volume in one second as a percentage of the predicted value; CAT, COPD assessment test; mMRC, modified medical research council dyspnea scale; COPD severity was classified using Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria; LABA, long-acting β2-agonist; LAMA, long-acting muscarinic antagonist; ICS, inhaled corticosteroid.*

51% of patients treated with umeclidinium/vilanterol, umeclidinium, or salmeterol achieved a clinical important improvement at week 24 (Vogelmeier et al., 2021). The benefits of triple treatment compared with mono and dual therapy are obvious in prospective clinical studies. Lee et al. demonstrated better improvements in St George's Respiratory Questionnaire (SGRQ) scores in patients on inhaled triple therapy (tiotropium plus budesonide/formoterol) compared with those on monotherapy (tiotropium) (Lee et al., 2016). In the IMPACT study, fluticasone furoate/umeclidinium/vilanterol (FF/UMEC/VI) single-inhaler triple therapy was associated with a better clinically meaningful improvement in SGRQ score (defined as a decrease ≥4 units from baseline) compared with ICS/LABA (FF/VI) (Lipson et al., 2018). In the EMAX study, UMEC/VI showed greater improvements in the proportion of CAT responders versus UMEC at week 12 and week 24 (Maltais et al., 2019). A network meta-analysis demonstrated that LABA/LAMA combinations were associated with a greater improvement in SGRQ scores and the Transitional Dyspnea Index (TDI) than monotherapy (Oba et al., 2016). Our study provides consistent evidence in the real world that confirms the benefits of dual bronchodilation on symptom improvement compared with mono-bronchodilator therapy in symptomatic patients with COPD.

An RCT showed that the improvement over time in the total score on the SGRQ was greater in the LABA/LAMA group than in the ICS/LABA group, which is consistent with our results (Wedzicha et al., 2016). We found no difference in the MCID response rate between LAMA/LABA and triple inhalation therapy for symptomatic COPD patients. However, we also had results inconsistent with the Germany DACCORD real-world observational study, in which the response rate of patients with a clinically relevant improvement (CAT score ≥2-unit change from baseline) was higher in patients receiving LAMA/LABA compared with triple therapy patients (62 vs. 47%, respectively; $p < 0.001$) (Buhl et al., 2018). Poverty, a high rate of smoking, and indoor biomass burning are traditionally considerable issues in Asia (Gordon et al., 2014; Kim et al., 2019). COPD phenotypes in Asia may be somewhat different from those in Western countries (Kim et al., 2019). We believe that this difference may be due to the heterogeneity of the region and the study participants.

Our logistic regression model showed that female patients had a higher incidence of MCID. In Asian cities, the characteristics of COPD patients vary and the history of exposure to biomass fuels is related to frequency of symptoms and severe airflow limitation (Oh et al., 2013). Our previous study showed that nearly 70% of female in COPD patients were exposed to biomass smoke exposure alone. It has also been demonstrated that COPD patients with biomass exposure alone have higher CAT scores than patients with only smoke or occupational exposure (Duan et al., 2020). These previous reports also show that female COPD patients have more severe symptoms. We consider that these factors lead to higher MCID, because patients with more severe symptoms are more likely to obtain a 2 units reduction in the CAT score. We also found that patients treated with ICS/LABA/LAMA or LABA/LAMA were more likely to attain MCID than patients treated with LAMA. We have discussed this before, so we will not repeat it here.

In our study, we chose the MCID, which was defined as attaining a CAT decrease ≥2 during the 6 months follow-up, as our main effectiveness indicator in patients treated with inhalation bronchodilators. In clinical practice, it is time-consuming and impractical to monitor several different patient-reported outcome (PRO) measures such as CAT, SGRQ, self-administered computerized-Transition Dyspnea Index (SAC-TDI), and Evaluating Respiratory Symptoms (E-RS) (Vogelmeier et al., 2021). Previous systematic reviews supported the reliability and validity of the CAT and concluded that the tool is responsive to interventions. Furthermore, the correlation between CAT and SGRQ scores is typically quite high (convergent validity using Pearson's correlation coefficient: 0.69–0.82 and 0.63), which has also been demonstrated in a systematic review (Gupta et al., 2014). Moreover, a large variety of questionnaires brings many difficulties to clinical practice and popularization. We think two or more PRO measures are more suitable for RCTs. A single CAT score for assessing systemic symptoms is more operable in real-world clinical practice and has been used in previous studies (Buhl et al., 2018).

TABLE 2 | Effectiveness of different inhalation therapy options during 6 months follow-up.

Outcome	Total (*N* = 695)	LAMA (*N* = 169)	LAMA/LABA (*N* = 149)	ICS/LABA (*N* = 72)	ICS/LABA/LAMA (*N* = 245)	Others (*N* = 60)	*p*-value
Δ CAT, Median (IQR)	2 (8)	0 (7)	4 (8.5)	0 (7.75)	3 (9)	−0.5 (4)	<**0.001**
MCID of CAT, *n* (%)							<**0.001**
Yes	341 (49.1)	54 (32.0)	98 (65.8)	23 (31.9)	150 (61.2)	16 (26.7)	
No	354 (50.9)	115 (68.0)	51 (34.2)	49 (68.1)	95 (38.8)	44 (73.3)	
AE during 6 months follow-up, Median (IQR)	0 (1)	1 (2)	0 (0)	0 (1.75)	0 (2)	0 (2)	<**0.001**
AE during 6 months follow-up, *n* (%)							0.932
Yes	154 (22.2)	37 (21.9)	31 (20.1)	14 (19.4)	58 (23.7)	14 (23.3)	
No	541 (77.8)	132 (78.1)	118 (79.9)	58 (80.6)	187 (76.3)	46 (76.7)	
Severe AE during 6 months follow-up, *n* (%)							**0.011**
Yes	60 (8.6)	8 (4.7)	23 (15.4)	4 (5.6)	21 (8.6)	4 (6.7)	
No	635 (91.4)	161 (95.3)	126 (84.6)	68 (94.4)	224 (91.4)	56 (93.3)	
Prescription outcome, *n* (%)							<**0.001**
Continuous using	571 (82.1)	129 (76.3)	145 (97.3)	47 (65.3)	191 (78.0)	59 (98.3)	
De-escalation therapy	66 (9.5)	0 (0)	3 (2.0)	9 (12.5)	54 (22.0)	0 (0)	
Escalation therapy	6 (0.9)`	4 (2.4)	1 (0.7)	0 (0)	0 (0)	1 (1.7)	
Augmented	52 (7.5)	36 (21.3)	0 (0)	16 (22.2)	0 (0)	0 (0)	

Note: *For comparison, Chi-square or Fisher's test was used for categorical variables, and Kruskal-Wallis H test were used for continuous variables; the bold* p*-values indicate statistical significance.*

Abbreviations: *CAT, COPD assessment test; Δ CAT was calculated by subtracting the baseline CAT score from the follow-up CAT score; MCID, minimum clinically important difference, defined as attaining minimum clinically important differences of CAT (decrease ≥2) assessed at 6 months follow-up; AE, acute exacerbation; LABA, long-acting β2-agonist; LAMA, long-acting muscarinic antagonist; ICS, inhaled corticosteroid.*

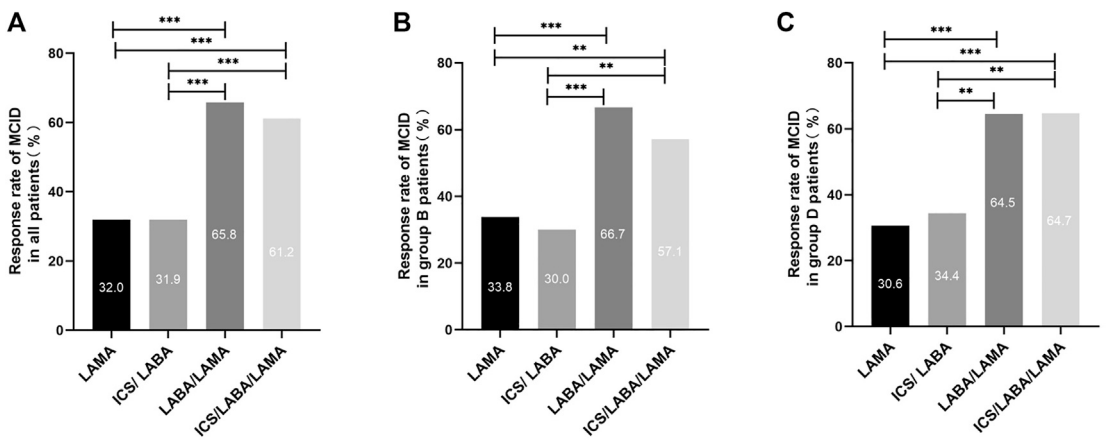

FIGURE 2 | Comparison of the MCID response rate between different main inhalation therapy in patients with symptomatic COPD. Note: **(A)** MCID response rate in all patients with symptomatic COPD. **(B)** MCID response rate in group B patients with symptomatic COPD. **(C)** MCID response rate in group D patients with symptomatic COPD. For comparison, the chi-squared test was used for categorical variables. ** indicates *p*-values <0.01, *** indicates *p*-values <0.001. Abbreviations: MCID, minimum clinically important difference; LABA, long-acting β2-agonist; LAMA, long-acting muscarinic antagonist; ICS, inhaled corticosteroid.

We found no significant differences in the incidence of acute exacerbations during the 6 months follow-up period between different inhalation therapies in symptomatic COPD patients. In the past, there has been controversy regarding the risk of acute exacerbations after treatment with different inhalation therapies (Wedzicha et al., 2016; Lipson et al., 2018; Papi et al., 2018; Maltais et al., 2019; Suissa et al., 2019; Wang et al., 2021). However, we found that there were certain differences in the incidence of hospitalization-related acute exacerbations during the 6-months follow-up period between different inhalation therapies in symptomatic COPD patients. We found that COPD patients treated with LAMA had a lower incidence of severe exacerbations than LABA/LAMA patients. A network meta-analysis showed that all LAMAs are equally effective in preventing moderate-to-severe exacerbations, but the concomitant use of LABA may not enhance the efficacy of LAMAs in preventing COPD exacerbations (Oba and Lone, 2015). The EMAX randomized trial conducted in low exacerbation risk patients with COPD not receiving ICS showed that there was no difference in the occurrence of

TABLE 3 | Response rate of MCID between different clinical features for symptomatic COPD patients.

Clinical feature	Total, *N*	Patients with MCID, *n* (%)	Patients without MCID, *n* (%)	*p*-value
Age (year)				0.131
<65	387	180 (46.5)	207 (53.5)	
≥65	308	161 (52.3)	147 (47.7)	
Sex				**0.004**
Male	630	298 (47.3)	332 (52.7)	
Female	65	43 (66.2)	22 (33.8)	
BMI (kg/m^2)				0.575
<24	500	242 (48.4)	258 (51.6)	
≥24	195	99 (50.8)	96 (49.2)	
Smoking history				0.290
Never smoker	134	66 (49.3)	68 (50.7)	
Former smoker	274	125 (45.6)	149 (54.4)	
Current smoker	287	150 (52.3)	137 (47.7)	
Occupational exposure				0.218
Yes	242	111 (45.9)	131 (54.1)	
No	453	230 (50.8)	223 (49.2)	
Biofuel exposure				0.813
Yes	219	106 (48.4)	113 (51.6)	
No	476	235 (49.4)	241 (50.6)	
AE in the past 1 year				0.738
0	285	142 (49.8)	143 (50.2)	
≥1	410	199 (48.5)	211 (51.5)	
COPD severity				0.212
Mild	40	20 (50.0)	20 (50.0)	
Moderate	282	126 (44.7)	156 (55.3)	
Severe	288	147 (51.0)	141 (49.0)	
Very severe	85	48 (56.5)	37 (43.5)	
Group B/D				0.673
Group B	344	166 (48.3)	178 (51.7)	
Group D	351	175 (49.1)	176 (50.9)	
Treatment status at baseline				0.073
Initial treatment	624	299 (47.9)	325 (52.1)	
Adjust treatment	71	42 (59.2)	29 (40.8)	

Note: *For comparison, Chi-square was used for categorical variables; the bold* p*-values indicate statistical significance.*
Abbreviations: *MCID, minimum clinically important difference; BMI, body mass index; AE, acute exacerbation; COPD severity was classified using Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria.*

TABLE 4 | Multiple logistic regression for factors correlated with the response rate of MCID.

Characteristics (*N* = 695)	cOR	c95%IC	*p*-value	aOR	a95%IC	*p*-value
Sex			**0.005**			**0.024**
male	Reference			Reference		
female	2.18	1.27–3.73		1.93	1.09–3.42	
Inhalation therapy			<**0.001**			
LAMA	Reference			Reference		
LAMA/LABA	4.09	2.56–6.54	<**0.001**	3.97	2.48–6.35	<**0.001**
ICS/LABA	1.00	0.55–1.81	0.999	0.90	0.49–1.64	0.726
ICS/LABA/LAMA	3.36	2.23–5.08	<**0.001**	3.17	2.09–4.80	<**0.001**
Others	0.77	0.40–1.49	0.446	0.78	0.41–1.51	0.462

Note: *Factors in the logistic model: sex, age, smoking status, treatment status at baseline, exacerbation history in the past 1 year, Gold stage, group B/D, Inhalation therapy; the bold* p*-values indicate statistical significance.*
Abbreviations: *MCID, minimum clinically important difference; LABA, long-acting β2-agonist; LAMA, long-acting muscarinic antagonist; ICS, inhaled corticosteroid; cOR, crude odds ratio; c95% CI, crude 95% confidence interval; aOR, adjusted odds ratio; a95% CI, adjusted 95% confidence interval.*

severe exacerbations between the umeclidinium/vilanterol and umeclidinium treatment groups (Maltais et al., 2019). It is known that previous exacerbation history is a reliable predictor of future exacerbations (Singh et al., 2019; Singh et al., 2020). In our study, the LAMA group had a higher proportion of patients with a history of exacerbations during the previous year than patients treated with LABA/LAMA (65.7 vs. 52.3%, $p = 0.016$). We think that this difference in the history of acute exacerbation between the LAMA and LABA/LAMA groups may be the main reason for this result.

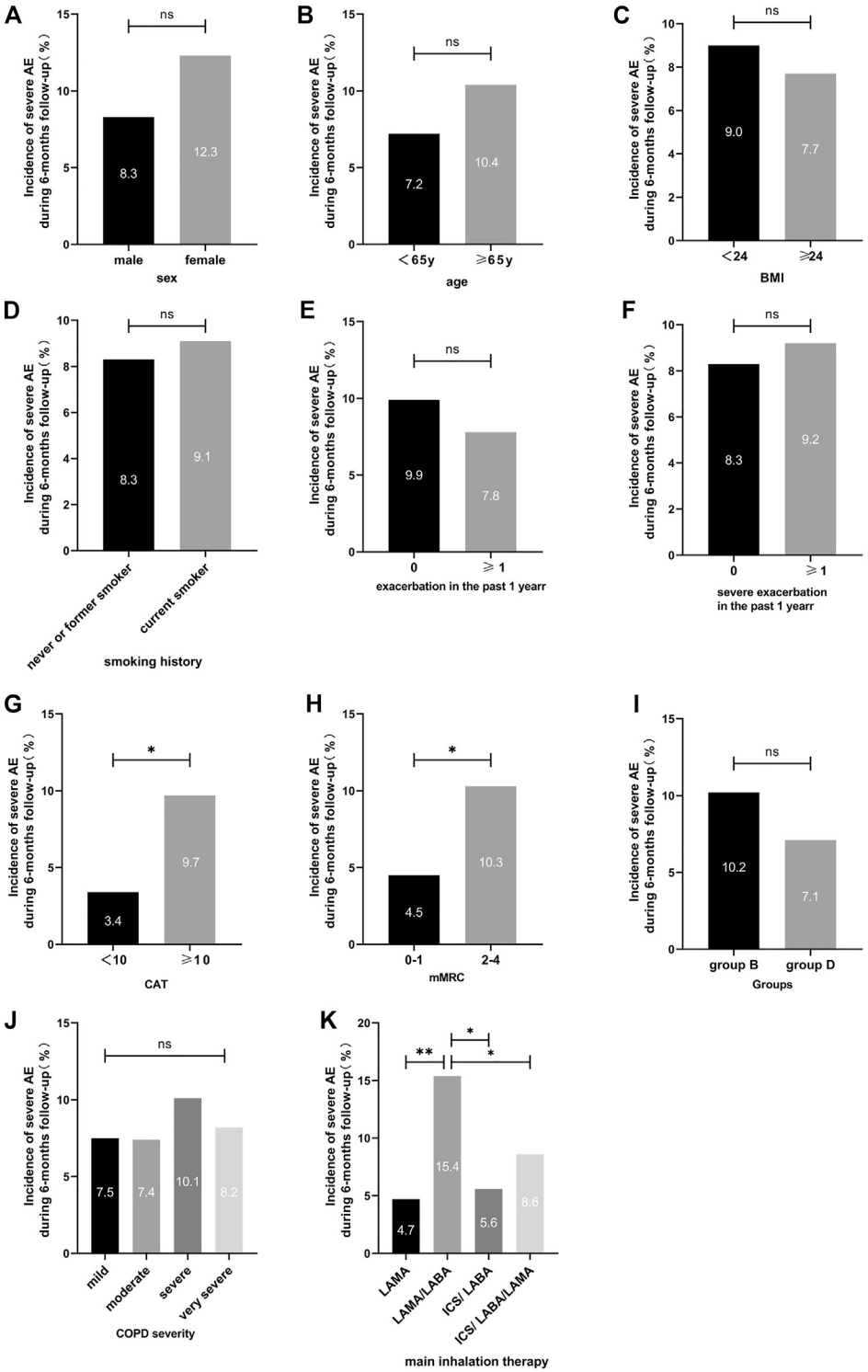

FIGURE 3 | Incidence of severe exacerbations during the 6-months follow-up between different clinical features for symptomatic COPD patients. Note: For comparison, the chi-squared test was used for categorical variables. ns indicates p-values ≥0.05, * indicates p-values <0.05, ** indicates p-values <0.01. Abbreviations: BMI, body mass index; CAT, COPD assessment test; mMRC, modified medical research council dyspnea scale; COPD severity was classified using Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria; LABA, long-acting β2-agonist; LAMA, long-acting muscarinic antagonist; ICS, inhaled corticosteroid.

TABLE 5 | Multiple logistic regression for factors correlated with the incidence of severe exacerbation during 6 months follow-up.

Characteristics (*N* = 695)	aOR	a95%IC	*p*-value
Inhalation therapy			
ICS/LABA/LAMA	Reference		
LAMA	0.53	0.23–1.23	0.138
LAMA/LABA	1.95	1.04–3.66	**0.038**
ICS/LABA	0.63	0.21–1.89	0.408
Others	0.76	0.25–0.31	0.631

Note: *Factors in the logistic model: sex, age, treatment status at baseline, exacerbation in the past 1 year, severe exacerbation in the past 1 year, CAT score, mMRC score, Gold stage, group B/D, inhalation therapy; the bold* p*-values indicate statistical significance.*
Abbreviations: *CAT, COPD assessment test; mMRC, modified medical research council dyspnea scale; LABA, long-acting β2-agonist; LAMA, long-acting muscarinic antagonist; ICS, inhaled corticosteroid; aOR, adjusted odds ratio; a95% CI, adjusted 95% confidence interval.*

Furthermore, inhalation treatment of COPD patients with ICS/LABA presented a lower incidence of severe exacerbations than LABA/LAMA. In previous research, there has been controversy regarding the risk of severe exacerbations between different inhalation treatments. In a real-world clinical practice setting of COPD treatment, the hazard ratio (HR) of severe COPD exacerbations associated with LABA/LAMA relative to ICS/LABA was 0.94. This study showed that combined LABA/LAMA inhalers appear to be as effective as combined ICS/LABA inhalers in preventing COPD exacerbations (Suissa et al., 2019), but an RCT demonstrated that the time to the first severe exacerbation was longer in the LABA/LAMA group than in the ICS/LABA group (HR 0.81; 95% CI, 0.66 to 1.00; $p = 0.046$) (Wedzicha et al., 2016). Another RCT showed that the annual rate of severe exacerbations during treatment was 0.15 among those assigned to ICS/LABA and 0.19 among those assigned to LABA/LAMA (Lipson et al., 2018). We consider that this difference may be due to the heterogeneity of the study population and the history of severe exacerbations between LABA/LAMA and ICS/LABA groups in the previous year. In our study, we also found that patients treated with LABA/LAMA had a higher incidence of severe exacerbation than those on triple inhalation therapy, which was consistent with a previous study. A matched cohort of 1,647 patients with COPD in a UK primary care database found that triple therapy reduced the exacerbation risk (HR 0.87, 95% CI 0.76–0.99) compared with LAMA/LABA dual therapy (Voorham et al., 2019). In the IMPACT study, triple therapy resulted in a lower rate of hospitalization due to COPD than LABA/LAMA (rate ratio with triple therapy, 0.66; 95% CI, 0.56 to 0.78; 34% difference; $p < 0.001$), but the rate was not significantly lower with triple therapy than with ICS/LABA (rate ratio with triple therapy, 0.87; 95% CI, 0.76 to 1.01; 13% difference; $p = 0.06$), which is consistent with our study (Lipson et al., 2018). This trial also demonstrated that these benefits were observed regardless of the patients' blood eosinophil levels at randomization. We think this difference may be related to the effect of ICS on exacerbation prevention (Singh et al., 2019). Finally, in the multivariate analysis, we showed that the incidence of severe exacerbations in patients receiving LABA/LAMA treatment was higher than that of patients on triple inhalation therapy, which indirectly reflects the differences in the rate of severe exacerbations in other treatment groups, which may be related to the history of exacerbation before treatment.

There are some limitations to this study. First, the study did not correct the Charlson comorbidity index due to the limitations of real-world studies, but we evaluated other chronic pulmonary diseases, concurrent malignancy, severe heart, liver, or kidney diseases based on actual diagnoses from case records, which may reduce the confounding deviation of comorbidities to a certain extent. Second, according to current COPD treatment guidelines, blood eosinophil counts should be taken into consideration when deciding whether to initiate ICS treatment in combination with a LABA and/or LAMA (Singh et al., 2019). Our study did not include blood eosinophils in the multivariate analysis, which could cause a certain selection bias. However, it is likely that blood eosinophil counts were not considered in the treatment decisions observed in the current study, since the study was conducted prior to the inclusion of this recommendation. We also excluded patients diagnosed with asthma in our study, which may reduce this bias. Third, our study may have a relatively low incidence of exacerbation due to the short follow-up time. In the future, we may need to further explore and carry out follow-up studies on the acute exacerbations of these patients. Additionally, we did not include COPD patients in the less symptomatic groups into the study due to the fact that there are fewer COPD patients in groups A and C (8.7%) in these 12 comprehensive hospitals (**Supplementary Table S3**). In the future, we may need to cooperate with community hospitals to further expand the number of patients in groups A and C to supplement real-world data. Finally, we did not discuss the impact of the different types of inhalers, which may have influenced the selection of medications based on patient preference. However, these patients received inhalation training at the patient health management office after receiving the inhaler at their first visit. Therefore, each of our participants was able to use the inhaler correctly after assessment and inhalation training.

CONCLUSION

The incidence of MCID in symptomatic COPD patients treated with inhalation therapy was nearly 50%. Patients treated with LABA/LAMA or ICS/LABA/LAMA were more likely to attain MCID than patients treated with LAMA. Patients treated with LABA/LAMA had a higher incidence of severe exacerbations than patients given ICS/LABA/LAMA.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The local Ethics Committee of the Second Xiangya Hospital of Central South University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.753653/full#supplementary-material

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Association Between Air Pollution and Lung Lobar Emphysema in COPD

*Nguyen Thanh Tung[1,2†], Shu-Chuan Ho[3†], Yueh-Hsun Lu[4,5], Tzu-Tao Chen[6], Kang-Yun Lee[6,7], Kuan-Yuan Chen[6], Chih-Da Wu[8,9], Kian Fan Chung[10], Han-Pin Kuo[7], Huynh Nguyen Xuan Thao[11], Hoang Ba Dung[2], Tran Phan Chung Thuy[12], Sheng-Ming Wu[6,7], Hsiao-Yun Kou[3], Yueh-Lun Lee[13] and Hsiao-Chi Chuang[3,6,14]**

[1] International Ph.D. Program in Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan, [2] Otorhinolaryngology Department, Cho Ray Hospital, Ho Chi Minh City, Vietnam, [3] School of Respiratory Therapy, College of Medicine, Taipei Medical University, Taipei, Taiwan, [4] Department of Radiology, Shuang Ho Hospital, Taipei Medical University, New Taipei City, Taiwan, [5] Department of Radiology, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan, [6] Division of Pulmonary Medicine, Department of Internal Medicine, Shuang Ho Hospital, Taipei Medical University, New Taipei City, Taiwan, [7] Division of Pulmonary Medicine, Department of Internal Medicine, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan, [8] Department of Geomatics, National Cheng Kung University, Tainan City, Taiwan, [9] National Institute of Environmental Health Sciences, National Health Research Institutes, Miaoli, Taiwan, [10] National Heart and Lung Institute, Imperial College London, London, United Kingdom, [11] Otorhinolaryngology Department, Ho Chi Minh City University of Medicine and Pharmacy, Ho Chi Minh City, Vietnam, [12] Otorhinolaryngology Department, Faculty of Medicine, Vietnam National University Ho Chi Minh City, Ho Chi Minh City, Vietnam, [13] Department of Microbiology and Immunology, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan, [14] Cell Physiology and Molecular Image Research Center, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan

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Amany Fathy Elbehairy, Alexandria University, Egypt

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***Correspondence:**
Hsiao-Chi Chuang
r92841005@ntu.edu.tw

[†] These authors have contributed equally to this work and share first authorship

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The development of emphysema has been linked to air pollution; however, the association of air pollution with the extent of lobar emphysema remains unclear. This study examined the association of particulate matter $<2.5\,\mu m$ in aerodynamic diameters ($PM_{2.5}$) ($\leq 2.5\,\mu m$), nitrogen dioxide (NO_2), and ozone (O_3) level of exposure with the presence of emphysema in 86 patients with chronic obstructive pulmonary disease (COPD). Exposure to the air pollution estimated using the land-use regression model was associated with lung function, BODE (a body mass index, degree of obstruction, dyspnea severity, and exercise capacity index) quartiles, and emphysema measured as low-attenuation areas on high-resolution CT (HR-CT) lung scans. Using paraseptal emphysema as the reference group, we observed that a 1 ppb increase in O_3 was associated with a 1.798-fold increased crude odds ratio of panlobular emphysema ($p < 0.05$). We observed that $PM_{2.5}$ was associated with BODE quartiles, modified Medical Research Council (mMRC) dyspnea score, and exercise capacity (all $p < 0.05$). We found that $PM_{2.5}$, NO_2, and O_3 were associated with an increased degree of upper lobe emphysema and lower lobe emphysema (all $p < 0.05$). Furthermore, we observed that an increase in $PM_{2.5}$, NO_2, and O_3 was associated with greater increases in upper lobe emphysema than in lower lobe emphysema. In conclusion, exposure to O_3 can be associated with a higher risk of panlobular emphysema than paraseptal emphysema in patients with COPD. Emphysema severity in lung lobes, especially the upper lobes, may be linked to air pollution exposure in COPD.

Keywords: air pollution, BODE, computed tomography, COPD, LAA

DEFINITION

Lobar percent emphysema, the voxel numbers <-950 Hounsfield units (HUs) in a lung lobe field divided by the total voxel numbers in that lung lobe field; centrilobular emphysema, low attenuated areas (LAAs) located within the central portion of the pulmonary lobe; paraseptal emphysema, LAA in the more distal alveoli adjacent to the visceral pleura or interlobular septa; and panlobular emphysema, diffuse LAA that distributes throughout the pulmonary lobe.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is an irreversible and progressive respiratory condition. COPD is currently the chronic disease associated with one of the highest mortality in the world, and also ranks fifth worldwide in terms of disease burden (1, 2). In previous studies, air pollution has been associated with increased risk of COPD and reduced lung function (3–6). Specifically, a 5 $\mu g/m^3$ increase in 1-year particulate matter $<2.5\,\mu m$ in aerodynamic diameters ($PM_{2.5}$ concentrations has been associated with 1.52-fold increased odds ratio (OR) of COPD prevalence (95% CI: 1.42–1.62), while a 10 $\mu g/m^3$ increase in 1-year nitrogen dioxide (NO_2) concentrations was associated with 1.12-fold increased OR of COPD prevalence (95% CI: 1.10–1.14) (7). Therefore, air pollution can be considered as a risk factor for the development of COPD by the Chronic Obstructive Lung Disease (GOLD) guideline (8).

The hybrid kriging/land-use regression (LUR) combined two methods (i.e., kriging interpolation and LUR) to detect small-scale variation in air pollution. This model also takes into consideration the local emission sources (i.e., temples and restaurants) to achieve a more accurate prediction of annual NO_2 variability ($R^2 = 0.90$) (9). Another study in Taiwan captured 85% of annual $PM_{2.5}$ variation by using this method (10). Utilizing estimates from the hybrid kriging/LUR, our previous study showed that 0.99 $\mu g/m^3$ increase in $PM_{2.5}$ resulted in 0.011 kg increase in right arm fat mass, whereas 2.45 ppb increase in NO_2 resulted in 0.181 kg decrease in muscle mass ($p < 0.05$) (11).

Lung emphysema is characterized by the destruction of alveolar septal structures and the loss of lung parenchyma (12). Emphysema is categorized into three major subtypes based on its distribution in secondary lung lobules: centrilobular, paraseptal, and panlobular emphysema (13–15). Centrilobular emphysema has been characterized as low attenuated areas (LAAs) in the central portion of the pulmonary lobe, paraseptal emphysema as LAA in the more distal alveoli adjacent to the visceral pleura or interlobular septa, and panlobular as diffuse LAA that distributes throughout the pulmonary lobe (14, 16, 17). Previous studies have reported an association between smoking and increased risk of centrilobular emphysema (14, 18).

Emphysema can be examined quantitatively on high-resolution CT (HRCT) imaging by measuring the LAA of the lung. The percentage of LAA (or percent emphysema) on HRCT scans has been associated with the decrease in lung function in patients with COPD (19). It was reported that cigarette smoke affected emphysema preferentially in the upper lobes (14, 20). Previous studies have demonstrated the association of air pollution with emphysema severity of the total lung (21, 22). However, the link between exposure to air pollution and the degree of emphysema at the lobular level has not been reported. We hypothesized that exposure to air pollution was associated with the emphysema severity at the lung lobular level. Meanwhile, lung function and BODE index [a composite measure of body-mass index (BMI), degree of obstruction, dyspnea severity, and exercise capacity (23)] were considered clinical outcomes related to the severity of COPD and emphysema. Therefore, we examined the association of 1-year exposure to $PM_{2.5}$, NO_2, and ozone (O_3) with the extent of emphysema in different lung lobes of COPD subjects, while also assessing the associations between 1-year exposure to air pollutants with lung function and the degree of incapacity as measured by the BODE index.

MATERIALS AND METHODS

Study Subjects

We conducted a retrospective cross-sectional study in 86 patients with COPD recruited from the COPD clinic of a respiratory department of a hospital in New Taipei, Taiwan. The patients underwent HRCT of the thorax between April 2010 and October 2019. The inclusion criteria in this study were: (1) having been diagnosed with COPD by a post-bronchodilator forced expiratory volume in the first second (FEV_1)/forced vital capacity (FVC) ratio of <70% (1) and (2) being between 40 years old and 90 years old. The smoking statuses of the patients were collected by oral questionnaire. Patients with a known malignancy, progressive inflammatory condition (i.e., bronchiectasis, asthma, or other non-COPD-related diseases), or exacerbation during the 3 months before the study were excluded. The Ethics Committee of the Taipei Medical University-Joint Institutional Review Board approved this study (Approval No. N202003075).

Ambient Air Pollution Exposure

Individual-level exposure to air pollutants ($PM_{2.5}$, NO_2, and O_3) was predicted by a hybrid kriging/LUR approach, which was previously demonstrated (9, 10). Briefly, mean air pollutant data were obtained from Taiwan Environmental Protection Administration (EPA) air quality monitoring stations (https://airtw.epa.gov.tw/). The Environment Resource Database, Point of Interest, Land-use Investigation of Taiwan, Traffic Network Digital Map, Digital Terrain Model, Industrial Development Bureau Database, and Normalized Difference Vegetation Index were included to build the model. The regression model takes into consideration the traffic intensity, weather, population density, industry emissions, elevation, vegetation distribution, the number of temples, and the number of restaurants to calculate residential air pollution levels. Daily $PM_{2.5}$, NO_2, and O_3 levels were then accumulated into 1-year average concentrations. Land-use predictors with a Spearman's correlation coefficient larger than 0.4 with an effect on air pollutants were entered into a stepwise linear regression. Furthermore, to improve the robustness of the LUR model, a set of pollutant levels was created through a leave-one-out kriging interpolation and added

to the model. Average individual exposure to air pollution was estimated for 1 year before the HRCT assessment.

Lung Function

Lung function data were collected from each subject retrospectively from the hospital records of the subject. Spirometry was conducted according to the American Thoracic Society/European Respiratory Society guidelines (24). Lung function tests were performed once right before conducting the HRCT.

BODE Index

BODE index, namely, BMI, degree of obstruction, dyspnea severity, and exercise capacity, has proven to be a predictor of COPD mortality and severity (23, 25). Previous studies reported that exposure to air pollution could cause adverse effects on dyspnea and exercise capacity (26, 27). A subgroup of four variables (i.e., the BMI scale, airflow obstruction index, modified Medical Research Council (mMRC) dyspnea scale (28), and the exercise capacity index) is included in the BODE index, which is shown in **Supplementary Table 1**. Airflow obstruction index was defined as the FEV_1 (% predicted). Exercise capacity index was defined as the distance walked in 6 min (meters). The BODE index was categorized into four quartiles: quartile 1 by a score of 0–2; quartile 2 by a score of 3–4; quartile 3 by a score of 5–6; and quartile 4 by a score of 7–10 as previously described (29). Quartile 1 represents the least severity, while quartile 4 represents the most severity.

Emphysema Severity

HRCT scans were acquired at suspended full inspiration. APOLLO version 1.2 software (VIDA Diagnostics, Coralville, IA, USA) was employed to assess the image attenuation on full-lung scans at a single reading center by trained readers without the knowledge of the characteristics of participants. The lung volume was calculated, and all voxels in the lung were identified. The percent emphysema (%LAA) on CT scans was determined as the voxel numbers <-950 Hounsfield units in a lung field divided by the total voxel numbers in that lung field based upon pathological comparisons (30, 31). Emphysema severity was categorized into three levels: level 1 if $1\% \leq \%LAA < 5\%$, level 2 if $5\% \leq \%LAA < 25\%$, and level 3 if $25\% \leq \%LAA < 50\%$.

Statistical Analysis

Tests of normality were used to determine if the data were normally distributed. The extremely low and high values outside percentiles 1 and 99 were replaced by using a winsorization approach to minimize the influence of severe outliers (32). Upper lobe LAA was defined as right upper lobe LAA plus left upper lobe LAA. Lower lobe LAA was defined as right lower lobe LAA plus left lower lobe LAA. We performed a generalized linear model, adjusted for age, sex, BMI, and smoking pack-years, to identify the associations of 1-year air pollution exposure in the five lung lobes with lung function, BODE quartiles, and the percent emphysema in the left upper lobe, left lower lobe, left lung, right upper lobe, right middle lobe, right lower lobe, right lung, upper lobes, lower lobes, and total lung. The beta coefficients (β) were calculated to estimate the contribution of each of the individual variables. Also, the crude OR of outcome variables of predominant centrilobular and panlobular emphysema with the reference group (paraseptal emphysema group) was investigated by a multinomial logistic regression model. To calculate the adjusted OR, we performed the multinomial logistic regression adjusting for age, sex, BMI, and smoking pack-years. SPSS version 22.0.0.0 for Windows statistical software (SPSS Inc., Chicago, IL, USA) was used for data analysis. The value of $p < 0.05$ was set as statistically significant.

RESULTS

Characteristics of the Study Subjects

The baseline characteristics of 86 patients enrolled in our study are summarized in **Table 1**. Overall, the patients had a mean age of 70.4 ± 7.9 years, and 91.9% were men. Their BMI was 23.3 ± 4.4 kg/m^2. About 40.7% of the subjects were current smokers, 51.2% were ex-smokers, and 8.1% were non-smokers. The average smoking pack-years was 50.4 ± 37.9 years. In terms of lung function, the patients had a mean FEV_1 (% predicted) of 56.6 ± 19.8%, an average FEV_1 of 1.3 ± 0.5 L, and FEV_1/FVC ratio of 52.3 ± 10.0%. Mean outcomes were as follows: BODE quartiles (1.8 ± 1.1 points), mean BMI scale (0.3 ± 0.5 points), mean airflow obstruction index (1.3 ± 1.1 points), mean mMRC dyspnea scale (0.7 ± 0.8 points), and mean exercise capacity index (0.7 ± 1.0 points).

Based on HRCT scans, pulmonary emphysema was further classified into different subtypes (i.e., centrilobular, paraseptal, and panlobular emphysema). The percentage of predominant centrilobular, paraseptal, and panlobular emphysema were 66.3, 22.1, and 11.6%, respectively. The mean degree of emphysema in the total lung was 15.6 ± 9.4%. The mean emphysema severity was 2.1 ± 0.5 points.

Air Pollution

One-year mean air pollution levels are depicted in **Table 1**. Levels of 1-year concentrations of $PM_{2.5}$, NO_2, and O_3 to which study subjects were exposed were 28.02 ± 3.38 $\mu g/m^3$, 18.20 ± 2.23 ppb, and 24.44 ± 3.31 ppb, respectively.

Association of O_3 With Predominant Panlobular Emphysema

The association of air pollution with emphysema subtypes is shown in **Table 2**. We observed that a 1 ppb increase in O_3 was associated with 1.798-fold increased crude OR of panlobular subtype (95% CI: 1.073, 3.013; $p < 0.05$). After adjusting for age, sex, BMI, and smoking pack-years, 1 ppb increase in O_3 was associated with 1.854-fold increased adjusted OR of panlobular subtype (95% CI: 1.069, 3.216; $p < 0.05$).

Associations of $PM_{2.5}$, NO_2, and O_3 With BODE and Degree of Emphysema

One-year mean air pollution levels and the association of $PM_{2.5}$, NO_2, and O_3 on BODE quartiles and percent emphysema are depicted in **Table 3**. Levels of 1-year concentrations of

TABLE 1 | Demographic characteristics of study subjects.

Characteristics	Mean ± SD
Total	*N* = 86
Age, years	70.4 ± 7.9
Male, % (*n*)	91.9 (79)
Body mass index, kg/m^2	23.3 ± 4.4
Smoking, % (*n*)	
Current	40.7 (35)
Ex-smoker	51.2 (44)
Non-smoker	8.1 (7)
Lung function	
FEV_1, %	56.6 ± 19.8
FEV_1, L	1.3 ± 0.5
FEV_1/FVC, %	52.3 ± 10.0
BODE quartiles, point	1.8 ± 1.1
BMI scale, point	0.3 ± 0.5
Airflow obstruction index, point	1.3 ± 1.1
mMRC dyspnea scale, point	0.7 ± 0.8
Exercise capacity index, point	0.7 ± 1.0
Emphysema subtypes	
Centrilobular, % (*n*)	66.3 (57)
Paraseptal, % (*n*)	22.1 (19)
Panlobular, % (*n*)	11.6 (10)
Percent emphysema	
Left upper lobe LAA, %	17.0 ± 11.7
Left lower lobe LAA, %	14.0 ± 11.1
Left lung LAA, %	15.8 ± 10.8
Right upper lobe LAA, %	16.5 ± 11.2
Right middle lobe LAA, %	17.3 ± 10.4
Right lower lobe LAA, %	13.1 ± 9.1
Right lung LAA, %	15.4 ± 9.0
Upper lung LAA, %	33.5 ± 22.1
Lower lung LAA, %	27.2 ± 18.8
Total lung LAA, %	15.6 ± 9.4
Emphysema severity, point	2.1 ± 0.5
Air pollution	
$PM_{2.5}$, μg/m^3	28.02 ± 3.38
NO_2, ppb	18.20 ± 2.23
O_3, ppb	24.44 ± 3.31

BMI, body mass index; FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity; LAA, low attenuation area; mMRC, modified Medical Research Council.

$PM_{2.5}$, NO_2, and O_3 to which study subjects were exposed were 28.02 ± 3.38 μg/m^3, 18.20 ± 2.23 ppb, and 24.44 ± 3.31 ppb, respectively. After adjusting for age, sex, BMI, and smoking pack-years, we observed significant associations between $PM_{2.5}$ and left upper lobe LAA (β = 1.476), right upper lobe LAA (β = 1.296), left lower lobe LAA (β = 1.293), right middle lobe LAA (β = 1.202), right lower lobe LAA (β = 0.978), and emphysema severity (β = 0.059). Furthermore, we observed significant associations between $PM_{2.5}$ and BODE quartiles, mMRC dyspnea scale, and exercise capacity scale.

Similarly, significant associations were observed between NO_2 and right upper lobe LAA (β = 1.946), left upper lobe LAA (β = 1.434), right lower lobe LAA (β = 0.883), and emphysema severity (β = 0.055).

Meanwhile, we observed significant associations between O_3 and right upper lobe LAA (β = 1.560), left upper lobe LAA (β = 1.492), right middle lobe LAA (β = 1.126), left lower lobe LAA (β = 0.866), right lower lobe LAA (β = 0.860), and emphysema severity (β = 0.044).

Furthermore, we observed that an increase in $PM_{2.5}$, NO_2, and O_3 was associated with greater increases in upper lobe LAA than in lower lobe LAA.

DISCUSSION

We showed an association between O_3 exposure and panlobular emphysema subtype. Importantly, air pollution ($PM_{2.5}$, NO_2, and O_3) was associated with an increased degree of lobar emphysema, especially in the upper lobes. Our results suggest that particulate and gaseous pollution could have distinct impacts on lung lobes emphysema.

The annual $PM_{2.5}$, NO_2, and O_3 levels in our study were following previous studies conducted in Taipei (33, 34). However, the mean $PM_{2.5}$ levels in our study were nearly 3-fold higher than the WHO acceptable upper limit (annual $PM_{2.5}$ of 10 μg/m^3) (35). Meanwhile, the mean O_3 levels in our study were lower than the acceptable upper limit of the United States EPA (annual O_3 of 70 ppb) (36). Using paraseptal emphysema as the reference group, we found that exposure to O_3 was associated with a higher OR of panlobular emphysema. O_3 is formed by the photochemical dissociation of molecular oxygen into two oxygen atoms, followed by a combination between the oxygen atom and the molecular oxygen. Previous studies reported the role of apoptosis of alveolar epithelial cells and endothelial cells in the pathogenesis of emphysema (37–39). O_3-induced oxidative stress could induce the activation of proteases (caspase-3) (40–42). Moreover, a previous *in vivo* study reported that exposure to 2.5 ppm O_3 for 6 weeks resulted in alveolar enlargement and airway wall destruction associated with an increase in matrix metalloproteinase-12 (MMP-12) and caspase-3 (42). It was also reported that interleukin (IL)-13 may modulate O_3-induced neutrophilic inflammation (43). Furthermore, IL-13 could promote alveolar macrophage elastase (MMP-12) upregulation, thus leading to emphysema (44–46). Moreover, previous studies reported that O_3 was diffused in the alveolar periphery (47–49). It is also suggested that panlobular emphysema represents a more advanced phase of emphysema and COPD (14, 50). Together, this suggests that exposure to O_3 can be associated with a higher risk of panlobular emphysema than paraseptal emphysema in patients with COPD. However, the association of O_3 with panlobular emphysema warrants further investigations.

Next, we examined the association between air pollution and BODE quartiles and demonstrated that exposure to $PM_{2.5}$ was associated with the increased BODE quartiles, the mMRC dyspnea scale, and the exercise capacity index. Our findings are

TABLE 2 | Associations [odds ratio (OR)] of centrilobular and panlobular emphysema subtypes with paraseptal emphysema (reference) by 1-year average air pollution concentrations of $PM_{2.5}$, NO_2, and O_3.

	Crude OR (95% CI)			Adjusted OR (95% CI)		
Air pollution	**Paraseptal**	**Centrilobular**	**Panlobular**	**Paraseptal**	**Centrilobular**	**Panlobular**
$PM_{2.5}$, $\mu g/m^3$	1	0.937 (0.799, 1.098)	1.151 (0.922, 1.436)	1	0.923 (0.784, 1.087)	1.110 (0.883, 1.395)
NO_2, ppb	1	0.868 (0.683, 1.103)	0.870 (0.613, 1.236)	1	0.865 (0.680, 1.101)	0.850 (0.587, 1.232)
O_3, ppb	1	0.933 (0.788, 1.106)	**1.798 (1.073, 3.013)***	1	0.923 (0.774, 1.100)	**1.854 (1.069, 3.216)***

NO_2, nitrogen dioxide; O_3, ozone; $PM_{2.5}$, particulate matter <2.5 μm in aerodynamic diameters.
Adjusted for age, sex, body mass index, and smoking pack-years.
*Values in bold characters are deemed statistically significant. *$p < 0.05$.*

consistent with previous studies showing that mMRC scores of patients with COPD when the air quality index (AQI) > 100 were higher than when AQI ≤ 100 (27). Moreover, exposure to diesel engine exhaust significantly decreased the exercise capacity compared to exposure to clean air in heart failure patients (26). The results indicate that exposure to air pollution increased the risk of COPD severity. Together, our data further suggested the adverse effects of exposure to $PM_{2.5}$ to the BODE quartiles (i.e., BMI, airflow obstruction, dyspnea, and physical activities) in patients with COPD.

We identified air pollution (i.e., $PM_{2.5}$, NO_2, and O_3) associated with increased percent emphysema of the total lung and emphysema severity. Previous studies showed that air pollutants may penetrate deeply into the lung and destroy the alveolar septa through the excessive reactive oxygen species (ROS) generation (51). It was reported that the ROS played an important role in the apoptosis of alveolar epithelial cells (52–55). ROS was necessary to activate the BCL2-associated X (Bax) protein, leading to cell death (53, 56, 57). Furthermore, the ROS can cause endothelial cell apoptosis (58, 59). Our results are consistent with previous studies (21, 22). Using 1-year average air pollution exposure, a previous study found that 5 $\mu g/m^3$ increase in $PM_{2.5}$ and 25 ppb increase in oxides of nitrogen (NO_x) were associated with 0.6 (95% CI: 0.1, 1.2%) and 0.5 (95% CI: 0.1, 0.9%) increase in percent emphysema (21). A cohort study involving 19-year exposure to air pollutants demonstrated that 2 $\mu g/m^3$ increase in $PM_{2.5}$, 10 ppb increase in NO_x, and 3 ppb increase in O_3 resulted in 0.11 (95% CI: 0.03, 0.19), 0.06 (95% CI: 0.01, 0.12), and 0.13 (95% CI: 0.03, 0.24) increase in percent emphysema, respectively (22). In a study including 10-year exposure to O_3, 5 ppb increase in O_3 concentration was associated with increased emphysema severity ($\beta = 0.94$; 95% CI: 0.25, 1.64; $p < 0.05$) (60). However, the associations between air pollution and lobar emphysema are still unclear.

Due to the lung anatomy and aerodynamic motion of inhaled particles, we suspect that air pollution (especially particulate pollution) may have different impacts on the lung lobes. Our results further showed that air pollution was associated with lobar emphysema, especially in the upper lobes, by $PM_{2.5}$, NO_2, and O_3. Our previous study found that a 1 $\mu g/m^3$ increase in $PM_{2.5}$ deposition in each lung lobe was associated with increases in %LAA (beta coefficient) of the same lung lobe ($p < 0.05$) (61). Because of particle physicochemical characteristics, lung geometric difference, and breathing pattern, the associations between air pollutants with lung lobe percent emphysema could be associated with our findings. In this study, we observed that an increase in $PM_{2.5}$, NO_2, and O_3 was associated with greater increases in upper lobe LAA than in lower lobe LAA. This was consistent with a previous study showing that $PM_{2.5}$ deposition was associated with higher emphysema severity in upper lobes than in lower lobes (61). This suggested that upper lobes might be preferentially impacted by air pollution than lower lobes. Furthermore, smoking-induced emphysema was also commonly observed in the upper lung lobes (13, 18). Smoking was also reported to be associated with centrilobular emphysema, which is mostly observed in upper lobe emphysema (14, 18, 20). The reasons for this upper lobe predominant distribution may include regional differences in lung physiology (i.e., ventilation/perfusion ratio, lymphatic flow, particle clearance, and intrapleural pressure) (13, 62). Although the ventilation predominated in the lower lung lobes, the ratio of ventilation to perfusion was higher in the upper lobes than in the lower lobes, which could favor the pathogenesis of upper lobe emphysema (63, 64). Meanwhile, the decrease in the lymphatic drainage in the upper lobes due to gravity could result in a decline in particle clearance, thus facilitating the development of emphysema (18, 63). Furthermore, the higher mechanical stress and more intrapleural pressures in the upper lung lobes may also result in the high distribution of emphysema in the upper lung lobes (65–67). However, this needs further investigations in future studies. Together, our data suggest that upper lung lobes could be more susceptible to impairment by air pollution.

The limitation of this study included its small sample size because the subjects recruited for this study depended on the number of admissions diagnosed with COPD during the study period. Furthermore, the lack of female representation in our study could be a limitation. The chemical components of $PM_{2.5}$ (i.e., water-soluble ions, heavy metals, and polycyclic aromatic hydrocarbon) were not examined in our study. The effects of indoor pollution should also be clarified in future studies. We observed the associations between air pollution and the extent of emphysema, but the

TABLE 3 | Associations between lung function, BODE quartiles, and percent emphysema (95% CI) with 1-year average air pollution concentrations of $PM_{2.5}$, NO_2, and O_3.

Air pollution	$PM_{2.5}$ β coefficient (95% CI)	NO_2 β coefficient (95% CI)	O_3 β coefficient (95% CI)
Lung function			
FEV_1, %	−0.724 (−1.976, 0.529)	−0.135 (−2.013, 1.742)	0.468 (−0.817, 1.753)
FEV_1, L	−0.022 (−0.053, 0.010)	−0.003 (−0.050, 0.044)	−0.006 (−0.038, 0.027)
FEV_1/FVC, %	−0.358 (−0.995, 0.278)	0.038 (−0.915, 0.992)	0.148 (−0.506, 0.801)
BODE quartiles, point	**0.099 (0.024, 0.175)***	−0.066 (−0.182, 0.050)	0.054 (−0.027, 0.135)
BMI scale, point	0.003 (−0.021, 0.027)	−0.015 (−0.060, 0.031)	0.003 (−0.029, 0.034)
Airflow obstruction, point	0.024 (−0.052, 0.100)	−0.085 (−0.197, 0.026)	0.007 (−0.069, 0.083)
mMRC dyspnea, point	**0.076 (0.023, 0.130)***	0.019 (−0.065, 0.102)	0.017 (−0.043, 0.077)
Exercise capacity, point	**0.083 (0.017, 0.150)***	0.046 (−0.056, 0.148)	0.031 (−0.040, 0.103)
Percent emphysema			
Left upper lobe LAA, %	**1.476 (0.813, 2.139)***	**1.434 (0.387, 2.481)***	**1.492 (0.813, 2.171)***
Left lower lobe LAA, %	**1.293 (0.660, 1.927)***	0.922 (−0.086, 1.929)	**0.866 (0.185, 1.546)***
Left lung LAA, %	**1.414 (0.811, 2.018)***	**1.236 (0.269, 2.203)***	**1.206 (0.568, 1.845)***
Right upper lobe LAA, %	**1.296 (0.665, 1.927)***	**1.946 (1.008, 2.883)***	**1.560 (0.939, 2.180)***
Right middle lobe LAA, %	**1.202 (0.604, 1.800)***	0.687 (−0.269, 1.642)	**1.126 (0.507, 1.746)***
Right lower lobe LAA, %	**0.978 (0.481, 1.474)***	**0.883 (0.106, 1.661)***	**0.860 (0.342, 1.378)***
Right lung LAA, %	**1.138 (0.640, 1.635)***	**1.242 (0.462, 2.021)***	**1.209 (0.705, 1.712)***
Upper lobe LAA, %	**2.772 (1.535, 4.009)***	**3.380 (1.473, 5.286)***	**3.052 (1.812, 4.291)***
Lower lobe LAA, %	**2.271 (1.238, 3.303)***	**1.805 (0.154, 3.455)***	**1.726 (0.621, 2.830)***
Total lung LAA, %	**1.283 (0.769, 1.797)***	**1.282 (0.461, 2.102)***	**1.198 (0.662, 1.735)***
Emphysema severity, point	**0.059 (0.029, 0.089)***	**0.055 (0.008, 0.103)***	**0.044 (0.012, 0.076)***

BMI, body mass index; FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity; LAA, low attenuation area; mMRC, modified Medical Research Council; NO_2, nitrogen dioxide; O_3, ozone; $PM_{2.5}$, particulate matter <2.5 μm in aerodynamic diameters.
Adjusted for age, sex, body mass index, and smoking pack-years.
*Values in bold characters are deemed statistically significant. *p < 0.05.*

inflammatory responses and underlying mechanisms need to be investigated in the future. Because the sample size in our study was small, we did not adjust for previous pulmonary infections, alpha-1 antitrypsin levels, and socioeconomic status. These confounding factors should be included in future works. The subjects in our study were exposed to 1-year air pollution concentrations, which could be a limitation. Longitudinal analyses with long-term exposure to air pollution may better clarify these associations with percent emphysema.

CONCLUSIONS

In conclusion, exposure to air pollution was associated with the degree and type of lobar emphysema in COPD. Our findings suggested that exposure to O_3 was preferentially associated with panlobular emphysema than paraseptal emphysema in patients with COPD. Particulate and gaseous pollution may have distinct impact on the lung lobes. Moreover, this study showed an association between air pollution and the degree of emphysema, especially in the upper lobes. Air pollution could be associated with the severity of lobar emphysema in COPD.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Taipei Medical University-Joint Institutional Review Board approved this study (Approval No. N202003075). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

H-CC planned the study and designed the experiments. NT and S-CH completed the manuscript. Y-HL, T-TC, K-YL, K-YC, S-MW, and H-YK completed the COPD data collection. C-DW completed the personal exposure assessment. HT, HD, and TT conducted the Multiple-Path

Particle Dosimetry (MPPD) model. KC, H-PK, and Y-LL critically revised the manuscript. All authors analyzed and discussed the results, commented on the manuscript, and read and approved the final version of the manuscript for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.705792/full#supplementary-material

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Therapeutic Potential of Human Umbilical Cord-Derived Mesenchymal Stem Cells in Recovering From Murine Pulmonary Emphysema Under Cigarette Smoke Exposure

*Xiao-Yue Chen[1,2], Yi-Ying Chen[2], Willie Lin[3], Chien-Han Chen[3], Yu-Chieh Wen[3], Ta-Chih Hsiao[4], Hsiu-Chu Chou[5], Kian Fan Chung[6] and Hsiao-Chi Chuang[2,7,8]**

[1] Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, Taipei, Taiwan, [2] School of Respiratory Therapy, College of Medicine, Taipei Medical University, Taipei, Taiwan, [3] Meridigen Biotech Co., Ltd., Taipei, Taiwan, [4] Graduate Institute of Environmental Engineering, National Taiwan University, Taipei, Taiwan, [5] Department of Anatomy and Cell Biology, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan, [6] National Heart and Lung Institute, Imperial College London, London, United Kingdom, [7] Division of Pulmonary Medicine, Department of Internal Medicine, Shuang Ho Hospital, Taipei Medical University, New Taipei City, Taiwan, [8] Cell Physiology and Molecular Image Research Center, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan

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Edited by:
Sinéad Weldon,
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***Correspondence:**
Hsiao-Chi Chuang
r92841005@ntu.edu.tw

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Human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) were shown to have potential for immunoregulation and tissue repair. The objective of this study was to investigate the effects of hUC-MSCs on emphysema in chronic obstructive pulmonary disease (COPD). The C57BL/6JNarl mice were exposed to cigarette smoke (CS) for 4 months followed by administration of hUC-MSCs at 3×10^6 (low dose), 1×10^7 (medium dose), and 3×10^7 cells/kg body weight (high dose). The hUC-MSCs caused significant decreases in emphysema severity by measuring the mean linear intercept (MLI) and destructive index (DI). A decrease in neutrophils (%) and an increase in lymphocytes (%) in bronchoalveolar lavage fluid (BALF) were observed in emphysematous mice after hUC-MSC treatment. Lung levels of interleukin (IL)-1β, C-X-C motif chemokine ligand 1 (CXCL1)/keratinocyte chemoattractant (KC), and matrix metalloproteinase (MMP)-12 significantly decreased after hUC-MSC administration. Significant reductions in tumor necrosis factor (TNF)-α, IL-1β, and IL-17A in serum occurred after hUC-MSC administration. Notably, the cell viability of lung fibroblasts improved with hUC-MSCs after being treated with CS extract (CSE). Furthermore, the hUC-MSCs-conditioned medium (hUC-MSCs-CM) restored the contractile force, and increased messenger RNA expressions of elastin and fibronectin by lung fibroblasts. In conclusion, hUC-MSCs reduced inflammatory responses and emphysema severity in CS-induced emphysematous mice.

Keywords: cigarette smoke, COPD, emphysema, inflammation, stem cell

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is currently one of the world's highest causes of mortality and ranks fifth worldwide in terms of disease burden (1–3). About 80–90% of COPD patients are related to cigarette smoking (4). A previous study found that exposure to cigarette smoke (CS) for 12 weeks induced emphysematous lung lesions in rats (5). This irreversible alveolar destruction and emphysematous changes due to CS exposure resulted in higher mortality and difficulties in treating COPD.

Mesenchymal stem cells (SCs; MSCs), multipotent SCs, have high self-renewal and differentiation capacities (6). Recent studies demonstrated immunoregulatory functions of MSCs in treating graft vs. host disease (7, 8). Also, tissue-repair actions of MSCs through a paracrine mechanism were explored (9, 10). Notably, most intravenously (i.v.) administered MSCs were localized in the lungs (11). Recruitment of MSCs to the lungs provides new insights that MSCs may have greater paracrine effects in the lungs. Therefore, the effects of MSCs on lung disease treatment were recently noted (12, 13).

Human umbilical cord-derived (hUC)-MSCs have a higher differential capacity, lower immunogenicity, and less age-related dysfunction compared to adult SCs (14). Other advantages of hUC-MSCs are that there are fewer ethical issues associated with them and they can be non-invasively collected (15). Anti-inflammatory effects of hUC-MSCs were found in an acute lung injury mouse model (16). Moreover, it was demonstrated that hUC-MSCs prevented bleomycin-induced lung fibrosis *in vivo* (17).

Lung fibroblasts were shown to have an important role in repairing damaged lung tissues after CS exposure (18). However, a previous study found a decrease in the proliferation of lung fibroblasts in COPD (19). Recently, the senescence-associated secretory phenotype of lung fibroblasts was found in CS-induced emphysema (20). Consequently, the loss of the ability to repair alveoli due to CS was mainly because of lung fibroblast dysfunction (21, 22). MSCs were shown to mediate the proliferation and increase the pro-collagen expression of lung fibroblasts (23).

Despite the efficacy of MSCs in ameliorating acute lung damage, few studies have investigated the effects of hUC-MSCs on chronic CS-induced emphysema. The objective of this study was to investigate the therapeutic efficacy of hUC-MSCs in emphysema.

MATERIALS AND METHODS

Animals

The animal study was approved by the Animal and Ethics Review Committee of the Laboratory Animal Center at Taipei Medical University, Taipei, Taiwan (IACUC: LAC-2017-0231). Male C57BL/6JNarl mice (8 weeks, 20–25 g, $n = 8$–10 per group) were obtained from the National Laboratory Animal Center (Taipei, Taiwan). Mice were housed in plastic cages and supplied with Lab Diet 5001 (PMI Nutrition International, St. Louis, MO, USA) and water *ad libitum*. A light/dark cycle of 12 h/12 h was maintained. The room temperature was set to 22 ± 2°C, and relative humidity to 55 ± 10%.

CS-Induced Emphysema

An emphysema mouse model was established by whole-body exposure to CS for 4 months. Details of the CS exposure system were previously reported (24). Briefly, the system consisted of a CS generator, a whole-body exposure chamber (TECNIPLAST, VA, Italy), and a particulate matter (PM) monitor. A side-stream was introduced into the whole-body exposure chamber at a flow rate of 15 L/min. There were 16 commercial cigarettes (Longlife, Taipei, Taiwan; 11 mg of tar and 0.9 mg of nicotine) combusted for 8 h/day and 5 days/week for 4 months (**Figure 1A**). The mass concentration of PM of <2.5 μm in aerodynamic diameter ($PM_{2.5}$) was monitored using a DustTrak monitor (8530, TSI, Shoreview, MN, USA). **Figure 1B** shows the distribution of the $PM_{2.5}$ mass concentration during CS exposure. The average $PM_{2.5}$ mass concentration was 90.5 ± 40.6 mg/m^3 during the first 15 min. It reached a maximum level of about 154.3 ± 58.2 mg/m^3 after 4 min of cigarette combustion, and then the mass concentration declined to the baseline level after 16 min. Simultaneously, mice exposed to CS-free high-efficiency particulate air (HEPA)-filtered room air (RA) served as the control group.

hUC-MSC Preparation and Characterization

Details of hUC-MSC preparation were previously reported (24). Briefly, umbilical cords were aseptically harvested and digested with collagenase (SERVA, Heidelberg, Germany) at 37°C. The cell pellets were expanded in α-minimal essential medium (α-MEM, Invitrogen, Carlsbad, CA, USA), and cultured in an incubator with 5% CO_2 at 37°C for 3 days. hUC-MSCs were characterized using flow cytometry (BD Stemflow™ hMSC Analysis Kit; BD Biosciences, San Jose, CA, USA) to detect expressions of cluster of differentiation (CD) markers (CD11b, CD19, CD34, CD44, CD45, CD73, CD90, and CD105) and human leukocyte antigen–antigen D related (HLA-DR). As presented in **Supplementary Table 1**, hUC-MSCs exhibited positive expressions of SC-specific surface markers (CD44, CD73, CD90, and CD105) and negative expressions of CD11b, CD19, CD34, CD45, and HLA-DR, which followed International Society for Cellular Therapy Guidelines (25). hUC-MSCs were prepared in clinical-grade normal saline supplemented with 2% clinical-grade human serum albumin and 16.7% clinical grade CS10. This study was approved by the Ethics Committee of the National Cheng Kung University Hospital Institutional Review Board (Tainan, Taiwan; IRB no.: A-BR-104-045). All subjects received written and oral informed consent before inclusion. All study processes were conducted following the approved study protocol.

hUC-MSC Administration and Sample Collection

The experimental design is shown in **Figure 1A**. After 4 months of CS exposure, emphysematous mice were randomly divided into four groups: sham control (CS), low-dose group (CS + MSC-L), medium-dose group (CS + MSC-M), and high-dose group

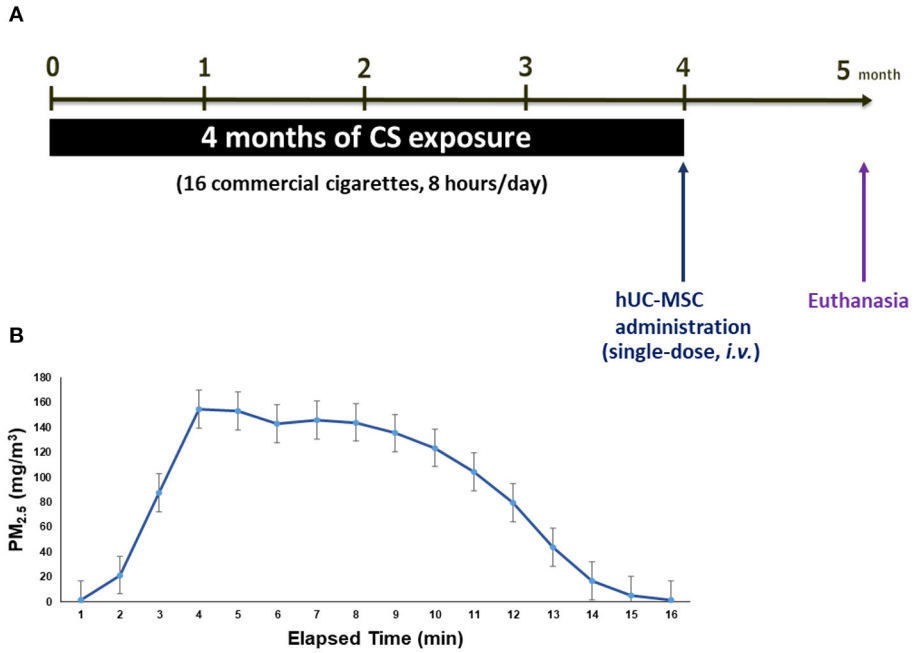

FIGURE 1 | (A) Schematic mice model of cigarette smoke (CS)-induced emphysema. **(B)** The distribution of particulate matter with an aerodynamic diameter of <2.5 μm ($PM_{2.5}$) mass concentration in the whole-body exposure system (mean ± SD). Mice (8 weeks old, 20–25 g, n = 8–10 per group) were exposed to CS for 4 months and received (i.v.) a single dose of human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) after CS exposure (CS + MSC-L: hUC-MSCs at 3 × 10^6 cells/kg body weight (BW) for low-dose, CS + MSC-M: 1 × 10^7 cells/kg BW for medium-dose, and CS + MSC-H: 3 × 10^7 cells/kg BW for high-dose).

(CS + MSC-H). Mice were intravenously (i.v.) administrated a single-dose of hUC-MSCs at 3 × 10^6 cells/kg body weight (BW) for CS + MSC-L, 1 × 10^7 cells/kg BW for CS + MSC-M, and 3 × 10^7 cells/kg BW for CS + MSC-H. The administered dose of hUC-MSCs was referenced to our previous reports (24, 26). The control and CS sham groups were i.v. administrated the same volume of vehicle. BW was measured once a week before and after hUC-MSC administration. Mice were euthanized 4 weeks after hUC-MSC administration. Bronchoalveolar lavage fluid (BALF), lung tissues, and serum were collected. For histological analyses, lung samples were inflated with 10% (m/v) paraformaldehyde in phosphate-buffered saline (PBS) at a pressure of 21 cm H_2O.

Emphysema Evaluation

Lung tissues were embedded in paraffin and sectioned into slices for staining with hematoxylin and eosin (H&E). The mean linear intercept (MLI) and destructive index (DI) were used to evaluate the presence of emphysema. The MLI was assessed by counting the number of the alveolar walls intercepted in the grid lines, according to previously described methods (27, 28). The DI for microscopic lung lesions was previously reported (27, 28). Emphysematous defects or intramural parenchyma in at least two intersections of alveoli were considered alveolar destruction.

Hematology

BALF was centrifuged at 1,500 rpm for 10 min at 4°C. Cell pellets were resuspended in PBS. Numbers of neutrophils, lymphocytes, monocytes, and eosinophils were quantified by a hematology analyzer (ProCyte Dx, IDEXX Laboratories, Westbrook, ME, USA). Data are expressed as percentages (%) of total cell counts.

Proteins Extracted From Lung Tissues

Lysis buffer was prepared from 490 μL of lysis reagent (Sigma-Aldrich, St. Louis, MO, USA) containing 5 μL of a protease inhibitor (Geno Technology, St. Louis, MO, USA) and 5 μL of ethylenediaminetetraacetic acid. Lung tissues were homogenized in lysis buffer using a homogenizer (Minilys® personal homogenizer, Bertin, Rockville, MD, USA).

Cytometric Bead Array (CBA) and Enzyme-Linked Immunosorbent Assay (ELISA)

A CBA (BD Biosciences, San Jose, CA, USA) was used to quantify levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, chemokine (C-X-C motif) ligand 1/keratinocyte chemoattractant (CXCL1/KC), and IL-17A in BALF, lung, and serum samples. Matrix metalloproteinase (MMP)-12 was determined in lung samples by an ELISA (Cloud-Clone, Katy, TX, USA). Quantification of these markers in lung samples was normalized to the total protein. All measurements were undertaken in accordance with the manufacturers' instructions.

Human Lung Fibroblasts

Human lung fibroblasts (MRC-5 cells) were obtained from the Food Industry Research and Development Institute (FIRDI,

Hsinchu, Taiwan) and cultured in T75 flasks with Eagle's minimum essential medium (EMEM, Lonza Group, Basel, Switzerland) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 0.1 mM non-essential amino acids, and 1 mM sodium pyruvate.

hUC-MSCs-Conditioned Medium (CM) Preparation

To collect hUC-MSCs-CM, hUC-MSCs (1.2×10^6 cells) were cultured in T75 flasks with 15 mL of hUC-MSC culture medium for 24 h. After being washed with PBS, the culture medium was replaced with 10 mL of α-MEM basal medium (Invitrogen, Carlsbad, CA, USA) and incubated for 48 h. The subsequent serum-free culture medium was collected and served as hUC-MSCs-CM.

CS Extract (CSE)

CSE was prepared from the combustion of three cigarettes (Marlboro, Philip Morris, VA, USA) by impinging onto 30 mL of α-MEM (Invitrogen) with a firm filter. The cigarette contained 10 mg of tar and 0.8 mg of nicotine. Fresh CSE was collected to serve as 100% CSE and immediately used for cell experiments.

Cell Viability of Human Lung Fibroblasts by hUC-MSCs After CSE Exposure

MRC-5 cells were treated with 8% CSE for 24 h and then indirectly cocultured with hUC-MSCs for another 48 h. Cell viability of MRC-5 cells was determined by a cell counting kit-8 (Merck, Darmstadt, Germany).

Cell Contractile Force and Elastin and Fibronectin of Human Lung Fibroblasts by hUC-MSCs-CM After CSE Exposure

MRC-5 cells (2×10^5/cells) seeded in six-well plates were treated with 8% CSE for 24 h. After CSE exposure, cells were cultured in hUC-MSCs-CM for 24 h. The cell contractile force was measured using a collagen-based cell contraction assay kit (CellBiolabs, San Diego, CA, USA). Messenger (m)RNA expressions of elastin and fibronectin were analyzed by a quantitative polymerase chain reaction (qPCR), according to the manufacturer's instructions.

Statistical Analysis

Data are presented as the mean ± standard deviation (SD). Multiple groups were compared by an analysis of variance (ANOVA) with Tukey's *post-hoc* test. An unpaired *t*-test was used for comparisons between continuous variables. All analyses were performed using GraphPad vers. 6 (San Diego, CA, USA). $p < 0.05$ was considered statistically significant.

RESULTS

hUC-MSCs Mitigated Emphysema Severity

Results of the histological analysis are shown in **Figure 2**. A significant decrease in the MLI by hUC-MSCs was observed compared to the CS group (low-dose: 87.08 ± 14.20, medium-dose: 82.34 ± 7.50, and high-dose MSCs: 79.32 ± 7.14 vs. the CS group: 103.10 ± 11.52 μm, $p < 0.001$). Furthermore, the DI (%) significantly decreased after hUC-MSC administration (medium-dose: 15.67 ± 3.30% and high-dose MSCs: 12.05 ± 2.65% vs. the CS group: 24.30 ± 2.85%, $p < 0.001$).

Reduction of Lung Infiltration by hUC-MSCs

As shown in **Figure 3A**, a significant decrease in the percentage of neutrophils was observed in the hUC-MSC group compared to the CS group (low-dose: 35.83 ± 9.50%, medium-dose: 20.64 ± 12.44%, and high-dose MSCs: 23.05 ± 12.54% vs. the CS group: 57.29 ± 27.45%, $p < 0.001$). In contrast, lymphocytes (%) significantly increased after hUC-MSC administration compared to the CS group (low-dose: 44.47 ± 13.17%, medium-dose: 65.44 ± 13.29%, and high-dose MSCs: 63.73 ± 13.08% vs. the CS group: 24.77 ± 18.41%, $p < 0.001$). There was no statistical difference in monocytes (%) or eosinophils (%) among the groups. Also, we observed no statistical difference in TNF-α, IL-1β, CXCL1/KC, or IL-17A in BALF after hUC-MSC administration (**Figure 3B**).

hUC-MSCs Decreased Levels of IL-1β, CXCL1/KC, and MMP-12 in the Lungs

Levels of IL-1β (low-dose: 0.70 ± 0.42 and medium-dose MSCs: 0.76 ± 0.42 vs. the CS group: 1.28 ± 0.47 pg/mg, $p < 0.05$) and CXCL1/KC (medium-dose: 8.20 ± 4.14 and high-dose MSCs: 9.92 ± 9.47 vs. CS group: 41.61 ± 21.56 pg/mg, $p < 0.001$) in lung lysates significantly decreased after hUC-MSC administration compared to the CS group (**Figure 4A**). Also, we found that MMP-12 in lungs of mice was significantly reduced by hUC-MSCs (low-dose: 3.83 ± 0.92, medium-dose: 3.14 ± 0.89, and high-dose MSCs: 3.13 ± 1.03 vs. the CS group: 6.40 ± 2.20 pg/mg, $p < 0.001$). There was no significant change in TNF-α or IL-17A levels among all groups.

hUC-MSCs Reduced Levels of TNF-α, IL-1β, and IL-17A in Serum

TNF-α, IL-1β, CXCL1/KC, and IL-17A levels in serum of mice were examined (**Figure 4B**). hUC-MSCs significantly reduced levels of TNF-α (low-dose MSCs: 6.49 ± 2.48 vs. the CS group: 14.71 ± 9.34 pg/mL, $p < 0.01$), IL-1β (low-dose: 14.16 ± 22.48, medium-dose: 17.69 ± 10.86, and high-dose MSCs: 21.4 ± 15.27 vs. the CS group: 56.31 ± 47.24 pg/mL, $p < 0.05$), and IL-17A (low-dose: 0.75 ± 0.59 and high-dose MSCs: 0.90 ± 0.44 vs. the CS group: 2.02 ± 1.34 pg/mL, $p < 0.05$) compared to the CS group. No significant reduction in CXCL1/KC was found when compared among all groups.

Proliferation of Lung Fibroblasts by hUC-MSCs

As shown in **Figure 5A**, the cell viability of MRC-5 cells significantly increased by hUC-MSCs after CSE treatment compared to the CSE group ($p < 0.05$). The contractile force of MRC-5 cells as determined by the collagen gel surface area was significantly reduced by hUC-MSCs-CM treatment compared to the CSE group ($p < 0.05$; **Figure 5B**). A significant increase in mRNA expressions of elastin and fibronectin were observed

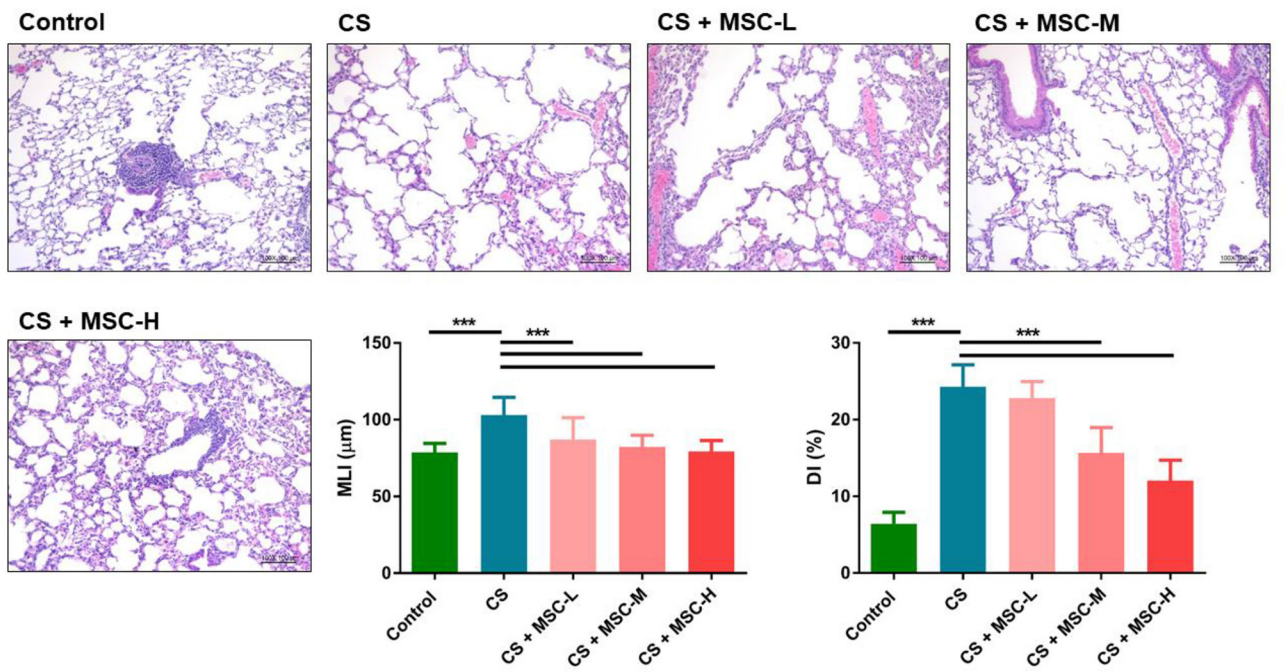

FIGURE 2 | Repair of alveolar structures by human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) in a mice model of cigarette smoke (CS)-induced emphysema. Lung tissue sections were stained with hematoxylin and eosin (H&E). Lung lesions were quantified by measuring the mean linear intercept (MLI) and destructive index (DI). Significant reductions of the MLI and DI (%) were observed by hUC-MSC administration after CS exposure for 4 months. Results were determined by a one-way ANOVA with Tukey's test. $n = 8–10$ per group. $^{***}p < 0.001$.

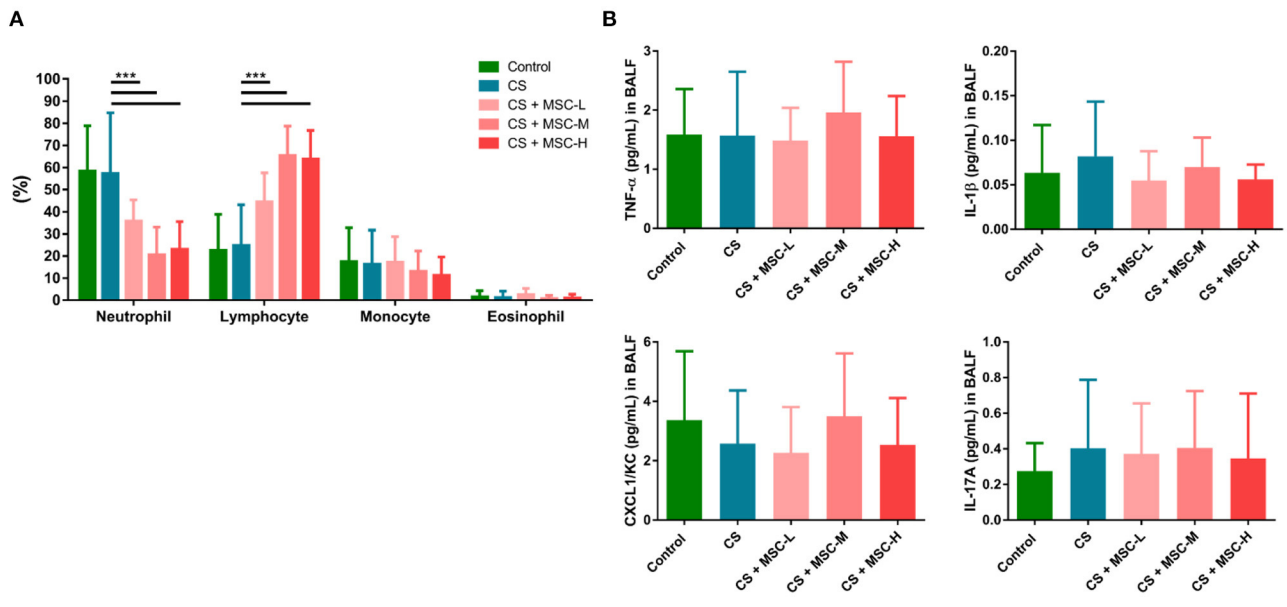

FIGURE 3 | (A) The human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) reduced neutrophils and increased lymphocytes in bronchoalveolar lavage fluid (BALF) of mice. **(B)** Regulation of cytokine production (tumor necrosis factor (TNF)-α, interleukin (IL)-1β, C-X-C motif chemokine ligand 1 (CXCL1)/keratinocyte chemoattractant (KC), and IL-17A) by hUC-MSCs in BALF. A significant decrease in neutrophils (%) was observed by hUC-MSC administration, whereas lymphocytes (%) increased after hUC-MSC administration. There was no significant difference in cytokine production in BALF after hUC-MSC administration. The results were determined by a one-way ANOVA with Tukey's test. $n = 8–10$ per group. $^{***}p < 0.001$.

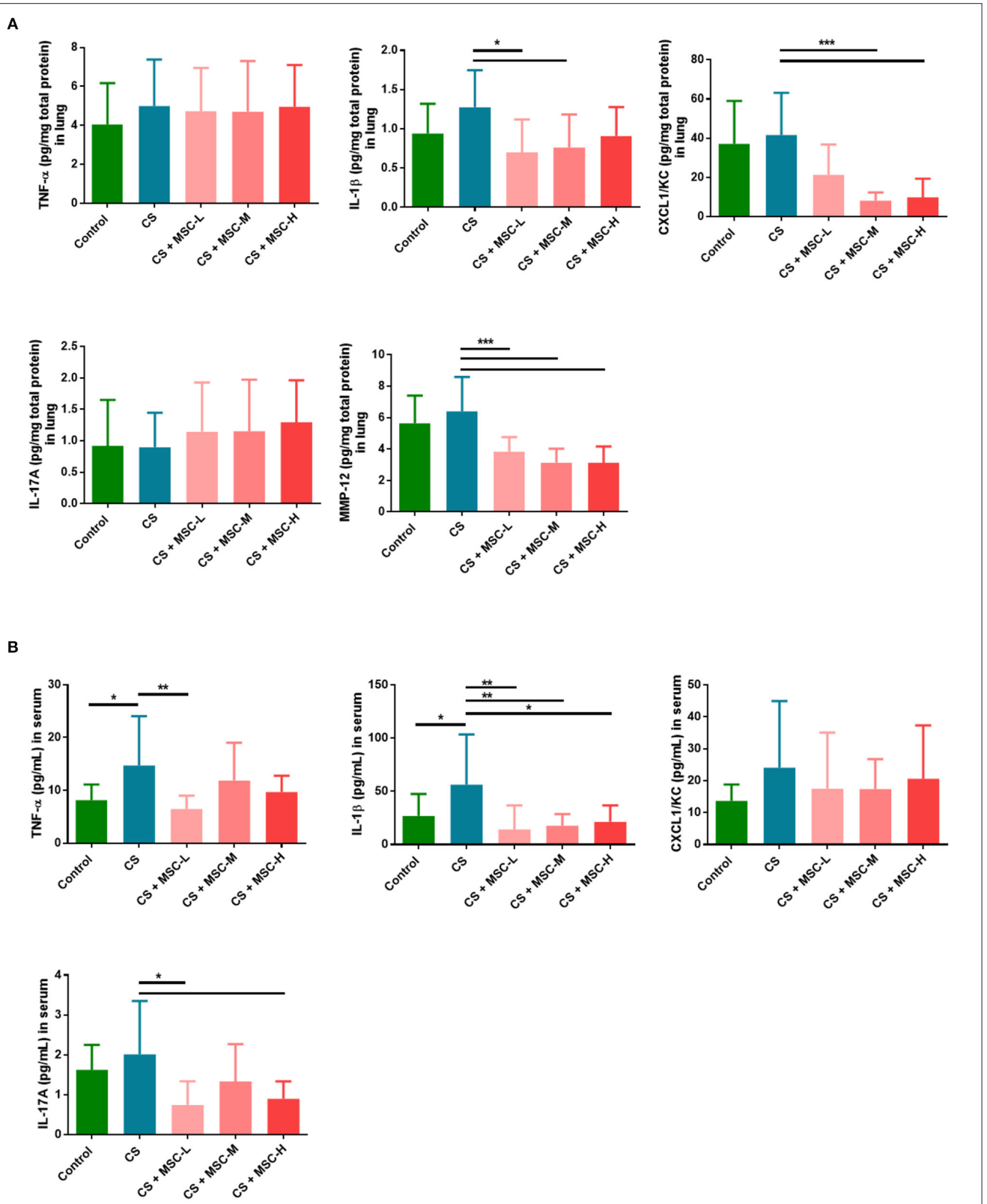

FIGURE 4 | (A) Human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) downregulated interleukin (IL)-1β, C-X-C chemokine ligand 1 (CXCL1)/keratinocyte chemoattractant (KC), and matrix metalloproteinase (MMP)-12 in lung lysates. **(B)** hUC-MSCs decreased systemic cytokine production (tumor

(Continued)

FIGURE 4 | necrosis factor (TNF)-α, IL-1β, and IL-17A) in serum. Mice lungs were homogenized, and then lung lysates and facial blood of mice were measured by a CBA or ELISA. IL-1β, CXCL1/KC, and MMP-12 in the lungs of mice were significantly reduced by hUC-MSCs. Significant decreases in TNF-α, IL-1β, and IL-17A in the serum of mice by hUC-MSCs were seen, and data were determined by a one-way ANOVA with Tukey's test. $n = 8$–10 per group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

by hUC-MSCs-CM treatment compared to the CSE group ($p < 0.001$; **Figure 5C**).

DISCUSSION

MSCs were shown to have the potential for immunomodulation and tissue regeneration in different diseases (29–31). We observed that hUC-MSCs decreased the emphysema severity and reduced lung and systemic inflammatory infiltration in mice with CS-induced emphysema. Moreover, we observed that hUC-MSCs increased the proliferation of lung fibroblasts after CSE exposure. hUC-MSCs may ameliorate emphysematous lung lesions in COPD.

Mice were exposed to CS for 4 months at an average mass concentration of 90.5 ± 40.6 mg/m^3 $PM_{2.5}$ to induce development of emphysema in the present study. The CS-exposure system in this study was described previously (24). Previous reports also showed that CS exposure for 12–14 weeks was able to induce an emphysema model (32–35). During the CS exposure, the mice were significantly decreased in body weight and a significant increase in the serum level of TNF-α as compared to the control before hUC-MSC administration (**Supplementary Figures 1A,B**). After 4 months of exposure to CS, we observed significantly increased emphysema severity (MLI and DI) and elevation of pro-inflammatory factors (TNF-α and IL-1β) in serum without a significant change in BW (**Supplementary Figure 2**). The observation suggests that a mouse model of CS-induced emphysema was successfully established in the present study. However, it is worth to note that the mice were euthanized 4 weeks after the CS exposure. This may result in the decrease of inflammatory responses in the CS group.

The lungs are an important organ for accumulation of hUC-MSCs after their administration (36–38). Lung inflammatory infiltration was mitigated by hUC-MSCs in emphysematous mice. First, neutrophils were significantly reduced in BALF by hUC-MSCs. Previous studies showed that neutrophils or polymorphonuclear cells decreased in BALF by MSC administration after CS exposure for 7∼16 weeks *in vivo* (35, 39). Pulmonary neutrophil activation by CS is reported to be associated with pro-inflammatory activation and alveolar destruction by releasing neutrophil elastase in COPD (40–42). Therefore, hUC-MSC administration is able to reduce increasing levels of neutrophilic inflammation. Next, we observed that lymphocytes significantly increased in BALF after hUC-MSC administration. Another study showed that intranasal delivery of MSCs slightly increased lymphocytes in BALF of mice compared to the intraperitoneal route in mice with CS-induced emphysema (43). Those results pointed out that different routes and timing of MSC administration could have distinct effects on regulating immune cell populations. MSCs transiently activate T cells to preserve the antiapoptotic function (44). For example, higher lymphocyte counts were more efficient in activating MSCs in the treatment of graft vs. host disease (45). A previous study showed that that hUC-MSCs recruited the regulatory T cells in the damaged lung (46). Together, hUC-MSCs could regulate lung neutrophil infiltration and lymphocyte activation in emphysematous mice. However, more experiments should be conducted in the future to support this.

We observed that inflammatory responses of the lungs, including IL-1β, CXCL1/KC, and MMP-12, by CS decreased after administration of hUC-MSCs. Consistent with a previous study, pro-inflammatory cytokines (TNF-α, IL-1β, and monocyte chemoattractant protein-1) and proteases (MMP-9 and−12) in the lungs of rats decreased by MSC administration after CS exposure for 11 weeks (47). In addition, we found that serum levels of TNF-α, IL-1β, and IL-17A significantly decreased by hUC-MSC administration after CS exposure. TNF-α, IL-1β, and IL-17A were shown to be key mediators in recruiting neutrophils to the lungs after CS exposure (48–53). Previous studies have found that the MMP-12 liberated the neutrophil chemoattractants (e.g., TNF-α) from the macrophage, which recruited the neutrophils and released the elastase that contributes to the lung damage (41, 54–56). It was hypothesized that MSCs may protect the pulmonary matrix structure by reducing MMP and elastase productions in alveolar macrophages and neutrophils, respectively (41, 57–59). Our results showed that decreases in serum levels of neutrophil chemotactic factors, including TNF-α, IL-1β, and IL-17A by hUC-MSCs may possibly be associated with the reduction in neutrophils in the BALF of mice after CS exposure.

The emphysema severity was significantly decreased by hUC-MSCs in emphysematous mice based on the MLI and DI results. Previous studies showed a decrease in emphysematous lesions in the lungs of mice due to bone marrow (BM)-MSCs (60, 61). Other studies found that MSCs induced neutrophil apoptosis and decreased protease secretions resulting in reduced severity of COPD (62–64). In our study, one explanation for the mitigation of the emphysema was decreased levels of pro-inflammatory factors in the lungs (IL-1β and CXCL1/KC) and circulation (TNF-α, IL-1β, and IL-17A) by hUC-MSCs which may associate with the reduction of the neutrophil infiltration in emphysematous mice. In addition, the decrease in protease secretion (MMP-12) by hUC-MSCs contributed to reducing alveolar destruction. Our results suggest that hUC-MSCs may ameliorate alveolar destruction in mice after CS-induced emphysema. However, the underlying mechanisms should be investigated in the future.

Fibroblasts play an important role in regulating COPD severity. We observed that the cell viability of lung fibroblasts increased by hUC-MSC administration after CSE exposure. In addition, hUC-MSCs-CM restored collagen's contractile force in lung fibroblasts after treatment with the CSE.

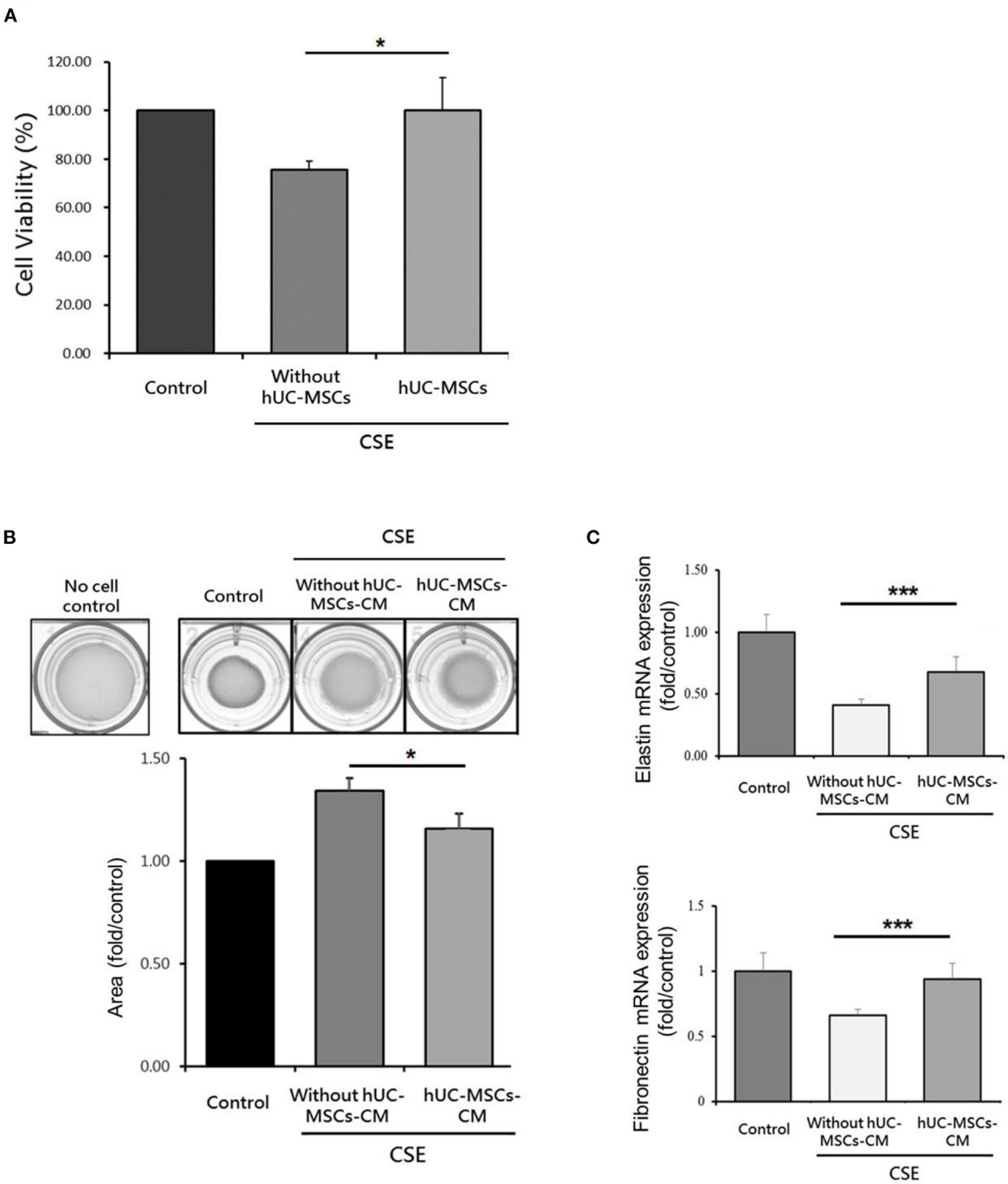

FIGURE 5 | (A) Increased cell viability of human lung fibroblasts by human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) after cigarette smoke extract (CSE) exposure. **(B)** Restoration of the contractile force of lung fibroblasts by hUC-MSCs-conditioned medium (hUC-MSCs-CM). **(C)** hUC-MSCs-CM increased mRNA expressions of elastin and fibronectin in lung fibroblasts after CSE treatment. A transwell coculture system was used to determine cell viability of human lung fibroblasts (MRC5 cell line). A significant increase in cell viability (%) of MRC5 cells by hUC-MSCs was observed after CSE treatment for 24 h. MRC-5 cells were treated by CSE for 24 h and then received hUC-MSCs-CM for 24 h afterward. The contractile force was evaluated by a collagen-based cell contraction assay. The mRNA expressions of elastin and fibronectin were quantified by a qPCR. Results were examined by an unpaired *t*-test. Four independent experiments in each group. $^{*}p < 0.05$, $^{***}p < 0.001$.

MSCs-conditioned medium (MSCs-CM) was compatible with MSCs in attenuating inflammation in bronchopulmonary dysplasia (65). A previous study showed that MSCs-CM induced lung fibroblast proliferation and restored their repair function after CSE exposure (66). Consistent with previous findings, our results showed that mRNA expressions of elastin and fibronectin

by lung fibroblasts significantly increased after treatment with hUC-MSCs-CM compared to the group treated with CSE alone. Collectively, these results suggested that paracrine factors secreted by hUC-MSCs to lung fibroblasts may be partly involved in the alveolar repair process after CS exposure.

There are a few limitations in this study. We observed an increase in lymphocytes in BALF of mice due to hUC-MSC administration. The different subgroups of lymphocytes, including regulatory T cells, were not determined in our study. In addition, interactions of hUC-MSCs with lymphocytes are not fully understood. The pulmonary function and the underlying mechanism of the hUC-MSCs in COPD will be determined in the future. Moreover, the adverse effects of fibroblasts by hUC-MSCs *in vivo* are still unclear, which should be evaluated in future work.

CONCLUSIONS

In conclusion, hUC-MSCs reduced the emphysema severity and inflammatory responses in mice with CS-induced emphysema. hUC-MSCs increased the proliferation of fibroblasts after CSE exposure. hUC-MSCs may mitigate COPD in mice after CS exposure.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the National Cheng Kung University Hospital Institutional Review Board (Tainan, Taiwan; IRB no.: A-BR-104-045). The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by Animal and Ethics Review Committee of the Laboratory Animal Center, Taipei Medical University, Taipei, Taiwan (IACUC: LAC-2017-0231).

AUTHOR CONTRIBUTIONS

H-CC and X-YC contributed to interpretation of the data and completion of the manuscript. H-CC, WL, and KC contributed substantially to the concept, design, interpretation of the data, and completion of the study and manuscript. Y-YC and C-HC contributed substantially to the completion of the study. T-CH contributed to the establishment of the cigarette smoke generation system and particle measurement. All authors contributed to critically revising the manuscript for important intellectual content and read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.713824/full#supplementary-material

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Potential Value of Expiratory CT in Quantitative Assessment of Pulmonary Vessels in COPD

Xianxian Cao[1,2†], Xiaoyan Gao[1,3†], Nan Yu[4], Meijuan Shi[5], Xia Wei[6], Xiaoqi Huang[7], Shudi Xu[6], Jiantao Pu[8], Chenwang Jin[1] and Youmin Guo[1]*

[1] Department of Radiology, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China, [2] Department of Diagnostic Imaging, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, [3] Medical Imaging Center, Shaanxi Provincial People's Hospital, Xi'an, China, [4] Department of Radiology, The Affiliated Hospital of Shaanxi University of Traditional Chinese Medicine, Xianyang, China, [5] Department of Radiology, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China, [6] Department of Respiratory Medicine, The Ninth Hospital of Xi'an Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China, [7] Department of Radiology, The Affiliated Hospital of Yan'an University, Yan'an, China, [8] Departments of Radiology and Bioengineering, University of Pittsburgh, Pittsburgh, PA, United States

Objective: To investigate the associations between intrapulmonary vascular volume (IPVV) depicted on inspiratory and expiratory CT scans and disease severity in COPD patients, and to determine which CT parameters can be used to predict IPVV.

Methods: We retrospectively collected 89 CT examinations acquired on COPD patients from an available database. All subjects underwent both inspiratory and expiratory CT scans. We quantified the IPVV, airway wall thickness (WT), the percentage of the airway wall area (WA%), and the extent of emphysema ($LAA\%_{-950}$) using an available pulmonary image analysis tool. The underlying relationship between IPVV and COPD severity, which was defined as mild COPD (GOLD stage I and II) and severe COPD (GOLD stage III and IV), was analyzed using the Student's *t*-test (or Mann-Whitney *U*-test). The correlations of IPVV with pulmonary function tests (PFTs), $LAA\%_{-950}$, and airway parameters for the third to sixth generation bronchus were analyzed using the Pearson or Spearman's rank correlation coefficients and multiple stepwise regression.

Results: In the subgroup with only inspiratory examinations, the correlation coefficients between IPVV and PFT measures were $-0.215 \sim -0.292$ ($p < 0.05$), the correlation coefficients between IPVV and WT_{3-6} were $0.233 \sim 0.557$ ($p < 0.05$), and the correlation coefficient between IPVV and $LAA\%_{-950}$ were $0.238 \sim 0.409$ ($p < 0.05$). In the subgroup with only expiratory scan, the correlation coefficients between IPVV and PFT measures were $-0.238 \sim -0.360$ ($p < 0.05$), the correlation coefficients between IPVV and WT_{3-6} were $0.260 \sim 0.566$ ($p < 0.05$), and the correlation coefficient between IPVV and $LAA\%_{-950}$ were $0.241 \sim 0.362$ ($p < 0.05$). The multiple stepwise regression analyses demonstrated that WT were independently associated with IPVV ($P < 0.05$).

Conclusion: The expiratory CT scans can provide a more accurate assessment of COPD than the inspiratory CT scans, and the airway wall thickness maybe an independent predictor of pulmonary vascular alteration in patients with COPD.

Keywords: chronic obstructive pulmonary disease (COPD), computed tomography, intrapulmonary vessels, inspiratory, expiratory

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***Correspondence:**
Chenwang Jin
jcw76@163.com

† These authors have contributed equally to this work

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is very prevalent worldwide and carries high mortality and morbidity rates (1, 2). Among COPD patients, 30–70% have clinically significant pulmonary vascular disease (3–5). The major vascular alterations are vascular remodeling and vasoconstriction caused by emphysema and/or hypoxemia, and often cause pulmonary hypertension (6, 7). There are investigations showing that pulmonary vascular alterations were found in patients with mild COPD, even in non-smokers with normal lung function (8, 9). All these suggest that pulmonary vascular alterations may persist throughout the entire progress of COPD, and it is important to develop methods to quantitatively assess the pulmonary vascular alterations in COPD.

The high-resolution characteristic of computed tomography (CT) makes it possible to visualize very detailed lung structures and quantify a variety of lung abnormalities, such as emphysema, airway remodeling, and pulmonary vascular alterations in COPD (10, 11). There have been investigative efforts made to quantitatively assess pulmonary vascular alterations in COPD. Matsuoka et al. (12) proposed the total cross-sectional area (CSA) of small pulmonary vessels as an index of pulmonary vascular alterations. They reported that %CSA<5 mm^2 had a significant correlation with forced expiratory volume in 1 s (FEV_1) and FEV_1/forced vital capacity (FVC) as well as $\%LAA_{-950}$ in severe COPD. Previous studies (13–15) have demonstrated that there were quantitative pulmonary

TABLE 1 | Patient Characteristics and PFT results in the COPD subjects.

Characteristic	COPD subjects ($n = 89$)
Age (years)	63.6 ± 9.4
Sex, %female	19 (21.35%)
BMI (kg/m^2)	22.76 ± 3.59
GOLD stage I:II:III:IV	12:31:28:18
FEV_1/FVC%	51.45 ± 9.75
FEV_1%	47.00 (32.85)

BMI, body mass index; FEV_1/FVC, ratio of forced expiratory volume in 1 s to forced vital capacity; FEV_1, percentage predicted forced expiratory volume in 1 s.

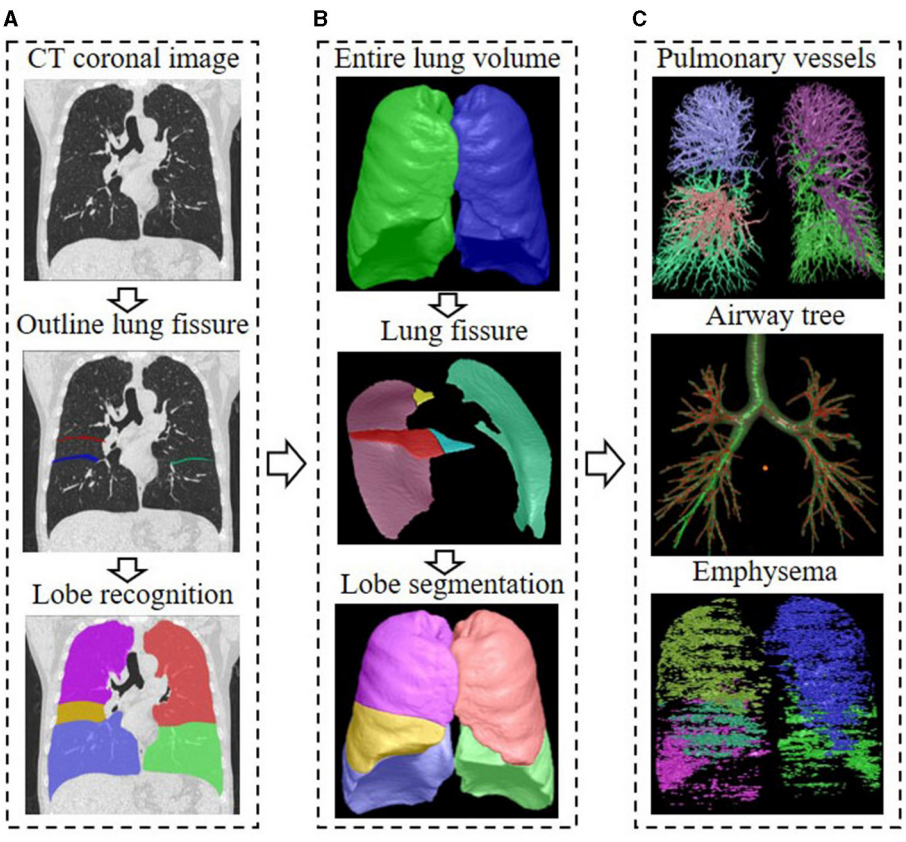

FIGURE 1 | Flow chart of CT quantitative parameter measurement. **(A)** The delineation of lung fissures and the identification of lung lobes on CT images; **(B)** The total lung volume identified by the 3D adaptive border marching algorithm, the lung fissure segmented by the computational geometry approach, and the five lung lobes segmented by implicit surface functions; **(C)** The principal curvatures and the principal directions were used to distinguish pulmonary vessels from lung tissue, and the vascular tree was automatically extracted and segmented to calculate IPVV in the whole lung and each individual lobe; The differential geometric approach to segment the airway tree, and the average values of the measurements for the 3–6th bronchial generation were automatically calculated; The extent of emphysema under the threshold of −950 HU was automatically computed, the area shown in color.

TABLE 2 | Comparisons of IPVV between mild and severe COPD.

		Mild COPD ($n = 43$)	Severe COPD ($n = 46$)	t/z-value	P-value
Inspiration IPVV					
	WL	168.94 ± 44.12	176.78 ± 48.61	−0.796	0.428
	RL	87.66 (35.42)	92.83 ± 25.05	−0.452	0.652
	LL	77.12 ± 22.36	85.15 ± 24.04	−1.628	0.107
	RUL	33.68 (15.16)	33.07 (15.26)	−0.164	0.87
	RML	12.36 (5.69)	14.13 ± 5.42	−1.355	0.176
	RLL	43.41 ± 12.46*	43.69 ± 13.11*	−0.105	0.917
	LUL	38.37 (14.95)	40.29 ± 12.98	−0.435	0.663
	LLL	37.58 ± 13.24	45.65 ± 13.84	−2.809	0.006
Expiration IPVV					
	WL	145.37 ± 49.68	171.18 ± 45.11	−2.568	0.012
	RL	79.46 ± 25.73	90.46 ± 22.90	−2.133	0.036
	LL	65.91 ± 26.06	81.93 ± 22.98	−3.081	0.003
	RUL	29.57 (16.28)	31.50 (11.32)	−1.067	0.286
	RML	10.87 (5.00)	14.32 ± 5.54	−2.451	0.014
	RLL	35.69 ± 15.26	41.58 ± 12.56*	−1.993	0.049
	LUL	34.97 ± 13.99	39.53 ± 12.33	−1.635	0.106
	LLL	30.94 ± 16.01	43.34 ± 14.83	−3.793	<0.001
Difference Value					
	WL	17.19 (32.77)	4.72 (16.95)	−3.966	<0.001
	RL	11.00 (16.12)	1.71 (10.60)	−3.834	<0.001
	LL	8.82 (13.97)	1.00 (8.16)	−3.53	<0.001
	RUL	3.15 (4.82)	0.10 (3.23)	−3.875	<0.001
	RML	0.72 (1.76)	−0.19 ± 1.24	−3.654	<0.001
	RLL	4.80 (12.39)*	0.93 (5.09)	−3.296	0.001
	LUL	4.58 ± 5.60	0.78 (3.82)	−3.851	<0.001
	LLL	5.54 (7.52)	2.07 (6.17)	−3.206	0.001
Relative Value					
	WL	1.10 (0.24)	1.03 (0.10)	−4.335	<0.001
	RL	1.14 (0.25)	1.02 (0.12)	−4.171	<0.001
	LL	1.11 (0.30)	1.02 (0.09)	−3.966	<0.001
	RUL	1.10 (0.21)	1.00 (0.10)	−3.982	<0.001
	RML	1.09 (0.13)	1.01 (0.11)	−3.752	<0.001
	RLL	1.14 (0.36)	1.02 (0.12)	−3.465	0.001
	LUL	1.11 (0.20)	1.02 (0.10)	−3.998	<0.001
	LLL	1.14 (0.55)	1.04 (0.13)	−3.563	<0.001

IPVV, intrapulmonary vascular volume; COPD, chronic obstructive pulmonary disease; WL, the whole lung; RL, the right lung; LL, the left lung; RUL, the right upper lobe; RML, the right middle lobe; RLL, the right lower lobe; LUL, the left upper lobe; LLL, the left lower lobe.
**Difference of IPVV between RUL and RLL, $P < 0.05$.*

vascular features, such as the percentage of total vessel area and the number of small vessels, closely associated with survival and PFT measures in COPD patients. It is notable that most of the available investigations about pulmonary vascular alternation were limited to the inspiratory CT scans. Although there are studies (16–18) demonstrating the unique value of expiratory CT examinations in assessing COPD, it is unclear whether the expiratory CT scans have any advantage over inspiratory CT scans in assessing pulmonary vascular alternation.

In this study, we proposed to quantify the intrapulmonary vascular volume (IPVV) depicted on CT images in COPD patients. The objective is to study whether pulmonary vascular alternations in COPD subjects are associated with emphysema extent, pulmonary functions, and airway abnormalities, and to determine which parameter can be used as predictor of IPVV in COPD patients. In particular, we performed the analyses on both inspiratory and expiratory CT scans, aiming to clarify the potential of expiratory CT examinations in assessing pulmonary vascular alternations in COPD. For this purpose, we established a dataset consisting of 89 paired inspiration-expiration CT scans. A detailed description of our dataset, methods, and experimental results follows.

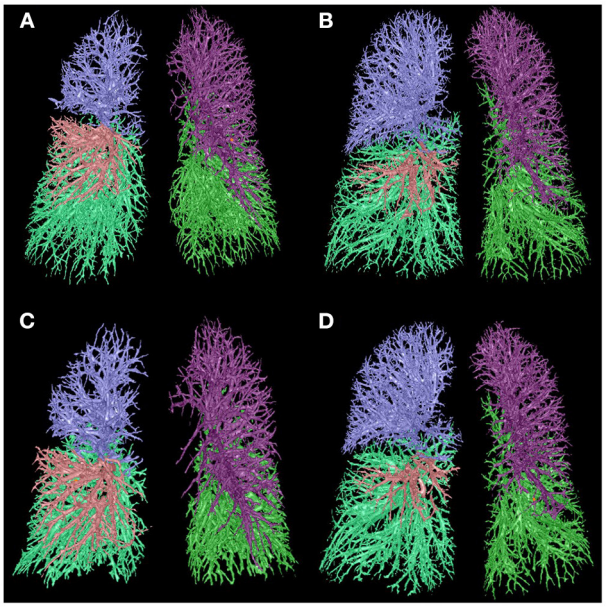

FIGURE 2 | Comparison of IPVV between mild and severe COPD in inspiratory and expiratory CT. **(A,C)** A male with mild COPD (GOLD II, 68 years): a, the IPVV of the whole lung in inspiratory CT is 209.95 ml; b, the IPVV of the whole lung in expiratory CT is 169.10 ml. **(B,D)** A male with severe COPD (GOLD III, 64 years): a, the IPVV of the whole lung in inspiratory CT is 208.31 ml; b, the IPVV of the whole lung in expiratory CT is 201.18 ml.

MATERIALS AND METHODS

Study Population

We retrospectively identified 92 patients from the "Digital Lung" Respiratory Disease Evaluation System and Diagnostic Criteria (201402013). These subjects were diagnosed with COPD and underwent both inspiratory and expiratory CT examinations. COPD was diagnosed on the basis of past history, physical examination, and spirometry data by following the Global Initiative for Chronic Obstructive Lung Disease (GOLD) (1) diagnostic criteria ($FEV_1/FVC < 70\%$ bronchodilators inhaled). Among the collected subjects, three were excluded, because of the involved issues: (1) concomitant lung diseases such as interstitial lung disease, lung cancer, infectious pneumonia, and pulmonary tuberculosis; (2) previous lung surgery; (3) insufficient CT quality of analysis; and (4) unable to complete the pulmonary function test. As a result, we have 89 subjects involved in this study and the demographics information was summarized in **Table 1**. All subjects were divided into subjects with mild COPD (GOLD I and II, $n = 43$) and subjects with severe COPD (GOLD III and IV, n=46) for comparison of IPVV. This retrospective study was approved by the Chinese Clinical Research Registry (Grant No.: ChiCTR-OCH-14004904), and written informed consent was obtained from all subjects.

Pulmonary Function Tests

All subjects underwent spirometry according to American Thoracic Society/European Thoracic Society guidelines (19). PFT measurements included forced expiratory volume during the first second of exhalation (FEV_1) percent to the predicted value (FEV_1%predicted) post inhalation of 200 μg salbutamol, FEV_1/forced vital capacity ratio (FEV_1/FVC), the ratio of residual volume to total lung capacity(RV%TLC) and the diffusing capacity for carbon monoxide (DL_{CO}). Referring to previous studies (20, 21), we only used the FEV_1% predicted and FEV_1/FVC in the subsequent analysis in this study.

CT Scan Acquisition

The CT examinations were performed at full inspiration and expiration states for the involved subjects in the supine position using 64-slice multi-detector CT scanners (SOMATOM Definition AS; Siemens, Erlangen, Germany). All subjects were given breathing training prior to examination. The scan parameters were as follows: tube voltage: 100 or 120 KV tube current, autoexposure control, exposure time: 0.5 s, and the matrix size: 512 × 512 pixels. Images were reconstructed with a 1 mm slice thickness (with 0.625 mm overlap) using a standard kernel algorithm.

Image Processing

We analyzed the CT scans using the FACT-Digital Lung Workstation (Dexin, Xi'an, China), which have both US FDA 510 K and CFDA cleared. This software system enables automated segmentation of a variety of lung structures, including right/left lungs, lung vessels, airway trees, inner/outer airway walls. On the basis of the segmentations, an automatically 3D approach was used to reconstruct the pulmonary vasculature and calculate the entire volume of the intrapulmonary vascular volume (IPVV) in the whole lung or each individual lobe. In inspiratory and expiratory CT, the measures of IPVV all includes the vascular wall and lumen of both arteries and veins, which is specified in milliliter (ml). We also measured the airway wall thickness (WT), and the percentage of the airway wall area (WA%) of the 3–6th generations and the extent of emphysema in each individual lobe of both inspiratory and expiratory CT examinations. The extent of emphysema, which is defined as the percentage of lung area with CT attenuation values <-950 HU at inspiration ($LAA\%_{-950}$), was also automatically computed at a threshold of −950 Hounsfield Unit (HU). The difference in the values between inspiratory and expiratory scans was defined as difference value, the ratio of inspiratory scans to expiratory was defined as relative value. Detailed descriptions of these computerized schemes have been reported elsewhere (22–24), and the segmentation results were shown in **Figure 1**.

Statistical Analysis

We assessed the correlations of IPVV with PFT measures, WT_{3-6}, and $LAA\%_{-950}$ using Pearson or Spearman's rank correlation analysis and multiple linear regression analysis with step-wise selection method for inspiratory and expiratory CT. Continuous data were tested for normality using the Shapiro-Wilk or Kolmogorov-Smirnov test according to the number of subjects. Data meeting the normal distribution were expressed as mean ± SD. Non-normally distributed data were expressed as median (interquartile range). The comparison of IPVV between mild COPD (GOLD stage I and II) and

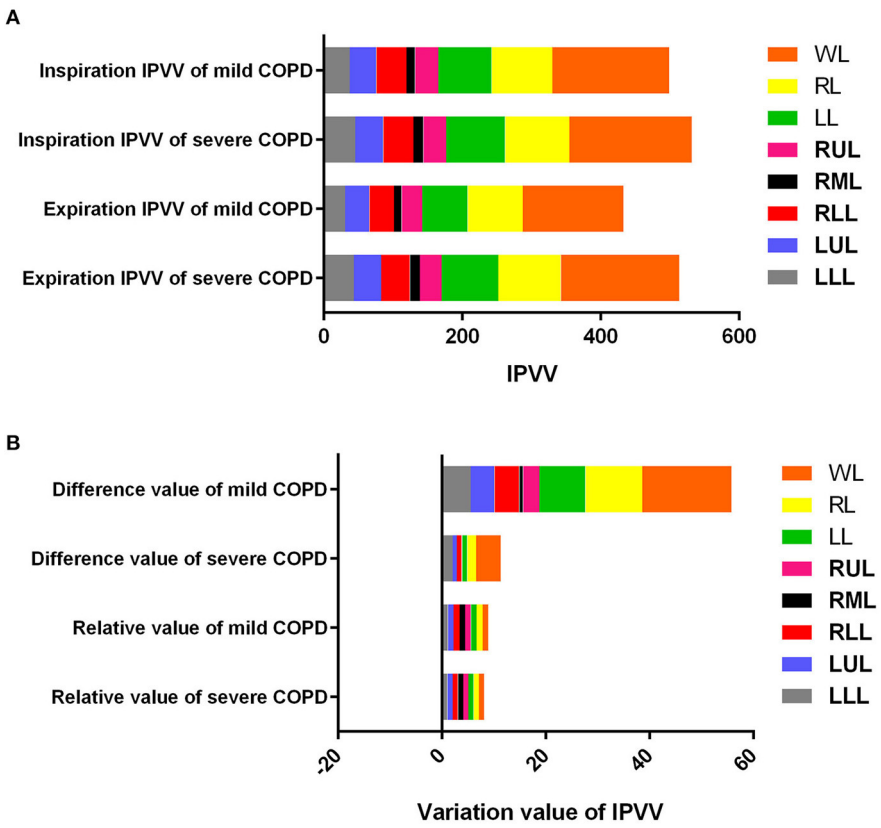

FIGURE 3 | Comparison of IPVV between mild and severe COPD in inspiratory, expiratory CT, difference value and relative value. **(A)** Comparison in inspiratory and expiratory CT; **(B)** Comparison in difference value and relative value.

severe COPD (GOLD stage III and IV) was analyzed using the Student's *t*-test or Mann-Whitney *U*-test. Statistical analysis was performed using SPSS 20.0. A p-value < 0.05 was considered statistically significant.

RESULTS

The comparison of IPVV between mild and severe COPD in inspiratory and expiratory CT were summarized in **Table 2** and **Figures 2**, **3**. In the subgroup with only expiratory CT examinations, there were significant differences of IPVV between mild and severe COPD groups, except for the right upper lobe (RUL, $p = 0.286$) and left upper lobe (LUL, $p = 0.106$). In contrast, in the subgroup with only inspiratory CT examinations, only the IPVV value in the left lower lobe (LLL, $p = 0.006$) showed a difference regardless COPD severity. The IPVV values of the lower lobes were consistently higher than those of the upper lobes in both inspiratory and expiratory CT scans. For the difference values and relative values, the changes of IPVV in the severe COPD groups were significantly less than the mild.

TABLE 3 | Correlation between IPVV and PFT.

Pulmonary vascular measurement	Spirometry	
	FEV_1/FVC	$FEV_1\%$
Inspiration		
$IPVV_{RUL}$	−0.289 (0.006)	−0.046 (0.666)
$IPVV_{RML}$	−0.280 (0.008)	−0.246 (0.020)
$IPVV_{RLL}$	−0.215 (0.043)	−0.062 (0.562)
$IPVV_{LUL}$	−0.283 (0.007)	−0.094 (0.383)
$IPVV_{LLL}$	−0.292 (0.005)	−0.230 (0.030)
Expiration		
$IPVV_{RUL}$	−0.318 (0.002)	−0.168 (0.117)
$IPVV_{RML}$	−0.346 (0.001)	−0.360 (0.001)
$IPVV_{RLL}$	−0.238 (0.024)	−0.202 (0.057)
$IPVV_{LUL}$	−0.326 (0.002)	−0.243 (0.022)
$IPVV_{LLL}$	−0.292 (0.005)	−0.297 (0.005)
Difference Value		
$IPVV_{RUL}$	0.157 (0.143)	0.393 (<0.001)

(Continued)

TABLE 3 | Continued

Pulmonary vascular measurement	Spirometry	
	FEV_1/FVC	FEV_1%
$IPVV_{RML}$	0.137 (0.202)	0.353 (0.001)
$IPVV_{RLL}$	0.176 (0.099)	0.338 (0.001)
$IPVV_{LUL}$	0.162 (0.130)	0.406 (<0.001)
$IPVV_{LLL}$	0.173 (0.106)	0.311 (0.003)
Relative Value		
$IPVV_{RUL}$	0.200 (0.060)	0.412 (<0.001)
$IPVV_{RML}$	0.211 (0.047)	0.391 (<0.001)
$IPVV_{RLL}$	0.197 (0.064)	0.367 (<0.001)
$IPVV_{LUL}$	0.226 (0.033)	0.444 (<0.001)
$IPVV_{LLL}$	0.228 (0.032)	0.359 (0.001)

FEV_1/FVC, ratio of forced expiratory volume in 1 s to forced vital capacity; FEV_1, percentage predicted forced expiratory volume in 1 s; IPVV, the intrapulmonary vascular volume; All P-values were presented in parentheses.

The correlations between IPVV and PFT measures were presented in **Table 3**. For the inspiratory CT scan, there were mild negative correlations between IPVV and FEV_1/FVC in each individual lobes ($r = -0.215$ to -0.292, all $p < 0.05$), between IPVV and FEV_1% in right middle lobe (RML, $r = -0.246$, $p = 0.020$) and LLL($r = -0.230$, $p = 0.030$). $LAA\%_{-950}$ ($r = 0.221$ to 0.409, all $p < 0.05$) and WT_{3-6th} ($r = 0.233$ to 0.557, all $p < 0.05$) were significantly associated with IPVV in all lobes (see **Figures 4**, **5** and **Table 4**). In particular, the strongest correlation was consistently observed for right lower lobe (RLL) and LLL. IPVV had no association WA%, except for $WA\%_{4-5th}$ in RML ($r = -0.272$, -0.236, respectively, $p < 0.05$) and $WA\%_{6th}$ in LUL($r = -0.219$, $p = 0.045$).

For expiratory CT scans, FEV_1/FVC ($r = -0.238$ to -0.346, $p < 0.05$) and FEV_1% ($r = -0.243$ to -0.360, all $p < 0.05$) had a significant, mild-to-moderate negative correlation with IPVV, except for FEV_1% in RUL and RLL. $LAA\%_{-950}$ and WT_{3-6th} (except for WT_{5-6th} in RML) positively correlated with IPVV (see **Figures 6**, **7** and **Table 4**). Similar to the inspiratory

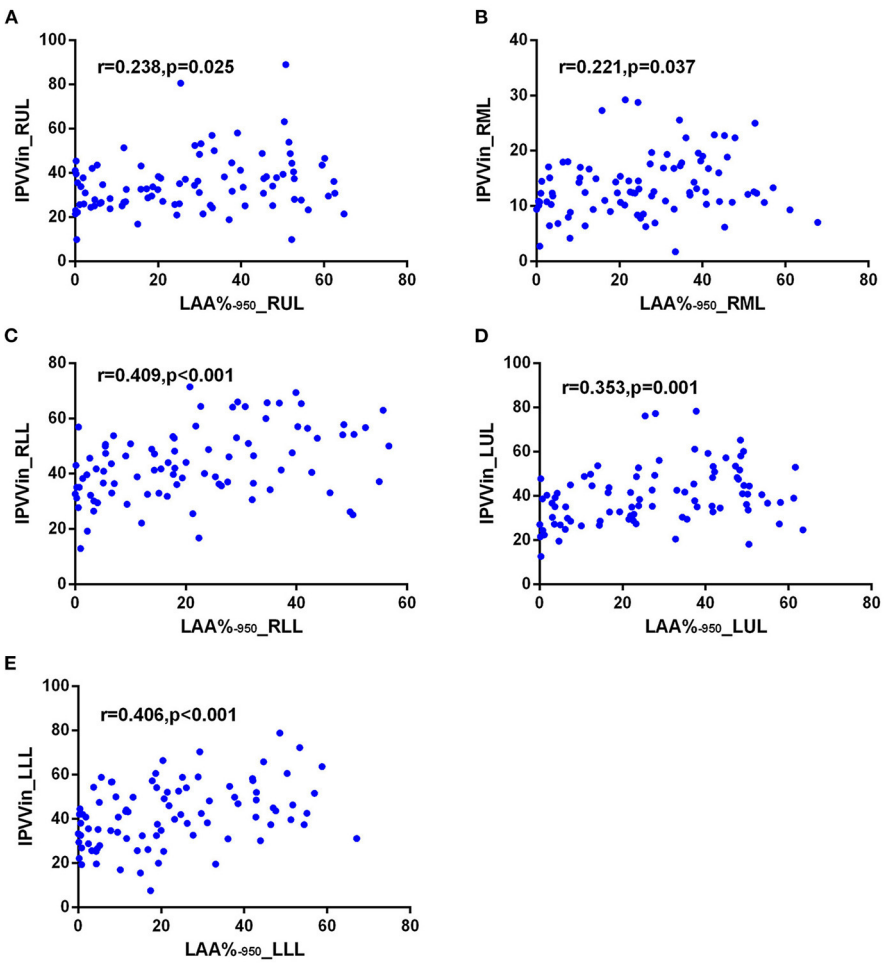

FIGURE 4 | Correlations of IPVV in individual lobes with $LAA\%_{-950}$ in the inspiratory CT scan. **(A)** RUL; **(B)** RML; **(C)** RLL; **(D)** LUL; **(E)** LLL.

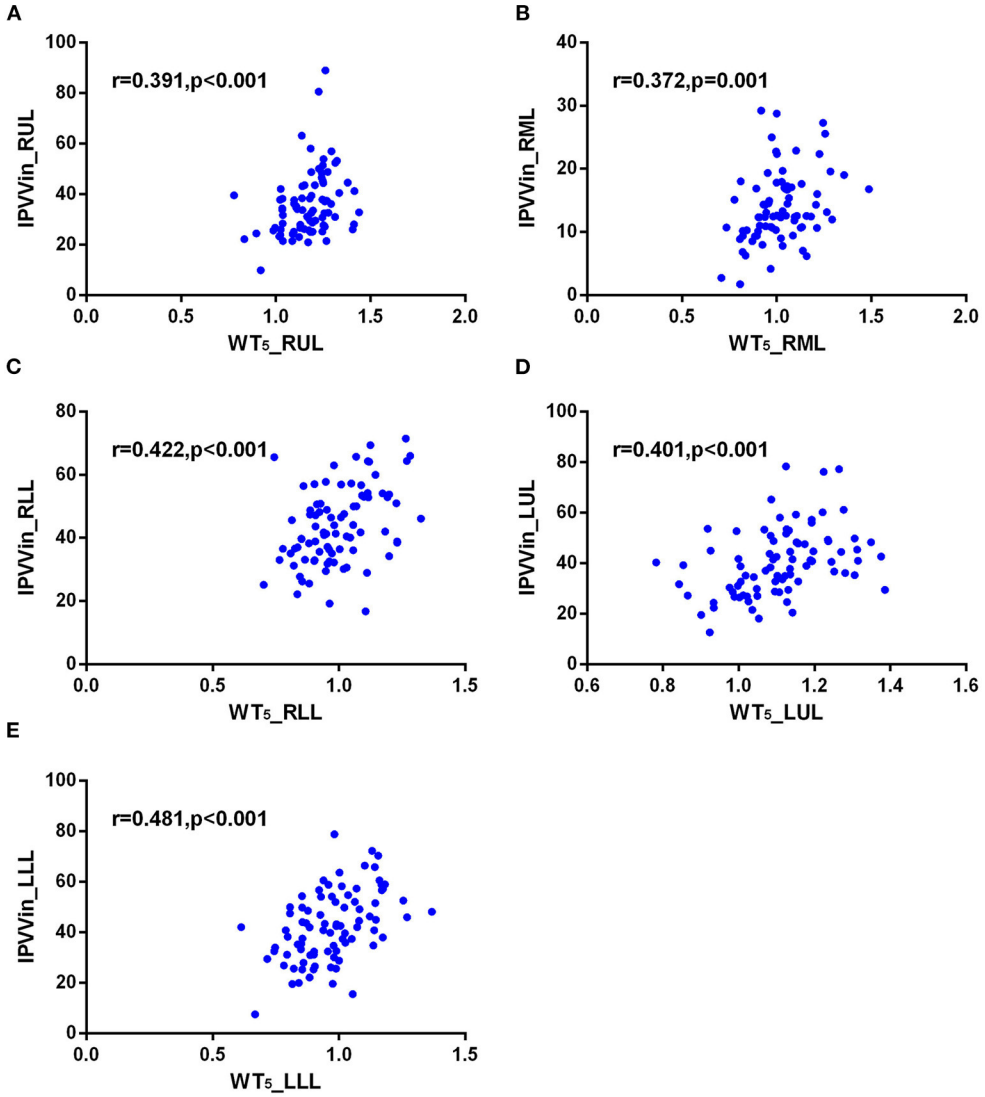

FIGURE 5 | Correlations of IPVV in individual lobes with WT_5 in the inspiratory CT scan. **(A)** RUL; **(B)** RML; **(C)** RLL; **(D)** LUL; **(E)** LLL.

CT, IPVV had no association with WA%, except for $WA\%_{3th}$ ($r = -0.266$, $p = 0.016$) in LLL. The correlation coefficients of the expiratory CT were slightly higher than that of the inspiratory CT.

For the respiratory variation, $FEV_1\%$ showed significant, moderate positive correlations with the difference value and relative value ($r = 0.350$–0.463, all $p < 0.05$), and FEV_1/FVC showed mild positive correlations with the relative value (except for RUL and RLL, $p > 0.05$). There was no correlation between FEV_1/FVC and the difference value.

Table 5 shows the results of multiple linear regression analysis, where IPVV was the dependent variable, age, BMI and other CT parameters were the independent variables. In inspiratory CT, the R^2 values of each pulmonary lobe regression equation were within the range of 0.075–0.426, while the R^2 was 0.165–0.559 in the expiratory, except for RML. The R^2 value of each lobe in the expiratory was higher than the inspiratory. The largest R^2 was observed at LLL in two respiratory phases, while the least is RML. The multiple regression analysis for IPVV revealed that WT was a significant independent predictor of IPVV at the inspiratory and expiratory CT, particularly in difference value and relative value.

DISCUSSION

In this study, we quantified IPVVs depicted on both inspiratory and expiratory CT scans and investigated their associations with pulmonary functions, airway remodeling, and disease severity in

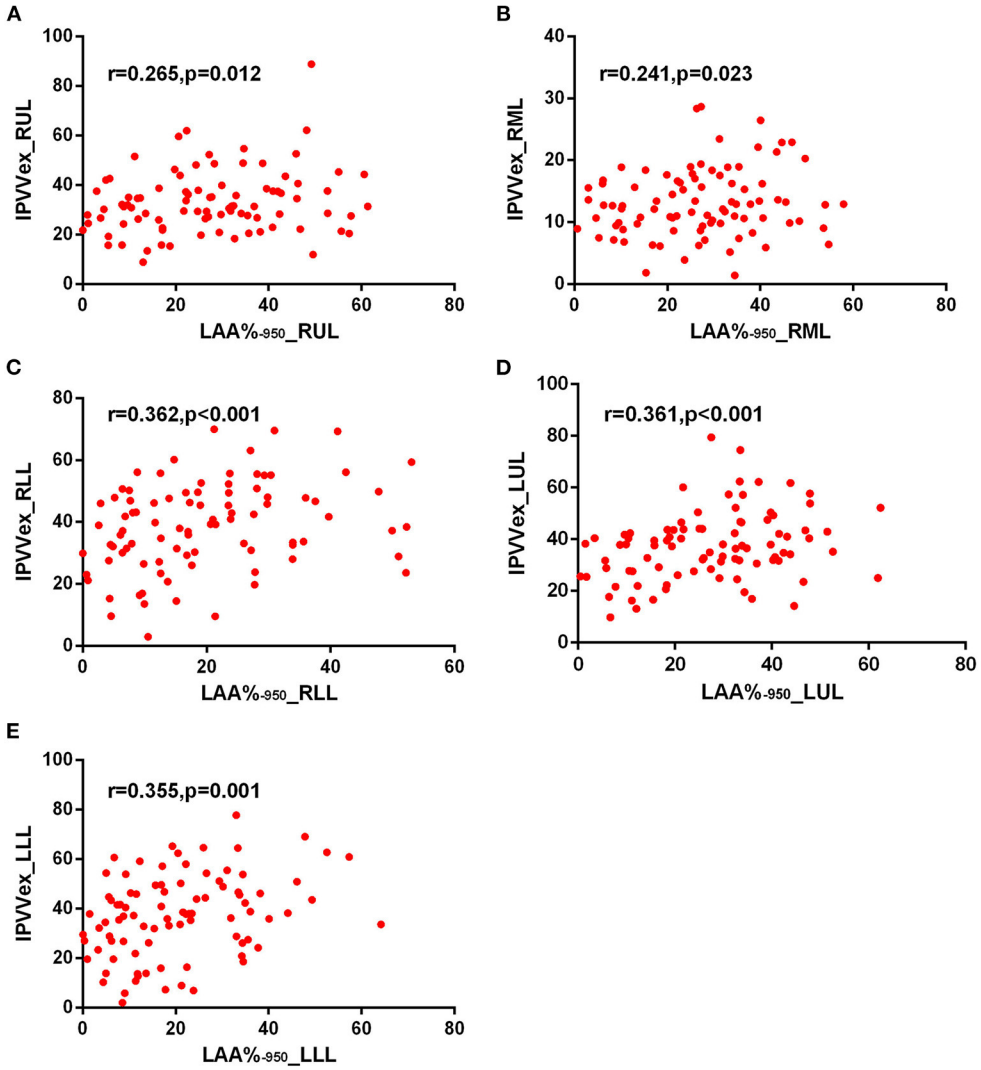

FIGURE 6 | Correlations of IPVV in individual lobes with LAA%$_{-950}$ in the expiratory CT scan. **(A)** RUL; **(B)** RML; **(C)** RLL; **(D)** LUL; **(E)** LLL.

COPD patients. The analyses were performed at the levels of the entire lungs and individual lobes. Our experimental results showed that the IPVV could serve as a quantitative index for pulmonary vascular alternations in COPD patients. In particular, the IPVVs quantified using expiratory CT examinations does not only provide a more reliable and accurate assessment of pulmonary vascular alternations and COPD as well as their progression than the inspiratory CT examinations, but also allows the calculation of the difference and relative value to show the dynamic changes of IPVV during respiration. Additionally, the multiple linear regression analyses showed that bronchial wall thickness had significant correlation with IPVV and suggested that WT might be an independent predictor of pulmonary vascular alteration in COPD.

Our findings are in consistent with Estepar et al.'s investigation (25), where there was no association between COPD severity and total blood vessel volume depicted on inspiratory CT scans. In contrast, in the expiratory CT examinations, the IPVV in severe COPD subgroup were significantly higher than those in the mild COPD subgroup at the levels of in the entire lung and individual lobes except for RUL and LUL. The difference value and relative value between inspiratory and expiratory CT revealed that the alteration of severe COPD was less than the mild. The results demonstrated that pulmonary vascular alternations between breaths decreased with the increase of COPD severity.

Stronger correlations were found between IPVV and PFT in the expiratory CT compared with the inspiratory CT. This finding reinforces the viewpoint about the value of expiratory CT in COPD patients proposed by previous studies (17, 26). Matsuoka et al. (27) reported that the correlation coefficients between airway luminal area measured at expiratory CT and PFT

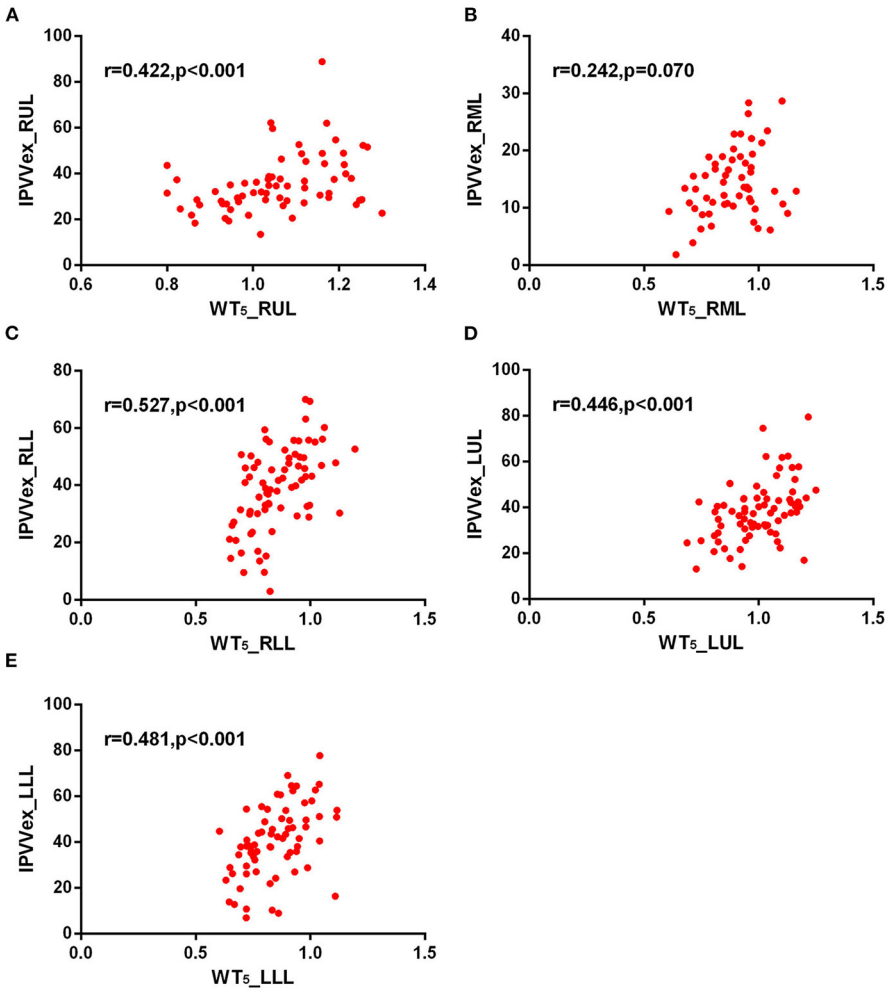

FIGURE 7 | Correlations of IPVV in individual lobes with WT_5 in the expiratory CT scan. **(A)** RUL; **(B)** RML; **(C)** RLL; **(D)** LUL; **(E)** LLL.

TABLE 4 | Correlations between IPVV and other parameters.

Pulmonary vascular measurement	Emphysema index	Airway measurements							
	$LAA\%_{-950}$	$WA\%_3$	$WA\%_4$	$WA\%_5$	$WA\%_6$	WT_3	WT_4	WT_5	WT_6
Inspiration									
$IPVV_{RUL}$	0.238 (0.025)	−0.099 (0.362)	−0.124 (0.256)	−0.062 (0.575)	−0.157 (0.176)	0.233 (0.030)	0.257 (0.017)	0.391 (<0.001)	0.415 (<0.001)
$IPVV_{RML}$	0.221 (0.037)	−0.142 (0.192)	−0.272 (0.013)	−0.236 (0.037)	−0.101 (0.437)	0.280 (0.009)	0.258 (0.019)	0.372 (0.001)	0.257 (0.043)
$IPVV_{RLL}$	0.409 (<0.001)	−0.109 (0.311)	0.045 (0.684)	0.100 (0.364)	0.219 (0.045)	0.448 (<0.001)	0.460 (<0.001)	0.422 (<0.001)	0.557 (<0.001)
$IPVV_{LUL}$	0.353 (0.001)	−0.027 (0.802)	−0.046 (0.678)	0.031 (0.776)	−0.021 (0.852)	0.383 (<0.001)	0.351 (0.001)	0.401 (<0.001)	0.425 (<0.001)
$IPVV_{LLL}$	0.406 (<0.001)	−0.036 (0.745)	0.079 (0.470)	0.181 (0.096)	0.087 (0.434)	0.440 (<0.001)	0.487 (<0.001)	0.481 (<0.001)	0.432 (<0.001)
Expiration									
$IPVV_{RUL}$	0.265 (0.012)	0.137 (0.218)	−0.030 (0.793)	−0.144 (0.248)	0.073 (0.589)	0.370 (0.001)	0.394 (<0.001)	0.422 (<0.001)	0.418 (0.001)
$IPVV_{RML}$	0.241 (0.023)	−0.160 (0.157)	−0.186 (0.124)	−0.167 (0.213)	−0.093 (0.631)	0.308 (0.005)	0.260 (0.030)	0.242 (0.070)	0.256 (0.181)
$IPVV_{RLL}$	0.362 (<0.001)	−0.027 (0.807)	−0.091 (0.425)	0.153 (0.184)	0.158 (0.182)	0.529 (<0.001)	0.383 (<0.001)	0.527 (<0.001)	0.504 (<0.001)
$IPVV_{LUL}$	0.361 (0.001)	0.040 (0.717)	0.088 (0.437)	−0.051 (0.665)	−0.048 (0.718)	0.417 (<0.001)	0.455 (<0.001)	0.446 (<0.001)	0.566 (<0.001)
$IPVV_{LLL}$	0.355 (0.001)	−0.266 (0.016)	0.139 (0.238)	−0.178 (0.140)	0.199 (0.146)	0.474 (<0.001)	0.441 (<0.001)	0.481 (<0.001)	0.535 (<0.001)

IPVV, the intrapulmonary vascular volume; $LAA\%_{-950}$, the percentage of lung area with CT attenuation values<-950HU; WT_{3-6}, airway wall thickness of the 3–6th generations; $WA\%_{3-6}$, the percentage of the airway wall area of the 3–6th generations. All P-values were presented in parentheses.

TABLE 5 | Multiple linear regression analysis of IPVV in inspiratory CT, expiratory CT, difference value and relative value.

Inspiratory IPVV		***β***	**CI**	***P*-value**
RUL ($R^2 = 0.160$)	BMI	−0.88	(−1.75, −0.02)	0.045
	WT_6	39.86	(14.95, 64.77)	0.002
RML ($R^2 = 0.075$)	WT_5	10.05	(0.40, 19.69)	0.042
RLL ($R^2 = 0.373$)	$LAA\%_{-950}$	0.21	(0.06, 0.35)	0.006
	WT_6	46.45	(27.91, 65.00)	<0.001
LUL ($R^2 = 0.189$)	WT_5	46.80	(24.17, 69.44)	<0.001
LLL ($R^2 = 0.426$)	Age	−0.38	(−0.66, −0.11)	0.007
	$LAA\%_{-950}$	0.18	(0.04, 0.33)	0.016
	$WA\%_4$	−46.00	(−81.93, −10.07)	0.013
	WT_4	50.17	(30.98, 69.37)	<0.001
Expiratory IPVV		***β***	**CI**	***P*-value**
RUL ($R^2 = 0.165$)	WT_6	41.22	(15.11, 67.33)	0.003
RLL ($R^2 = 0.439$)	Age	−0.32	(−0.63, −0.01)	0.045
	$LAA\%_{-950}$	0.29	(0.08, 0.50)	0.007
	$WA\%_4$	−61.44	(−108.07, −14.81)	0.011
	WT_5	67.73	(43.21, 92.24)	<0.001
LUL ($R^2 = 0.330$)	WT_5	61.69	(37.69, 85.68)	<0.001
LLL ($R^2 = 0.559$)	Age	−0.50	(−0.83, −0.16)	0.005
	$WA\%_3$	−95.39	(−144.02, −46.75)	0.001
	WT_6	67.83	(41.88, 93.79)	<0.001
	$LAA\%_{-950}$	0.24	(0.04, 0.45)	0.021
Difference Value		***β***	**CI**	***P*-value**
RUL ($R^2 = 0.196$)	WT_6	18.42	(7.60, 29.24)	0.001
RML ($R^2 = 0.174$)	WT_3	4.97	(0.30, 9.65)	0.038
RLL ($R^2 = 0.247$)	WT_5	27.92	(15.73, 40.10)	<0.001
LUL ($R^2 = 0.191$)	WT_4	14.05	(5.93, 22.17)	0.001
LLL ($R^2 = 0.283$)	Age	0.22	(0.04, 0.41)	0.017
	WT_4	22.50	(10.71, 34.29)	<0.001
Relative Value		***β***	**CI**	***P*-value**
RUL ($R^2 = 0.359$)	WT_6	0.39	(0.03, 0.76)	0.035
	$LAA\%_{-950}$	−0.11	(−0.19, −0.04)	0.004
	WT_4	0.40	(0.03, 0.76)	0.035
RML ($R^2 = 0.385$)	WT_3	0.79	(0.32, 1.26)	0.002
	$WA\%_3$	−0.93	(−1.70, −0.17)	0.019
RLL ($R^2 = 0.119$)	WT_5	1.54	(0.49, 2.59)	0.005
LUL ($R^2 = 0.253$)	WT_3	0.49	(0.25, 0.72)	<0.001
LLL ($R^2 = 0.311$)	WT_5	1.47	(0.79, 2.15)	<0.001
	$WA\%_5$	−1.11	(−1.78, −0.45)	0.002

BMI, body mass index; IPVV, the intrapulmonary vascular volume; $LAA\%_{-950}$, the percentage of lung area with CT attenuation values<-950 HU; WT_{3-6}, airway wall thickness of the 4–6th generations; $WA\%_{3-4}$, the percentage of the airway wall area of the 3–4th generations.

were higher than those for inspiratory CT. Gawlitza et al. (17) demonstrated that quantitative CT parameters of emphysema such as mean lung density and low attenuation volume in expiratory phase show stronger correlation with lung function testing than the inspiratory. Nevertheless, there are few studies on the expiratory CT involving pulmonary blood vessels. Our study not only verified higher correlations in expiratory CT but also found significant correlations between $FEV_1\%$ and the difference, relative values. Compared with the difference value, the relative value may be the better indicator of changes in pulmonary vessels during respiration. From a pathophysiological standpoint, this may be explained by promotion of pulmonary vasoconstriction and remodeling by expiratory state in patients with airflow obstruction (6).

We in particular investigated the association between pulmonary vascular disease and airway disease. Very limited investigations (28) have been conducted in this regard. We found that the airway wall thickness correlated positively with the IPVV on both inspiratory and expiratory CT. Our finding indicated that vascular alteration in COPD was influenced by

both emphysema and airway remodeling, and the multiple linear regression analyses of inspiratory, expiratory CT, the difference and relative value showed that the main parameter able to explain pulmonary vascular alteration in patients with COPD was WT, which was similar in part to the result reported by Coste et al. (29). Furthermore, the higher correlations in the expiratory CT demonstrated that the expiratory CT had potential value in quantitative pulmonary vascular disease and evaluating the severity and progress of COPD, compared with the inspiratory CT.

When evaluating the distribution of pulmonary vascular alteration in different lobes, Wrobel et al. (30) quantified the percentage wall thickness to vessel diameter and showed that there was increased pulmonary arterial remodeling in the upper lobes compared with the lower lobes in subjects with COPD. Our results were in consistent with Estepar et al.'s (25) but contradict with Wrobel et al.'s (30) in that the IPVV of the lower lobes was higher than that of the upper lobes. However, this study failed to observe significant difference in IPVV between LUL and LLL. This may be due to the influence of cardiac motion in the left lung, resulting in some errors in IPVV measurement (31) and the limitation of the small datasets. Additional efforts are needed to verify this.

We are aware that the primary limitations with this study is the relatively small dataset for the analyses. There is significant imbalance with the study population in many aspects, such as gender, disease severity, and lung functions. All these along with other potential confounding factors (e.g., image quality and acquisition protocols) could unavoidably lead to some biases in both conclusion and analyses, and this may also be the reason why the correlation coefficient of this study is small. Nevertheless, the findings in this study suggest the unique potential of expiratory CT scans in analyzing pulmonary vascular alternations and the potential association of pulmonary vascular alternations with COPD and other airway diseases.

In conclusion, the quantitative parameter IPVV demonstrated significant associations with PFT, emphysema and airway disease in patients with COPD, the expiratory CT and the relative values showed potential values in quantifying pulmonary vascular alterations and evaluating the severity of COPD. Additionally, the airway wall thickness may be the independent predictor of pulmonary vascular alteration in COPD. Further work is required to clarify and validate the exact value of expiratory CT in quantitative pulmonary vessels in COPD with advanced quantitative technique.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Chinese Clinical Research Registry (Grant No.: ChiCTR-OCH-14004904) and written informed consent was obtained from all subjects. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XC, CJ, and YG conceived of the idea. XC conducted statistical analyses. XG, NY, XW, and XH collected the data. XC and XG wrote the manuscript with inputs from all authors. All authors contributed to the article and approved the submitted version.

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Effects of Exercise Intervention on Peripheral Skeletal Muscle in Stable Patients With COPD: A Systematic Review and Meta-Analysis

Peijun Li[1], *Jian Li*[1], *Yingqi Wang*[1], *Jun Xia*[1]* *and Xiaodan Liu*[2,3]*

[1] *Department of Sports Rehabilitation, Shanghai University of Sport, Shanghai, China,* [2] *School of Rehabilitation Science, Shanghai University of Traditional Chinese Medicine, Shanghai, China,* [3] *Institute of Rehabilitation Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai, China*

Objectives: Peripheral skeletal muscle dysfunction is an important extrapulmonary manifestation of chronic obstructive pulmonary disease (COPD) that can be counteracted by exercise training. This study aimed to review the effect of three major exercise training modalities, which are used in pulmonary rehabilitation to improve on skeletal muscle mass, function, and exercise capacity in COPD.

Methods: PubMed, Embase, EBSCO, Web of Science, and the PEDro database were searched on April 25, 2020. Only randomized controlled studies published in English evaluating the effects of exercise interventions on peripheral skeletal muscle mass, strength, and exercise capacity in stable COPD patients were included. The quality of included studies was evaluated using the PEDro scale. The mean difference (MD) or the standardized mean difference (SMD) with 95% CI was calculated to summarize the results. Subgroup meta-analysis was used to investigate the effects of different exercise training modalities and different outcome measures. The Grading of Recommendations Assessment, Development, and Evaluation guidelines were used to rate evidence quality.

Results: A total of 30 randomized controlled trials involving 1,317 participants were included. Data from trials investigating endurance exercise (EE), resistance exercise (RE), and combined aerobic and resistance exercise (CE) were pooled into a meta-analysis, and the differences compared with the non-exercising COPD control were improvement in the muscle strength and exercise capacity in stable COPD patients. Subgroup meta-analysis for different exercise training modalities showed that RE significantly improved muscle strength (SMD = 0.6, 95% CI 0.35–0.84, I^2 = 61%), EE and CE significantly increased VO_{2peak} (EE: MD = 3.5, 95% CI 1.1–5.91, I^2 = 92%; CE: MD = 1.66, 95% CI 0.22–3.1, I^2 = 1%). Subgroup meta-analysis for different outcome measures showed that only isotonic strength was improved after exercise interventions (SMD = 0.89, 95% CI 0.51–1.26, I^2 = 71%).

Conclusion: Moderate evidence supports that exercise training in stable COPD patients has meaningful and beneficial effects on peripheral skeletal muscle strength and exercise capacity. Peripheral skeletal muscle shows a higher response to RE, and the isotonic test

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***Correspondence:**
Xiaodan Liu
hzhp403@126.com
Jun Xia
dx00122@163.com

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is relatively sensitive in reflecting muscle strength changes. The proportion of aerobic and resistance exercise components in a combined exercise program still needs exploration.

Systematic Review Registration: The review was registered with the PROSPERO: (The website is https://www.crd.york.ac.uk/PROSPERO/, and the ID is CRD42020164868).

Keywords: chronic obstructive pulmonary disease, exercise training, meta-analysis, skeletal muscle dysfunction, exercise capacity

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a common disease characterized by persistent respiratory symptoms and expiratory flow limitation (1). Furthermore, many patients with COPD experience systematic symptoms, including impaired cardiopulmonary and skeletal muscle function (2, 3). Skeletal muscle dysfunction is one of the significant systemic manifestations of COPD, characterized by the loss of muscle mass, a transition of the fiber type proportion, a decrease in the capillary to fiber ratio, and muscle strength and endurance (4, 5). In most patients with COPD, the observed decrease in muscle strength is proportional to muscle mass loss, suggesting that the onset of skeletal muscle dysfunction is caused by paralleled chronic inactivity and muscle deconditioning rather than myopathy (6). The existence of dyspnea in COPD decreases physical activity, and the decrease in physical activity induces and accelerates skeletal muscle dysfunction, worsening the dyspnea in patients, forming a vicious cycle that causes further deconditioning on COPD (7). Recently, lower limb muscle function has been associated with exercise capacity in COPD (8). Previous studies have confirmed that skeletal muscle dysfunction is an additional important contributor to COPD exercise restriction and function impairments (9, 10), and it is closely related to the quality of life, readmission rate, and mortality (11, 12).

Pulmonary rehabilitation is a comprehensive management program designed for COPD and has significant clinical effects in improving dyspnea, quality of life, and exercise capacity (1). As the cornerstone of pulmonary rehabilitation, exercise training can effectively reverse or at least stabilize the loss of skeletal muscle mass and strength in patients with COPD, and it is considered currently the most effective non-pharmaceutical intervention for COPD skeletal muscle dysfunction (13). The American Thoracic Society/European Respiratory Society (ATS/ERS) statement provided a short overview of the effects of exercise interventions on the muscle function and mass in COPD, showing that exercise interventions can improve the morphology and function of COPD skeletal muscle (12), but the included literatures are extensive and heterogeneous. Another international guideline described and analyzed the effects of different exercise modalities in COPD skeletal muscle dysfunction and provided a GRADE scale for evidence quality (4). In 2018, a review included 70 English language literature to be analyzed and concluded that exercise intervention could improve COPD skeletal muscle strength, endurance, and mass, despite the fact that intervention programs and outcome measures were heterogeneous (14). Therefore, although previous international guidelines and recent reviews have consistently concluded that exercise training improves COPD skeletal muscle dysfunction, it is still difficult to clarify the degree of real benefit due to the diversity and heterogeneity of exercise intervention programs and outcome measures. Previous meta-analysis of exercise in COPD explored the effects of resistance exercise (RE) on exercise capacity (15), endurance exercise (EE) vs. RE (16), and combined aerobic and resistance exercise (CE) vs. EE on lower limb muscle strength and exercise capacity (17). However, these studies focused on the effects of single exercise modality or the compared effects of two exercise modalities. There is still a lack of comprehensive quantitative effect of exercise on peripheral skeletal muscle mass, strength, and exercise capacity in COPD.

In this systematic review and meta-analysis, the effects of exercise interventions on peripheral skeletal muscle mass, strength, and exercise capacity in COPD were determined. The characteristics of different exercise modalities were further discussed to provide a theoretical reference for developing a targeted COPD exercise rehabilitation program.

METHODS

Search Strategy and Selection Criteria

This systematic review and meta-analysis was registered (PROSPERO registration number: CRD42020164868) and conducted according to the preferred reporting items for systematic reviews and meta-analysis (PRISMA) recommendations (18). According to the principle of population intervention comparison outcomes, the inclusion criteria were as follows: (a) participants diagnosed with stable COPD, and without gender and age restrictions; (b) EE and or RE was used for intervention; (c) a comparable control group applied with other treatments, including health education and sham training;

Abbreviations: 6MWD, 6-min walking distance; ATS/ERS, the American Thoracic Society/European Respiratory Society; BMI, body mass index; CE, combined aerobic and resistance exercise; COPD, chronic obstructive pulmonary disease; CPET, cardiopulmonary exercise test; CSA, cross-sectional area; EE, endurance exercise; FEV_1, forced expiratory volume in 1 s; FFM, fat-free mass index; GOLD, Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease; GRADE, the Grading of Recommendations Assessment, Development and Evaluation; MD, mean difference; MeSH, medical subject headings; OR, odds ratio; PEDro, the Physiotherapy Evidence Database; PRISMA, the Preferred Reporting Items for Systematic Reviews and Meta-Analysis; RE, resistance exercise; SMD, standardized mean difference; VO_{2peak}, peak oxygen consumption.

(d) outcomes including skeletal muscle mass related parameters (body mass index, BMI; fat-free mass index, FFM; and cross-sectional area, CSA), strength-related parameters (isometric, isotonic, and isokinetic strength), endurance exercise capacity (6-min walking distance, 6MWD), and peak exercise capacity (peak oxygen consumption, VO_{2peak}); and (e) randomized controlled study published in English. The exclusion criteria were as follows: (a) the immediate response to a single exercise test or exercise session was studied; (b) the follow-up effects of previous exercise program were studied; (c) traditional Chinese exercise and yoga were used for interventions; (d) animal trials, observational trials, expert opinions, literature reviews, comments, and letters were involved; (e) regular exercise programs were utilized in control groups (e.g., breath training, ≥twice a week); and (f) data could not be extracted.

Electronic searches of PubMed, Embase, EBSCO, Web of Science, and PEDro database were conducted from inception to April 25, 2020 using Medical Subject Headings (MeSH) terms and free-text keywords. In addition to the PEDro database, the following search terms were used: (COPD OR chronic obstructive pulmonary disease OR chronic obstructive lung disease OR chronic obstructive airway disease) AND (exercise OR exercise training OR rehabilitation OR pulmonary rehabilitation OR aerobic exercise OR endurance exercise OR resistance exercise OR strength training OR combined exercise) AND (muscle OR skeletal muscle). Search filters were applied, including article type (randomized controlled trials), species (humans), and language (English). In the PEDro database, the search terms were as follows: topic (chronic respiratory disease), method (clinical trial), therapy (fitness training), and abstract and title (COPD). Searches were supplemented by reviewing the reference lists of the included studies, previous review, meta-analysis, and guidelines.

To determine the eligibility of identified studies, two investigators independently conducted the process of study selection. Cohen's kappa was used to quantify the interrater agreement. Discrepancies of opinion between authors about study eligibility were resolved through discussions with a third investigator.

Data Analysis

Two investigators independently extracted data on study design, sample characteristics, intervention programs, and effects of exercise from included studies. Discrepancies were resolved through discussions with a third investigator. The studies were described in terms of study design (sample size, and PEDro score), sample characteristics (age, sex, FEV1%pred for forced expiratory volume in 1 s, and BMI), intervention programs (site, exercise modality, intensity, frequency, and duration), effects of exercise (outcome measures and change data), and adherence to the program. For trials with more than one exercise intervention group, the effects of each exercise intervention were evaluated. For trials with more than one outcome measures, the data of each outcome measures was included and analyzed. For trials with multiple time points, only the pre-intervention and post-intervention outcomes were extracted.

Predetermined primary outcomes included skeletal muscle mass (BMI, FFM, and CSA), strength (isometric, isotonic, and isokinetic strength), endurance exercise capacity (6MWD), and peak exercise capacity (VO_{2peak}). Secondary outcomes were attrition rate and severe adverse events. The change in mean and SD were calculated for each outcome and used to estimate the effects of the exercise. Summary measures for continuous outcomes were mean difference (MD) or standard mean difference (SMD) with 95% CI, and odds ratio (OR) with 95% CI for the attrition rate.

Review Manager (version 5.3) provided by Cochrane was used for meta-analysis. Random-effects model was used for analyzing. The I^2 statistic, representing the percentage of variation across studies due to heterogeneity, was used to assess heterogeneity between studies. Planned subgroup analyses were conducted in terms of exercise modalities (EE, RE, and CE) and outcome measures (isometric, isotonic, and isokinetic strength test). Sensitivity analyses were performed to check the heterogeneity source based on the intervention program and characteristics of the participants when subgroup analysis could not determine the source of substantial heterogeneity. Visual inspection of funnel plots and Egger's test were undertaken in Stata (version 15) to assess publication bias. Trim and fill method was used when there was a publication bias. The methodological quality of randomized controlled trial (RCTs) was assessed using the physiotherapy evidence database (PEDro) scale. When available, the PEDro rating and score were obtained from the PEDro database. Otherwise, two investigators independently rated and scored the publications; discrepancies were resolved through discussions with a third investigator. The PEDro scale includes 11 items with 10 scores, and a higher score means better quality (19). It should be noted that the eligibility criteria item does not contribute to the total score. PEDro scale 9–10 was considered high quality, 6–8 was generally high quality, 4–5 was moderate quality, and <4 was low quality. The quality of evidence was assessed according to the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) recommendations (limitation of study design, inconsistency, indirectness, imprecision, and publication bias) (20).

RESULTS

A total of 2,665 records were identified, and 30 RCTs were included in the quantitative analysis (**Figure 1**). A strong agreement was observed with respect to the interrater reliability of study selection (kappa = 0.89, $P < 0.001$). The PEDro scale of all included studies is 5.7 ± 1.4 (**Supplementary Table S1**), and the characteristics of participants of each included study are reported in **Table 1**. A total of 1,317 participants with stable COPD (age range from 46 to 79.8 years) were included, and 675 (51%) participants accepted exercise intervention. According to the criteria of Global strategy for the diagnosis, management, and prevention of COPD (GOLD), majority of the participants showed moderate to severe airflow restriction (30% ≤ FEV1%pred ≤ 80%), and four studies did not provide the

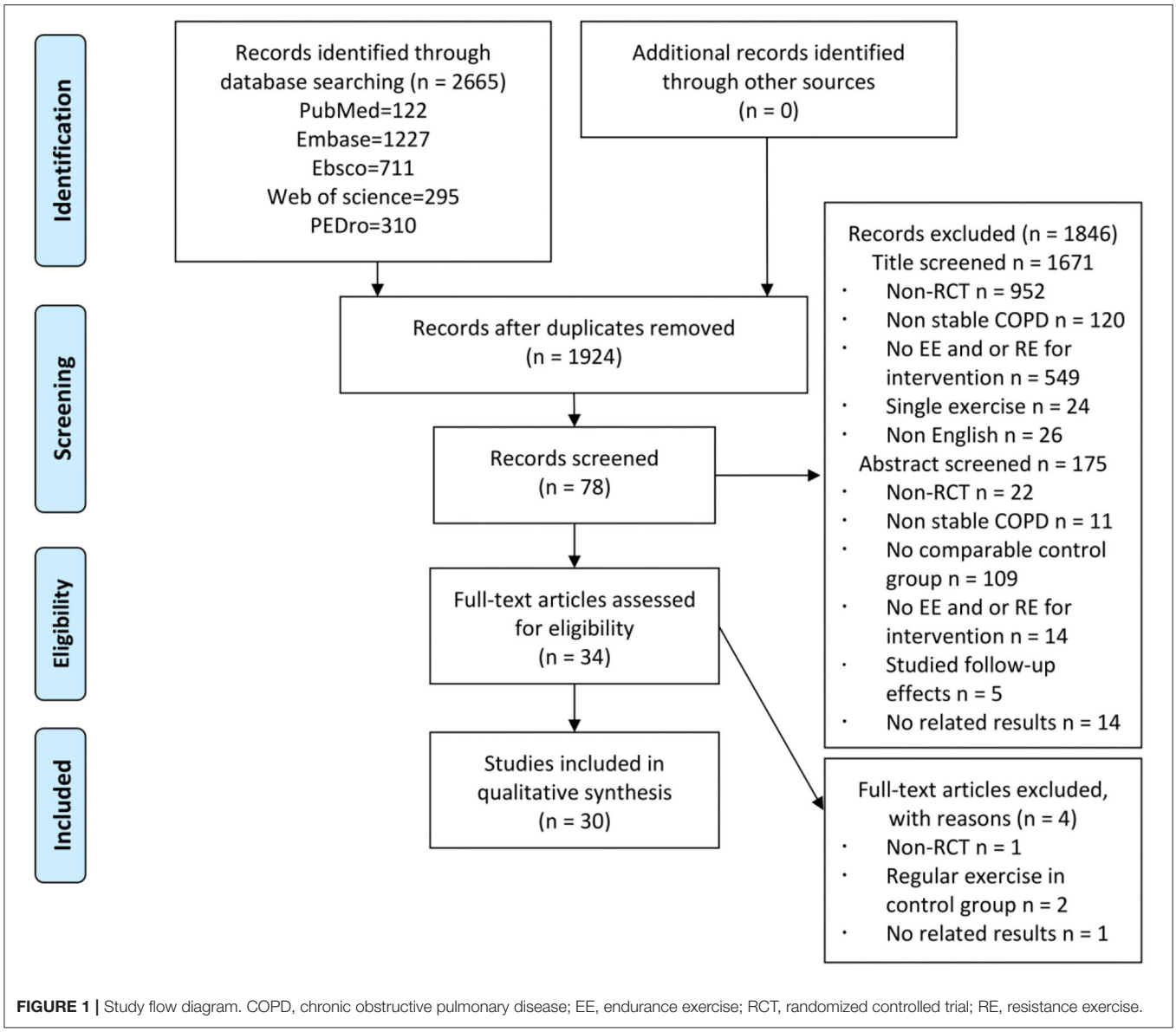

FIGURE 1 | Study flow diagram. COPD, chronic obstructive pulmonary disease; EE, endurance exercise; RCT, randomized controlled trial; RE, resistance exercise.

baseline data of FEV1%pred (27, 30, 38, 41). Most participants were normal to overweight (BMI: 18.5–29.9 kg/cm^2), while five studies did not provide this data (27, 38, 39, 42, 49). In addition, exercise intervention programs of all the included studies are presented in **Table 2**. Most trials were conducted in a hospital, at home, or at both the places, while six studies did not report a place (31, 35, 38, 40, 45, 50). Most studies applied exercise program duration ranges from 6 to 12 weeks, while some studies applied 14 weeks (22), 16 weeks (47), and 24 weeks (38). EE was mainly performed in the form of treadmill, cycling, or walking with a moderate to vigorous exercise intensity (Borg 4–6, even exhaustion, despite indexes used to assess were various) for two to three sessions per week. RE was mainly performed on weight machines, free weights, and elastic bands through the movements of the upper and lower limbs. One study performed RE only through the upper limbs (35) and three studies conducted RE only through the lower limbs (31, 33, 34). Exercise intensity of RE ranged from 50 to 85% 1-repetition maximum (1RM) or Borg 4–6, and exercise frequency was two to three sessions per week. The performance of CE was consistent with EE and RE. The exercise intensity of EE was Borg 4–6, while the exercise intensity of RE was often unclear. The characteristics of muscle strength testing relative to the variety of muscle strength testing methods and programs are summarized in **Table 3**.

Five studies (21, 31, 32, 34, 47) provided data on skeletal muscle mass, assessed by mid-thigh CSA, BMI, FFMI, and total lean mass. In the meta-analysis, the estimated results showed that exercise intervention did not have a significant effect on changes in BMI (MD = −0.11, 95% CI: 1.13–0.91, I^2 = 84%, **Figure 2**). Considering the high heterogeneity detected, we excluded studies with PEDro < 6, and found a significant improvement in BMI (MD = 0.26, 95% CI 0.23–0.29, I^2 = 0%). In addition, a CE

TABLE 1 | Characteristics of included studies.

Author, Country	I/C sample size/Male%	Age	FEV1%pred	BMI	Outcome			Attrition number*	PEDro
					Mass	Strength	Exercise capacity		
Endurance exercise									
Alcazar et al. (21), Spain	14(79%)/ 15(87%)	77.7 ± 7.9/ 79.8 ± 6.4	47.4 ± 18.1/ 58.7 ± 15.2	28.8 ± 3/ 32.5 ± 5.9	Mid-thigh CSA	Leg press	6MWD VO_{2peak}	5/1*	4
Barakat et al. (22), France	40(85%)/ 40(83%)	63.7 ± 11.9/ 65.9 ± 10.3	41.9 ± 2.6/ 43.33 ± 3.6	24.2 ± 6.4/ 25.6 ± 4.3	/	/	6MWD	5/4*	6
Borghi-silva et al. (23), USA	20(65%)/ 14(86%)	67 ± 10/ 67 ± 10	33 ± 9/ 35 ± 11	25 ± 4/ 24 ± 5	/	/	6MWD VO_{2peak}	0/6	5
Borghi-silva et al. (24), USA	10(70%)/ 10(50%)	67 ± 7/ 66 ± 10	32 ± 11/ 35 ± 12	23.4 ± 4.4/ 27.2 ± 4.7	/	/	6MWD VO_{2peak}	7/5	5
de Souto Araujo et al. (25), Brazil	13(62%)/ 11(73%)	56.9 ± 7.9/ 71.1 ± 10.1	39.2 ± 11.4/ 45.1 ± 12.6	30 ± 10.1/ 24.4 ± 6.7	/	/	6MWD	1/3	4
Gallo-Silva et al. (26), Brazil	10/9	66.3 ± 6.5/ 66.5 ± 9.5	47.9 ± 20.5/ 47.8 ± 26.2	23.2 ± 2.6/ 25.7 ± 6.1	/	/	6MWD	2/3	6
Mehri et al. (27), Iran	20(55%)/ 18(39%)	52.1 ± 10.7/ 52.17 ± 11.6	/	/	/	/	VO_{2peak}	0/0	5
Petersen et al. (28), Denmark	9(22%)/ 10(40%)	67 ± 3/ 66 ± 3	33 ± 5/ 30 ± 4	23 ± 2/ 26 ± 2	/	/	VO_2 max	0/4	5
Pradella et al. (29), Brazil	29(79%)/ 15(87%)	62.4 ± 10.7/ 65.3 ± 8	43.9 ± 16.2/ 54 ± 22.2	25.2 ± 5/ 26.7 ± 5.3	/	/	6MWD	3/3	5
Wiyono et al. (30), Indonesia	27(93%)/ 29(96%)	64.3 ± 6.3/ 67.2 ± 4.5	/	19.7 ± 8.5/ 20.2 ± 6.4	/	/	6MWD VO_2 max	3/1	5
Resistance exercise									
Casaburi et al. (31), USA	12 (100%)/ 12(100%)	68.9 ± 9.8/ 67.7 ± 8.7	35.9 ± 9.2/ 38.6 ± 12.1	27.57/ 26.31	Total lean mass	Leg press	VO_{2peak}	1/1	5
Clark et al. (32), Scotland	26(58%)/ 17(59%)	51 ± 10/ 46 ± 11	76 ± 23/ 79 ± 23	26 ± 4/ 26 ± 4	BMI	Quadriceps	VO_2 max		5
Chen et al. (33), China	25(88%)/ 22(68%)	69.04 ± 8.07/ 64.95 ± 11.59	54.49 ± 23.62/ 54.93 ± 25.58	23.86 ± 3.9/ 24.15 ± 3.93	/	Quadriceps	6MWD	4/4	6
Hoff et al. (34), USA	6(67%)/ 6(67%)	62.8 ± 1.4/ 60.6 ± 3	32.9 ± 3.3/ 39.5 ± 6.4	26.27/ 26.65	BMI	Leg press	VO_{2peak}	0/0	6
Janaudis-Ferreira et al. (35), Canada	17(53%)/ 19(37%)	67 ± 11/ 67 ± 11	37.8 ± 16.2/ 32.5 ± 14.1	27.9 ± 7.9/ 25.7 ± 8.2	/	Biceps Triceps Anterior Middle deltoids	/	4/1*	9
Nyberg et al. (36), Sweden	22(55%)/ 22(50%)	69 ± 5/ 68 ± 6	59 ± 11/ 55 ± 15	26 ± 4/ 25 ± 5	/	Shoulder flexion Knee extension	6MWD	2/2*	8
O'shea et al. (37), Australia	27/27	66.9 ± 7/ 68.4 ± 9.9	49 ± 25/ 52 ± 22	25.5 ± 5.1/ 27.8 ± 7.9	/	Knee extension Hip abduction Shoulder horizontal flexion Shoulder flexion	6MWD	7/3*	7
Thabitha et al. (38), India	30	/	/	/	/	/	6MWD VO_{2peak}	/	4
Simpson et al. (39), Canada	14(35%)/ 14(71%)	73 ± 4.8/ 70 ± 5.7	39.5 ± 18.96/ 39.2 ± 21.39	/	/	Elbow flexion Quadriceps Leg press	6MWD VO_2 max	3/3	6
Zambom-Ferraresi et al. (40), Spain	14(100%)/ 8(100%)	68 ± 7/ 69 ± 5	48 ± 12/ 39.7 ± 5	28.5 ± 3.9/ 25.7 ± 4.6	/	Leg press Chest press	6MWD VO_{2peak}	1/1	7
Combined exercise									
Cameron-Tucker et al. (41), Australia	43(53%)/ 41(54%)	64.5 ± 9.3/ 67.1 ± 9.41	/	28.4 ± 7.63/ 29.7 ± 6.5	/	/	6MWD	5/10*	6
Emery et al. (42), USA	30(50%)/ 24(42%)	65.4 ± 6.4/ 67.4 ± 5.9	43 ± 18/ 43 ± 18	/	/	/	VO_2 max	4/2*	6

(Continued)

TABLE 1 | Continued

Author, Country	I/C sample size/Male%	Age	FEV1%pred	BMI	Outcome			Attrition number*	PEDro
					Mass	Strength	Exercise capacity		
Lahham et al. (43), Australia	29(59%)/ 29(59%)	68 ± 9/ 67 ± 10	90 ± 8/ 92 ± 7	28 ± 4.5/ 28 ± 4.3	/	/	6MWD	3/4*	8
Mendes et al. (44), Brazil	23(83%)/ 29(66%)	71.3 ± 6.7/ 70.8 ± 8.7	51.5 ± 23.9/ 41.4 ± 18.4	23.5 ± 4.2/ 24.6 ± 6.3	/	/	6MWD	23/0	4
Nakamura et al. (45), Japan	10/ 10	69 ± 8.7/ 69.9 ± 7.1	53.2 ± 15.1/ 48.2 ± 20.1	21.9 ± 3.5/ 21.6 ± 3	/	HGF	6MWD VO_{2peak}	/	5
Tsai et al. (46), Australia	19(63%)/ 17(35%)	78 ± 3/ 75 ± 9	60 ± 23/ 68 ± 19	28 ± 4/ 28 ± 5	/	/	6MWD	1/0	8
van Wetering et al. (47), Netherlands	102(71%)/ 97(71%)	65.9 ± 8.8 / 67.2 ± 8.9	58 ± 17/ 60 ± 15	26.1 ± 4.4/ 27.3 ± 4.7	BMI FFMI	HGF Quadriceps	6MWD	15/9*	7
Wadell et al. (48), Canada	17(53%)/ 24(54%)	68 ± 6/ 66 ± 7	48 ± 12/ 48 ± 19	26.7 ± 4.9/ 28.9 ± 4.3	/	Knee extension	6MWD	3/ 4*	6
Wadell et al. (49), Sweden	15(33%)/ 13(54%)	65 ± 7/ 63 ± 7	53 ± 12/ 49 ± 12	/	/	/	VO_{2peak}	1/ 1*	6
Weiner et al. (50), Israel	18/ 5	63.2 ± 2.3/ 60.1 ± 2.8	35 ± 2.2/ 36 ± 1.9	23.84/ 24.84	/	/	6MWD	1/1*	5
Zambom-Ferraresi et al. (40), Spain	14(100%)/ 8(100%)	68 ± 7/ 69 ± 5	44.3 ± 11.9/ 39.7 ± 5	29.3 ± 6.4/ 25.7 ± 4.6	/	Leg press Chest press	6MWD VO_{2peak}	2/1	7

6MWD, 6-min walking distance; HGF, Handgrip force; I/C, Intervention group/Control group; RM, Repetition maximum; VO_2, Oxygen uptake.
*/Not accessible; *Attrition number is included in the sample size.*

program significantly improved FFMI ($P = 0.01$) (47), an EE program significantly improved the mid-thigh CSA (+4.5%, $P < 0.05$) of elderly patients with COPD (age: 77.7 ± 7.9 years old) (21), an RE program only found an increasing trend in the total lean mass (31). A total of 13 studies (21, 31–37, 39, 40, 45, 47, 48) with 27 data on skeletal muscle strength were provided, demonstrating a significant improvement after exercise intervention (SMD = 0.58, 95% CI 0.21–0.95, $I^2 = 89\%$). Considering the high heterogeneity detected, we first excluded studies with PEDro < 6, and found a consistent result with high heterogeneity (SMD = 0.62, 95% CI 0.19–1.05, $I^2 = 91\%$). Then, we only pooled data in kilograms unit, and found a consistent result (MD = 0.78, 95% CI 0.64–0.92, $I^2 = 0\%$) besides the isometric strength test. Finally, subgroup analysis for different exercise modalities (**Figure 3**), muscle strength measures (**Figure 4**), and upper or lower limbs muscle strength found that RE provided significant benefits (SMD = 0.6, 95% CI 0.35–0.84, $I^2 = 61\%$), isometric strength significantly improved (SMD = 0.89, 95% CI 0.51–1.26, $I^2 = 71\%$), and both upper and lower limbs muscle strength significantly improved (SMD = 0.78, 95% CI 0.4–1.17, $I^2 = 79\%$; SMD = 0.67, 95% CI 0.12–1.22, $I^2 = 91\%$).

A total of 22 studies (21–26, 29, 30, 33, 36–41, 43–48, 50) provided data on endurance exercise capacity, demonstrating a significant improvement in 6MWD after exercise intervention (MD = 26.64, 95% CI 15.38–37.91, $I^2 = 77\%$). Subgroup analysis for different exercise modalities showed a consistent result, namely that all EE, RE, and CE can improve 6MWD significantly (EE: MD = 40.99, 95% CI 34.65–47.32, $I^2 = 0\%$; RE: MD = 22.32, 95% CI 6.76–37.89, $I^2 = 0\%$; CE: MD=11.89, 95% CI 10.81–12.97, $I^2 = 0\%$, **Figure 5**). A total of 13 studies (21, 23, 27, 28, 30, 32, 34, 36, 38, 40, 42, 45, 49) provided data on the peak exercise capacity, demonstrating a significant improvement in VO_{2peak} after exercise intervention (MD = 1.82, 95% CI 0.62–3.02, $I^2 = 77\%$). Subgroup analysis for different exercise modalities showed that EE and CE can improve VO_{2peak} significantly (EE: MD = 3.5, 95% CI 1.1–5.91, $I^2 = 92\%$; CE: MD = 1.66, 95% CI 0.22–3.1, $I^2 = 1\%$, **Figure 5**). Considering that the methodological quality of included studies in EE was relatively low (PEDro < 6), the results need to be carefully considered.

There was no difference of attrition number between exercise and control group (OR = 1.12, 95% CI 0.75–1.67, $I^2 = 15\%$, **Supplementary Figure S1**). The reasons for attrition in the exercise and control groups were similar (**Supplementary Table S2**).

Funnel plots are presented in **Supplementary Figure S2**. The results of Egger's test showed a significant publication bias in the results of skeletal muscle strength and 6MWD ($P = 0.031$ and $P = 0.018$, respectively). Then, the trim and fill method was used to adjust the impact of publication bias, and the results showed 0 missing studies for skeletal muscle strength results, and five missing studies for 6MWD results were merged to diminish the publication bias (**Supplementary Figure S3**). The certainty of the evidence for endurance and peak exercise capacity was deemed moderate, for skeletal muscle strength was deemed low, and for BMI was deemed very low (**Table 4**).

DISCUSSION

This systematic review and meta-analysis confirmed that regular exercise intervention for more than 6 weeks can effectively

TABLE 2 | Characteristics of intervention protocols.

Author,Country	Setting	Intervention contents	Intervention intensity	Intervention duration/ frequency	Control group
Endurance exercise					
Alcazar et al. (21), Spain	Outpatient	First 3 weeks: HIIT (5 sets of 90 s at light intensity plus 30 s at heavy intensity) + power training (2–3 sets of 8–12 reps) Week 4–12: HIIT (10stes) + 3sets of 8reps with the optimal load	First 3 weeks: HIIT (heavy-80%W_{peak}, light-40% W_{peak}) + power training (50–60% 1RM) Week 4–12: HIIT (augmented) + power training (optimal load)	2 sessions/week, 12 weeks	Usual care
Barakat et al. (22), France	Outpatient	30 min cycling + 30 min aerobic activity (5 min warm-up, 10 min aerobic activity, 15 min cool-down)	Cycle: 80%VO_2 max	3 sessions/week, 14 weeks	Routine outpatient attendance
Borghi-Silva et al. (23), USA	Outpatient	30 min stretching + treadmill ambulation	70% of the maximal speed	3 sessions/week, 6 weeks	Usual care
Borghi-Silva et al. (24), USA	Outpatient	5 min warm-up + 30 min treadmill	70% of the peak speed/Borg 4	3 sessions/week, 12 weeks	Respiratory therapy, 1session/week
de Sauto Araujo et al. (25), Brazil	Outpatient	15 min callisthenic activities + 30 min unsupported upper limb exercise using weights + 30 min bicycle + 15 min cool-down	Upper: 50% of the maximum load; Lower: Borg 5	3 sessions/week, 8 weeks	No exercise
Gallo-Silva et al. (26), Brazil	Laboratory	60 min water aerobic interval exercise (10 min warm-up, 20–40min aerobic exercise, 10 min cool-down)	Borg 4–6	3 sessions/week, 8 weeks	Usual care
Mehri et al. (27), Iran	Outpatient	Treadmill exercise training with gradually increased speed	Exhaustion	2 sessions/week, 8 weeks	No exercise
Petersen et al. (28), Denmark	Outpatient	Walking with 85% maximal speed + progressive ergometer cycling	Exhaustion	2 sessions/week, 7 weeks	Usual daily activities
Pradella et al. (29), Brazil	Home	40 min walking + 15 min stair exercise + arm exercise with 1 kg load (3 sets of 30 movements)	Walking: 60–70% HR_{max}	3 sessions/week, 8 weeks	No exercise
Wiyono et al. (30), Indonesia	Outpatient	5 min cycling, and gradually increased for 5 min/week	/	3 sessions/week, 6 weeks	Routine outpatient attendance
Resistance exercise					
Casaburi et al. (31), USA	/	First 4 weeks: 3 sets of 12 reps; Week 5–10: 4 sets of 8–10 reps (seated leg press, seated leg curl, seated leg extension, standing calf raise, seated ankle dorsiflexion)	First 4 weeks: 60% 1RM Week 5–10: 80% 1RM	3 sessions/week, 10 weeks	No exercise
Clark et al. (32), Scotland	Outpatient	3 sets of 10 reps weight exercises (bench press/triceps, body squat/quadriceps, squat calf/medial and lateral gastrocnemiius soleus, latissimus/latissimus dorsi/arm curls/biceps, leg press/quadriceps hamstrings gluteals, knee flexion/quadriceps, hamstrings)	70% maximal load	2 sessions/week, 12 weeks	Usual daily activities
Chen et al. (33), China	Home	20–30min, 8–12 reps Thera-band exercise (straight-leg lifting, prone hip extension, thigh abduction, posterior muscle group exercises, anterior muscle group exercises, and standing calf raise)	Borg 5	3 sessions/week, 12 weeks	No exercise
Hoff et al. (34), USA	Laboratory	4 sets of 5 reps concentric contraction of quadriceps	85–90% 1RM	3 sessions/week, 8 weeks	Normal daily living
Janaudis-Ferreira et al. (35), Canada	/	10–12RM using free weights and a multistation gym (biceps brachii, triceps brachii, pectoralis major and minor, latissimus dorsi, deltoids, rhomoboids)	10–12RM	3 sessions/week, 6 weeks	Upper limb flexibility and stretching exercises
Nyberg et al. (36), Sweden	Outpatient	40 min, 2 sets of 25 reps Thera-band exercise (Latissimus row/chest press/leg extension/shoulder flexion/leg curl/elbow flexion/heel-raise/step up)	Borg 4	3 sessions/week, 8 weeks	No exercise

(Continued)

TABLE 2 | Continued

Author, Country	Setting	Intervention contents	Intervention intensity	Intervention duration/ frequency	Control group
O'shea et al. (37), Australia	1 hospital + 2 home	3 sets of 8–12 reps Thera-band exercise (hip abduction in standing, simulated lifting, SST, seated row, lunges, chest press)	12RM and gradually increased	3 sessions/week, 12 weeks	No exercise
Thabitha et al. (38), India	/	15–30min, 1–3 sets of 10 reps using multi exerciser (chest pull-lattismus dorsi, butterfly-pectoralis major muscle, neck press-triceps brachii and deltoid, leg flexion-biceps femoris and gastronemious, leg extension)	10RM and increased by 10%	2 sessions/day, 3 days/week, 24 weeks	No exercise
Simpson et al. (39), Canada	Outpatient	3 sets of 10 reps single limb weight lifting exercise (arm curl/leg extension/leg press)	50–85% 1RM	3 sessions/week, 8 weeks	No exercise
Zambom-Ferraresi et al. (40), Spain	/	90 min, 3–4 sets of 6–12 reps (chest press, seated row, shoulder press, leg press, knee extension and flexion)	50–70% 1RM	2 sessions/week, 12 weeks	Habitual physical activity
Combined exercise					
Cameron-Tucker et al. (41), Australia	Outpatient	1 h combine exercises, individualized for each participant	RPE 3–5	1 sessions/week, 6 weeks	No exercise
Emery et al. (42), USA	Outpatient	First 5 weeks: 45 min combine exercises on Nautilus equipment; Week 6–10: 60–90 min	/	First 5 weeks: every-day; Week 6–10: 3 sessions/week	No exercise
Lahham et al. (43), Australia	Home	Aerobic: 80% of walking speed from 6MWD + 30 min whole-body exercise; Resistance: using equipment available at home (stairs and sealed water bottles)	/	5 sessions/week, 8 weeks	No exercise
Mendes et al. (44), Brazil	Outpatient	Aerobic: 30 min treadmill walking; Resistance: 10 reps (hand weight, elbow flexion, elbow abduction, shoulder abduction, shoulder flexion, hip flexion, knee extension)	Aerobic: 60–80% HR_{max} Resistance: 50% 1RM with an increase of 0.5 kg every 2 weeks	3 sessions/week, 12 weeks	No exercise
Nakamura et al. (45), Japan	/	Aerobic: 20 min walking; Resistance: 30 min, 3 sets of 10 reps using self-weight or elastic bands (push-ups, leg squats, sit-ups, back extension)	Aerobic: Borg 3–5	12 weeks	No exercise
Tsai et al. (46), Australia	Home	Aerobic: 15–20 min cycling + 15–20 min walking Resistance: 3 sets of 10 reps SST and squats exercise	Cycle: 60–80% W_{peak} Walk: 80% of best 6MWD or Aerobic: Borg 3–4	3 sessions/week, 8 weeks	Usual care
van Wetering et al. (47), Netherlands	Community	Aerobic: 30 min cycling/walking Resistance: 4 specific exercises for upper and lower limbs	/	2 sessions/week, 16 weeks	Usual care
Wadell et al. (48), Canada	Outpatient	2.5 h combine exercise	Moderate intensity	3 sessions/week, 8 weeks	Usual care
Wadell et al. (49), Sweden	Outpatient	45 min, (4 min aerobic, 3 min leg resistance, 4 min aerobic, 3 min arm resistance, 4 min aerobic, 3 min torso resistance)	80–100%HR peak or Borg 5 or RPE 15	3 sessions/week, 12 weeks	No exercise
Weiner et al. (50), Israel	/	Aerobic: 30 min cycling; Resistance: 15 min rowing with low resistance +15 min resistance exercises for limbs and abdominal muscles	Aerobic: 50% W_{max}	3 sessions/week, 6 weeks	Sham training
Zambom-Ferraresi et al. (40), Spain	/	Aerobic: 20–35 min cycle Resistance: 90 min, 3–4 sets of 6–12 reps (chest press, seated row, shoulder press, leg press, knee extension and flexion)	Aerobic: 40–85% W_{max} Resistance: 50–70% 1RM	2 sessions/week for each exercise types, 12 weeks	Habitual physical activity

6MWD, 6-min walking distance; HIIT, high intensity interval training; HGF, Handgrip force; HR, Heart rate; reps, repetitions; RM, Repetition maximum; SST, sit to stand; W_{max}, Maximal work rate; VO_2, Oxygen uptake.
/Not accessible.

improve peripheral skeletal muscle strength and exercise capacity of patients with stable COPD. Furthermore, the greatest improvement in peripheral skeletal muscle strength appears in RE, the greatest improvement in endurance exercise capacity (6MWD: 40.99 m) appears in EE, and both EE and CE can significantly improve the peak exercise capacity.

TABLE 3 | Characteristics of skeletal muscle strength tests.

Type	Author, Country	Outcomes	Methods	Apparatus	Site
Isometric test	Alcazar et al. (21), Spain	Leg press (N)	Evaluate two legs performance, test for at least 4s	Force plate	Lower limb
	Chen et al. (33), China	Quadriceps (Nm)	Evaluate the maximal strength of dominant leg	Computerized dynamometer	Lower limb
	Janaudis-Ferreira et al. (35), Canada	Biceps (kg) Triceps (kg) Anterior (kg) Middle deltoids (kg)	Evaluate the dominant side by Micro FET2, the average of the highest 3 measures were used for analysis	Hand-held dynamometer	Upper limb
	Nakamura et al. (45), Japan	HGF (kg)	Evaluate the dominant side	Hand-grip dynamometer	Upper limb
	Wadell et al. (48), Canada	Knee extension (kg)		Fixed dynamometer	Lower limb
	van Wetering et al. (47), Netherlands	HGF (kg) Quadriceps (Nm)		Unknown device	Upper limb Lower limb
Isotonic test	Casaburi et al. (31), USA	Leg press (kg)	Evaluate two legs performance by 1RM test	Pneumatic device	Lower limb
	Clark et al. (32), Scotland	Quadriceps (kg)	1RM test	Multigym	
	Hoff et al. (34), USA	Leg press (kg)	1RM test	Force platform	
	O'shea et al. (37), Australia	Knee extension (kg) Hip abduction (kg) Shoulder horizontal flexion (kg) Shoulder flexion (kg)	Averaged across right and left limbs were used for analysis	Hand-held dynamometry	Lower limb Lower limb Upper limb Upper limb
	Simpson et al. (39), Canada	Elbow flexion (kg) Quadriceps (kg) Leg press (kg)	Unilateral 1RM test	Unknown device	Upper limb Lower limb
	Zambom-Ferraresi et al. (40), Spain	Leg press (kg) Chest press (kg)	1RM test	Force plate	Lower limb Upper limb
Isokinetic test	Chen et al. (33), China	Quadriceps (Nm)	Evaluate the maximal strength of dominant leg	Computerized dynamometer	Lower limb
	Nyberg et al. (36), Sweden	Shoulder flexion (Nm) Knee extension (Nm)	The highest of 5 maximal contractions was used for analysis	Computerized dynamometer	Upper limb Lower limb

Kg, Kilogram; HGF, Handgrip force; N, Newton; RM, Repetition maximum.

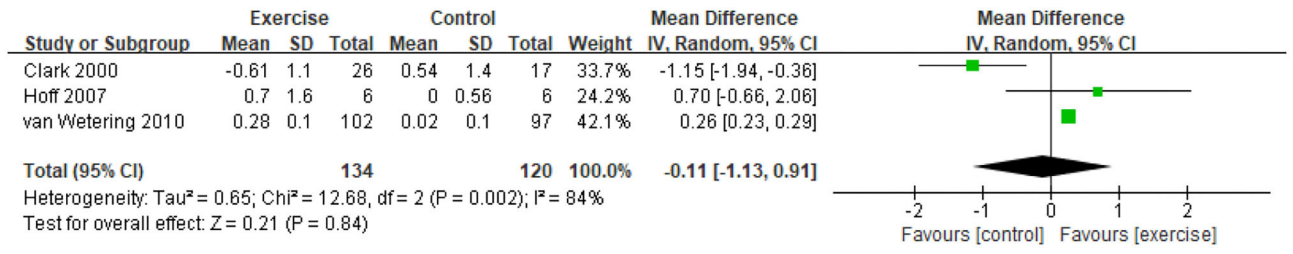

FIGURE 2 | Pooled effect of exercise on BMI in patients with COPD. BMI, body mass index (kg/m^2); CI, confidence interval; COPD, chronic obstructive pulmonary disease; SD, standard deviation.

In a previous study, skeletal muscle wasting could occur in the early COPD stages (51), and different exercise modalities could effectively improve lower limb muscle mass in COPD (14). However, in this study, exercise significantly improved the BMI of patients with COPD after excluding studies with PEDro < 6. Through the analysis of literature characteristics, we proposed that exercise improved the BMI of patients with COPD unrelated to exercise modalities, but it was more affected by age and FEV1%pred. That is, the younger the age and better FEV1%pred, the lower the potential for improvement by exercise intervention. A recent meta-analysis of clinical trials has found a negative correlation between the BMI and decline of FEV1 in patients with COPD (52). Age, severity of COPD, and dyspnea degree are closely and clinically related to the loss of skeletal muscle mass and the decline of muscle function in patients with COPD (51). The results from the above-mentioned cross-sectional trials

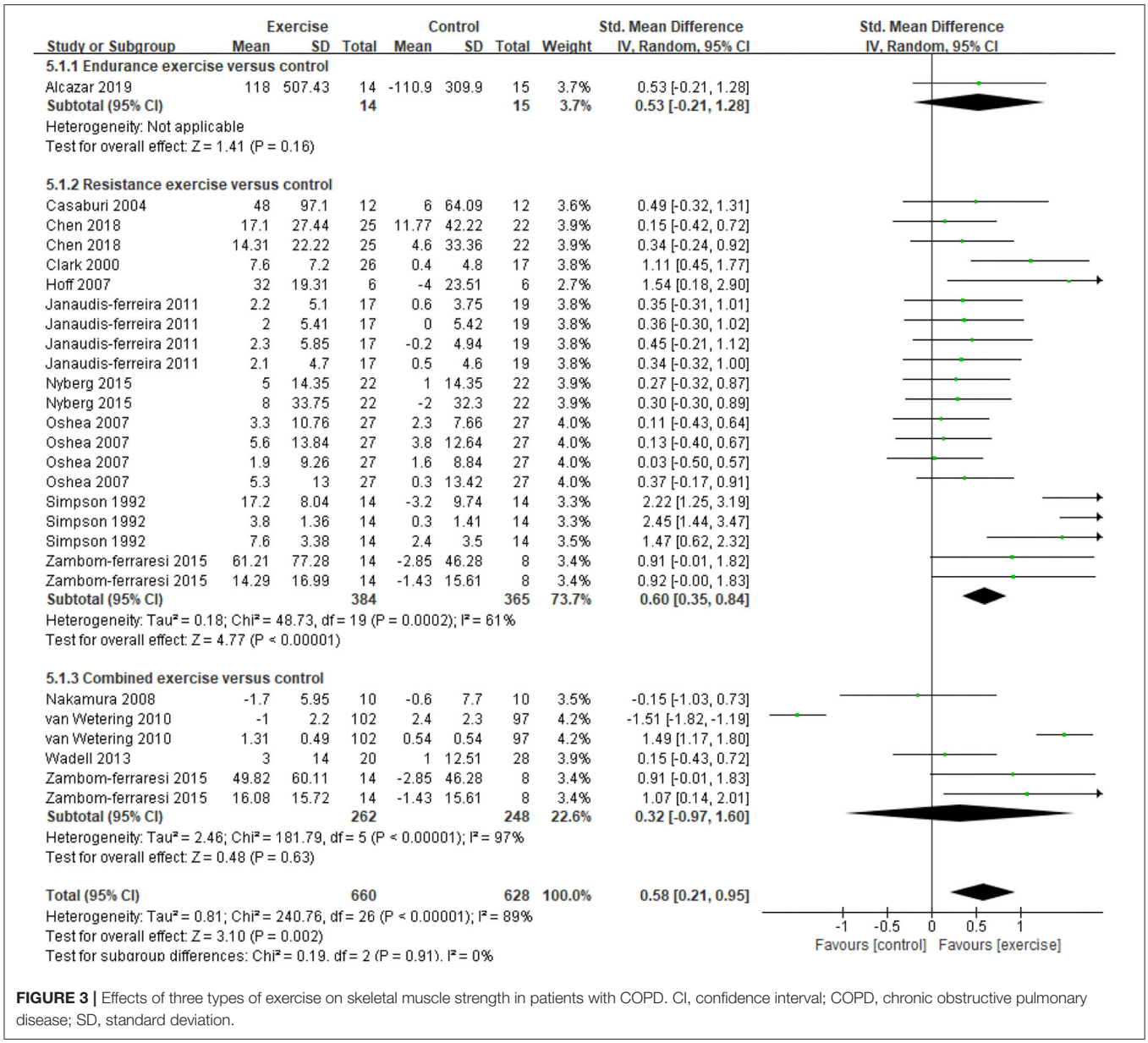

FIGURE 3 | Effects of three types of exercise on skeletal muscle strength in patients with COPD. CI, confidence interval; COPD, chronic obstructive pulmonary disease; SD, standard deviation.

supported the speculation, but the factors that modulated the effects of exercise in COPD skeletal muscle mass still need to be explored due to the small data size in this study. Furthermore, BMI is affected by adipose and connective tissues in the body and may inadequately reflect muscle mass changes. Previous studies have found that RE can significantly improve lower limb lean muscle mass, increase the CSA of the rectus femoris and quadriceps, and decrease the density of muscle fiber (which indicate increased muscle mass per unit area) in COPD (53, 54), but have no effects on the proportion of muscle fiber type and the CSA of different muscle fiber types (an increasing trend only be found in type IIx fibers) (54). Another trial compared the effects of EE and RE on quadriceps muscle morphology and found no significant change in proportion and CSA of type I fibers, intermediate fibers, type IIx fibers, and capillarization (expressed as capillary-to-fiber ratio capillary density) after both exercise modalities, while the proportion of type IIa fibers significantly decreases after EE (55). Consistent with the present study results, both EE and RE have a beneficial effect on the peripheral skeletal muscle mass of patients with COPD, and EE seems to bring more changes in the aerobic metabolism phenotype. The exercise intervention mechanism to improve COPD skeletal muscle mass may be related to inhibiting the level of systemic inflammation, promoting skeletal muscle protein synthesis, muscle hypertrophy and regeneration, and improving the skeletal muscle metabolic enzyme activity (56).

Although there was a high heterogeneity in the methods and programs used to assess muscle strength, the results of this

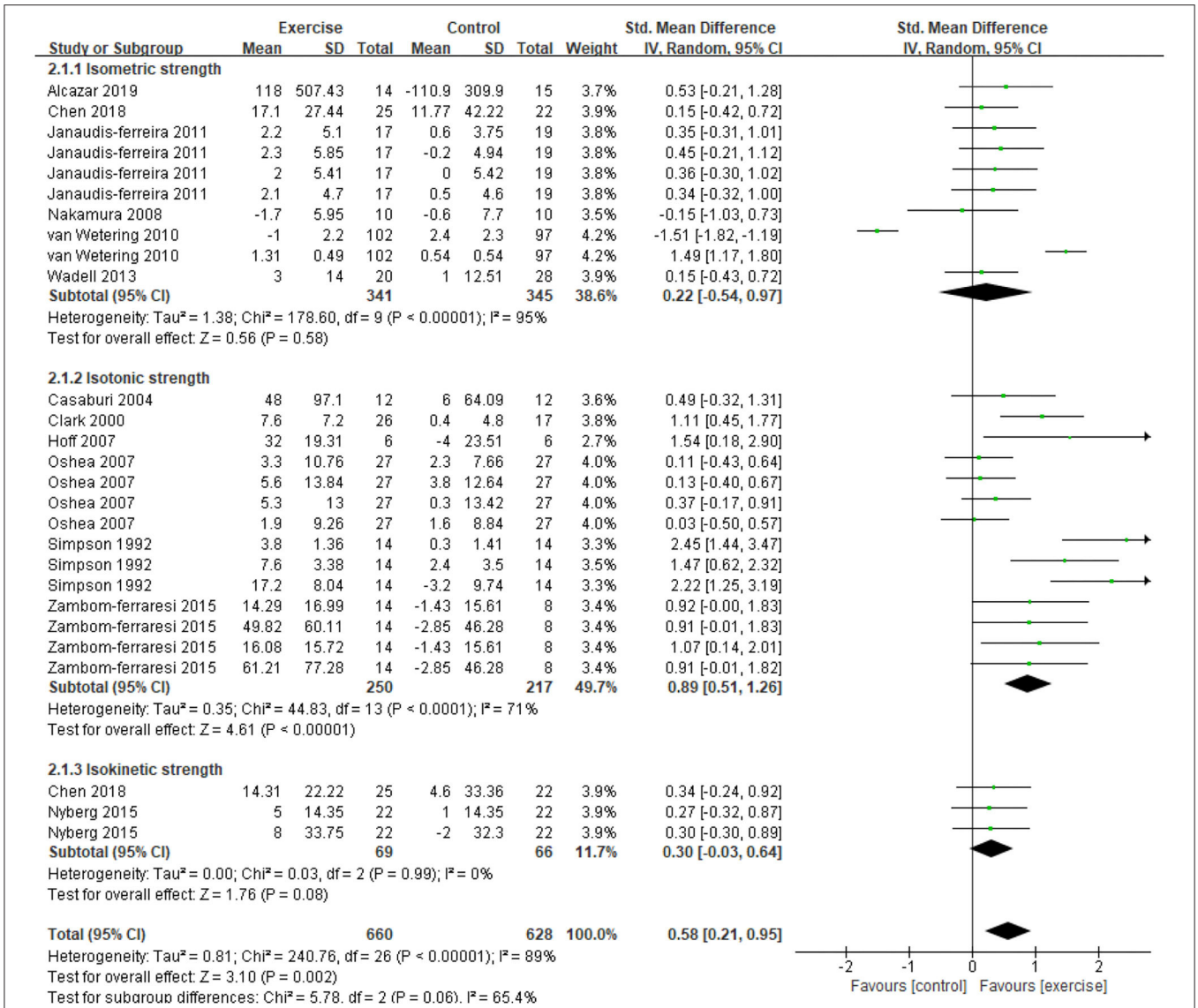

FIGURE 4 | Effects of exercise on skeletal muscle strength evaluated by three types of measurements in patients with COPD. CI, confidence interval; COPD, chronic obstructive pulmonary disease; SD, standard deviation.

study still confirmed the significant positive effect of exercise on improving peripheral skeletal muscle strength in stable COPD. Subgroup analysis for different exercise modalities found that RE showed significant effects. We speculated that RE was designed for specific muscle groups that have less pressure on ventilation load and can effectively improve neuromuscular adaptation (57). Previous studies hypothesized that high-intensity whole/local body EE is sufficient to induce changes in the morphology and function of peripheral skeletal muscles in COPD (14). In the present study, only Alcazar et al. applied a 12-week high-intensity interval training program (high intensity: 80–90% W_{peak} and low intensity: 40–50% W_{peak}) in stable COPD patients and found that the maximum isometric contraction strength and the force development rate of leg press significantly improved (21). Hence, the dose-response relationship between EE intensity and effect still needs to be determined. Also, there was a high heterogeneity in the pooled estimates of CE, and the heterogeneity decreased after a sensitivity analysis excluding the results from van Wetering et al., but still without reaching statistical significance. In the analysis of the literature characteristics, we found that the quadriceps muscle strength of the participants was 92–95% of the normal predicted value (47), which may lead to a small potential for improvement. However, the results are still inconsistent with speculations and previous research results, that is, CE has similar or even greater effects than EE and RE alone (16, 17, 40), which may be attributed to a variety of CE programs included in this meta-analysis. First, the proportion of EE and RE in CE programs. Most programs scheduled EE and RE in one session

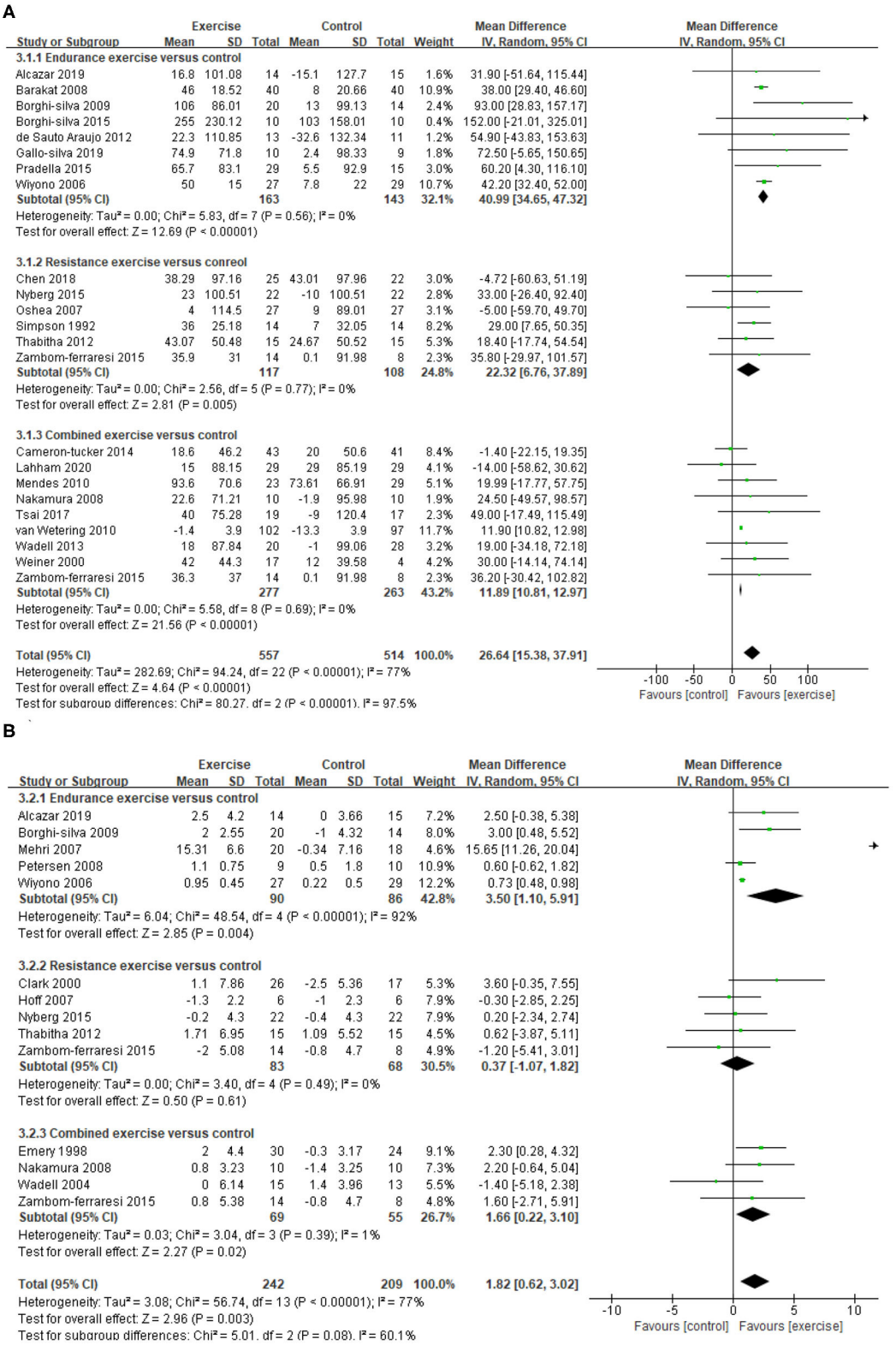

FIGURE 5 | Pooled effect of exercise on exercise capacity in people with COPD. **(A)** 6MWD, **(B)** VO_{2peak}. 6MWD, 6-min walking distance (m); CI, confidence interval; COPD, chronic obstructive pulmonary disease; SD, standard deviation; VO_{2peak}, peak oxygen consumption (ml/kg/min).

TABLE 4 | Grading of recommendations assessment, development, and evaluation summary of findings.

Outcomes	Certainty Assessment							No. of Patients		Effect		Certainty
	No. of Studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	EG	CG	Relative (95% CI)	Absolute (95% CI)	
BMI	3	Randomized trials	Serious[a]	Serious[b]	Not serious	Serious[c]	None	134	120	–	MD 0.11 lower (1.13 lower to 0.91 higher)	⊕◯◯◯ Very low
Skeletal muscle strength	13	Randomized trials	Serious[a]	Serious[b]	Not serious	Not serious	None	660	628	–	SMD 3.48 higher (1.81 to 5.15 higher)	⊕⊕◯◯ Low
6MWD	22	Randomized trials	Serious[a]	Not serious	Not serious	Not serious	None	557	514	–	MD 12.76 higher (11.69 to 13.82 higher)	⊕⊕⊕◯ Moderate
VO_{2peak}	13	Randomized trials	Serious[a]	Not serious	Not serious	Not serious	None	242	209	–	MD 1.82 higher (0.62 to 3.02 higher)	⊕⊕⊕◯ Moderate

[a] *Most of the studies are without allocation concealment, subject blinded and intention-to-treatment analysis.*
[b] *There was a substantial heterogeneity among the three studies according to the heterogeneity test.*
[c] *Only three studies were included in the analysis, and the sample size was relatively low.*

and two to three sessions a week, respectively, apart from the program in Zambom-Ferraresi et al. (scheduled EE in one session and RE in another session, only two sessions a week). Second, the range of exercise intensity was relatively extensive, which may play a role in maintenance but not in improvement. Therefore, in the CE program for improving COPD's skeletal muscle strength, the different proportions and intensities of EE and RE might have different effects, and it is still necessary to explore the best program.

Subgroup analysis for different muscle strength testing methods found that exercise can only significantly improve isotonic muscle strength. We speculated that the isotonic muscle strength test is more familiar to the participants and has a higher correlation with daily life than other tests (58). Considering that different strength units may be the source of heterogeneity, we pooled data units in kilograms and found that exercise significantly improved isometric muscle strength. Although the data of isokinetic muscle strength showed an increasing trend after exercise (33, 36), many studies are still needed to determine the degree of response. We also conducted subgroup analysis to determine the effects of exercise on upper limbs and lower limbs muscle strength and found that exercise can improve the muscle strength of both upper and lower limbs. Although subgroup analysis was performed, high heterogeneity still existed, and the source of heterogeneity was unclear. A standard and clinically feasible measurement program is needed to quantitatively evaluate the damage of peripheral skeletal muscle strength and the response to exercise in COPD.

Consistent with previous meta-analysis (15, 59), this study found that exercise can significantly improve 6MWD (26.64 m) in patients with COPD. However, only the EE improvement reached the minimal clinical important difference of 30 m (60), which may be attributed to EE bringing more aerobic metabolism changes and greater improvements in ventilation capacity; the relatively low proportion of EE in the CE program cannot bring significant improvement. The peak exercise capacity is often evaluated using a cardiopulmonary exercise test (CPET), which is considered the gold standard to assess the exercise capacity and closely related to COPD's prognosis (61, 62). A progressive incremental exercise protocol in a treadmill or cycle ergometer is often used for CPET, and the results can provide abundant physiological information related to exercise restriction, including the heart (e.g., heart rate, VO_{2peak}, and oxygen pulse), lung (e.g., inspiratory capacity, gas exchange, and dynamic inflation), muscle (e.g., power and lactic acid), dyspnea (Borg), and exercise initiative (62). A Cochrane review conducted in 2015 showed that pulmonary rehabilitation (at least 4 weeks of exercise training) is beneficial in improving maximal exercise capacity (measured by W_{max}) in patients with COPD, and the effect size exceeds the minimal clinically important difference (4 W) (63). Although a different outcome was used in this present study, the effect of exercise is confirmed. The comparison results of the effects of different modalities exercise showed no significant differences between RE vs. the control group (15, 64), RE vs. EE (16), and CE vs. EE (64) in improving the peak exercise capacity (VO_{2peak}, W_{peak}) of patients with COPD. It seems that a contradictory deduction

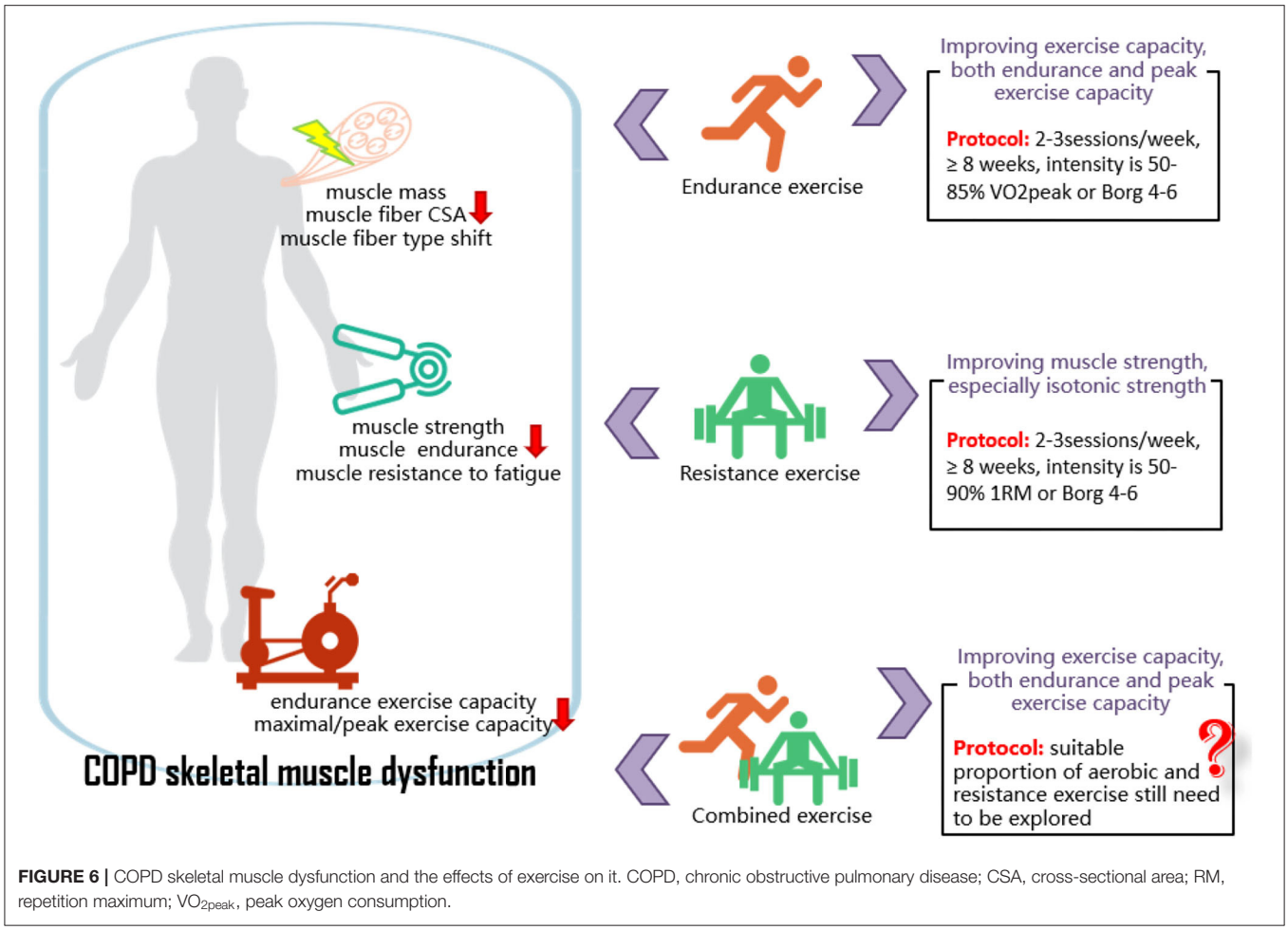

FIGURE 6 | COPD skeletal muscle dysfunction and the effects of exercise on it. COPD, chronic obstructive pulmonary disease; CSA, cross-sectional area; RM, repetition maximum; VO_{2peak}, peak oxygen consumption.

might be concluded that exercise does not have a significant positive effect on peak exercise capacity of patients with COPD. Based on the primary pathophysiological mechanisms of exercise limitation in patients with COPD undergoing CPET, including ventilatory abnormalities, pulmonary gas exchange abnormalities, and skeletal muscle dysfunction (61), exercise with different modalities seems beneficial in improving peak exercise capacity in patients with COPD. Consistent with the hypothesis, this meta-analysis showed that exercise could significantly improve COPD's peak exercise capacity (1.82 ml/kg/min), and both EE and CE have positive effects.

This systematic review and meta-analysis had some limitations. First, there were flaws in methodological quality of the original studies, namely the lack of subject blinding and evaluator blinding in exercise intervention trials. Second, one of the included literatures had an apparently large sample size, which may have had an impact on the research results. Sensitivity analysis was performed to reduce the impact when high heterogeneity was found. Third, we only analyzed the effects of exercise on skeletal muscle strength and still needed to explore the effects of exercise on skeletal muscle endurance and power. Fourth, the outcomes of skeletal muscle function were not assessed comprehensively in most of the included studies, which may cause a limitation. Fifth, trial designs were heterogeneous. For high heterogeneity, we used a random-effects model and subgroup analysis to analyse the source of heterogeneity, and the results were consistent.

CONCLUSIONS

Exercise with different modalities seems effective in improving peripheral skeletal muscle strength and exercise capacity in patients with stable COPD. Specifically, EE shows a greater improvement in endurance and peak exercise capacity, and RE shows a greater improvement in peripheral skeletal muscle strength, and the isotonic test is relatively sensitive in reflecting muscle strength changes. Therefore, for patients with COPD whose exercise limitation is caused by a decreased cardiorespiratory capacity, EE might be a suitable choice. EE can be conducted in cycling, running, and walking, with an intensity of 50–85% VO_{2peak}, 2–3 times/week, for at least 8 weeks. For patients with COPD whose exercise limitation is caused by an impaired peripheral skeletal muscle function, RE might be a preferable intervention. RE can be conducted in weight machines, free weights, and elastic bands, with an intensity of 50–90% 1RM, 2–3 times/week, for at least 8 weeks. The proportion

of EE and RE in CE programs still needs to be explored and analyzed (**Figure 6**). High methodological quality RCTs with a large sample size are still needed to verify the present study results because of the relatively small inclusion of literature on the peripheral skeletal muscle structure and function in patients with COPD. It is also necessary to explore the effect of exercise intervention on peripheral skeletal muscle in AECOPD or patients with COPD with different severity.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

XL and JX conceived of the idea for this review. JL and YW did the literature search. PL and YW collected the data. PL and JL did the quality assessment. PL did the statistical analyses and wrote the first draft of the manuscript. All authors analyzed and interpreted the data and revised and approved the final manuscript for submission.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.766841/full#supplementary-material

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Serum CYR61 Is Associated With Airway Inflammation and Is a Potential Biomarker for Severity in Chronic Obstructive Pulmonary Disease

Zhu-Xia Tan[1,2†], Lin Fu[1†], Wen-Jing Wang[1], Ping Zhan[1], Hui Zhao[1], Hua Wang[2] and De-Xiang Xu[2*]*

[1] Second Affiliated Hospital, Anhui Medical University, Hefei, China, [2] Department of Toxicology, Anhui Medical University, Hefei, China

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Edited by:
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United Kingdom

***Correspondence:**
De-Xiang Xu
xudex@126.com
Hua Wang
wanghuadev@126.com

[†] These authors have contributed equally to this work

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Background: Cysteine-rich 61 (CYR61) and inflammation was upregulated in the lungs of patients with chronic obstructive pulmonary disease (COPD). However, the association between CYR61 and inflammation was unclear in COPD patients. This study aimed to analyze the association of serum CYR61 with pulmonary inflammation and lung function indexes in COPD patients.

Methods: One hundred and fifty COPD patients and 150 control subjects were enrolled. Serum and pulmonary CYR61 was detected. Lung function indexes were evaluated in COPD patients.

Results: Serum CYR61 level was elevated and pulmonary CYR61 expression was upregulated in COPD patients. An increased CYR61 was associated with decreased pulmonary function indexes in COPD patients. Further analyses showed that nuclear factor-kappa B (NF-κB) p65-positive nuclei was elevated in the lungs of COPD patients with high level of CYR61. Accordingly, serum monocyte chemotactic protein (MCP)-1 and tumor necrosis factor α (TNF-α), two downstream inflammatory cytokines of NF-κB pathway, were increased in parallel with CYR61, among which serum MCP-1 and TNF-α were the highest in COPD patients with high level of CYR61. Moreover, a positive correlation, determined by multivariate regression that excluded the influence of age, gender and smoking, was observed between serum CYR61 and inflammatory cytokines in COPD patients.

Conclusion: These results provide evidence that an increased CYR61 is associated with pulmonary inflammation and COPD progression. Inflammatory cytokines may be the mediators between CYR61 elevation and COPD progression.

Keywords: CYR61, COPD, NF-κB, lung function, inflammatory cytokines

BACKGROUND

Chronic obstructive pulmonary disease (COPD) is a common respiratory disease, which is characterized by lung parenchyma damage and progressive decline in lung function (1–3). Cigarette smoking is a major risk factor for the occurrence and development of COPD (4). Chronic airway inflammation, accompanied by infiltration of numerous macrophages and lymphocytes, has been implicated in the progression of COPD (5–7). Accumulating data have demonstrated that chemokines, such as monocyte chemotactic protein (MCP)-1 and interleukin (IL)-8, are involved in the recruitment of inflammatory cells (8, 9). The activation of nuclear factor-kappa B (NF-κB) may play important roles in regulating cigarette smoke-evoked inflammatory chemokines (10–13).

Cysteine-rich 61 (CYR61), also named as CCN1, is a member of CCN protein family (14). Numerous data have demonstrated that CYR61 takes part in the process of angiogenesis, embryonic development, and tissue repair (15–17). Recently, the role of CYR61 in pulmonary diseases is concerned (18). Several studies indicated that CYR61 was involved in the pathogenesis of acute lung injury and acute respiratory distress syndrome (19, 20). Moreover, CYR61 aggravated transforming growth factor (TGF)-β-induced SMAD3 activation and lung fibrosis (21). An early report showed that pulmonary CYR61 expression was upregulated in COPD patients (22). Nevertheless, the association between upregulated CYR61 and COPD progression remains unknown.

In the current study, we aimed to analyze the association among serum CYR61, pulmonary inflammation and lung function indexes in COPD patients. We showed that serum CYR61 was elevated and pulmonary CYR61 expression was upregulated in COPD patients. Moreover, an elevation of serum CYR61 was associated with lung function decline in COPD patients. Our results provide evidence that inflammatory chemokine MCP-1 is a mediator between an increased CYR61 and lung function decline in COPD patients.

METHODS

Reagents and Chemicals

Antibodies against NF-κB p65 and CYR61 were purchased from Cell Signaling Technology (MA, USA). CYR61 ELISA kits were from Cusabio (TX, USA). MCP-1 ELISA kit was from Wuhan Colorful Gene Biological Technology (Hubei, China). Chemiluminescence (ECL) detection kits were from Advansta (CA, USA). All other regents and chemicals were from Sigma Chemical Co. (MO, USA) if not specifically noted.

COPD Patients and Lung Specimen

All COPD patients who were first time diagnosed were randomly selected from Anhui COPD Cohort (AHCC) that was a hospital-based prospective cohort established by the Second Affiliated Hospital of Anhui Medical University. For the matched case-control study, 150 COPD patients were recruited from AHCC. Pulmonary function was tested in all recruited COPD patients based on standardized methods. COPD was confirmed on basis of the American Thoracic Society criteria and the Global Initiative for COPD (GOLD) criteria (23), which forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) ratio was <70%. Total 150 sex- and age-matched control subjects were randomly collected from the physical examination center at the Second Affiliated Hospital of Anhui Medical University. To analyze the level of serum CYR61 and MCP-1, sera were collected from all COPD patients and controls. To measure pulmonary CYR61 and NF-κB, lung tissues were obtained from surgical operations between COPD patients and Controls. Lung specimens were collected from paracancerous tissue of lung cancer patients without other pulmonary disease were as controls at the Second Affiliated Hospital of Anhui Medical University. Each control subject was matched with one COPD patients in accord with age and gender (24). Finally, all 20 lung cancer patients without other pulmonary diseases and 20 COPD patients were enrolled. The current study was approved by the Ethics Committee of Anhui Medical University (2021030). All subjects have agreed and signed an informed consent.

Immunochemistry (IHC)

All lung specimens were fixed in formalin and embedded in paraffin. Lung section was dewaxed and rehydrated according to a conventional standard method (25). To punch cell membrane and suppress endogenous peroxidase, lung section was immersed in PBS containing 0.5% Triton X-100 and 3% H_2O_2 for 45 min. Antigen retrieval was performed in boiled citrate solution. After blocked, lung section was incubated with either CYR61 or NF-κB p65 antibody (1:200) at 37°C incubator for 3.5 h. After washed with PBS for three times, conjunction with streptavidin-HRP complex was incubated for 2.5 h at room temperature. Immunolabelling was evaluated using DAB solution. nucleus was stained with hematoxylin in a dark room. Pulmonary CYR61- and p65- positive cells were calculated by two independent pathologists.

Enzyme Linked Immunosorbent Assay

Serum concentrations of CYR61, monocyte chemoattractant protein 1 (MCP-1) and tumor necrosis factor α (TNF-α) were measured using enzyme linked immunosorbent assay (ELISA). CYR61 (CSB-E13884h) and MCP-1 (CSB-E04655h) ELISA kits were bought from Cusabio, Wuhan, China (https://www.cusabio.com/). TNF-α (JYM0110Hu) ELISA kits were obtained from Wuhan ColorfulGene Biological Technology Co (http://www.jymbio.com/product/286-cn.html). The detailed method referred to the reagent manual (26).

Statistical Analysis

The quantitative variables were expressed as means and standard error of mean. The categorical variables were expressed with frequencies and percentages. All statistical analysis was conducted in SPSS 21.0. Independent sample unpaired *t*-test was

Abbreviations: COPD, Chronic obstructive pulmonary disease; CYR61, Cysteine-rich 61; NF-κB, nuclear factor-kappa B; MCP-1, Macrophage chemoattractant protein-1; FEV1, Forced expiratory volume in 1 s; FVC, forced vital capacity.

TABLE 1 | Demographic information and clinical characteristics.

Variable	Control ($n = 150$)	COPD ($n = 150$)	*P*
Age (years)	67.9 ± 0.94	72.8 ± 0.61	0.132
Female, *n* (%)	51 (34.0)	38 (25.3)	0.100
Emphysema	N.A.	121 (80.7)	N.A.
Smoking status			<0.001
Never-smoker, *n* (%)	55 (36.7)	8 (5.3)	
Former-smoker, *n* (%)	65 (43.3)	123 (82.0)	
Current-smoker, *n* (%)	30 (20.0)	19 (12.7)	
WBC (10^9/L)	6.22 ± 0.121	7.45 ± 0.278	<0.01
Neutrophil (10^9/L)	3.24 ± 0.077	5.40 ± 0.270	<0.01
Lymphocyte (10^9/L)	1.83 ± 0.071	1.26 ± 0.047	<0.01
Eosinophil (10^9/L)	0.12 ± 0.010	0.18 ± 0.024	0.001
Monocyte (10^9/L)	0.41 ± 0.056	0.57 ± 0.033	<0.01
Basophil (10^9/L)	0.02 ± 0.002	0.03 ± 0.003	0.009
FEV1 (%)	N.A.	52.02 ± 2.659	N.A.
FEV1/FVC (%)	N.A.	57.79 ± 1.537	N.A.
FEV1 (L)	N.A.	1.16 ± 0.066	N.A.
FVC (L)	N.A.	1.88 ± 0.064	N.A.
PH	N.A.	7.39 ± 0.007	N.A.
PCO2 (mmHg)	N.A.	51.99 ± 2.107	N.A.
PO2 (mmHg)	N.A.	70.88 ± 2.588	N.A.
RV%TLC-SB (%)	N.A.	54.59 ± 1.564	N.A.
DLCO SB (mmol/min/kPa)	N.A.	3.82 ± 0.259	N.A.

WBC, White blood cell; FEV1, Forced expiratory volume in one second; FVC, Forced vital capacity; N.A., Not available.

used to evaluate the difference for continuous variables between two groups. The difference of continuous variables in three groups was determined through one-way ANOVA. Chi-square test was used to analyze the difference for count data. Pearson correlation analysis and linear regression analysis were used to evaluate the correlations among CYR61 and inflammatory cytokines. The association of serum CYR61 and hospital stays was accessed through logistical regression analysis. $P < 0.05$ was considered statistically significant.

RESULTS

Demographic Data and Clinical Characteristics

The demographic data and clinical characteristics were analyzed. As shown in **Table 1**, 150 COPD patients and 150 controls were recruited in this study. No significant difference on mean ages was observed between two groups (72.83 ± 0.61 in COPD patients vs. 67.94 ± 0.94 in controls, $P > 0.05$). In addition, no significant difference on sex ratio was shown between two groups (**Table 1**). There were 121 (80.7%) cases with emphysema in COPD patients (**Table 1**). Interestingly, there was obvious difference of smokers between COPD patients and control subjects. Blood routine indexes were then analyzed. As expected, the counts of white blood cell (WBC), neutrophil, eosinophil, monocyte and basophil were elevated in COPD patients (**Table 1**). By contrast, lymphocyte count was reduced in COPD patients (**Table 1**). The results of FEV1(%), FEV1/FVC, FEV1 (L), FVC (L), PH, PCO2, PO2, RV%TLC-SB (%), and DLCO SB in COPD patients was presented in **Table 1**. Not only that, the demographic data and clinical characteristics were further compared in COPD patients with different grades. As shown in **Supplementary Table 1**, no difference of emphysema and blood gas indicators was observed in COPD patients with different grades. RV%TLC-SB was lower and DLCO SB was higher in grade 1-2 (G 1-2) COPD patients than those in grade 3 and grade 4 (G 3 and G 4) COPD cases (**Supplementary Table 1**).

An Increased CYR61 Is Associated With the Severity of COPD

Serum CYR61 concentration was analyzed in COPD patients and controls. As shown in **Figure 1A**, serum CYR61 was elevated in COPD patients as compared with controls. Serum CYR61 was then compared among different grades of COPD patients. As shown in **Figure 1B**, serum CYR61 was gradually elevated in parallel with the grades of COPD patients, among which serum CYR61 level was the highest in patients with G 4. Pulmonary CYR61 was then detected in COPD patients and controls. As shown in **Figure 1C**, an obvious CYR61 staining was shown in the lungs from COPD patients. Quantitative analysis showed that pulmonary CYR61 was upregulated in COPD patients as compared with controls (**Figure 1D**). Moreover, pulmonary CYR61 was higher in G 3 and G 4 COPD patients than those in G 1-2 COPD patients (**Figures 1E,F**).

Serum CYR61 Is Negatively Correlated With Lung Function Indexes in COPD Patients

The correlation between serum CYR61 and FVC was analyzed among COPD patients. As expected, a negative correlation was observed between serum CYR61 and FVC (**Figure 2A**, $r = -0.328$, $P < 0.001$). The correlation between serum CYR61 and FEV1(L) was then analyzed among COPD patients. As shown in **Figure 2B**, there was a negative correlation between serum CYR61 and FEV1(L) ($r = -0.379$, $P < 0.001$). Next, the correlation between serum CYR61 and FEV1/FVC was evaluated among COPD patients. As shown in **Figure 2C**, there was a negative correlation between serum CYR61 and FEV1/FVC ($r = -0.144$, $P = 0.045$). The correlation between serum CYR61 and FEV1(%) is presented in **Figure 2D**. As expected, a negative correlation was observed between serum CYR61 and FEV1 (%) ($r = -0.507$, $P < 0.001$). Finally, regression analysis was used to evaluate the correlation between serum CYR61 and pulmonary function among COPD patients. Univariate regression analysis showed that there was a negative correlation between serum CYR61 and all lung function indexes in COPD patients (**Table 2**). Multivariate regression analysis was used to exclude the influence of age, gender and smoking on serum CYR61 level and pulmonary functions in COPD patients. Although no correlation between CYR61 and FEV1/FVC (%)

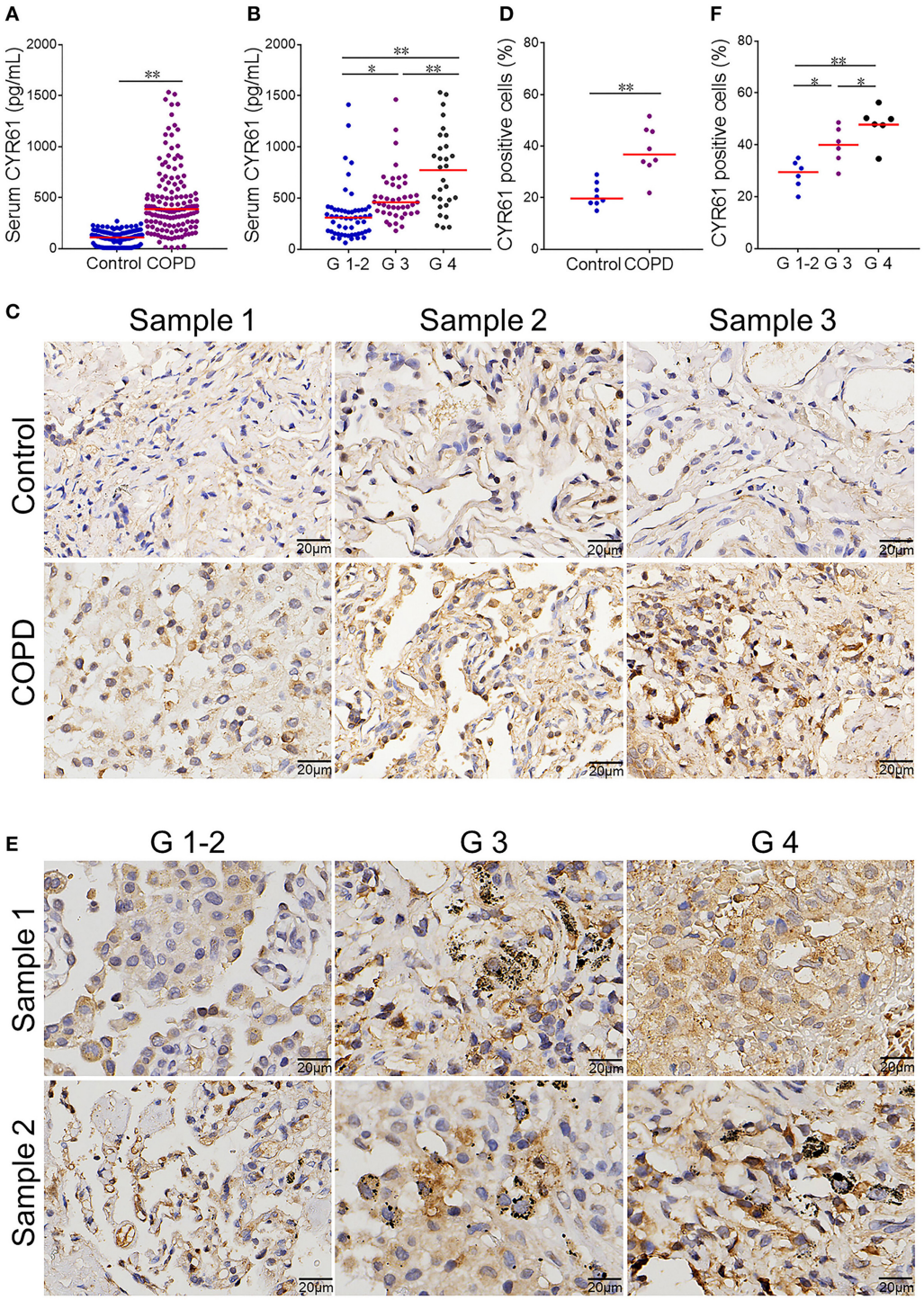

FIGURE 1 | Serum CYR61 level and its association with severity of COPD. Sera were collected from COPD patients and controls. **(A,B)** Serum CYR61 level was detected using ELISA. **(A)** Serum CYR61 level was compared between COPD patients and controls. All data were represented as means ± S.E.M. ($N = 150$). **(B)** Serum CYR61 level was compared among different grades of COPD patients. All data were represented as means ± S.E.M. ($N = 69$ for G 1-2 patients, $N = 48$ for G 3 patients, $N = 33$ for G 4 patients). **(C,D)** Pulmonary CYR61 expression was compared between COPD patients and controls. **(C)** Three representative pictures. **(D)** Quantitative analysis of CYR61-positive cells in COPD patients and controls. **(E,F)** Pulmonary CYR61 was compared among different grades of COPD patients. **(E)** Three representative pictures: arrows indicate CYR61-positive cell; **(F)** Quantitative analysis of CYR61-positive cells in COPD patients with different grades. All data were represented as means ± S.E.M. ($N = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$.

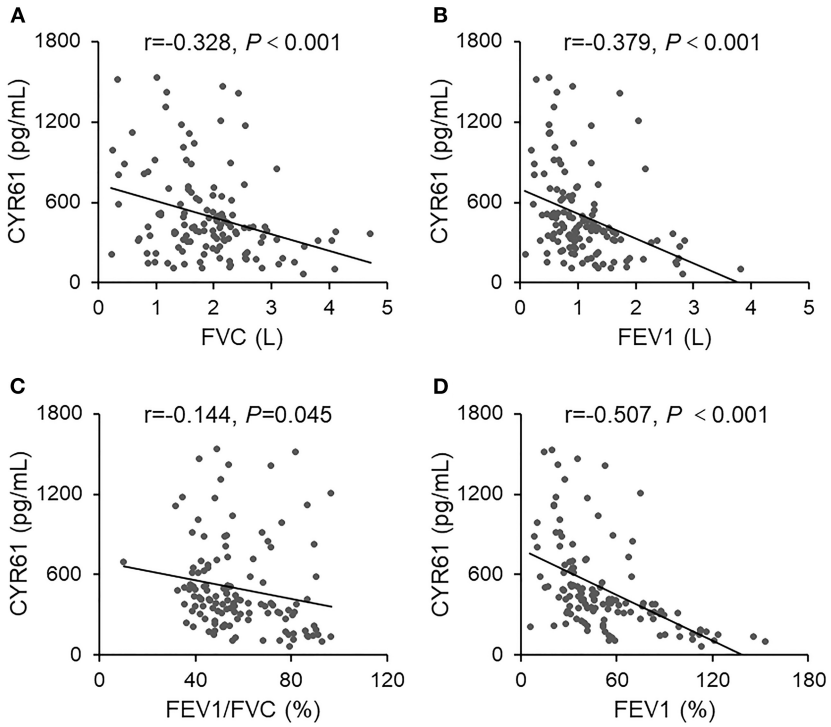

FIGURE 2 | The correlation between serum CYR61 and lung function indexes in COPD patients. Pulmonary function was measured in 150 COPD patients. Sera were collected and CYR61 was detected using ELISA. Correlation between serum CYR61 and lung function indexes was analyzed. **(A)** CYR61 vs. FVC(L); **(B)** CYR61 vs. FEV1(L); **(C)** CYR61 vs. FEV1/FVC (%); **(D)** CYR61 vs. FEV1(%).

was observed, there remains a negative correlation between serum CYR61 and other three lung function indexes in COPD patients (**Table 2**).

TABLE 2 | Association of serum CYR61 with lung function.

Variables	Multivariable, β (95% CI)*	*P*
FEV1 (%)	−0.466 (−0.054, −0.028)	<0.001
FEV1/FVC (%)	−0.050 (−0.011, 0.006)	0.547
FEV1 (L)	−0.408 (−0.001, 0.000)	<0.001
FVC (L)	−0.406 (−0.001, 0.000)	<0.001

**Age, gender, and smoking were adjusted.*

An Increased CYR61 Is Associated With Pulmonary NF-κB Activation in COPD Patients

Pulmonary NF-κB was evaluated among COPD patients and controls. As expected, numerous NF-κB p65-positive nuclei, as determined by IHC, were observed in the lung of COPD patients (**Figure 3A**). Quantitative analysis showed that NF-κB p65-positive nuclei were elevated in COPD patients as compared with controls (**Figure 3B**). The association between serum CYR61 and pulmonary NF-κB activation was analyzed in COPD patients. As expected, the numbers of pulmonary NF-κB p65-positive nuclei were more in COPD patients with high level of CYR61 than in COPD patients with low level of CYR61 (**Figures 3C,D**).

An Increased CYR61 Is Associated With Serum MCP-1 and TNF-α in COPD Patients

Firstly, the associations between serum CYR61 and inflammatory cytokines was analyzed among all subjects. As expected, serum MCP-1 was gradually elevated in parallel with CYR61, among which serum MCP-1 level was the highest in subjects with high level of CYR61 (**Figure 4A**). Correlation analysis showed a positive association between serum CYR61 and MCP-1 (**Figure 4D**, $r = 0.518$, $P < 0.001$). A positive correlation, as determined by multivariate regression analysis that excluded the influence of age, gender and smoking, was observed between serum CYR61 and MCP-1 in all subjects (**Table 3**). Next, the association between serum CYR61 and MCP-1 was analyzed among COPD patients. As expected, serum MCP-1 was gradually elevated in parallel with CYR61, among which serum MCP-1 level was the highest in subjects with high level of CYR61 (**Figure 4B**). Correlation analysis showed a positive association between serum CYR61 and MCP-1 (**Figure 4E**, $r = 0.456$, $P < 0.001$). Moreover, a positive correlation, as determined by multivariate regression analysis that excluded the influence

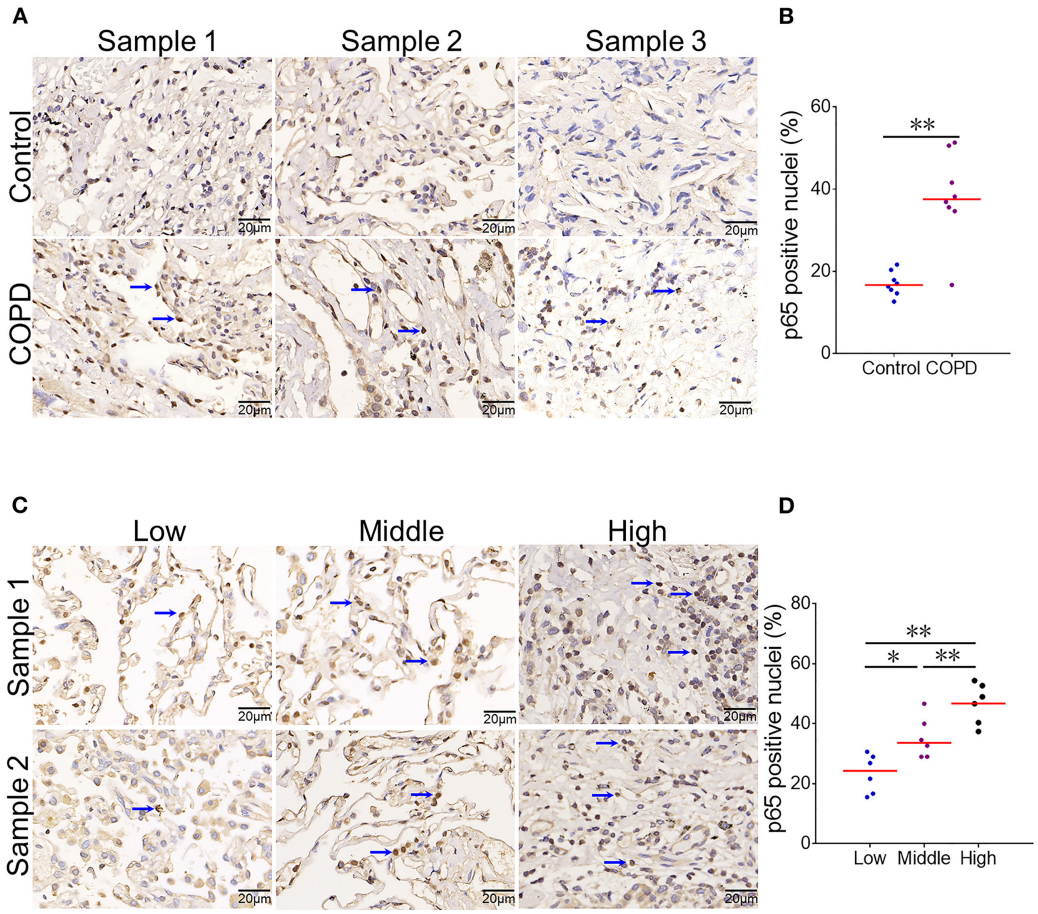

FIGURE 3 | The association between serum CYR61 and pulmonary NF-κB activation in COPD patients. Lung tissues were collected from COPD patients and controls. Pulmonary NF-κB p65 was detected using IHC. **(A,B)** Pulmonary NF-κB p65-positive nuclei were compared between COPD patients and control subjects. **(A)** Three representative pictures: arrows indicate p65-positive nuclei; **(B)** Quantitative analysis of p65-positive nuclei in COPD patients and controls. **(C,D)** Pulmonary NF-κB p65-positive nuclei were compared among COPD patients with different levels of CYR61. **(C)** Three representative pictures: arrows indicate p65-positive nuclei; **(D)** Quantitative analysis of p65-positive nuclei in COPD patients with different levels of CYR61. All data were represented as means ± S.E.M. ($N = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$.

of age, gender and smoking, was observed between serum CYR61 and MCP-1 in COPD patients (**Table 3**). Finally, the association between serum CYR61 and MCP-1 was analyzed among control subjects. As shown in **Figure 4C**, no significant difference on serum MCP-1 was observed among different groups. Correlation and multivariate regression analyses showed that there was no association on serum CYR61 and MCP-1 (**Figure 4F**; **Table 3**, $P > 0.05$). Moreover, the association of serum CYR61 and TNF-α was evaluated in all cases. As shown in **Figure 4G**, serum TNF-α was increased in high serum CYR61 group than these in low serum CYR61 group. Besides, serum TNF-α was gradually risen in line with serum CYR61 in COPD patients (**Figure 4H**). In addition, serum TNF-α was elevated in High group than in Middle group among control cases (**Figure 4I**). Although, there was no obvious correlation of serum TNF-α with CYR61 in control cases (**Figure 4L**), serum CYR61 was positively associated with TNF-α in all cases (**Figure 4J**, $r = 0.177$, $P = 0.001$) and COPD patients (**Figure 4K**, $r = 0.329$, $P < 0.001$).

The Mediating Effect of Inflammatory Cytokines Between Increased CYR61 and Decreased Lung Function Indexes in COPD Patients

First, the direct effect of increased CYR61 on pulmonary function decline was analyzed. As shown in **Figure 5**, serum CYR61 was negatively associated with FEV1 (%) ($\beta = -0.513$, $P < 0.01$) in COPD patients. The mediating effect of inflammatory cytokines between CYR61 and lung function indexes was then evaluated in COPD patients. As shown in **Figure 5**, obvious mediating effects between increased MCP-1 ($\beta = -0.300$, $P < 0.05$) and TNF-α ($\beta = -0.111$, $P < 0.05$) with decreased lung function were observed in COPD patients. The total effect of CYR61

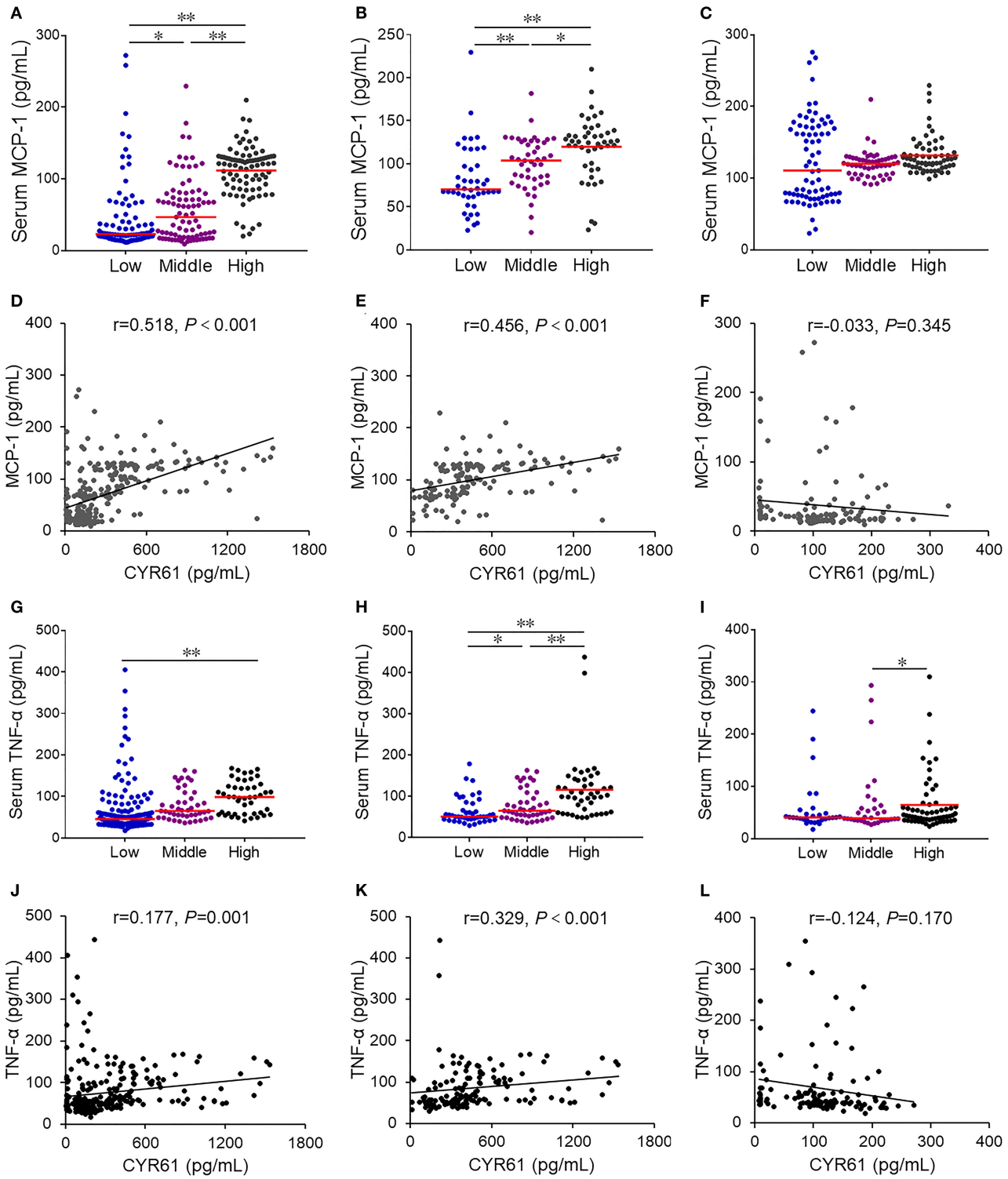

FIGURE 4 | The association of serum CYR61 level with serum inflammatory cytokines in COPD patients. Sera were collected from 150 COPD patients and 150 control subjects. Serum CYR61 and inflammatory cytokines were detected using ELISA. **(A,D)** Association between serum CYR61 and MCP-1 was analyzed among all subjects. **(A)** Serum MCP-1 was compared among subjects with different levels of CYR61. **(D)** Correlation analysis between serum CYR61 and MCP-1. **(B,E)** Association between serum CYR61 and MCP-1 was analyzed among COPD patients. **(B)** Serum MCP-1 was compared among COPD patients with different levels of CYR61. **(E)** Correlation analysis between serum CYR61 and MCP-1. **(C,F)** Association between serum CYR61 and MCP-1 was analyzed among control

(Continued)

FIGURE 4 | subjects. **(C)** Serum MCP-1 was compared among control subjects with different levels of CYR61. **(F)** Correlation analysis between serum CYR61 and MCP-1. **(G,J)** Association between serum CYR61 and TNF-α was analyzed among all subjects. **(G)** Serum TNF-α was compared among subjects with different levels of CYR61. **(J)** Correlation analysis between serum CYR61 and TNF-α. **(H,K)** Association between serum CYR61 and TNF-α was analyzed among COPD patients. **(H)** Serum TNF-α was compared among COPD patients with different levels of CYR61. **(K)** Correlation analysis between serum CYR61 and TNF-α. **(I,L)** Association between serum CYR61 and TNF-α was analyzed among control subjects. **(I)** Serum TNF-α was compared among control subjects with different levels of CYR61. **(L)** Correlation analysis between serum CYR61 and TNF-α. *$P < 0.05$, **$P < 0.01$.

TABLE 3 | Associations of serum CYR61 with MCP-1 and TNF-α.

Groups	MCP-1		TNF-α	
	Multivariable,β (95% CI)	***P***	**Multivariable,β (95% CI)**	***P***
All cases	0.479 (0.067, 0.102)	<0.001	0.177 (0.316, 1.685)	<0.001
Control	−0.027 (−0.145, 0.104)	0.747	−0.156 (−0.316, 0.020)	0.083
COPD	0.473 (0.041,0.077)	<0.001	0.327 (1.467, 4.365)	<0.001

Age, gender, and smoking were adjusted.

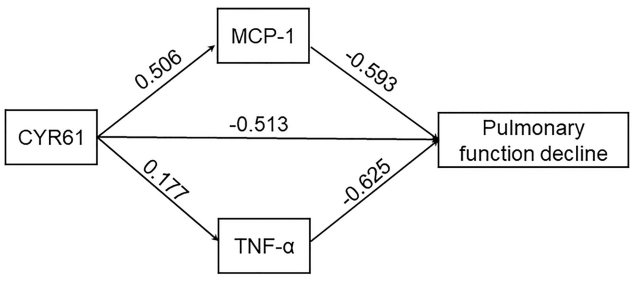

FIGURE 5 | Postulated mediational path model.

on lung function decline was −0.924 ($P < 0.01$) in COPD patients. The relative contribution of MCP-1 and TNF-α in CYR61 elevation-induced pulmonary function decline was 44.5% in COPD patients.

An Increased Serum CYR61 Is Positively Associated With Hospital Stays in COPD Patients

According to quartile, serum CYR61 was classified as Low group (<410.0 pg/ml) and High group (≥410.0 pg/ml), hospital stays were divided into Short group (<10 days) and Long group (≥10 days). Then, the effect of serum CYR61 at the early stage on hospital stay was evaluated in **Table 4**. Among 150 COPD patients, the hospital stays of 69 (46.0%) patients was longer than 10 days. The number of cases with higher hospital stays was more among COPD patients with high serum CYR61 than those with low serum CYR61 (55.1 vs. 25.9%; RR = 2.124, 95% Cl: 1.388∼3.250; $P < 0.001$).

TABLE 4 | Association of serum CYR61 and hospital stays in COPD patients.

CYR61	Cases, *n*	Longer hospital stays, *n* (%)	RR (95%)	*P*
Low	81	21 (25.9)	1	-
High	69	38 (55.1)	2.124 (1.388, 3.250)	<0.001

According to quartile, serum CYR61 was classified as Low group (<410.0 pg/ml) and High group (≥410.0 pg/ml), hospital stays were divided into Short group (<10 days) and Long group (≥10 days).
RR, relative risk.

DISCUSSION

The associations of serum CYR61 with pulmonary inflammation and lung function indexes were investigated in COPD patients. The major findings were as follow: Firstly, serum CYR61 level was elevated and pulmonary CYR61 expression was upregulated in COPD patients; Secondly, an increased CYR61 was associated with decreased lung function indexes in COPD patients; Thirdly, an increased CYR61 is associated with an elevation of serum MCP-1, TNF-α and activation of pulmonary inflammatory signaling in COPD patients. Fourthly, an increased CYR61 is associated with a longer hospital stays among COPD patients. Our results suggest that pro-inflammatory cytokine and chemokine are mediators between CYR61 elevation and COPD progression.

Accumulating data indicate that CYR61 is involved in the pathogenesis of COPD (18). An early study found that pulmonary CYR61 was upregulated in COPD patients as compared with controls (22). Indeed, cigarette smoking is a major risk factor for COPD (27). According to an earlier report, cigarette smoke extracts upregulated CCN1 through evoking excess reactive oxygen spices and endoplasmic reticulum stress (28). In the current study, we measured serum CYR61 level and pulmonary CYR61 expression in COPD patients. Our results showed that serum CYR61 was elevated and pulmonary CYR61 was upregulated in COPD patients. Interestingly, we found that serum CYR61 level and pulmonary CYR61 expression were positively associated with the severity of COPD. To further determine the role of CYR61 in COPD progression, the current study analyzed the association between serum CYR61 level and lung function indexes in COPD patients. We found that serum CYR61 level is negatively correlated with several lung function indexes in COPD patients. Not only that, the association of serum CYR61 and the prognosis was estimated in COPD patients. Our results indicated that serum higher CYR61 on admission prolonged the hospital stays among COPD patients. Our results provide evidence for

the first time that CYR61 may be a biomarker to predict COPD progression.

The mechanism by which CYR61 associates COPD progression remains unclear. Numerous studies demonstrated that chronic airway inflammation aggravated lung function decline in COPD patients (5, 29). On the other hand, several studies found that CYR61 had proinflammatory activities (30–34). An earlier study showed that CYR61 mediated cigarette smoke extracts evoked IL-8 secretion by lung epithelial cells (28). The current study investigated the association of serum CYR61 with serum inflammatory cytokines and inflammatory signaling in the lungs of COPD patients. Our results showed that pulmonary NF-κB p65-positive nuclei were higher in COPD patients with high CYR61 than with low CYR61. Accordingly, serum MCP-1 and TNF-α, the downstream pro-inflammatory cytokine and chemokine of NF-κB signaling, were increased in parallel with CYR61, among which serum inflammatory cytokines were the highest in COPD patients with high CYR61. To determine the mediating effect of inflammatory cytokines between increased CYR61 and decreased lung function indexes, we analyzed the link between serum CYR61 and inflammatory cytokines in COPD patients. Despite no association between serum CYR61 and inflammatory cytokines among control subjects, a positive correlation of serum CYR61 with TNF-α and MCP-1, as determined by multivariate regression analysis that excluded the influence of age, gender and smoking, was observed among COPD patients. Further analysis showed an obvious mediating effect between an increased inflammatory cytokines with a decreased lung function indexes in COPD patients.

There are several flaws in the current study. First, the results of the current study were from a cross-sectional analysis, in which all COPD patients were from Anhui COPD Cohort. The causal link among CYR61, pulmonary inflammation and COPD progression was not clear. Thus, further follow-up observation and animal experiments are needed to determine the influence of CYR61 on pulmonary inflammation and lung function indexes in COPD patients. Second, the current study has not clarified the underlying mechanism through which CYR61 upregulates inflammatory cytokines in COPD patients. Indeed, an earlier study found that CYR61 upregulated MCP-1 through activating NF-κB pathway in vascular endothelial cells (33). In addition, the previous study has revealed that CYR61 elevated TNF-α via NF-κB signal in murine macrophages (30). Additional experiment is required to explore the exact mechanism by which CYR61 activates NF-κB in the lungs of COPD patients. Third, CYR61 was only detected in lung tissues and sera. But, the level of CYR61 was unclear in bronchoalveolar lavage of COPD patients. In addition, cell localization of CYR61 was not conducted in COPD patients. Further experiments are required to resolve this puzzle in the future work.

CONCLUSION

In summary, the current study investigated the association among serum CYR61, pulmonary inflammation and lung function indexes among COPD patients. Our results showed that serum CYR61 and MCP-1 were elevated in COPD patients. We found that an increased CYR61 was correlated with decreased lung function indexes in COPD patients. Moreover, an increased CYR61 was associated with pulmonary NF-κB activation and serum MCP-1 increase in COPD patients. In addition, an increased CYR61 is associated with a longer hospital stays among COPD patients. Our results provide evidence that CYR61 can be used as a biomarker to predict COPD progression. Inflammatory chemokine MCP-1 may be a mediator between CYR61 and lung function decline in COPD patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The current study was approved by the Ethics Committee of Anhui Medical University (2021030). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

D-XX, HW, Z-XT, and LF contributed to the study design, analyzing data, and preparation manuscript. W-JW, PZ, and HZ were involved in the acquisition of data. D-XX and LF worked on the study concept, design, and final proof. All authors have read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.781596/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

ORIGINAL RESEARCH
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Host Factor Interaction Networks Identified by Integrative Bioinformatics Analysis Reveals Therapeutic Implications in COPD Patients With COVID-19

Wenjiang Zheng[1†], Ting Wang[1†], Peng Wu[1†], Qian Yan[1], Chengxin Liu[1], Hui Wu[1], Shaofeng Zhan[2], Xiaohong Liu[2], Yong Jiang[3]* and Hongfa Zhuang[2]**

[1]The First Clinical Medical School, Guangzhou University of Chinese Medicine, Guangzhou, China, [2]The First Affiliated Hospital of Chinese Medicine, Guangzhou University of Chinese Medicine, Guangzhou, China, [3]Shenzhen Hospital of Integrated Traditional Chinese and Western Medicine, Shenzhen, China

Background: The COVID-19 pandemic poses an imminent threat to humanity, especially for those who have comorbidities. Evidence of COVID-19 and COPD comorbidities is accumulating. However, data revealing the molecular mechanism of COVID-19 and COPD comorbid diseases is limited.

Methods: We got COVID-19/COPD -related genes from different databases by restricted screening conditions (top500), respectively, and then supplemented with COVID-19/COPD-associated genes (FDR<0.05, |LogFC|≥1) from clinical sample data sets. By taking the intersection, 42 co-morbid host factors for COVID-19 and COPD were finally obtained. On the basis of shared host factors, we conducted a series of bioinformatics analysis, including protein-protein interaction analysis, gene ontology and pathway enrichment analysis, transcription factor-gene interaction network analysis, gene-microRNA co-regulatory network analysis, tissue-specific enrichment analysis and candidate drug prediction.

Results: We revealed the comorbidity mechanism of COVID-19 and COPD from the perspective of host factor interaction, obtained the top ten gene and 3 modules with different biological functions. Furthermore, we have obtained the signaling pathways and concluded that dexamethasone, estradiol, progesterone, and nitric oxide shows effective interventions.

Conclusion: This study revealed host factor interaction networks for COVID-19 and COPD, which could confirm the potential drugs for treating the comorbidity, ultimately, enhancing the management of the respiratory disease.

Keywords: COPD, COVID-19, comorbidity, bioinformatics analyses, host factor interaction networks

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Edited by:
Chia-Li Han,
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Nirmal Kumar Ganguly,
Indraprastha Apollo Hospitals, India
Emily Chia-Yu Su,
Taipei Medical University, Taiwan

****Correspondence:***
Xiaohong Liu
rsclxhgzucm@163.com
Yong Jiang
jiangyongszzxy@163.com
Hongfa Zhuang
zhuanghongfa@163.com

[†]These authors have contributed equally to this work and share first authorship

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic resulting from the highly contagious severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a dramatic increase in hospitalizations for pneumonia with multiple organ dysfunction. It's reported that approximately 60–90% of hospitalized infected patients have comorbidities, most of which include hypertension, diabetes, cardiovascular disease, chronic pulmonary disease and so forth (Garg et al., 2020; Richardson et al., 2020). Given that Chronic Obstructive Pulmonary Disease (COPD) patients are prone to viral exacerbations (Bafadhel et al., 2011; George et al., 2014; Wilkinson et al., 2006) and the devastating impact the COVID-19 may have on the lungs, it is natural for them to fear in the context of the COVID-19 pandemic. The prevalence of COPD amongst hospitalized COVID-19 patients have been reported in many countries or regions, with estimates ranging from 0 to 10% in China (Guan et al., 2020a; Cai et al., 2020; Lian et al., 2020; Liu et al., 2020; Yan et al., 2020), 2.4–14% in New York City (Goyal et al., 2020; Kuno et al., 2020; Palaiodimos et al., 2020; Richardson et al., 2020), and 5.6–9.2% in Italy (Cecconi et al., 2020; Inciardi et al., 2020; Lagi et al., 2020). What's more, several studies have found that pre-existing COPD greatly increases the risk of severe disease and death in COVID-19 patients. A Chinese multicenter study involving 1590 COVID-19 patients showed that COPD carried an odds ratio of 2.681 (95% CI 1.424–5.048; $p = 0.002$) for ICU admission, mechanical ventilation, or death; 62.5% of severe cases had a history of COPD and 25% of those who died were COPD patients (Guan et al., 2020b). Feng et al. (2020) has also found significant differences ($p < 0.001$) between critically ill (15.7%) and moderate (2.3%) patients in the subgroup of COPD.

Currently, the interaction mechanism between COPD and COVID-19 remains unclear and there is little direct evidence about the management of COPD in people with COVID-19 (Halpin et al., 2021). It seems that the highly expressed angiotensin converting enzyme 2 (ACE2) receptors in the COPD airway, the SARS-CoV-2 receptor, were to blame, but evidence has not been shown yet to confer increased susceptibility or increased severity of disease (Leung et al., 2020; Yao et al., 2020). Moreover, COPD patients also feature endothelial cell dysfunction and increased coagulopathy, which may provide explanations for the increased risk of worse outcomes from COVID-19 (Kasahara et al., 2001; Minakata et al., 2005; Husebø et al., 2021).

Host factor networks, based on the integration of systems biology and bioinformatics, serves as a critical strategy for exploring viral diseases as well as non-viral diseases. On one hand, since viruses are obligate intracellular parasites and depend on the host to complete their life cycle, the goal of regulating virus replication can be achieved by changing the expression level of host factors closely related to virus survival. Thus, the identification of host factors involved in regulating the virus life cycle can help reveal the virus-host interaction mechanism. On the other hand, host factor networks can also further enhance our understanding of COPD, the complex and heterogeneous disease both in the clinical and biological aspects. For example, a series of studies on genome-scale identification of SARS-CoV-2 host factor networks reveals new insights into SARS-CoV-2 biology and inform ongoing drug development efforts (Daniloski et al., 2021; Hoffmann et al., 2021; Wei et al., 2021); Morrow and others (Morrow et al., 2015) used integrative genomics to identify host factors associated with specific COPD phenotypes and described a network-co-expression module that was related to the frequency of COPD exacerbations. Obeidat and others reported three co-expression modules (including interleukin 8 and 10 related pathways) associated with the severity of airflow limitation, which reveals novel gene signatures in peripheral blood for COPD patients (Obeidat et al., 2017). In short, host interaction networks allow the identification of subnetworks corresponding to the functional units of a living system, which can help us explore the pathophysiology of the disease from multiple levels, and provide insights for clarifying the virus-host immune interaction mechanism, identifying the host's gene function, predicting underlying drugs and patient classification (Tan et al., 2007).

Therefore, we have adopted a strategy of integrative bioinformatics analysis to explore the host factor networks of COVID-19 and COPD comorbid diseases. Here, several online databases and bio-datasets were employed to identify the co-factors of COPD and COVID-19. On this basis, a series of biological information analyses were performed in an attempt to clarify the shared pathogenic molecular mechanism of comorbidities and to predict potential therapeutic drugs. Our results provide a new perspective of comorbidity interaction and identify host-derived therapeutic targets for COVID-19 and COPD.

MATERIALS AND METHODS

Collection of COVID-19 and COPD-Related Genes

The data source for COVID-19/COPD consists of two parts, namely, databases and data sets. For COVID-19-associated genes, we referred the PubChem (https://pubchem.ncbi.nlm.nih.gov/#query=covid-19), CTD (http://ctdbase.org/), DisGeNET (https://www.disgenet.org/covid/diseases/summary/), baillielab net (https://baillielab.net/maic/covid19) and KEGG DISEASE (https://www.genome.jp/kegg/disease/) databases and data sets of (Grant et al., 2021; Blanco-Melo et al., 2020; Mahmud et al., 2021; Singh et al., 2021; Overmyer et al., 2021; Mo et al., 2021). Regarding COPD-related genes, we considered DisGeNET (https://www.disgenet.org/), CTD (http://ctdbase.org/) and GeneCards (https://www.genecards.org/) databases and data sets of (O'Beirne et al., 2020; Han et al., 2021; Morrow et al., 2017; Hu et al., 2018; Raman et al., 2009; Huang et al., 2019; Morrow et al., 2019; Shen et al., 2021). These selected data sets above represent different clinical samples of COVID-19/COPD, specifically including alveolar lavage fluid, lung tissue, airway and peripheral blood.

The top 500 genes of each database were gathered according to their ranking rules. The data sets were analyzed using GEO2R, R and limma package (Ritchie et al., 2015). Genes from data sets that meet the Benjamini–Hochberg adjusted p-values (False discovery rate, FDR) < 0.05 and $|\log2FC| \geq 1$ were selected as

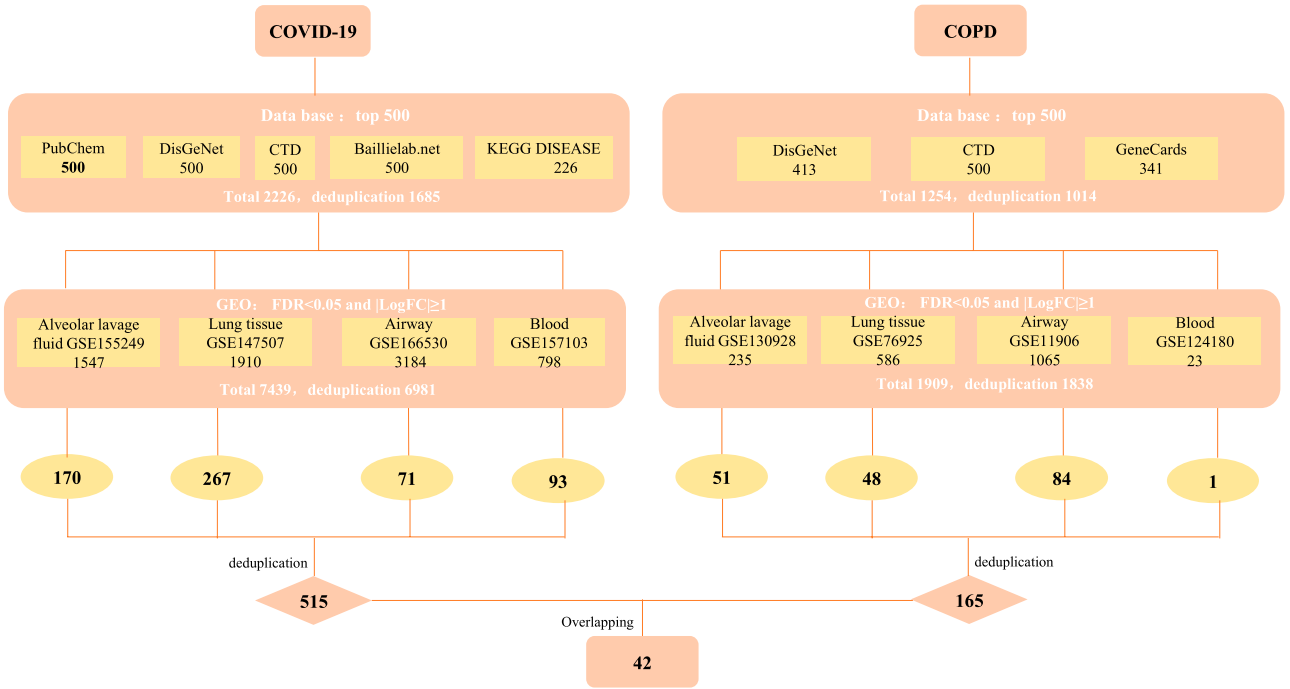

FIGURE 1 | The screening process of obtaining common targets between COVID-19 and COPD. CTD: Comparative Toxicogenomics Database (http://ctdbase.org/), DisGeNET: a platform containing genes associated to human diseases (https://www.disgenet.org/), PubChem: a collection of accessible chemical information (https://pubchem.ncbi.nlm.nih.gov/), KEGG DISEASE: indicates association of genes to diseases (https://www.genome.jp/kegg/disease/), Baillielab.net:genes implicated in SARS-CoV2 infection (https://baillielab.net/), GeneCards: The Human Gene Database (https://www.genecards.org/).

differentially expressed genes (DEGs). Subsequently, intersection genes of the two parts were selected as candidate targets for further analysis. The date of access to these websites was October 6, 2021.

Analysis of TF-Gene Interactions and Gene-miRNA Coregulatory Network

The NetworkAnalyst tool (version 3.0, https://www.networkanalyst.ca/) (Zhou et al., 2019a) was used to evaluate the interaction of TF genes with common genes associated with COVID-19 and COPD comorbidities, as well as gene-miRNA interactions. The basic data of the TF-gene interaction network comes from the ENCODE ChIP-seq database (https://www.encodeproject.org/), using only peak intensity signals <500 and predicted regulatory potential score <1 (using the BETA Minus algorithm). The basic data of gene-miRNA interaction comes from miRNA-gene interaction data collected by miRTarbase comprehensively verified by experiments. Relevant results were visualized by Cytoscape (version 3.8.1, https://cytoscape.org/) (Shannon et al., 2003).

Protein-Protein Interaction Analysis and Network Construction

Common host factors were uploaded to STRING (version 11.0, https://string-db.org/) (Szklarczyk et al., 2019) for generating PPIs network. Here, we set the minimum interaction score required by the PPI network to a medium confidence level: 0.4, and the *p*-value for PPI enrichment: 1.0e-16. The PPI results were analyzed and visualized through Cytoscape. And MCODE analysis of PPI network was subsequently performed and visualized through Metascape (https://metascape.org/) (Zhou et al., 2019b).

Gene Ontology and Pathway Enrichment Analysis

We conducted gene ontology (GO) analysis and pathway enrichment analysis to characterize the biological mechanisms and signaling pathways of common host factor networks. GO biological processes and GO molecular functions are drawn by the WEB-based genome analysis toolkit webgestalt (Hu et al., 2021) (http://www.webgestalt.org/), and the KEGG pathway analysis results are generated by R and clusterprofiler (Yu et al., 2012) package. A cutoff of Benjamini-Hochberg adjusted *p*-values < 0.05 was adopted in this apart.

Tissue Specific Enrichment Analysis of Top Genes

In this study, we used the multigene query function available on GTEx (Sun et al., 2021) (https://www.gtexportal.org/home/multiGeneQueryPage, accessed October 16, 2021) to perform tissue-specific enrichment analysis of 42 COVID-19 genes that overlap with COPD.

TABLE 1 | Sources of genetic selection.

Disease	Database or GEO	Data sources	Amount of raw data	Filter condition	Amount of data after filtering and deduplication	Merge		Overlapping genes
COVID	Data base	PubChem	629	If the raw data is greater than 500, then take	500	1,685	515	42
		DisGeNet	1843	500; if the raw data is less than 500, then all	500			
		CTD	500	are included	500			
		Baillielab.net	2000		500			
		KEGG DISEASE	231		226			
	GEO	Alveolar lavage fluid GSE155249	57,928	FDR <0.05 and |LogFC|≥1	1,547	6,981	—	—
		Lung tissue GSE147507	23,710		1910			
		Airway GSE166530	3,188		3,184			
		Blood GSE157103	1,054		798			
COPD	Data base	DisGeNET	448	If the raw data is greater than 500, then take	413	1,014	165	—
		CTD	53,814	500; if the raw data is less than 500, then all	500			
		GeneCards	341	are included	341			
	GEO	Alveolar lavage fluid GSE130928	54,675	FDR <0.05 and |LogFC|≥1	235	1838	—	—
		Lung tissue GSE76925	32,831		586			
		Airway GSE11906	54,675		1,065			
		Blood GSE124180	31,786		23			

Candidate Drugs Analysis

Overlapping genes were uploaded to ShinyGO (Ge et al., 2020) v0.741 (http://bioinformatics.sdstate.edu/go/) for further candidate drug prediction. Preset all available gene sets, *p*-value cutoff (FDR, adjusted in the hypergeometric test) < 0.05 and show the top 30 pathways. Finally, the candidate drugs from the STITCH database are screened out from the enrichment results.STITCH (Li et al., 2021) (http://stitch.embl.de/) is a powerful search tool for predicting drug-target relationships. In this analysis, we used 42 genes shared by COVID-19 and COPD to predict drug candidates for COVID-19 and COPD comorbidities.

RESULTS

Identification of Common Host Factors Between COVID-19 and COPD

We strictly screened the host factors of COVID-19. First, we searched COVID-19-related host factors from PubChem, DisGeNET, CTD, baillielab net and KEGG DISEASE, respectively. In order to improve the credibility of the data, we choose to filter the first 500 entries in each database. If it is less than 500 entries, all retrieved data will be included. Based on this, we obtained 500 host factors (after deduplication) in PubChem, CTD, DisGeNET, and baillielab net, respectively, and 226 host factors (after deduplication) in KEGG DISEASE. The host factors of the five databases are combined to a total of 1,685 after deduplication. At the same time, we also searched for COVID-19 factors in data sets that contain clinical samples. According to the screening thresholds of FDR<0.05 and |LogFC|≥1, 1,547, 1910, 3,184, and 798 differentially expressed genes (DEGs) were obtained in GSE155249, GSE147507, GSE166530 and GSE157103 after deduplication. The host factors of the four data sets are combined and deduplicated into a total of 6,981. In the end, there were 515 overlapping genes in the COVID-19 databases and data sets (**Figure 1** and **Table 1**).

Similarly, to determine the host factors of COPD, we searched DisGeNET, CTD, and GeneCards to get the top 500 genes of these databases. After combining the database genes and deduplication, a total of 1014 COPD host factors were gained. In addition, we also supplemented the COPD host factors in the data sets and got 235, 586, 1,065 and 23 DEGs in GSE130928, GSE76925, GSE11906 and GSE124180 respectively. A total of 1838 host factors were obtained after merging and removing duplicates. Finally, we combined the host factors obtained by the two methods, and selected overlapping genes as the disease host factors for COPD, a total of 165. Additionally, after collecting data from the COVID-19 and COPD datasets, we sorted out the overlapping genes between different tissues, as shown in **Table 2**. At the same time, in order to understand more intuitively which genes are included in each database or data set, we have also traced the source distribution of 42 genes (see **Supplementary Table S1**).

Finally, we cross-processed the overlapping factors that were strictly screened for the two diseases and finally got 42 common host factors.

TF-Gene Interaction and Gene-miRNA Interaction

The TF-gene interaction network consists of 285 nodes and 717 edges (**Figure 2** and **Supplementary Table S2**). Among them, CFB is regulated by 58 TF-genes, FOS is regulated by 51 genes,

TABLE 2 | Overlapping genes in different tissues.

Gene source	Overlapping genes
Alveolar lavage fluid	MMP2, MMP7, RTN1, S100P, SLC22A4, RNASE6, EPS8, PRKCB, TIMP3, HS3ST1, GCLM, RASSF5, AFAP1L1, MERTK, MCOLN2, SPRY2, PLXNC1, CHST13, IFITM2, BNIP3, AOC3, CDK6, ANKRD22, SCD, SPP1, SECTM1, OSM, SPRED1, IGFBP2, GALM, GCH1, TNS1, SNCA, SLC26A11, TRERF1, SOCS3, ZC3H12C, CCL2, DFNA5, MMP12, FLT1, IFITM3, MARCKS, FAM198B, CYTL1, ADAM28, VNN1, MCOLN3, RASSF2, SLC20A1, ISG20, TRPC6, CADM1, TMEM163, SERPINE1, VCAN, SLC39A8, RASAL2, HS3ST2, CD84, SH3RF1, LINC01010, MLLT11, CYBRD1, GATM, FAM101B, AKT3, CYP1B1, XYLT1, ACKR3
Lung tissue	NOL8, TLR1, SMC3, TRAF5, SELL, CCL19, CCAR1, ARL13B, SAMSN1, PIK3AP1, DNAJB4, APOBEC3A, HPGDS, FCGR3A, ANP32A, CHIT1, CARD16, P2RY14, CTR9, DYRK3, MPHOSPH10, SH3PXD2B, GLT8D1, FAHD2A, GBP1P1, EVI2B, CWC22, MPLKIP, PI4K2B, DCAF13, IRF2, LUC7L3, TMEM133, SYAP1, ACAD8, PLCG1, ZC3H7A, POU2AF1, RTN3, HMGN3, PPIG, PLAGL1, ILK, SMAD7, FAM26F, HNRNPC, MCTS1, CAPZA1, POLR2K, GIMAP7, C1D, CYP51A1, ITM2A, GBP3, CBY1, DENND4C, SREK1, FCRLA
Airway	KCNK3, LOC101927769, CPNE4, VGLL3, AQP2, NMNAT2, IFITM10, AHRR, JPH2, PSD2, CDH11, DCTN1AS1, FGF22, SMIM1, SYNPO2L, LOC101927914, ELFN2, TAL1, FRMD8P1, TSPAN18, CLEC5A, GRP, JAKMIP3, LOC102546299, SLC30A3, PLK5, LCN8, GBX1, LINC00269, ITLN1, KCNIP3, EWSAT1, PITX2, TPH1, CDH6, PRICKLE2AS3, SULT4A1, SOX9AS1, C1QTNF4, SEMA5B, FRMD1, KCNJ4, CLEC14A, NAT16, KCNQ2, LINC00942, CBLN4, LOC101927870, GLB1L3, PITX3, PSMA8, NR1I2, ARHGEF10, ELAVL3, LOC400622, KCNA1, NKD1, SCUBE3, LOC101929552, MAPK12, OBP2A, RPL13AP17, OR5K1, NHLH2, PAX1, TCF4AS1, SGK2, PTGIR, GFRA2, COL8A1, GREM2, LINC00652, UNC5C, GPBAR1, LOC254028, VWC2, HHLA1, MYOZ3, KIZAS1, ABCB6, DKKL1, ATP8B5P, ADAM11, FAM167AAS1, HAP1, SYT16, PIK3CDAS1, PHACTR3, LOC158434, HIF3A, OR5H1, BDNF, CALCA, APLP1, ZIC1, LRRN4, FBXO17, BMP4, KLC3, MEIS3, NTRK3, SYT1, MIR924HG, DDN, AVPR1A, C10orf126, BRSK2, LOC101927636, LHX6, CYP1B1AS1, INMT, CTD2350J17.1, ART3, LINC01056, C1orf127, RAMP2, ATOH7, LHX9, CNPY1, DHRS2
Blood	CCL3L1, FCER1A, TRIM6

and FKBP5 is regulated by 43 TF-genes. See **Supplementary Table S2** for details. In addition, the gene-miRNA interaction network has a subnet with at least 3 nodes as shown below. Subnet 1 (**Figure 3** and **Supplementary Table S3**) is composed of 638 nodes and 879 edges, and subnet 2 (**Figure 4** and **Supplementary Table S3**) is composed of 6 nodes and 5 edges.

Protein-Protein Interaction Network and MCODE Analysis

The PPI network in this study was generated by string based on 42 common host genes and then introduced into Cytoscape for visual representation and network topology analysis. In the end, we get 42 nodes, 199 edges, and the average node degree is 9.48. In this study, we rank the nodes in the PPI network according to their degree values. The top ten targets are CCL2, MMP9, IL1A, LEP, SERPINE1, CXCL10, EGF, CCL4, STAT1, and HIF1A (**Figure 5**). In addition, set the cluster finding parameters (node score cutoff: 0.2, k-core: 2, max depth: 100), through MCODE analysis (**Figure 6**), we classify 42 host factors, and finally get three different biological functions subnet. Module A mainly reflects the interleukin-1 receptor binding function. In its visualization diagram, we can clearly see that MMP9, IL1A, FOS, LEP, EGF, HIF1A and other nodes occupy important positions. Module B mainly functions as the Chemokine activity. Among them, the degree of CXCL10, CXCL5, and CCL4 is higher. In module C, the active function of estrogen 16-alpha-hydroxylase is more prominent. The analysis of its function shows that the signal receptor binding function mediated by CCL2 and MMP9 is the potential mechanism of the host factor interaction network between COVID-19 and COPD.

GO, KEGG Enrichment Analysis

The directed acyclic graph (DAG) analysis of GO's biological process shows that the biological processes (**Figure 7** and **Table 3**) in COVID-19 and COPD comorbidities mainly have 5 branches, of which angiogenesis, cytokine-mediated signaling pathway, and cell migration are separate Branch. The defense response is immediately followed by the inflammatory response. At the same time, immune response and response to biotic stimulus are carried out in parallel, and response to biotic stimulus links response to external biotic stimulus and response to oxygen-containing compound processes, and finally ends with response to other organism. The molecular functions in COVID-19 and COPD comorbidities (**Figure 8** and **Table 4**) mainly have two major branches, among which the enrichment ratio of chemokine activity is the highest. Receptor regulator activity, receptor ligand activity, cytokine activity and chemokine activity belong to the same branch; signaling receptor binding, cytokine receptor binding, chemokine receptor binding and chemokine activity belong to the same branch; serine hydrolase activity, serine-type peptidase activity and serine-type endopeptidase activity both belong to another branch.

At the same time, pathway enrichment analysis showed (**Table 5**) that cytokine-cytokine receptor interaction, AGE-RAGE signaling pathway in diabetic complications, viral protein interaction with cytokine and cytokine receptor, osteoclast differentiation and IL-17 signaling pathway play an important role between COVID and COPD.

Tissue Specific Enrichment Analysis of Host Factor Interaction Network

Tissue specific enrichment analysis showed that the co-host factors of COVID-19 and COPD comorbidities were most densely distributed in the lungs, spleen, liver, blood, small salivary glands, breast-breast tissue, prostate and vagina (**Figure 9**).

Drug Prediction Through Common Host Factors

Based on a series of bioinformatics explorations on the interaction network between COVID-19 and COPD host factors, we finally made predictions about possible effective intervention drugs. Our research found that dexamethasone,

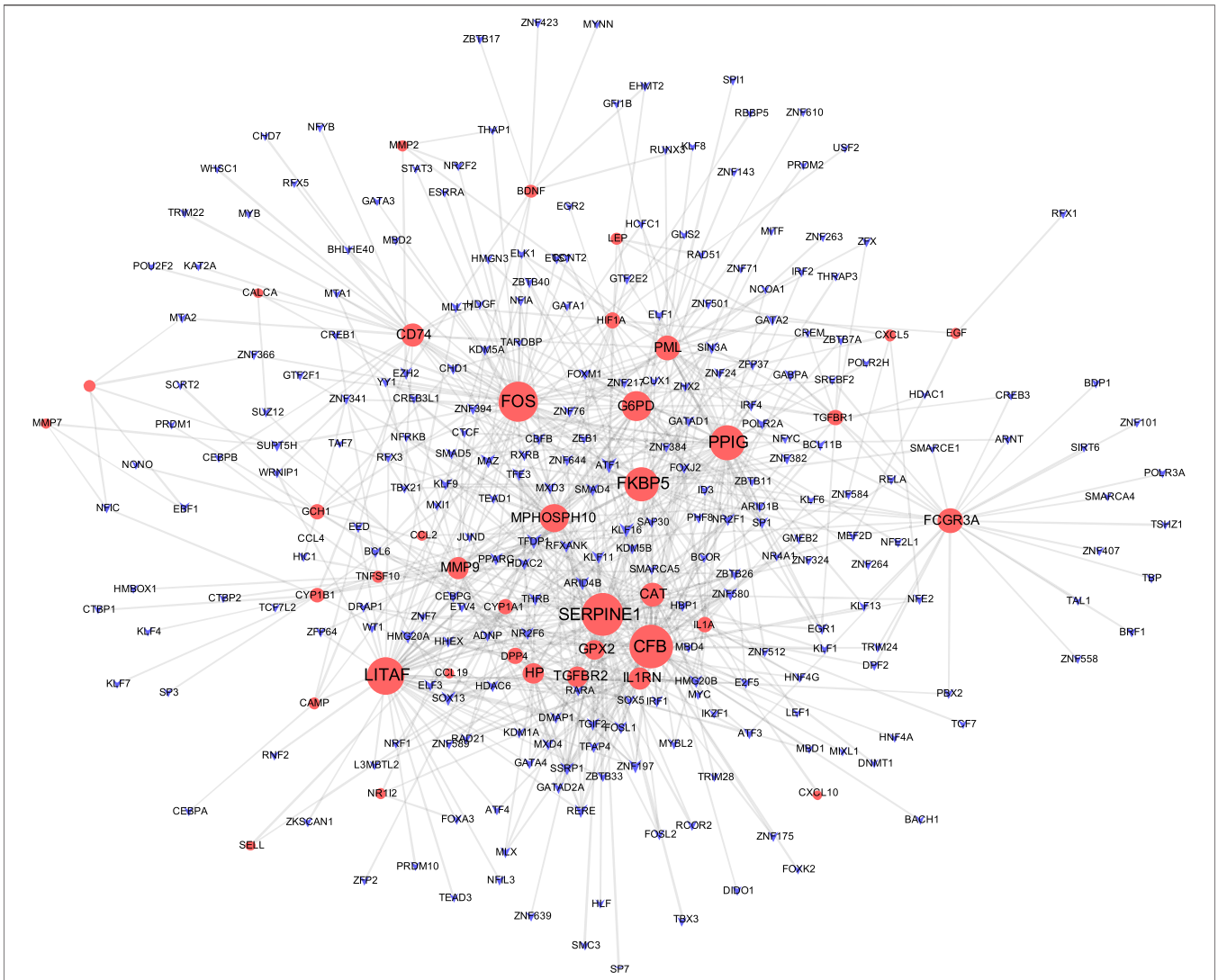

FIGURE 2 | Network of TF gene interpaly with shared targets. The red node is the shared target, and the purple node shows TF-gene.

estradiol, progesterone, and nitric oxide have certain intervention effects, as shown in **Table 6**.

DISCUSSION

All the work done in this research is to explore possible interaction pathways between COVID-19 and COPD from the perspective of host factors. On the basis of the front, we tried to find supporting evidence for the increased risk of pneumonia and poor prognosis when COPD patients were simultaneously infected with the SARS-COV-2 virus, and finally made reasonable predictions about the drug components that may be effective for intervention. The first step of our work was to screen out appropriate disease datasets/databases, find out the potential host factor of COVID-19/COPD, and then count the overlapping genes to obtain 42 common host factors. Based on the common host factors, a series of bioinformatics analyses were carried out.

Intersection Genes of COVID-19 and COPD Show the Key Host Factors of Comorbidity

After rigorously screening and processing genes, we have identified multiple shared genes exposed in the immune response of COVID-19 with COPD conditions (including CCL2, MMP9, IL1A, LEP, SERPINE1, CXCL10, EGF, CCL4, STAT1, and HIF1A). Most of these common genes have been shown to be related to the strong biological relevance of pathogenesis and pathology of COVID-19 and COPD.

TF-Gene Interaction and Gene-miRNA Interaction Analysis

Transcriptome analysis of host cells after virus infection is helpful to identify the dynamics of host immune response, so it is necessary to understand the expression of host factors after co-infection of COVID-19 and COPD. The TF gene acts as a regulator based on

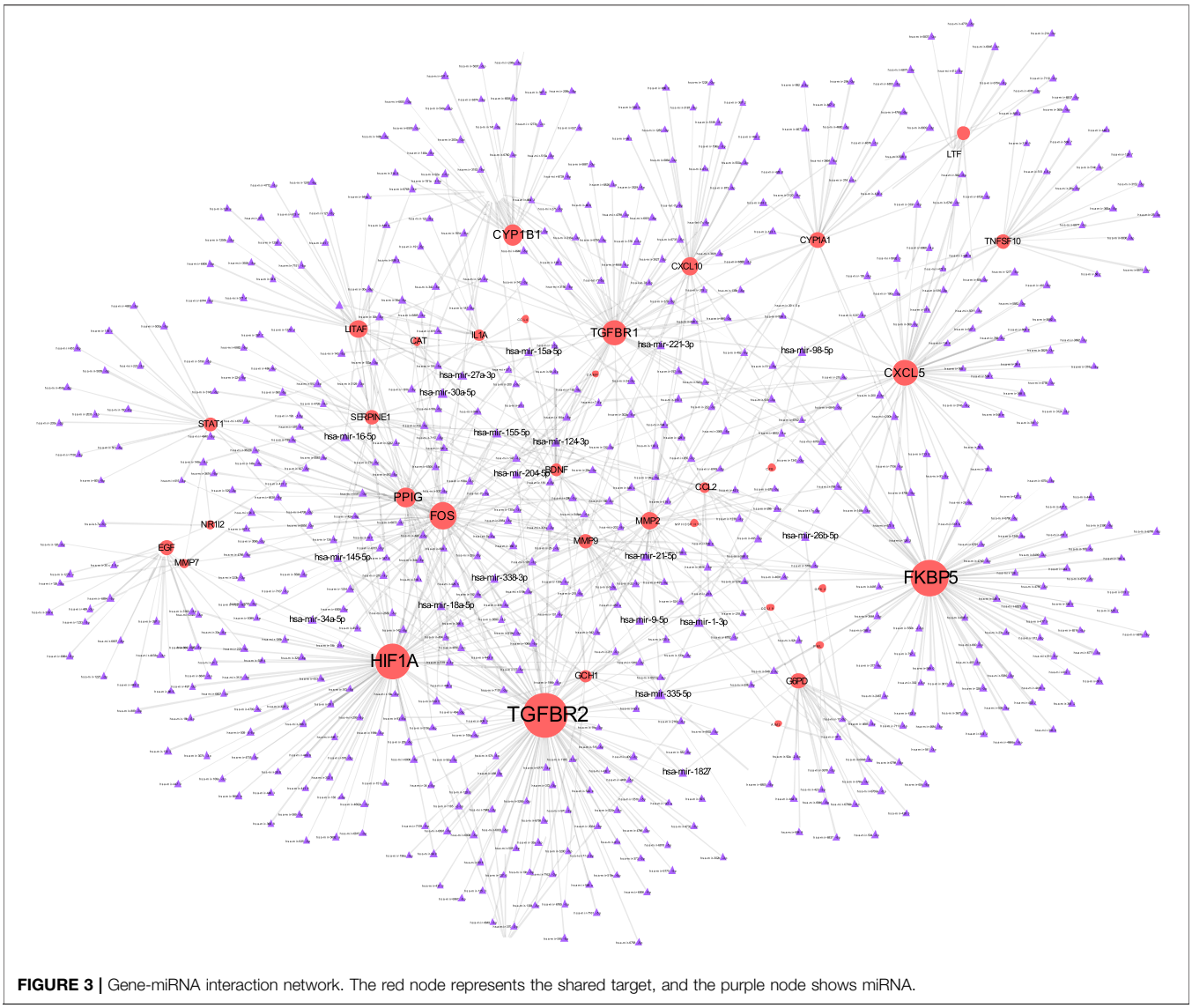

FIGURE 3 | Gene-miRNA interaction network. The red node represents the shared target, and the purple node shows miRNA.

genetic expression. In this study, we found that CFB, FOS and FKBP5 showed a high degree of interaction with other TF genes. CFB is one of the highly induced supplemental genes in response to SARS-CoV-2. The cell penetration inhibitor of CFB may block the active supplement C3a of respiratory epithelial cells produced by SARS-CoV-2 infection (Yan et al., 2021). Not only that, our research shows that the hypoxia-inducible factor HIF1A occupies an important position in the gene-miRNA network. Previous studies have also shown that HIF dysfunction is closely related to inflammatory airway conditions, and HIF1A plays a central role in the development of COPD and other lung diseases (Kelchtermans et al., 2021).

PPI and MCODE Analysis Reveal Essential Host Genes and Distinct Biological Functions for Comorbidity

Chemokines are small molecules (8–12 kDa) of a large family of cytokines associated with various biological functions, and several studies have now established the critical role of chemokines in the development and progression of chronic obstructive pulmonary disease (COPD). Cigarette smoke or other irritants can activate alveolar macrophages and airway epithelial cells, releasing chemokines that attract circulating leukocytes to the lungs. In addition, various factors such as air pollution can induce the release of chemokines from resident cells by triggering the release of damage-associated molecular patterns (DAMP) that bind to specific pattern recognition receptors (PRR) (Ko et al., 2016). Chemokines (CCL2, CCL4) also occupy an important position in this study. In the lung, CCL2 is mainly produced by lung macrophages, T cells and endothelial cells and is involved in endothelial and pulmonary epithelial cell proliferation, migration and wound closure, and is associated with a variety of diseases with disorders of lung inflammation, including COPD (Henrot et al., 2019), acute respiratory distress syndrome, allergic asthma and idiopathic pulmonary fibrosis (Rose et al., 2003). In contrast, for patients with COVID-19, the elevated cytokines in the blood

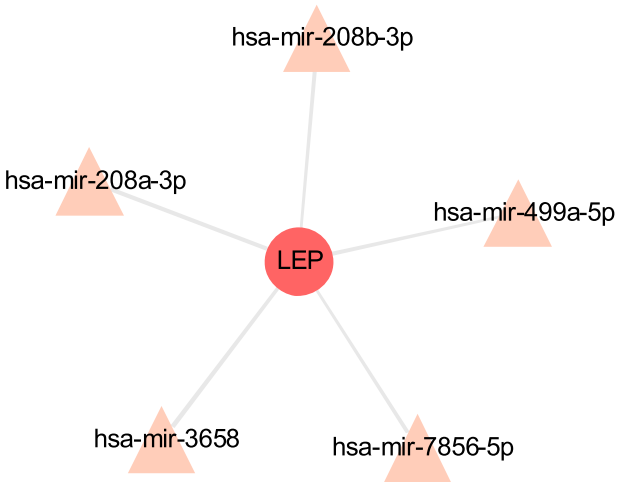

FIGURE 4 | Gene-miRNA interaction sub-network. The red node is the shared target, and the pink node shows miRNA.

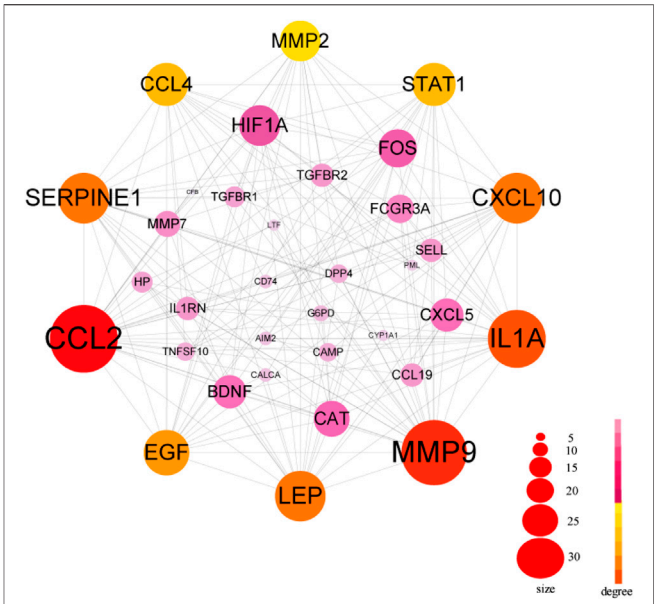

FIGURE 5 | PPI network of common host factors for COVID-19 and COPD. In this figure, the circled nodes represent host factors, and the edges represent the interactions between nodes. The larger the circle, the darker the color, the higher the importance, the thicker the line, the greater the interaction. The top ten genes with degree value are highlighted in another color, and other genes are shown in pink.

lie in the over-induction of the cytokine storm. In addition, the interaction between SARS-CoV spiking protein and ACE2 receptor mediated the phosphorylation of ERK1/2, which also led to the upregulation of CCL2 expression (Chen et al., 2010). In the early stages of SARS-CoV-2 infection, chemokine (CCL2) is broadly up-regulated by pro-inflammatory cytokine stimulation as a chemoattractant for effector cells such as monocytes, neutrophils, and leukocytes from the blood to the site of tissue injury. In addition, CCL2 can act as an autocrine factor that promotes viral replication in infected macrophages (Sabbatucci et al., 2015). In a high-density antibody microarray study of serum proteins from COVID-19 patients, a significant correlation between CCL2 and CXCL10-mediated cytokine signaling pathways has been demonstrated (Hou et al., 2020). This study suggests that CCL2 and CXCL10 have the potential to be used as anti-inflammatory targets for COVID-19 therapy (Zhang et al., 2020).

Matrix metalloproteinase 9 (MMP9) is particularly associated with COPD pathophysiology characterized by tissue remodeling. MMP9 mediates pulmonary inflammation through neutrophil chemotaxis, extracellular matrix degradation and enhanced inflammation, which is a key feature of the acute exacerbation phase of COPD (Mercer et al., 2005; Wells et al., 2015). Earlier reports suggested that human coronavirus infection increases MMP9 secretion (Desforges et al., 2007). Similarly, recent studies suggest that MMP9 stimulates the migration of inflammatory cells and further exacerbates lung tissue destruction by promoting inflammation and degradation of the pulmonary capillary barrier (Davey et al., 2011), which may serve as one of the early indicators of respiratory failure in patients with COVID-19 (Ueland et al., 2020).

The MCODE analysis unearthed modules with potentially different biological functions in the network nodes, providing a clearer direction for bioinformatics analysis. In this study, 3 subnetworks were obtained after dividing the 42 obtained host factors for COVID-19 and COPD comorbidity. Among them, module A focused on receptor binding, including interleukin-1 receptor binding, the RNA polymerase II core promoter sequence-specific DNA binding, the histone acetyltransferase binding, metalloendopeptidase activity, growth factor receptor binding, cytokine receptor binding, receptor ligand activity, receptor regulator activity and signaling receptor binding, etc. CCL2, MMP9, IL1A, HIF1A, and LEP occupy a prominent position in the whole module. Accumulating evidence suggests that infection with viruses activates extracellular signaling and induces IL-1 production (Liu et al., 2013). IL1A is upregulated in patients with mild COVID-19 and also enriched in alveolar lavage fluid of severe patients, playing an important role in innate immune virus infection (Shaath et al., 2020) and IL1A, as a pro-inflammatory cytokine, plays an important role in smoke-induced neutrophilic inflammation, dendritic cell recruitment and activation in CODP patients also plays a central role (Botelho et al., 2011).

GO, KEGG Highlights the Immune Mechanisms of Host Factors and Significant Shared Signaling Pathways

The directed acyclic graph (DAG), as a visual representation of the causal hypothesis (Suttorp et al., 2015), has clear advantages in estimating the effect of one variable on another and is a common tool for determining appropriate adjustment

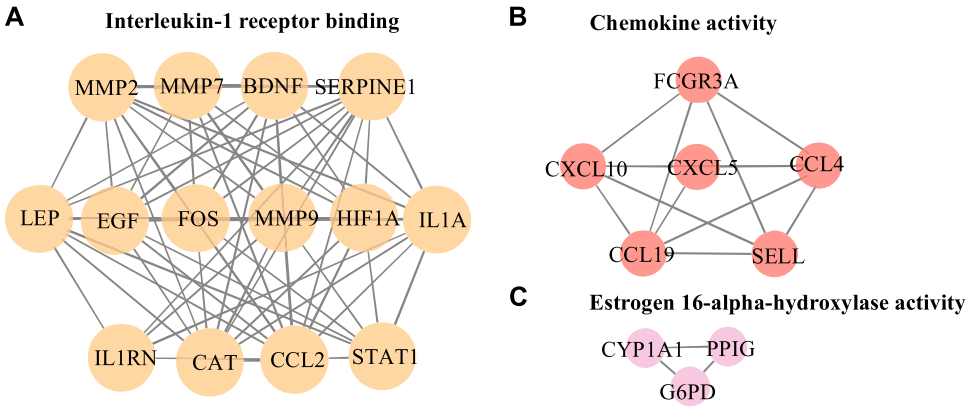

FIGURE 6 | Further MCODE analysis based on PPI network. The nodes circled in the figure represent the host factor, and the edges represent the interaction between the nodes. Different colors represent different modules.

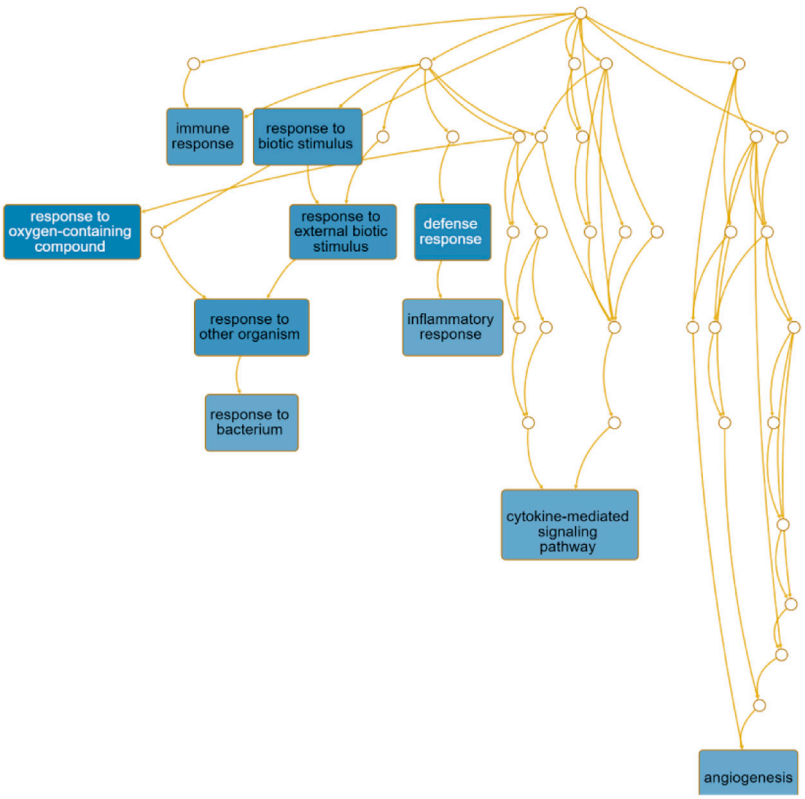

FIGURE 7 | GO biological process analysis. DAG maps the causal relationship between the arrows between the variables and the nodes. The absence of arrows between nodes means that there is no causality and precedence, and the nodes can be measured or cannot be measured. The node whose position is in the front is the parent node, and the one in the back is the child node.

strategies for epidemiological analyses (Ferguson et al., 2020). This structured approach facilitates visual clarification of the underlying relationships and serves as a visual aid in scientific discussions. Therefore, the GO analysis in this study abandoned the traditional network diagram format and adopted a DAG visualization approach to elucidate the mechanisms of comorbidity between COVID-19 and COPD.

The importance of angiogenesis is underscored by its separate branch in the biological processes of comorbidity, which is also the highest enrichment ratio of all genes. A 7-person clinical trial (Ackermann et al., 2020) had reported severe SARS-CoV-2 virus-associated endothelial damage and extensive vascular thrombosis in the lung cells of Covid-19 patients due to excessive cytokine storm, and significant neointimal growth in the lungs of Covid-19

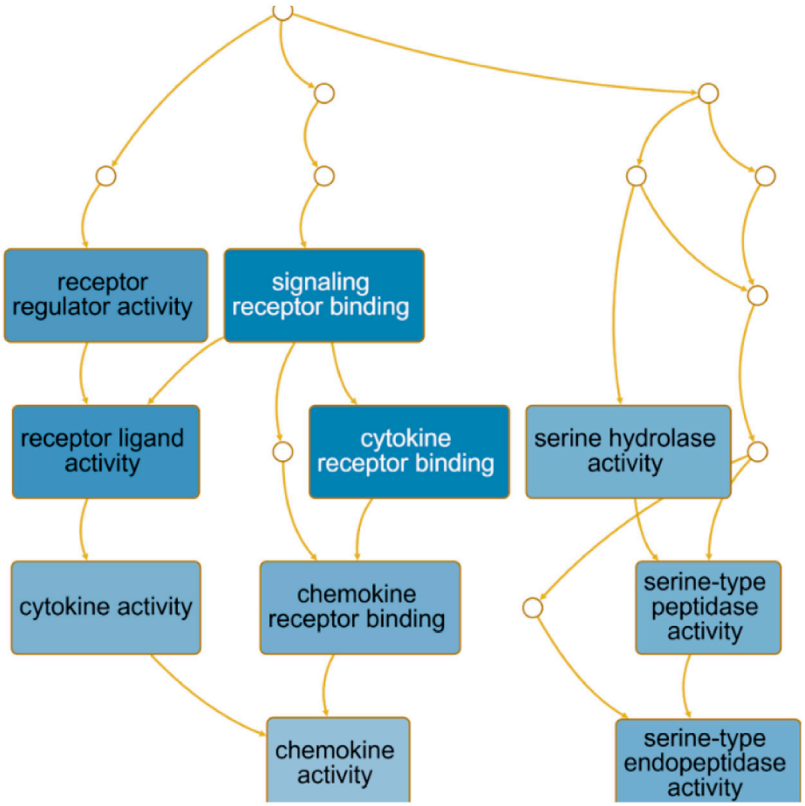

FIGURE 8 | GO molecular function analysis. DAG maps the causal relationship between the arrows between the variables and the nodes. The absence of arrows between nodes means that there is no causality and precedence, and the nodes can be measured or cannot be measured. The node whose position is in the front is the parent node, and the one in the back is the child node.

patients through an infected angiogenic mechanism (Magro et al., 2020). In contrast, COPD, a pulmonary and systemic inflammatory process with progressive obstruction of pulmonary airflow, epithelial-mesenchymal transition and extracellular matrix remodeling similarly affects pulmonary and airway angiogenesis (Eapen et al., 2018). Our findings suggest that chemokine activity also plays an important role in the biological function of comorbidities. Chemokines recruit innate and adaptive immune cells to sites of inflammation, enhance their cytotoxic function and inhibit viral host responses, limiting viral infection (Melchjorsen et al., 2003). At the same time, viruses link innate and adaptive immune responses by inducing the production of inflammatory chemokines and promoting Th1-polarized immune responses. For COVID-19, CCL2 recruits neutrophils, monocytes, and macrophages, and CXCL9 and CXCL16 recruit T cells and NK cells to the site of viral infection (Proudfoot, 2002; Xu et al., 2020). Interestingly, CXCL10 increases with disease severity and is a key marker for detection in asymptomatic infected individuals (Chi et al., 2020). And chemoreceptors have long been a fertile area for research as anti-inflammatory therapeutic targets in COPD (Donnelly and Barnes, 2006).

In addition, KEGG enrichment analysis revealed important shared signaling pathways, cytokine-cytokine receptor interactions, viral protein interactions with cytokines and cytokine receptors, and IL-17 signaling pathways in diabetic complications. The No. 1 cytokine-cytokine receptor interaction pathway is enriched with 11 host factors, mainly chemokine ligands and transforming growth factor β receptors. Viral infection and inflammation will cause changes in TGF-β activity (Xia et al., 2017).

Tissue Specific Enrichment Analysis Indicates the Expression of Certain Specific Host Factors

Our results suggest that the common host factors for COVID-19 and COPD comorbidity are most densely distributed in the lung, spleen, liver, blood, minor salivary glands, breast tissue, prostate, and vagina. As to why genes selected from alveolar lavage fluid, lung tissue, airway and blood samples are enriched in other organs and tissues, we think it may be due to the flow of blood that leads to the linkage between different tissues, or the intersection genes may also be derived from other tissues at the same time, because the genes from the database are not limited to samples of blood, lung tissue, alveolar lavage fluid and airway.

The Intervention Drug Reveals Therapeutic Implications for COPD Patients With COVID-19

As shown in the potential intervention drugs or ingredients for COVID-19 and COPD comorbidities (**Table 6**), our study found

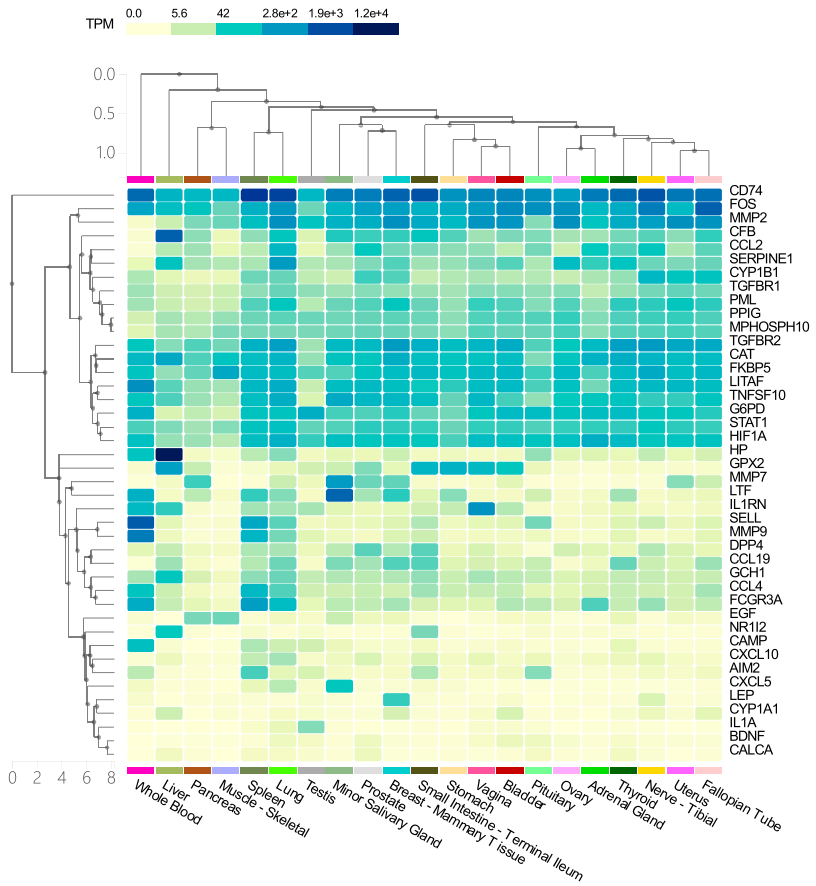

FIGURE 9 | Tissue specific enrichment analysis graph. The horizontal axis in the figure represents different tissues, and the vertical axis represents the corresponding distribution of host factors in the tissues. The darker the vertical axis, the higher the specific distribution density of the host factor in the corresponding tissue.

TABLE 3 | GO-BP enrichment analysis.

GeneSet	Description	Size	Overlap	Expect	Enrichment Ratio	*p* Value	FDR
GO:1901700	response to oxygen-containing compound	1,556	24	3.82837254	6.268982	5.11E-15	4.64E-11
GO:0006952	defense response	1,518	23	3.73487758	6.158167	4.02E-14	1.83E-10
GO:0051707	response to other organism	897	18	2.206973116	8.155967	7.61E-13	1.80E-09
GO:0043207	response to external biotic stimulus	899	18	2.211893903	8.137823	7.90E-13	1.80E-09
GO:0009607	response to biotic stimulus	926	18	2.278324532	7.900543	1.30E-12	2.37E-09
GO:0006955	immune response	1919	23	4.721495439	4.871338	5.78E-12	8.76E-09
GO:0019221	cytokine-mediated signaling pathway	705	15	1.734577532	8.647639	4.89E-11	6.35E-08
GO:0006954	inflammatory response	717	15	1.764102256	8.502908	6.20E-11	6.88E-08
GO:0009617	response to bacterium	595	14	1.463934229	9.563271	6.81E-11	6.88E-08
GO:0001525	angiogenesis	487	13	1.198211714	10.8495	8.08E-11	7.30E-08

that dexamethasone, estradiol, progesterone, and nitric oxide, etc. all demonstrated effective intervention. In a randomized controlled trial, the RECOVERY Collaborative Group (Horby et al., 2021) found that using dexamethasone at a daily dose of 6 mg for 10 consecutive days reduced mortality for 28 days in patients receiving respiratory support for COVID-19. Glucocorticoids are also recommended in the updated guidelines of the United Kingdom chief medical officers and the National Institutes of Health in the United States for inpatient use of COVID-19. There is no definitive clinical data on the clinical outcomes of the use of glucocorticoids in COPD patients who are infected with COVID-19 at the same time (Halpin et al., 2020; Halpin et al., 2021), but our results suggest that there is bioinformatics evidence for the use of dexamethasone for treatment. Nevertheless, more laboratory and clinical trials are needed before dexamethasone becomes a potential therapeutic

TABLE 4 | GO-MF enrichment analysis.

GeneSet	Description	Size	Overlap	Expect	Enrichment Ratio	*p* Value	FDR
GO:0005102	signaling receptor binding	1,538	19	3.783177	5.022233	6.55E-10	8.21E-07
GO:0005126	cytokine receptor binding	274	10	0.673986	14.8371	8.75E-10	8.21E-07
GO:0048018	receptor ligand activity	468	11	1.151188	9.555347	1.12E-08	7.03E-06
GO:0030545	receptor regulator activity	514	11	1.264339	8.700199	2.95E-08	1.38E-05
GO:0004252	serine-type endopeptidase activity	182	7	0.447684	15.63602	2.71E-07	1.02E-04
GO:0042379	chemokine receptor binding	61	5	0.150048	33.32267	3.76E-07	1.18E-04
GO:0008236	serine-type peptidase activity	204	7	0.5018	13.94978	5.86E-07	1.57E-04
GO:0017171	serine hydrolase activity	208	7	0.511639	13.68152	6.68E-07	1.57E-04
GO:0005125	cytokine activity	217	7	0.533777	13.11408	8.88E-07	1.85E-04
GO:0008009	chemokine activity	47	4	0.115611	34.59886	5.21E-06	9.70E-04

TABLE 5 | KEGG enrichment analysis.

ID	Description	GeneRatio	*p* value	p.adjust	Qvalue	Count
hsa04060	Cytokine-cytokine receptor interaction	11	6.17E-08	9.44E-06	6.37E-06	11
hsa04933	AGE-RAGE signaling pathway in diabetic complications	7	3.25E-07	2.49E-05	1.68E-05	7
hsa04061	Viral protein interaction with cytokine and cytokine receptor	6	6.08E-06	0.00031	0.000209	6
hsa04380	Osteoclast differentiation	6	2.52E-05	0.000963	0.000649	6
hsa04657	IL-17 signaling pathway	5	6.99E-05	0.00214	0.001443	5
hsa05142	Chagas disease	5	0.000103	0.002589	0.001746	5
hsa05164	Influenza A	6	0.000132	0.002589	0.001746	6
hsa04659	Th17 cell differentiation	5	0.000135	0.002589	0.001746	5
hsa04668	TNF signaling pathway	5	0.000161	0.002733	0.001842	5
hsa04062	Chemokine signaling pathway	6	0.00024	0.00367	0.002474	6
hsa04926	Relaxin signaling pathway	5	0.000311	0.004262	0.002874	5
hsa04068	FoxO signaling pathway	5	0.000334	0.004262	0.002874	5
hsa05212	Pancreatic cancer	4	0.000414	0.004483	0.003022	4
hsa05140	Leishmaniasis	4	0.000435	0.004483	0.003022	4
hsa05418	Fluid shear stress and atherosclerosis	5	0.000439	0.004483	0.003022	5
hsa05208	Chemical carcinogenesis - reactive oxygen species	6	0.000536	0.005122	0.003453	6
hsa05210	Colorectal cancer	4	0.000662	0.005962	0.00402	4
hsa05235	PD-L1 expression and PD-1 checkpoint pathway in cancer	4	0.000754	0.00641	0.004322	4
hsa05161	Hepatitis B	5	0.000883	0.006531	0.004403	5
hsa05323	Rheumatoid arthritis	4	0.00089	0.006531	0.004403	4
hsa05219	Bladder cancer	3	0.000896	0.006531	0.004403	3
hsa00380	Tryptophan metabolism	3	0.000962	0.006692	0.004512	3
hsa04620	Toll-like receptor signaling pathway	4	0.00135	0.008984	0.006057	4
hsa05152	Tuberculosis	5	0.001416	0.009024	0.006084	5
hsa04010	MAPK signaling pathway	6	0.002243	0.01373	0.009257	6

drug in the future. In addition, studies have found that 17β-estradiol administration can effectively reduce the up-regulation of ACE2-dependent NOX2, MCP-1 and ROS, and alleviate endothelial dysfunction and multiple organ failure mediated by COVID-19 inflammation during the pathogenesis (Youn et al., 2021). Experiments have pointed out that the combination of progesterone and glucocorticoids can synergistically reduce lung inflammation in mice caused by chronic ozone exposure (Fei et al., 2017). And, progesterone has a certain role in COPD airway remodeling (Zhang et al., 2018). In addition, estrogen can also promote the separation of endothelial nitric oxide synthase (eNOS) from plasma membrane acupoints, thereby activating NO pathways and vascular adsorption, and playing a role in regulating blood vessels (Hisamoto and Bender, 2005). These data provide support for our research results, but there are no relevant clinical and experimental studies on the use of this ingredient in COVID-19 and COPD comorbidities. This will be one of the contents of our future work research.

CONCLUSION

In order to explore the mechanism of co-morbidity between COVID-19 and COPD, after carefully screening the COVID-19 and COPD data sets and strictly processing co-host genes, we conducted a series of bioinformatics analyses from the perspective of host factor interactions, and initially discovered drugs or active ingredients for potential interventions. We found that the main biological process of COPD patients infected with COVID-19 is angiogenesis, and the main molecular functionis chemokine activity. In addition, we also found that the cytokine-cytokine receptor interactions signaling pathway is a common pathway for the progression of the two diseases. Finally, we

TABLE 6 | Drug stitch enrichment analysis.

Enrichment FDR	Genes in list	Total genes	Functional category
1.02E-25	23	409	STITCH dexamethasone (CID000005743)
1.02E-25	22	340	STITCH dexamethasone (CID100003003)
2.36E-18	18	367	STITCH estradiol (CID100000450)
4.94E-18	17	310	STITCH progesterone (CID000005994)
3.30E-16	14	194	STITCH nitric oxide (CID100000945)

concluded that dexamethasone, estradiol, progesterone, and nitric oxide are potentially effective therapeutic drugs, providing a clearer direction for future clinical research.

LIMITATION

First of all, our research is based on co-expressed genes, involving non-coding RNA, but we have not conducted studies on post-translational modification and interference with other metabolites. This is related to the content of our research, but they are not the subject of this research. Therefore, we will supplement the research in future work. Secondly, although the study selected sample data from airway, lung, and peripheral blood for tissue-specific enrichment analysis, the results showed that the co-host factors of COVID-19 and COPD comorbidities were also enriched in spleen, liver, blood, minor salivary glands, breast tissue, prostate, and vagina. We speculate that the peripheral blood may mediate this process, or it may be because we also selected genes from the database. The genes in the database are not distinguished according to the source of the tissue, so there is a certain amount of confounding. Finally, given the limitations of bioinformatics predictions, candidate drugs may also affect counter-regulatory genes not identified in this study. We must admit that this is the limitation of our research. Therefore, we are trying to find positive intermediary evidence to support the prediction results, and we also look forward to future *in vivo* and *in vitro* experiments to prove this.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/ **Supplementary Material**.

AUTHOR CONTRIBUTIONS

WZ conceived and designed the study, plotted the figures based on network pharmacology, using online databases. TW conducted data analysis and wrote discussions and abstracts. PW conducted a literature search on the background of this disease and wrote part of the article. QY is responsible for most of the image processing. CL organized the data and standardized the image format. HW annotated the picture and wrote the conclusion. SZ reviewed the manuscript. HZ, YJ and XL reviewed and revised the manuscript. All authors read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.718874/full#supplementary-material

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Dysregulated Cell Signaling in Pulmonary Emphysema

Chih-Ru Lin[1,2], *Karim Bahmed*[2,3] *and Beata Kosmider*[1,2*]

[1] Department of Microbiology, Immunology, and Inflammation, Temple University, Philadelphia, PA, United States, [2] Center for Inflammation and Lung Research, Temple University, Philadelphia, PA, United States, [3] Department of Thoracic Medicine and Surgery, Temple University, Philadelphia, PA, United States

Pulmonary emphysema is characterized by the destruction of alveolar septa and irreversible airflow limitation. Cigarette smoking is the primary cause of this disease development. It induces oxidative stress and disturbs lung physiology and tissue homeostasis. Alveolar type II (ATII) cells have stem cell potential and can repair the denuded epithelium after injury; however, their dysfunction is evident in emphysema. There is no effective treatment available for this disease. Challenges in this field involve the large complexity of lung pathophysiological processes and gaps in our knowledge on the mechanisms of emphysema progression. It implicates dysregulation of various signaling pathways, including aberrant inflammatory and oxidative responses, defective antioxidant defense system, surfactant dysfunction, altered proteostasis, disrupted circadian rhythms, mitochondrial damage, increased cell senescence, apoptosis, and abnormal proliferation and differentiation. Also, genetic predispositions are involved in this disease development. Here, we comprehensively review studies regarding dysregulated cell signaling, especially in ATII cells, and their contribution to alveolar wall destruction in emphysema. Relevant preclinical and clinical interventions are also described.

Keywords: lung, alveolar epithelium, alveolar type II cells, emphysema, oxidative stress, tissue homeostasis

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Shu-Chuan Ho,
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Manish Bodas,
University of Oklahoma Health Sciences Center, United States
Krishna Prahlad Maremanda,
Brigham and Women's Hospital and Harvard Medical School, United States
Yong-Xiao Wang,
Albany Medical College, United States

***Correspondence:**
Beata Kosmider
beata.kosmider@temple.edu

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INTRODUCTION

Over 300 million people suffer from chronic obstructive pulmonary disease (COPD) worldwide (1). It is the third leading cause of death, resulting in ∼3 million deaths every year, according to World Health Organization (WHO) (2). COPD includes lung parenchymal destruction (emphysema) and airway disease (chronic bronchitis). The extent of lung tissue damage in emphysema is measured by chest computed tomography (CT) density. Emphysema is a progressive and irreversible disease with limited therapeutic strategies. Lung volume reduction surgery and lung transplantation represent promising options for end-stage disease (3). At the cellular level, emphysema is characterized by alveolar epithelial cell death and impaired re-epithelialization (**Figure 1**), which causes alveolar wall destruction and decreased surface area in the lung parenchyma for gas exchange (4). Pulmonary vasculature is linked to alveolar architectures and function, whereas endothelial dysfunction and vascular abnormalities were observed in emphysema (5, 6). Furthermore, extracellular matrix (ECM), including elastin, collagen, and proteoglycans, tethers the parenchymal compartments to the airway and affects the tissue mechanics and airway smooth muscle contraction. The process of mechanotransduction provides mechanical cues of the microenvironment to control many cellular events such as proliferation and differentiation and maintain tissue integrity (7). Changes in ECM composition in emphysema may impact

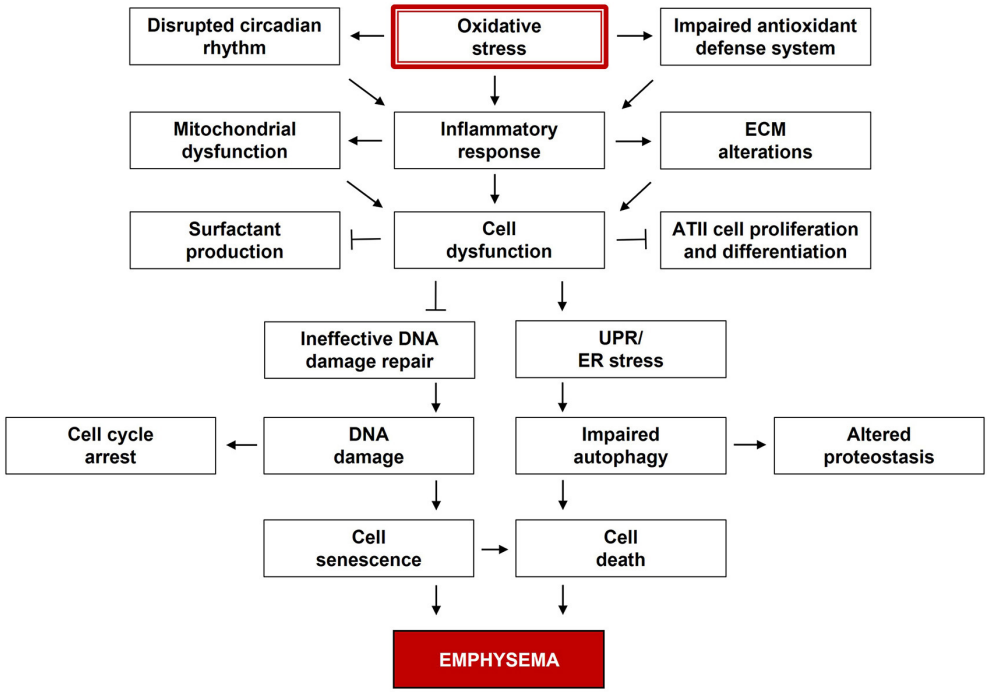

FIGURE 1 | Multiple dysregulated signaling pathways in the pathogenesis of emphysema. Oxidative stress is the major contributor to emphysema. Reactive oxygen species (ROS) and related species disturb cell signaling and impair cell functions, while the antioxidant defense system is overwhelmed. These components are dynamically and progressively interactive over time, and their alterations can lead to emphysema development.

airway smooth muscle cells function, including hypertrophy and hyperplasia, resulting in airway remodeling and obstruction. Also, the loss of elastic recoil leads to airspace enlargements and irreversibly weakens respiratory airflow (8). Increased lung volume, gas trapping, and reduced alveolar units are observed in emphysema patients compared to controls (4).

Environmental irritants, especially cigarette smoke, are the main risk factors of emphysema development. Cigarette smoke contains thousands of chemicals and oxidants that can induce oxidative stress and modify biomolecules, including proteins, lipids, nucleic acids, or carbohydrates, thus affecting lung physiology and tissue homeostasis (9). Surfactant is essential for alveoli and smoking has adverse effects on its function and composition through oxidation and altered proteostasis (10). For instance, Takamiya et al. showed that cigarette smoke extract and its component acrolein modified the surfactant protein (SP)-A in the alveolar epithelium (11). The ability of SP-A to inhibit bacterial growth and macrophage phagocytosis *in vitro* was attenuated due to its conformational changes. The antioxidant defense systems can protect from damage, whereas they are overwhelmed by persisting oxidative stress (9). Cigarette smoke-induced reactive oxygen species (ROS) disturb cell signaling and impair cell functions, including inflammatory responses and protease-antiprotease balance, resulting in ECM destruction and alveolar epithelial cell injury and death (12). Furthermore, emerging senescent cells and vascular dysfunction in the lungs fail the microenvironment for alveolar re-epithelialization.

Besides environmental factors, genetic susceptibility has a significant impact on the morbidity of COPD, such as alpha-1 antitrypsin (AAT) deficiency (13). Studies have identified multiple genetic loci related to this disease susceptibility, e.g., *HHIP, FAM13A, IREB2, MMP12, MMP1, RAGE, SFTPD, FBLN5* in humans and mice (14). These genes are associated with surfactant function, tissue growth, remodeling, and homeostasis. The large complexity of lung pathophysiological processes implicated in emphysema contributes to its heterogeneity. Genetic susceptibility, age, sex, and race-dependent differences may also contribute to this disease development.

DYSREGULATION OF CELL SIGNALING IN EMPHYSEMA DEVELOPMENT

Oxidative Stress

Cigarette smoke-induced oxidative stress and redox imbalance are major contributors to emphysema (15). ROS and related species act as second messengers in cell signal transduction and participate in cellular physiological responses, including the inflammatory immune system and mitochondrial respiration. Oxidative stress refers to high ROS levels that overwhelm the antioxidant defense system. It can alter the activities and functions of redox-sensitive molecules and metabolic enzymes such as p53, NRF2, NF-κB, JNK, MAPK, protein tyrosine phosphatases, glutathione, thioredoxin, peroxiredoxins, and histone deacetylase (15, 16). High oxidative stress and the

defective antioxidant defense system were detected in the lungs and alveolar type II (ATII) cells in emphysema patients (15, 17). Especially reduced NRF2 (18) and FOXO3 (19) levels were observed, which are important transcription factors regulating multiple antioxidant genes such as *HO-1*, *NQO1*, and *GPX1*. Also, NF-E2 was downregulated in ATII cells in this disease (20). Several NRF2 activators and different classes of antioxidants have been developed and tested in clinical trials, however, without promising results (15). Increasing evidence shows that the small redox protein thioredoxin relieved animal emphysema and pulmonary inflammation through multiple mechanisms (21). Its therapeutic potential was recently proposed.

We have demonstrated that DJ-1 protein modulates the NRF2-mediated antioxidant defense system in human primary ATII cells after exposure to cigarette smoke (22). Altered DJ-1 function, its overoxidation to sulfonate form ($-SO_3^-$) at the cysteine-106 and ubiquitination, were reported in the pathophysiological response to oxidative damage and emphysema patients (17). Of note, DJ-1 oxidation to sulfinate form ($-SO_2^-$) was detected in smokers, which reflected its cytoprotective activity. Oxidative stress-induced DJ-1 overoxidation and cleavage in mitochondria were observed in A549 cells treated with hydrogen peroxide. DJ-1 ablation resulted in mitochondrial dysfunction. We also showed impaired S100A8 function in ATII cells in emphysema (23). S100A8 belongs to the S100 protein family and responds to oxidative stress. ATII cell death in this disease correlated with decreased S100A8 sulfination (24). Furthermore, the cytoprotective function of S100A8 compensated for the absence of DJ-1 *in vitro* and *in vivo*. Targeting redox regulation appears to be an ideal approach to this disease yet challenging, possibly due to delicate redox balance, disparities between animal models and human diseases, species differences, compound bioavailability, and other variables.

Pro-inflammatory Response

Environmental irritants can promote the recruitment of inflammatory cells into the lungs. The pro-inflammatory and immune responses are evident in emphysema pathophysiology. Glucocorticoids and bronchodilators are commonly used to treat COPD, although both have many side effects, including immunosuppression (21). The PDE4 inhibitor roflumilast is an approved drug that selectively inhibits PDE4 and increases cAMP levels, leading to an anti-inflammatory response. Numerous PDE inhibitors are in clinical testing. Dual PDE3/4 inhibitors such as RPL554 have gained interest in enhanced efficacy as PDE3 is expressed in vascular smooth muscle cells, and its inhibitor induces bronchodilation (25). Similarly, drug combinations may have a synergistic effect over mono-components. For example, combination of PDE4 and PI3Kδ inhibitors significantly increased protection against cigarette smoke extract-induced apoptosis of lung epithelial cells and reduced inflammatory responses of neutrophils and macrophages *in vitro*.

Under normal conditions, the immune cells balance lung defense, tolerance, or tissue repair. Particularly, alveolar macrophages (AM) interact with pulmonary surfactants in the innate immune responses. AM are potent phagocytes, regulate adaptive immunity and recruit neutrophils and monocytes into the lungs. They secrete inflammatory mediators and proteases such as ECM degrading enzymes and cathepsins upon activation, resulting in elastolysis, alveolar tissue damage, and remodeling of alveoli (8, 12). AM show plasticity based on the microenvironment. Lechner et al. indicated that M2-like macrophages and bone marrow-derived monocytes constitute a regenerative ATII cell niche component, modulating their proliferation and differentiation (26). Using knockout mice and adoptive transfer studies, they found that macrophages accumulated in the lung *via* the CCL2-CCR2 chemokine recruitment axis during lung regeneration after pneumonectomy. F4/80+ M2-like macrophage and ATII cell co-culture supported the formation of pneumospheres.

Nevertheless, high inflammation in smokers correlated with emphysema development (8, 27). Inflammatory responses downregulated *SFTPB, SFTPD, SCNN1A,* and *SCNN1B* gene expression, related to surfactant production and alveolar fluid clearance in ATII cells (28). Decreased SP-D expression was detected in the lungs of emphysema patients (29), indicating the defects in ATII cell function and innate immunity. Especially the number of AM is negatively related to alveolar parenchymal tissue density (27), and impaired phagocytosis is positively associated with the severity of emphysema (30). Increased microRNA (miR)-155 expression was recently identified in the smokers' lungs and in AM of mice exposed to cigarette smoke (31), contributing to inflammation and this disease development. ATII cells isolated from subjects with this disease showed high oxidative stress (17, 32) and ECM degradation with the elevation of matrix metalloproteinase (MMP) 9, CD147, and cathepsin B (20). Other proteases elevated in patients with emphysema include neutrophil elastase, dipeptidyl peptidase-4, proteinase-3, MMPs 1, 8, 12, and 13, cathepsins C, D, E, G, K, and S, and caspases 1, 3, 7, 8, 9, and 11 (33). Several studies have shown the efficacy of antiprotease therapy in emphysema; however, without promising results in clinical trials (21). On the other hand, ADAM8 belongs to a disintegrin and metalloproteinase family. It was reported as a protective proteinase negatively related to emphysema development (34). ADAM8 levels were decreased in macrophages and alveolar epithelial cells in patients with this disease and mice exposed to cigarette smoke. Moreover, ADAM8 knockout mice displayed higher inflammatory and oxidative stress levels, mucus cell metaplasia, alveolar septal cell death, and reduced ATII cell proliferation and repair, contributing to the lung destruction caused by cigarette smoke exposure. Accordingly, pro-inflammatory mediators, oxidative stress, protease-antiprotease imbalance, and impaired pathogen phagocytosis contribute to the destruction of lung tissue and this disease progression (12). Many anti-inflammatory drugs have been developed but have limited efficacy (21). Further studies of immune responses and homeostasis can provide a clue for therapeutic interventions and lung regeneration.

Increasing evidence indicates that the nuclear hormone receptor PPARγ, a regulator of lipid metabolism, adipogenesis, and inflammation, represents a potential therapeutic target for emphysema. It was downregulated in antigen-presenting cells (APCs) isolated from the lungs of patients with this disease and mice exposed to cigarette smoke, thus

directing Th1 and Th17 cell differentiation (35, 36). Also, overexpression of dominant-negative PPARγ in murine ATII cells induced emphysema with increased inflammatory cytokines, MMPs, and accumulation of myeloid-derived suppressor cells (MDSCs) in the lungs and circulation system (37). As expected, treatment with a PPARγ agonist ciglitazone inhibited pathogenic lung APCs *in vitro* in humans and mice. Further, it attenuated cigarette smoke-induced emphysema in mice (35, 36). Thiazolidinediones (TZDs), including ciglitazone, pioglitazone, rosiglitazone, and troglitazone, are valuable drugs for type 2 diabetes and have shown anti-inflammatory effects through selective stimulation of PPARγ. Their application to emphysema needs to be further evaluated.

Chronic Inflammation

Chronic inflammation can increase susceptibility to lung infection. Cigarette smoke-induced emphysema in animals was challenged with bacterial or viral infections to study the mechanism of this disease exacerbation (38). H3N1 influenza A virus infection caused higher virus titers in the lung, inflammation around bronchi and in the parenchyma, and mucus exudates in the airways in cigarette smoke-exposed mice compared to the influenza virus alone (39). Also, *Pseudomonas aeruginosa* infection resulted in impaired autophagy with an increased number of aggresomes, cell senescence, and alveolar space enlargement in mice exposed to cigarette smoke compared to controls (40). Cysteamine, an antioxidant drug with autophagy-inducing properties, alleviated these changes. *Nippostrongylus brasiliensis,* a nematode in rodents, was used to develop a mouse model of progressive emphysema (38). This infection induced a long-lasting Th2 immune response and alternatively activated M2 macrophages accompanied by substantially upregulated MMP12 expression, resulting in alveolar wall destruction and airspace enlargement. Increasing evidence shows the formation of autoimmune components, including autoantibodies and key mediators (such as BAFF and IL-17A), increased B cell counts, and B cell-rich lymphoid follicles in emphysema patients, which were associated with this disease severity (41, 42). Zhou et al. exposed mice to 150–180 mg/m^3 cigarette smoke for 2 weeks followed by 2 weeks of rest before the elastin challenge to study the autoimmunity in emphysema development (43). MMP12-generated elastin fragments are self-antigens that induce monocyte chemotactic activity and contribute to this disease pathology. Exposure to cigarette smoke sensitized mice to elastin and elicited IL17A-predominant autoimmune processes leading to neutrophilic airway inflammation, mucus hyperproduction, airspace enlargement, and lung function decline. Of note, elastin-specific T cell response was also observed in COPD patients. The correlation of B cell adaptive immune responses with late-stage emphysema introduces opportunities for new therapeutic interventions. However, the crucial role of B cells in the defense against pathogenic infections and immune-regulatory activities limits anti-B cell therapies. Further characterization of B cell subsets and their contribution to disease progression are needed.

Altered Cellular Homeostasis

Cigarette smoke-induced oxidative stress increases protein misfolding (44), thus altering lung homeostasis. It induces endoplasmic reticulum (ER) stress and unfolded protein response (UPR), leading to inflammation and cell apoptosis. ER stress was observed in emphysema patients and animals (45). Activation of UPR with the upregulation of GRP78 was detected in human small airway epithelial cells and ATII cells obtained from smokers (46). The aggresome formation, involved in the cellular response to misfolded proteins and their clearance, was also observed in the lungs of smokers, emphysema patients, mice exposed to cigarette smoke, and aged mice (40). Specifically, the levels of aggresome correlated with smoking history and emphysema severity in humans. Cigarette smoke increased aggresome formation by accumulating ubiquitinated proteins and autophagy proteins p62 and valosin-containing protein (VCP) in murine lungs, indicating aberrant autophagy (40). Intraperitoneal administration with the autophagy-inducing drug cysteamine reduced cigarette smoke-induced aggresome formation in the lungs, inflammatory responses in bronchoalveolar lavage fluid (BALF), and emphysematous changes in mice. Similarly, inhibition of ER stress by treatment with 4-phenylbutyric acid reduced cigarette smoke-induced inflammatory response, alveolar cell apoptosis, and airspace enlargement in mice (47). Therefore, the ER stress-autophagy pathway represents a potential therapeutic intervention.

Several studies have pointed out the deleterious impact of ceramides or lactosylceramides accumulation in the human lung in emphysema progression (48), likely due to impaired autophagy resulting in sphingolipids imbalance. They were accumulated in different cell types, including alveolar epithelial and endothelial cells and macrophages in patients with this disease (49). Ceramides are components of sphingolipids and serve as second messenger lipids. Their upregulation leads to inflammatory responses, which can cause alveolar epithelial cell apoptosis and emphysema development in animal models (49). Cigarette smoke promoted the activation of membrane-localized acid sphingomyelinase, which catalyzes the hydrolysis of sphingomyelin to ceramide. This thereupon increased membrane and intracellular ceramide accumulation in aggresomes (48). Cysteamine reduced cigarette smoke-induced ceramide accumulation in Beas2b cells (48). It improved efferocytosis, cell viability, and decreased cell senescence. Also, intraperitoneal injection of autophagy-inducer gemfibrozil significantly reduced ceramide accumulation in murine lungs after cigarette smoke exposure.

Circadian Rhythm Disruption

The human respiratory system functions with a daily circadian rhythm, affecting airway resistance, ventilatory controls, and immune function, yet it changes with age and smoking (50, 51). Smokers have marked diurnal changes in pulmonary function tests compared to non-smokers (51). The lungs' molecular clock is also altered in response to environmental factors, including allergens, pollutions, pathogens, infections, oxidative stress, hypoxia/hyperoxia, jet lag, and shift work (52). Importantly, circadian gene expression positively connects to cell cycle

and immune regulation in mice and humans (53). Increasing evidence shows a link between circadian rhythm disruption and susceptibility to lung infection (54) and inflammatory diseases (55), including asthma and COPD with severe symptoms in the early morning, which correlated with disease exacerbations.

Pekovic-Vaughan et al. have demonstrated the circadian regulation of the NRF2 pathway in the murine lung, mainly in the bronchial and alveolar epithelium (56). The loss of NRF2-dependent antioxidant defense system and reduced-glutathione levels increased oxidative damage in the lungs of $Clock^{\Delta 19}$ mutant mice compared to wild-type mice. SIRT-1 functions as NAD^+-dependent protein deacetylases and regulates many pathophysiological processes. It controls the circadian clock through binding with CLOCK-BMAL1 complexes and promoting the deacetylation and degradation of PER2 proteins (57). In the lungs of mice exposed to cigarette smoke, altered rhythms of SIRT-1 protein and core clock genes including *Bmal1*, *Clock*, *Per1*, *Per2*, *Cry1*, *Cry2*, *Nr1d1*, *Nr1d2*, and *Rora* were observed in a circadian manner, increasing the susceptibility to inflammation and emphysema development (58). Similarly, BMAL1 protein was down-regulated in the lungs of smokers and individuals with this disease (58). BMAL1 deficiency accelerated senescence and caused age-associated shrinkage of major organs, including lungs, in 40-week-old mice, which correlated with increased ROS levels and inflammation (59). Melatonin, a natural hormone that regulates sleep-wake cycles, has shown beneficial effects on lung diseases through anti-oxidation and anti-inflammaton (60). It increased SIRT-1 expression *in vitro* and *in vivo* (61, 62). Intraperitoneal injection with melatonin decreased inflammatory response in BALF, and the lungs of mice challenged with cigarette smoke and lipopolysaccharide (61). It also reduced ER stress and bronchial and alveolar epithelial cell apoptosis, and protected alveolar architecture in a rat model of emphysema (62). The daily rhythm of SP-A, SP-B, and SP-C gene expression in the rat lungs was reported (63). This indicates the critical role of circadian rhythm in ATII cell function; however, its alterations in lung pathophysiology are still poorly understood.

Cellular Senescence

Cigarette smoke can cause DNA damage, while the impairment of double-strand DNA break repair in ATII cells was shown in emphysema patients (32). Oxidative stress and persistent DNA damage are associated with stress-induced premature senescence (SIPS) (64). SIPS induces irreversible cell cycle arrest, chromatin changes, and resistance to apoptosis. It also drives a senescence-associated secretory phenotype (SASP) in cells, including secretion of inflammatory cytokines IL-6 and IL-8 and remodeling factors, thereby affecting tissue microenvironment and promoting senescence in an autocrine and paracrine manner (65). DNA damage response (DDR) is required for SASP, including cell cycle regulatory protein NBS1 and checkpoint kinases ATM and CHK2 (66). Transient exposure to SASP promoted stem cell function and tissue regeneration, whereas prolonged exposure had an opposite effect in mice (67). Mice with GFP-expressing ATII cells (CBA/Ca × C57BL6J) exposed to cigarette smoke for over 3 months displayed airspace enlargement and alveolar epithelial cell apoptosis (68). The surviving ATII cells showed higher resistance to apoptosis, which was likely related to circadian rhythm. This suggests the contribution of ATII cell senescence and the circadian clock's regulation to emphysema development. Reduced anti-aging protein SIRT-1 levels were found in the lungs of smokers and patients with emphysema, including AM, airway, and alveolar epithelium (69, 70). Particularly, oxidative stress-induced miR-34a expression was detected in lung tissue in these patients leading to suppression of SIRT-1 and SIRT-6 expression *via* the PI3K pathway (70). Furthermore, increased lipofuscin levels, a marker of senescence, and cell cycle inhibitors p16 and p21 were mainly detected in ATII cells and endothelial cells in emphysema patients compared to controls (71). Telomere signal intensity in these cells was lower in smokers and emphysema than in controls. A negative correlation between p16 and proliferation cell nuclear antigen (PCNA) was also reported. Similarly, other cells displayed characteristics of cellular senescence, including airway epithelial cells, fibroblasts, and immune cells (72). Their accumulation indicates the loss of tissue homeostasis and an altered environment for alveolar re-epithelialization in emphysema. Senotherapies are effective in animal models, including SIRT-1 activators and inhibitors of mTOR, JAK, FOXO4, and anti-apoptotic proteins (72). Drugs targeting anti-apoptotic proteins in senescent cells such as dasatinib and quercetin were well-tolerated in patients with age-related diseases. Senotherapy seems promising since the mechanism of cellular senescence is likely shared between different diseases.

Furthermore, mitochondria generate ATP for cell metabolism, and their dysfunction has been linked to age-related lung diseases. Several mitochondria-targeted antioxidants, including mitoQ, mito-TEMPO, pyrroloquinoline quinone, and SkQl are in clinical trials for other age-related diseases (72). Increased mitochondrial (mt)DNA copy number has been detected in the blood of patients with COPD along with a positive correlation to the number of pack-years smoking (73). It was also found in urine and was associated with respiratory symptoms and emphysema severity (74). Importantly, elevated mitochondrial ROS levels, low mitochondrial amount, accumulation of mtDNA damage, and mitochondrial dysfunction were detected in human ATII cells in this disease progression (75). It has been shown that electronic (e)-cigarette aerosols containing nicotine caused airway hyperreactivity, alveolar destruction, reduced lung function, and emphysematous changes in mice (76). Nicotine altered calcium levels and mitochondrial membrane potential in human primary ATII cells with DJ-1 knockdown (77). Dysregulation of mitochondrial oxidative phosphorylation (OXPHOS) complexes was observed in the lungs of DJ-1 knockout mice exposed to aerosolized nicotine, which disrupted the nuclear/mitochondrial stoichiometry resulting in mitochondrial dysfunction. This was associated with increased AM number and ATII cell apoptosis. Both ER stress and mitochondrial dysfunction were detected in emphysema, however, with unknown integration. It was recently demonstrated that ER stress led to mitochondrial UPR in an ATF4-dependent manner in MLE-12 cells (78). Mitochondrial

UPR has contributed to different disease pathogenesis (79), although its activation is also known to lifespan extension in both *Caenorhabditis elegans* and mice (80). Improving our understanding of the mitochondrial role in cellular senescence can greatly benefit treating emphysema.

Apoptosis

The correlation of alveolar cell apoptosis and decreased lung surface area have been shown in emphysema (81). Intratracheal instillation of active caspase-3 protein led to alveolar epithelial cell apoptosis and this disease development in mice (82). Increased active caspase 3 levels were detected in ATII cells in smokers and individuals with emphysema (24). Histone deacetylase inhibition altered chromatin remodeling leading to ATII cell apoptosis and alveolar structure destruction in trichostatin A-treated rats through increased miR34a, p53, cleaved caspase 3, and microtubule-associated protein-1 light chain 3 (LC3) levels (83). Decreased hypoxia-inducible factor-1α (HIF-1α), vascular endothelial growth factor (VEGF), lysyl oxidase, and collagen expression in this animal model pointed out the involvement of angiogenic factors in the alveolar structure. Subcutaneous injection of VEGF receptor (VEGFR) blocker led to emphysema without the infiltration of inflammatory cells in rats (84). Treatments targeting VEGF signaling have beneficial effects against emphysema development in animal models (85–87). Alveolar and endothelial cells can sense microenvironment changes; therefore, they serve as niches for regulating lung repair and integrity. Angiogenesis impairment by subcutaneous injection of sodium glutamate enhanced lung inflammation and emphysema in mice induced by intratracheal instillation of cigarette smoke extract, which was related to insufficient migration of pericytes and smooth muscle cells in lung tissue (88). Surprisingly, intravenous administration of healthy lung endothelial cells ameliorated emphysematous phenotype and lung function in an elastase-induced mouse model (89). It was recently reported that endothelial cells released angiocrine sphingosine-1-phosphate (S1P) after *Pseudomonas aeruginosa* infection in mice which promoted alveolar re-epithelialization *via* S1PR2-YAP signaling (90). Supplementation of S1P prevented alveolar cell apoptosis in VEGFR blockade-induced emphysema in mice (91). This suggests that sphingolipid balance is important for the maintenance of alveolar septal integrity.

Smooth Muscle Cell Proliferation

In addition to endothelial dysfunction, pulmonary vascular remodeling characterized by increased smooth muscle cell proliferation and narrowing of the vascular lumen has been shown in smokers and to precede airspace enlargement in animal models of emphysema (92). It can subsequently lead to vascular wall thickening, pulmonary hypertension, and right heart failure. Bronchodilators that target the smooth muscles were beneficial in reducing COPD exacerbation risk vs. placebo in large-scale randomized trials (93). Significant improvements in forced expiratory volume (FVC) were reported after bronchodilator administration in subjects with emphysema (95, 96). Treatment with complementary bronchodilators or a combination of inhaled corticosteroids with bronchodilators was more effective than monotherapy (94). Pharmacological lung volume reduction through bronchodilator therapy is an important goal in these patients.

Smooth muscle cells are the mechanical sculptor of the epithelium (97). Nitric oxide (NO) production by nitric oxide synthase (NOS) regulates the degree of contraction of vascular smooth muscle cells and their proliferation. ATII cells express constitutive NOS as well as inducible NOS (iNOS) during inflammatory states, suggesting their interaction with vascular smooth muscle cells. The absence of SP-D in mice displayed iNOS-related chronic inflammation, alterations of surfactant homeostasis, and emphysematous changes. This elucidates the contribution of immune responses to NO/iNOS regulation (98). iNOS inactivation by genetic deficiency and pharmacological inhibition prevented cigarette smoke-induced pulmonary hypertension and emphysema, including structural and functional alterations of the lung vasculature and alveoli in mice (99). In clinical studies, elevated alveolar NO has been associated with COPD severity (100). These patients have higher numbers of $iNOS^+$ ATII cells related to increased protein nitration and decreased lung function (101). Especially, the lung tissue from patients with emphysema has a higher ratio of the number of alveoli/vessels and an increased degree of vessel muscularization. Nitrated proteins in vasculature and alveoli were increased in these patients (99). NO can be toxic through combination with superoxide to generate peroxynitrites leading to nitration of biomolecules, altering their structure and function. Further, peroxynitrites induced alveolar epithelial and endothelial cell apoptosis (99). However, chronic exposure to the NOS inhibitor N^{ω}-nitro-L-arginine methyl ester resulted in vascular senescence, hypertension, and emphysema development in mice (102). This emphasizes the importance of NO/NOS balance in vascular health and alveolar structure.

Chronic hypoxia in emphysema induces pulmonary artery smooth muscle cells proliferation and JAK2/STAT3 activation. JAK2 deficiency attenuated pulmonary vascular remodeling and smooth muscle hyperplasia in mice (103). Furthermore, HIF-1α promotes vascular smooth muscle cell proliferation under hypoxic conditions. Its increased expression was detected by immunohistochemistry in the lungs of emphysema patients, indicating vascular remodeling in this disease. Especially, HIF-1α was positively associated with disease severity (104). With oxygen treatment, the HIF-1α and erythropoietin decreased in COPD (105). It has been demonstrated that HIF-1α signaling in ATII cells promoted their proliferation after acute lung injury in mice induced by lipopolysaccharide or hydrochloric acid (106). ATII cell-specific HIF-1α deletion caused higher mortality in these mice. Also, HIF-1α is activated in the ATII to alveolar type I (ATI) cell transitional state (107). Together, these data show the intimate relationship between alveolar epithelium and smooth muscle cells for alveolar architecture and function.

Dysregulated Alveolar Re-epithelialization

ATII cells are progenitors in alveoli. Various ATII niches, mediators, and signaling support their functions for alveolar epithelial cell homeostasis and re-epithelialization. The interactions between signaling molecules arising from and acting on the alveolar epithelium, vasculature, mesenchyme, ECM, and immune cells institute ATII cell niches. Dynamic organization of WNT/β-catenin, TGF-β, YAP/TAZ, NOTCH, and TP53 signaling pathways participate in ATII cell growth and differentiation to ATI cells (108). The importance of WNT/β-catenin, YAP/TAZ, and TGF-β signaling in emphysema development has been demonstrated in animal models. Specifically, *TGF-β2* was identified as a significant emphysema-associated genetic variant by human genome-wide association studies (GWAS) (109). TGF-β regulates multiple context-dependent cellular processes associated with tissue remodeling and is crucial for epithelial-mesenchymal interactions. It includes the regulation of cell polarity, ECM turnover, ATII to ATI cell transdifferentiation, and differentiation of lung fibroblasts to myofibroblasts positive for α-smooth muscle actin (α-SMA). Leucine-rich α-2-glycoprotein-1 (LRG1), known to regulate TGF-β signaling, was increased in endothelial cells in patients with emphysema and correlated to its severity (89). Elevated YAP protein levels were detected in the lungs of patients with this disease compared to healthy donors (110). In contrast, reduced WNT/β-catenin pathway in ATII cells was observed in emphysema patients (111, 112) while the non-canonical WNT pathway was upregulated (113, 114). These data suggest signaling imbalance and ATII cell dysfunction in the emphysematous lung. It is known that WNT/β-catenin and anti-inflammatory PPARγ signaling pathways are integrated, and they work in opposition, highlighting the relationship between immune response and ATII cell fate. Feller et al. demonstrated that a non-canonical WNT ligand WNT5a and pro-inflammatory cytokines can be transported through extracellular vesicles, leading to systemic inflammation in COPD patients (115). This further points out the importance of the interactive signaling and microenvironment in the pathogenesis of this disease.

HHIP is a component of hedgehog signaling, which is important in many developmental processes (116). It is a genetic locus associated with emphysema susceptibility in humans and mice. Sonic hedgehog signaling is required for myofibroblast differentiation and mesenchymal proliferation during alveologenesis, while it maintains quiescence in the adult murine lung (117). A broad population of hedgehog-receptive mesenchymal cells surrounding airways and alveoli was identified in the murine lung (118). The transgenic activation of the hedgehog in the mesenchyme disrupted the alveolar niche. This impaired ATII cell proliferation, increased airspace, and emphysematous changes. Single-cell transcriptome analysis of the human lung shows that mesenchymal subsets are conserved across species. The intermediate mesenchymal subset was involved in cholesterol metabolism, suggesting its role in surfactant biosynthesis of the alveolar epithelium. In addition, Kato et al. demonstrated that the paracrine signaling capabilities of pericytes, specialized mesenchymal cells surrounding the capillary, are crucial for alveologenesis. Loss of pericyte-specific YAP/TAZ reduced endothelial and ATII cell proliferation through paracrine regulation, resulting in defective alveolarization and a severe emphysema-like morphology in mice (119). It changed the growth factor expression profiles of these cells in the lungs, including reduced *Angpt1*, *Tgfb2*, *Wnt11*, *Bmp4*, and *Hgf* (119). Treatments targeting ATII cell proliferation and differentiation to ATI cells have not been developed owing to plenty of unknowns regarding the mechanism of alveolar re-epithelialization.

Signaling Interplay

Oxidative stress, ER stress response, and inflammation are key contributors to emphysema development. Their interactive relationships have been reported (120). ROS provide an oxidizing environment and affect molecular chaperones and enzymes in the ER. In response to ER stress, the UPR helps cells adapt to and survive the stress condition by transcriptional and translational reprogramming. In contrast, programmed cell death signaling is activated when protein homeostasis cannot be achieved. UPR signaling is initiated by ER membrane-bound transducers: IRE1, ATF6, and PERK. Particularly, IRE1α regulates many cell functions, including metabolism, immunity, inflammatory cytokine production, cell differentiation, and apoptosis, through the RIDD mechanism, which induces the degradation of certain mRNAs or miRNAs. Of note, the IRE1α-mediated XBP1 pathway is essential for optimal expression of inflammatory cytokines and proangiogenic factors in human aortic endothelial cells (121, 122). Under chronic or severe ER stress, PERK-mediated phosphorylation of eIF2α induces expression of ATF4 and a proapoptotic factor CHOP/GADD153, resulting in ER stress-induced apoptosis. ATF4 can also induce growth arrest and DNA damage-inducible protein GADD34. Furthermore, ER and mitochondria are physically and functionally connected through mitochondria-associated ER membranes, which contain major calcium channels IP3R and VDAC. ROS can target ER calcium channels, leading to ER calcium release. It subsequently stimulates mitochondrial metabolism and ROS production. The delicate balance between different cell signaling can be the key to therapeutic benefit.

Given that ROS is involved in various physiological processes, the interplay between signaling potentially causes a cascade of cell dysfunction and imbalance under pathological stimuli, leading to tissue damage. Sirtuins (SIRT1-7) maintain cellular redox balance and are an important defense mechanism against oxidative stress according to the different subcellular localization of each sirtuin. Specifically, the ability of SIRT1 to intersect with different signaling, including the circadian clock, inflammation, cell cycle, and senescence, makes it a lucrative target for therapeutics. SIRT1 activators include baicalin, exendin-4, ginkgolide-B, pitavastatin, quercetin, SRT2104, and vitamin D. These medications are in clinical trials for other diseases. Of note, exendin-4 is a licensed medicine for patients with type 2 diabetes. Exendin-4 treatment improved lung function, mortality, and clinical appearance in mice with lipopolysaccharides-induced emphysema, possibly through bronchodilatory effects (123). The mechanistic links between diabetes and COPD imply the applicability of this medicine (124).

CONCLUSION

Despite numerous investigations, therapeutic strategies for pulmonary emphysema remain limited mainly due to the complexity and heterogeneity of disease manifestation. Multiple dysregulated signaling pathways affect physiological processes in emphysema development (**Figure 1**). Its pathological features include high inflammatory and immune response, oxidative stress, defective antioxidant defense system, altered protein homeostasis, cellular senescence, and apoptosis. Circadian rhythm disruption leads to abnormal cell cycle and immune dysregulation, which points out a poor lung repair in this disease. These components are dynamically and progressively interactive over time. Individual differences and genetic variations in surfactant function, tissue growth, remodeling, and homeostasis can also contribute to this disease development. Targeting the cellular processes to decrease alveolar injury and increase alveolar re-epithelialization may restore lung function. Various factors and signaling are interdependent in complex pathophysiology. Therefore, desired outcomes could be achieved by targeting multiple pathways. More systematic and comprehensive studies regarding interactions between different cell types, organelles, and signaling pathways are warranted to uncover new therapeutic strategies.

AUTHOR CONTRIBUTIONS

C-RL performed the literature search, prepared the figures, and wrote the manuscript. KB and BK provided significant works of literature, interpretations, and revision. All authors contributed to the article and approved the submitted version.

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Oral Health-Related Quality of Life in Patients With Chronic Respiratory Diseases—Results of a Systematic Review

Simin Li[1†], *Wanchen Ning*[1†], *Wei Wang*[1†], *Dirk Ziebolz*[2], *Aneesha Acharya*[3], *Gerhard Schmalz*[2‡], *Jianjiang Zhao*[4*‡], *Shaohong Huang*[1*‡] and *Hui Xiao*[1*‡]

[1] Stomatological Hospital, Southern Medical University, Guangzhou, China, [2] Department of Cariology, Endodontology and Periodontology, University Leipzig, Leipzig, Germany, [3] Dr. D. Y. Patil Dental College and Hospital, Dr. D. Y. Patil Vidyapeeth, Pune, India, [4] Shenzhen Stomatological Hospital, Southern Medical University, Shenzhen, China

Background: This systematic review evaluates the oral health-related quality of life (OHRQoL) of patients with chronic respiratory diseases.

Methods: A systematic literature search was performed based on the PubMed, Medline, Web of Science, and Scopus, using the search terms: "oral health-related quality of life" and "respiratory disease" or "lung" and "oral health-related quality of life." Full-text articles published until June 30, 2021 and reporting any OHRQoL measurement in children or adults with a chronic respiratory disease or condition were included and analyzed qualitatively.

Results: A total of seven out of 44 studies were included, of which four studies examined adults and three studies investigated children. The respective diseases were chronic obstructive pulmonary disease (COPD) ($n = 2$), sleep apnea ($n = 2$), severe asthma ($n = 1$), cystic fibrosis ($n = 1$), and lung transplantation ($n = 1$). Four studies confirmed a worse OHRQoL in the respiratory diseased group compared to healthy controls. The overall OHRQoL was reduced in the included studies. Oral health, health-related quality of life, and disease-related parameters were rarely examined with regard to OHRQoL.

Conclusion: Patients with chronic respiratory diseases show a reduced OHRQoL. Oral health should be fostered in these individuals to support their OHRQoL.

Keywords: oral health, oral health-related quality of life, respiratory disease, lung, COPD

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Kin-fai Ho,
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Reviewed by:
Vinay Jain,
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Sichuan University, China

***Correspondence:**
Shaohong Huang
hsh.china@tom.comz
Jianjiang Zhao
zjj2521@sina.com
Hui Xiao
zzmmxh@126.com

[†]*These authors share first authorship*
[‡]*These authors share senior authorship*

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BACKGROUND

Chronic diseases affecting the respiratory system cover a broad variety of different conditions; thereby, lifestyle or risk factor-associated, high prevalent diseases, such as chronic obstructive pulmonary disease (COPD), exist (1) alongside with very rare genetic diseases, such as cystic fibrosis (2). Other examples of respiratory diseases are asthma, which is a highly prevalent condition with primarily an autoimmune cause (3) or sleep apnea or sleep-disordered breathing as a complex and multifactorial condition (4). Especially, if breathlessness occurs, chronic respiratory diseases can

be related to a high morbidity and a negative impact on the quality of life of affected patients (5). Additionally, chronic respiratory diseases, such as COPD, can lead to different comorbidities and physical deconditioning, decreasing the health-related quality of life (HRQoL) of patients suffering from these diseases (6).

Oral health is often reported to be affected by respiratory diseases in respective patients; a recent meta-analysis showed that patients with asthma as well as COPD showed an association to the presence of periodontal diseases (7). Besides a direct association, medications, e.g., asthma medication including bronchodilators, corticosteroids, or anticholinergic drugs inhaled by the patients can increase the risk of dry mouth, dental caries, dental erosion, periodontal diseases, and oral candidiasis (8, 9). Especially for severely diseased patients, such as patients with cystic fibrosis or COPD, oral bacteria related to dental and periodontal diseases can also colonize the lungs, increasing the risk for complications (10, 11). Additionally, patients with obstructive sleep apnea, which is completely different from the other respiratory diseases described here, show affected oral health conditions as well as the relationship between oral diseases and the underlying disorder; this is heterogeneous, but obvious for both the children and adults (12). In the complex relationship between oral and respiratory health, the multifactorial character of oral diseases, as well as the anatomic proximity of the oropharyngeal and respiratory tract could be relevant.

A particular issue of interest in this context is the oral HRQoL (OHRQoL). As a part of the general HRQoL, the OHRQoL reflects the perceived affection of HRQoL by conditions related to the oral cavity including teeth, mouth, or dentures (13, 14). In other groups of systemically diseased patients, the OHRQoL showed interesting and clinically relevant results. For example, patients with rheumatic diseases show an impaired OHRQoL, whereby rheumatic disease-related parameters might be major influential factors (15). On the other hand, patients after solid organ transplantation were supposed to undergo a "response shift" of the perception of OHRQoL, i.e., reduced awareness of their insufficient oral health status (16). Considering the impaired oral health, reduced general HRQoL alongside the anatomic proximity between the oral cavity and the respiratory system, the OHRQoL of patients with different respiratory diseases appears a topic of clinical interest. Therefore, this study evaluates the OHRQoL of patients with chronic respiratory diseases. It was hypothesized that these patients would show a reduced OHRQoL.

METHODS

This study was performed in full accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (17).

Patients, Intervention, Comparison, and Outcome (PICO) Question

The PICO question was as follows: "Do patients with severe respiratory diseases show a reduced OHRQoL?". Thereby, "patients" were individuals with chronic respiratory diseases. The aspect "intervention" was not defined because it was expected to primarily include cross-sectional studies. "Comparison" was a healthy control group, if applicable. Otherwise, values should be compared to reference values or other groups of patients with systemic diseases. Finally, "outcome" was any applied OHRQoL measurement. The hypothesis was formed that the OHRQoL of patients with chronic respiratory diseases would be worse than in healthy individuals.

Eligibility Criteria

For inclusion in this study, several inclusion criteria were formulated:

- Publication until 30th of June, 2021.
- Examination of children or adults with a chronic respiratory disease or condition.
- Reporting of any OHRQoL measurement.
- Full text in English language.

Search Strategy

In July 2021, a systematic literature search was performed by two different and independent reviewers. As databases for literature search, the PubMed, Medline, Web of Science, and Scopus were chosen, using the search terms: "oral health-related quality of life" and respiratory disease or lung and "oral health-related quality of life." A manual search based on the references of findings complemented the systematic literature search. All the findings were checked and screened for their eligibility.

Data Extraction

After all the articles were screened and checked, qualitative data extraction was applied. Thereby, different issues were extracted from the respective articles.

- Form of respiratory disease, year of publication, number of participants, study type, age, gender, and smoking.
- Presence and characteristics of a healthy control group.
- Oral health findings, if applicable.
- Oral HRQoL assessment including the form of measurement and results.
- Potential relationship between OHRQoL and general parameters, disease-related parameters, or oral health findings, if applicable.
- Subscales of the OHRQoL measurements, if applicable.

Two independent reviewers executed the whole process of systematic search and study selection as well as qualitative analysis. Only studies explicitly reporting OHRQoL of patients with chronic respiratory diseases were considered within this study.

Quality Assessment

The 11-item checklist from the Agency for Healthcare Research and Quality (AHRQ) for cross-sectional studies was applied for quality assessment of the included studies (18). The answers "no" or "unclear" were rated as 0 and the answer "yes" was rated as 1 point for each question to

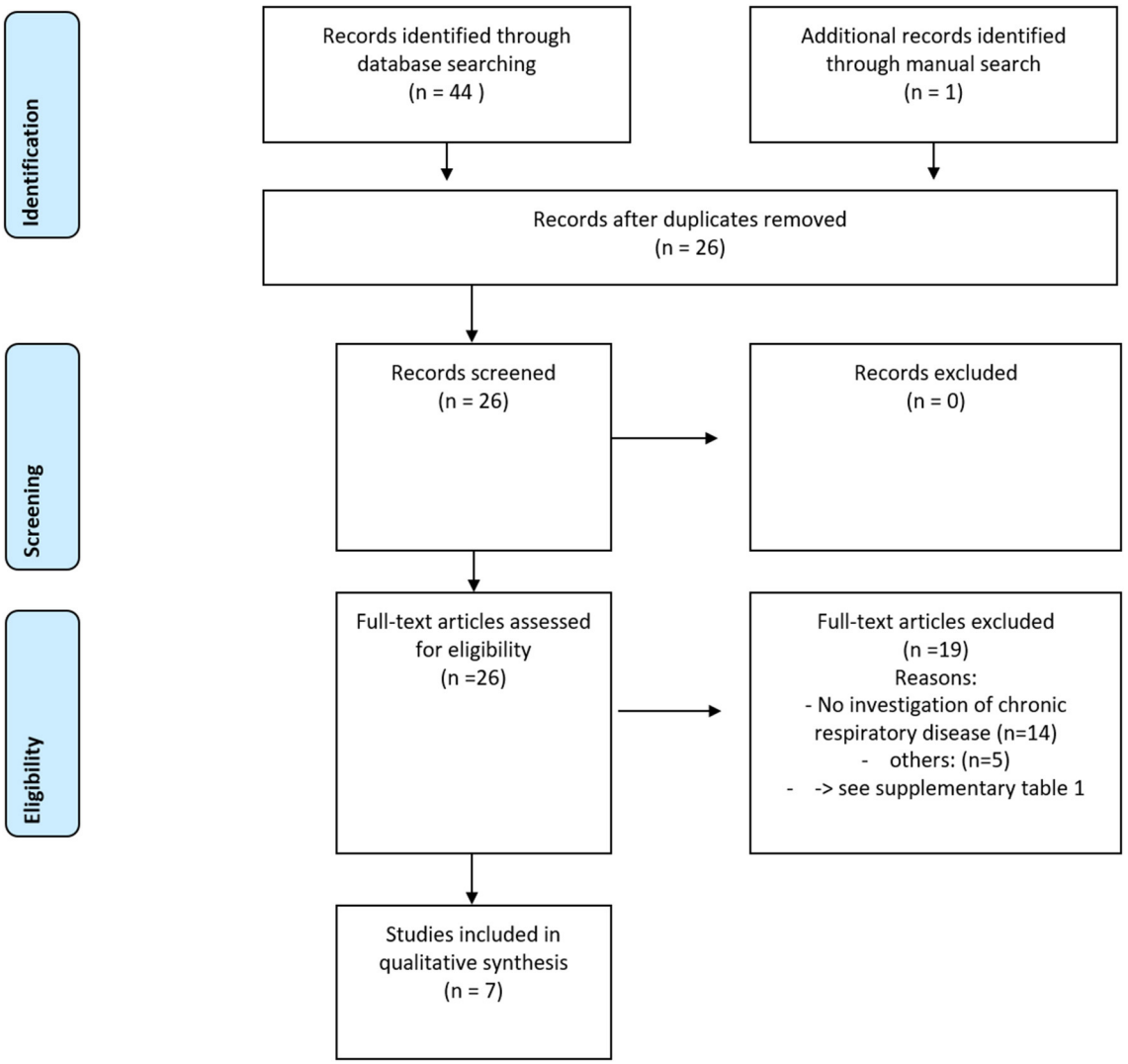

FIGURE 1 | The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) diagram for the systematic review process in this study (17).

estimate a score for the respective quality of the studies. A sum score of all the questions of 0–3 indicated low quality, 4–7 indicated moderate quality, and a score of 8–11 indicated high quality of this study. The quality appraisal was conducted by two independent reviewers, whereby any disagreements were discussed and resolved in the whole author group.

RESULTS

Search Findings

The PRISMA diagram, reflecting the findings of the systematic search, is given in **Figure 1**. Out of 44 database search findings, 26 full-text articles were assessed for their eligibility. During this, 19 articles were excluded (**Supplementary Table 1**). Finally, seven studies were included in the qualitative analysis.

Characteristics of the Included Studies

Out of the seven studies, two studies were on patients with COPD (19, 20), while each one study was on cystic fibrosis (21), lung transplantation (22), sleep-disordered breathing (23), obstructive sleep apnea (24), and severe asthma (25). Three studies were performed in children (21, 23, 24), while four studies were conducted in adult patients (19, 20, 22, 25). All the included studies had either a cross-sectional or an observational design. The healthy control group was examined in five of the included studies (20, 22–25). A full overview of the study characteristics is shown in **Table 1**.

Quality Assessment

Quality appraisal revealed that three studies were of high quality (19, 20, 23), while four studies were elevated with moderate

TABLE 1 | Overview of the included studies.

Author, year	Disease	Country	No. of patients	Study type	Subjects mean age in years	Smoking	Male (%)	Healthy control group for OHRQoL
Saltness et al. (19)	COPD	Norway	100	Monocentric cross-sectional study	65.9 ± 10.1	39%	56%	No
Patrick et al. (21)	Cystic fibrosis	USA	39	Multicentric cross-sectional	43.6% 8–12 years, 56.4% 13–17 years	n/a	53%	No
Schmalz et al. (22)	Lung transplantation	Germany	60	Monocentric cross-sectional study	54.03 ± 9.97	0%	50%	Yes: $n = 70$, age: 55.44 ± 8.54 years, 37% male
Gaeckle et al. (20)	COPD	USA	20	Monocentric prospective observational study (follow-up: 60 days)	60 (56–68)	50%	60%	Yes: $n = 10$, age: 54.5 (50-60) years, 60% male
Grillo et al. (23)	Sleep-disordered breathing	Italy	61	Monocentric cross-sectional study	12.4 ± 3.1	16.4%	54.1%	Yes: $n = 61$, age: 11.9 ± 2.8 years, 50.8% male
Tamsas et al. (24)	Obstructive sleep apnoea	USA	31	Monocentric cross-sectional study	12.8 ± 3.1	14%	52%	Yes: $n = 36$, age: 11.8 ± 2.2 years, 55% male
Brasil-Oliveira et al. (25)	Severe asthma	Brazil	40	Monocentric cross-sectional study	51.8 ± 10.8	0%	15%	Yes: $n = 50$, age: 48.2 ± 12.4 years, 42% male

Values are presented as the mean values ± SD, mean values (range), or percentages.
OHRQoL, oral health-related quality of life; n/a, not applicable; COPD, chronic obstructive pulmonary disease.

quality (21, 22, 24, 25) according to the AHRQ criteria for cross-sectional studies (18) (**Table 2**).

Oral Health Records and Findings

Reporting of oral health data was not very comprehensive in the majority of included studies. One study did not report on any oral health conditions of the participants (21). The four studies including adults reported on remaining teeth (19, 20, 22, 25). Moreover, four studies reported on dental as well as periodontal conditions, whereby the assessment method was quite different (22–25). The detailed oral health findings, if applicable, are shown in **Table 3**.

Oral Health-Related Quality of Life Measurements and Results

The four adult studies applied the Oral Health Impact Profile-14 (OHIP-14) for OHRQoL assessment, showing mean values between 1.7 and 12 points in sum scale (**Figure 2**) (19, 20, 22, 25). The three other studies applied the Child OHIP (COHIP), reporting average sum scores between 23.2 and 67.5 points (**Figure 3**) (21, 23, 24). Four studies (each two in children and adults) reported a worse OHRQoL in the respiratory disease compared to the healthy control group (20, 23–25), while only one study an adult lung transplant recipients did not show a difference against a healthy control (22). Associations between OHRQoL and HRQoL, disease-related parameters, or oral health conditions were rarely examined and reported, respectively (**Table 4**). Only one study examining adults (25) and two studies examining children (21, 24) reported on subscales of the OHIP-14 or the COHIP, respectively (**Table 5**).

DISCUSSION

This study hypothesized that patients with chronic respiratory diseases would show a reduced OHRQoL. Based on the findings of the literature search, this hypothesis can be confirmed, but several disease-specific and methodological issues need further discussion. Thereby, the following will provide: (I) an interpretation of the reduced OHRQoL in patients with chronic respiratory diseases in general and with respect to the different diseases, (II) a discussion of the applied methods alongside with (III) recommendations for further study in this field to strengthen the body of evidence and strive some clinical implications.

(I) Altogether, the OHRQoL of patients suffering from systemic diseases can be discussed controversially because several heterogeneous phenomena, depending on the form of the disease and/or therapy, exist. It is known that oral health can affect general HRQoL (26) and that reduced HRQoL can negatively affect OHRQoL (13, 27). Patients with severe chronic diseases or conditions, e.g., rheumatic diseases or hemodialysis show a reduced OHRQoL caused by their general disease burden including pain and impact on their daily life (15, 28). Thereby,

TABLE 2 | Quality assessment of the included studies according to the Agency for Healthcare Research and Quality (ARHQ) (18).

Item	Saltnes et al. (19)	Patrick et al. (21)	Schmalz et al. (22)	Gaeckle et al. (20)	Grillo et al. (23)	Tamasas et al. (24)	Brasil-Oliveira et al. (25)
(1) Define the source of information (survey, record review)	Yes	Yes	Yes	Yes	Yes	Yes	Yes
(2) List inclusion and exclusion criteria for exposed and unexposed subjects (cases and controls) or refer to previous publications	Yes	No	Yes	Yes	Yes	Yes	Yes
(3) Indicate time period used for identifying patients	Yes	No	Yes	Yes	Yes	No	Yes
(4) Indicate whether or not subjects were consecutive if not population-based	Yes	Yes	Yes	Yes	Yes	Yes	Yes
(5) Indicate if evaluators of subjective components of study were masked to other aspects of the status of the participants	No	No	No	No	No	No	No
(6) Describe any assessments undertaken for quality assurance purposes (e.g., test/retest of primary outcome measurements)	Yes	NA	Yes	Yes	Yes	NA	Yes
(7) Explain any patient exclusions from analysis	Yes	NA	NA	Yes	Yes	NA	NA
(8) Describe how confounding was assessed and/or controlled.	Yes	Yes	U	Yes	Yes	Yes	Yes
(9) If applicable, explain how missing data were handled in the analysis	NA	Yes	NA	NA	NA	NA	NA
(10) Summarize patient response rates and completeness of data collection	Yes	Yes	Yes	Yes	Yes	Yes	Yes
(11) Clarify what follow-up, if any, was expected and the percentage of patients for which incomplete data or follow-up was obtained	NA	NA	NA	Yes	NA	NA	NA
Total score	8	5	6	9	8	5	7

the OHIP-14 values for patients with respiratory diseases in this study were similar as for rheumatic diseased patients or individuals undergoing renal replacement therapy (15, 28). Thus, the HRQoL impairment due to the general disease could be one reason for the reduced OHRQoL in this study; especially, severely diseased individuals with proceeded COPD, cystic fibrosis, and severe asthma are impaired in their everyday life and show affected HRQoL (29–31). Thereby, both severe symptoms, such as breathlessness, as well as mental health issues, such as anxiety or depression, can affect HRQoL outcomes (6, 31), potentially

TABLE 3 | Oral health parameters and respective main results if they presented as the mean values ± SD, means (range), or percentages in the included studies.

References	Tooth loss, remaining teeth, dentures	Dental diseases, caries, dental treatment need	Oral hygiene indices	Periodontal parameters, periodontal treatment need	Further oral health parameters
Saltness et al. (19)	44% <20 teeth	n/a	n/a	n/a	9% hyposalivation, 39% oral health problems
Patrick et al. (21)	n/a	n/a	n/a	n/a	n/a
Schmalz et al. (22)	M-T: 8.17 ± 5.82*	DMF-T: 20.53 ± 5.09, D-T: 0.82 ± 1.85, F-T 11.55 ± 4.57	n/a	98% moderate to severe periodontitis	n/a
Gaeckle et al. (20)	Number of teeth: 16.5 (8.5–23.5)	n/a	PI: 2.2 (1.5–2.8)	n/a	n/a
Grillo et al. (23)	n/a	DMFS: 13.6 ± 4.7, dmfs: 8.5 ± 2.3	n/a	PPD: 2.4 ± 0.5, BOP: 0.9 ± 0.2	n/a
Tamsas et al. (24)	n/a	dmfs: 5.1 ± 8.5, DMFS: 15.2 ± 11.8	n/a	BOP: 87%, PPD mean 2.7 ± 1.3	Comprehensive information on oropharyngeal morphology reported
Brasil-Oliveira et al. (25)	M-T: 7.9 ± 7.2,	D-T: 1.4 ± 2.0, F-T: 4.2 ± 3.7, DMF-T: 13.5 ± 6.5	n/a	Periodontitis: 92.5%	Reduced salivary flow: 80%

*M-T, missing teeth; D-T, decayed teeth; F-T, filled teeth; DMF-T, decayed-, missing-, and filled teeth index; PI, plaque index; GI, gingival index; PPD, periodontal probing depth; n/a, not applicable; *inclusion criterion: at least 6 remaining teeth.*

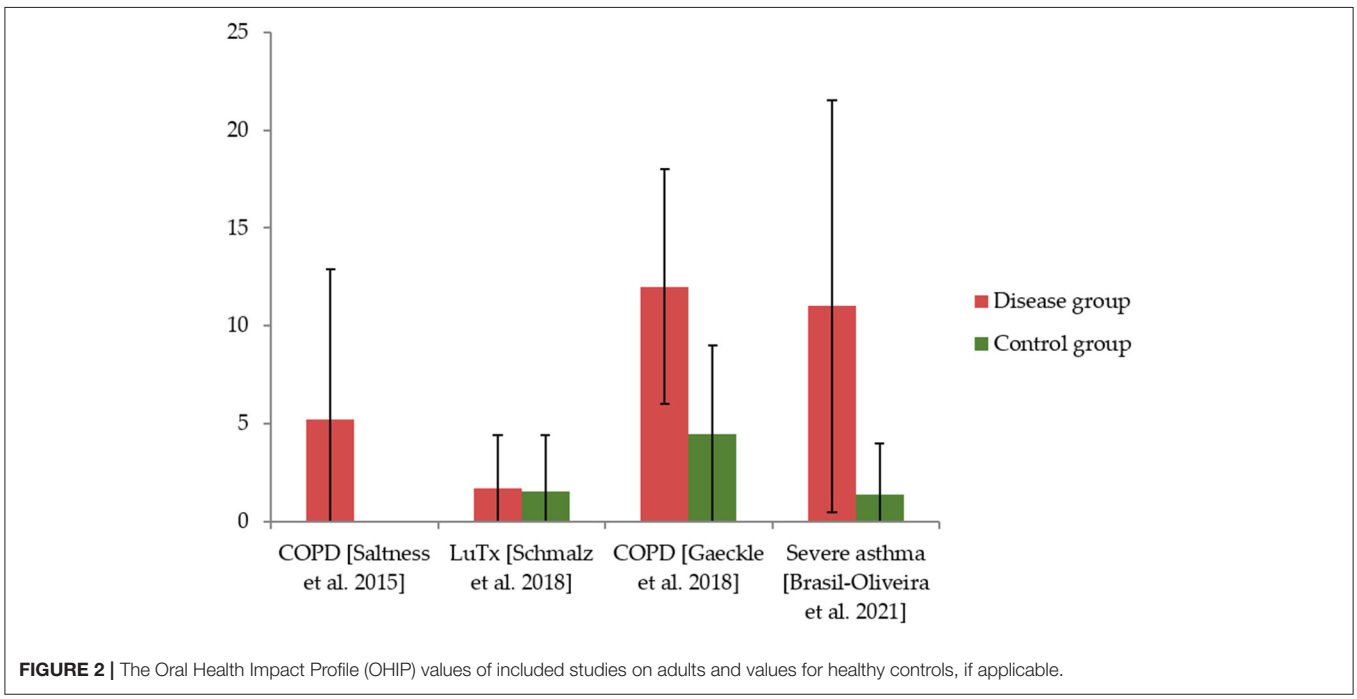

FIGURE 2 | The Oral Health Impact Profile (OHIP) values of included studies on adults and values for healthy controls, if applicable.

affecting OHRQoL of patients. A special position within this study is obstructive sleep apnea or sleep-disordered breathing, respectively. Although this is not an exclusive respiratory disease, it has been included in this study because it is a chronic condition affecting the respiratory system and is of high relevance for the dentist because oral health issues are common in these individuals (12). These sleep disorders lead to complex suffering of the patients, with an impairment of quality of life in both children and adults (12, 32). Furthermore, oral appliances for therapy or wearing an oxygen mask overnight could affect

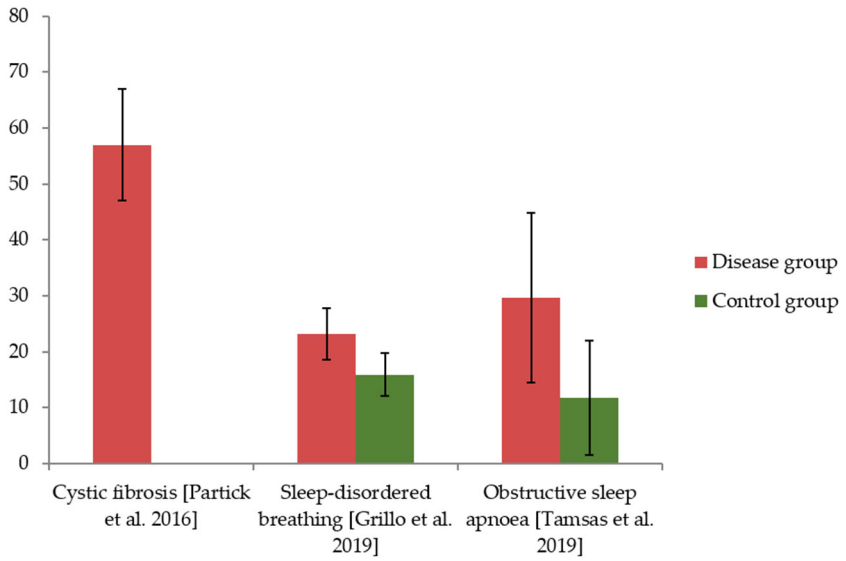

FIGURE 3 | The Child OHIP (COHIP) values for children in included studies and of healthy controls, if applicable.

TABLE 4 | Applied assessments for OHRQoL and relevant results for the included studies.

References	Assessment of OHRQoL	OHRQoL worse than healthy control (HC)	Association/correlation between OHRQoL and general HRQoL	Association/correlation between OHRQoL and oral health	Association and/or correlation between OHRQoL and disease-related parameters
Saltness et al. (19)	OHIP 14: 5.2 ± 7.7	n/a	Higher OHIP 14 related to worse MCS SF-36	Oral health problems related to poorer OHRQoL	n/a
Patrick et al. (21)	COHIP: A: 57.0 ± 10.0, B: 67.5 ± 14.2	n/a	n/a	n/a	Number of medications correlated with better COHIP
Schmalz et al. (22)	OHIP 14: 1.70 ± 2.70	No, OHIP 14: 1.54 ± 2.86	n/a	No associations detected	No
Gaeckle et al. (20)	OHIP 14: 12 (6–18.5)	Yes, OHIP 14: 4.5 (0–8)	n/a	n/a	No
Grillo et al. (23)	COHIP: 23.2 ± 4.6	Yes, COHIP: 15.9 ± 3.8	n/a	Malocclusion	Mallampati class and Obesity correlated with worse COHIP
Tamsas et al. (24)	COHIP: 29.7 ± 15.2	Yes, COHIP: 11.8 ± 10.2	n/a	n/a	n/a
Brasil-Oliveira et al. (25)	OHIP 14: 11.0 ± 10.5 (mild-to-moderate asthma: 6.2 ± 7.4)	Yes, OHIP 14: 1.4 ± 2.6	Higher OHIP 14 correlation with better PCS and MCS of SF-36	n/a	n/a

OHRQoL, oral health-related quality of life; n/a, not applicable; OHIP, Oral Health Impact Profile; COHIP, Child OHIP; PCS, physical compound summary; MCS, mental compound summary; SF-36, Short Form 36 Health Survey Questionnaire.

OHRQoL of respective individuals. Only one patient group, i.e., patients after lung transplantation, what rather represents a therapy than a respiratory disease, showed unaffected OHRQoL (22). It is known that patients after organ transplantation show a response shift, whereby oral health issues are pushed into the background because of the general disease burden (16, 33).

TABLE 5 | Subscales of OHRQoL in the included studies, if applicable.

	OHIP 14						
References, disease	**Functional limitation**	**Physical pain**	**Psycho-social discomfort**	**Physical disability**	**Psycho-logical disability**	**Social disability**	**Handicap**
Brasil-Oliveira et al. (25)	1.2 ± 2.0*	3.0 ± 2.4*	1.8 ± 2.5*	2.1 ± 2.3*	2.6 ± 2.3*	1.1 ± 0.8	0.6 ± 1.6
	OHIP 14						
References, disease	**Oral hgealth well-being**	**Functional well being**	**Social-emotional well-being**	**School environment**	**Self-image**	**Global health**	
Patrick et al. (21)**	A: 19.3 ± 4.3, B: 21.9 ± 6.4	A: 7.7 ± 1.4, B: 8.2 ± 3.2	A: 10.1 ± 3.1, B: 13.4 ± 5.2*	A: 4.5 ± 0.9, B: 4.9 ± 2.2	A: 12.3 ± 3.9, B: 14.8 ± 2.8*	A: 3.8 ± 1.6, B: 4.6 ± 1.4	
Tamasas et al. (24)	14.1 ± 5.5*	3.7 ± 3.0*	3.5 ± 5.6*	1.0 ± 1.7	7.3 ± 5.5*	n/a	

*The results are given as the mean values ± SD or otherwise as percentages. OHIP, Oral Health Impact Profile. *significant different from control. **Group A: 8–12 years (n = 17); group B: 13–17 years (n = 22).*

While this is the reason for the unaffected OHRQoL of patients after lung transplantation, the other groups did not show this response shift phenomenon. A reasonable explanation for that appears the anatomic proximity between the airways and the oral cavity. Moreover, the association between oral diseases and the included respiratory conditions and/or the respective medication (7–12) could explain the affected OHRQoL. Altogether, the reduced OHRQoL of patients with chronic respiratory diseases appears expedient, but complex. However, the reporting of oral health conditions and HRQoL in the included studies was inconsistent. However, the association between COPD (19) and severe asthma (25) with HRQoL supports the upper mentioned interpretation. The affected OHRQoL of patients with chronic respiratory diseases appears of clinical significance because appropriate management of oral conditions of these systemically ill patients will be needed to positively affect their quality of life. Therefore, sufficient multidisciplinary dental care concepts might be needed, as already demanded for other groups of at-risk patients (15, 16, 28). Until now, there is no specific approach available for a respective dental care concept. It is known that a simple allocation to the dentist does not significantly decrease the dental treatment need of patients with severe general diseases (34). Moreover, respective patients need to receive psychosocial support and sensibilization within a multidisciplinary care concept (16). Therefore, it appears of high importance to apply an individualized, prevention-oriented, and patient-centered dental care concept, focusing on the risk and needs of respective patients (35).

(II) The included studies applied two different methods for OHRQoL assessment, according to the age of included participants. For adult patients, the short form of the OHIP-14 was chosen, which is a validated questionnaire-based tool (36, 37). This questionnaire allows assessing the OHRQoL by 14 different questions, which can be answered on a five-point scale between 0 and 4, where higher values indicate worse OHRQoL. As a patient-reported outcome, the OHRQoL is part of the evidence-based dentistry, and applying the OHIP-14 allows conclusions on the impairment of the domains, such as oral function, psychosocial impact, oral pain, and orofacial appearance (14, 38). Although the OHIP-14 is a validated and commonly used instrument, it is not specific for generally diseased individuals, what potentially limits this method in that case (15, 16, 28). The other measurement, which was applied for children, was the COHIP. This instrument is also validated and was found to present reliable results by the assessment of 34 items and five conceptually distinct subscales: oral health, functional well-being, social/emotional well-being, school environment, and self-image (39). Similar as for the OHIP-14, higher values represent a worse OHRQoL. Thereby, the assessment of the OHRQoL of children is difficult because children often have difficulties to express their concerns in clinical environments and they are largely influenced by socioenvironmental factors of their family and caregivers (40, 41). Although the COHIP findings perceived by the caregivers are sometimes reported, this was not considered in this study because it was aimed to exclusively display the perspective of patient. Within the included studies, the COHIP values between children and parents did not differ in a clinically relevant manner, so it seems reasonable to omit this issue in this study (23, 24). Accordingly, the applied OHRQoL measurement appears reasonable in the included studies, but the major flaw appears the rarely applied investigation on the relationship between OHRQoL and HRQoL, disease-related parameters, and, particularly, oral health.

(III) Some recommendations can be provided for future study in the field. The comprehensive assessment of oral health parameters and their consideration as an influential factor in patients with respiratory diseases would be helpful to evaluate this issue. Additionally, assessment of HRQoL alongside with disease-specific parameters as well as mental problems or conditions would help to gain a deeper understanding. Thereby, the different dimensions/subcategories of OHRQoL measurement should be addressed. Multicenter, prospective study designs, especially considering any dental or medical intervention, would also bring benefit to the understanding of

OHRQoL and possibilities to its improvement in patients with respiratory diseases. Reference values and minimal important differences to interpret the clinical relevance of the data would also be of research interest in this study. Altogether, the body of evidence with respect to OHRQoL of patients with chronic respiratory diseases is quite weak, making more study in the field recommendable.

STRENGTHS AND LIMITATIONS

This is the first systematic study in this field. The methodology was sound, according to the PRISMA guidelines, and included a quality appraisal of the included studies. This quality appraisal revealed that the included studies were of moderate-to-high quality. The main points of criticism were missing blinding of examination and no consideration of any follow-up in most studies. Altogether, the included studies can be seen as of appropriate quality because the most relevant issues of quality appraisal were addressed by those investigations. The inclusion of such a heterogeneous combination of diseases and of children and adults together limits the comparability of findings. On one hand, the different diseases can cause different intra- and extraoral effects, potentially affecting OHRQoL of patients. For example, COPD and asthma are associated with periodontitis (7), while other included respiratory diseases are not associated with periodontitis. Periodontitis leads to reduced OHRQoL (42), what might be of relevance in the respective diseases, which are related to periodontal conditions. As this is just one potential example for the heterogeneity of the included diseases, the comparability of respective studies in this study is very limited. However, this study aimed to gain insight into the OHRQoL of patients with chronic respiratory diseases and not directly to compare the different diseases to each other. Similarly, the rationale for including children and adults together can be seen critically. Thereby, a direct comparison between the disease groups was not possible; but, due to the low number of studies on OHRQoL of the respective patient groups, it was decided to include studies on children, too. Of course, it is not possible to compare adults and children suffering from different respiratory diseases. To allow a comprehensive view on the OHRQoL of patients with chronic respiratory diseases, the inclusion appears reasonable, irrespective of the heterogeneity. Only seven studies were considered within this study, limiting the ability to draw meaningful conclusion. Moreover, the analysis was just qualitative. The low number of included studies is an important limitation, but is also an important result. The search terms were quite broad and a very comprehensive manual literature search was applied, checking the reference lists of all the included studies. Thereby, no additional findings could be detected. Therefore, this field of study appears understudied, yet, requiring an increased audience in the future. For this, this study provided several recommendations for future study in the field. More study will be necessary to gain insight into the OHRQoL of patients with respiratory diseases; this study can provide a basis for future study in the field.

CONCLUSION

Patients with chronic respiratory diseases show a reduced OHRQoL. This could be caused by a higher prevalence of oral diseases and underlying disease burden that need further clarification in future studies. An interdisciplinary dental care to support oral health and OHRQoL could be recommendable in individuals with chronic respiratory diseases.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

SL, WN, and WW conceptualized the research, conducted a systematic review, analyzed and interpreted the results, and wrote the manuscript. DZ and AA participated in data analysis and interpretation and revised the manuscript. GS, JZ, SH, and HX administrated and supervised the whole research project. All the authors have read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

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REVIEW
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Drug Therapies for COPD: A Bibliometric Review From 1980 to 2021

Gao Zhen[1], *Liu Yingying*[2]* *and Dong Jingcheng*[1]*

[1]Department of Integrated Traditional Chinese and Western Medicine, Huashan Hospital Affiliated to Fudan University, Shanghai, China, [2]Department of Retired Veteran Cadres, Putuo Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China

Objective: To analyze all chronic obstructive pulmonary disease (COPD) drugs-related articles that were indexed in the Web of Science Core Collection (WOSCC) database until August 28, 2021 using bibliometric analysis, in order to provide a reliable reference for the treatment of COPD.

Methods: A comprehensive search was conducted to analyze all COPD drugs-related articles using WOSCC database from inception to August 28, 2021. Abstracts and potentially eligible articles, which were retrieved during literature search, were screened by two reviewers. Besides, the CiteSpace (5.8.R1) software was utilized to analyze the overall structure of the network, the network clusters, the links between clusters, the key nodes or pivot points, and the pathways.

Results: A total of 2552 COPD-drugs related articles were retrieved. From the perspective of categorization of published articles based on country, the United States is the country with the largest number of published articles and completed clinical trials, highlighting the important role of this country in the treatment of COPD. However, in terms of the proportion of ongoing clinical trials, China has the highest proportion, suggesting that China will play a more pivotal role in the medication of COPD in the future. From the perspective of cooperation among countries, the cooperation among European countries was closer than that among Asian countries. In the recent three decades, the top 20 institutions, with a particular concentration on the treatment of COPD, were from North America and Europe. The co-citation analysis showed that, among 2,552 articles, 53154 citations were recorded, and the co-citation network indicated that 24 clusters could be achieved.

Conclusion: The administration of bronchodilators and pulmonary drug delivery systems, as well as consideration of elderly COPD patients remained the hotspots, while triple therapy and comorbidity of COPD, as well as the prevention and treatment of elderly COPD patients had been frontiers in recent years.

Keywords: COPD, medications, bibliometric analysis, pulmonary drug delivery systems, elderly

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Kin-Fai Ho,
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Reviewed by:
Luca Gallelli,
Magna Græcia University, Italy
Yahong Chen,
Peking University Third Hospital, China

***Correspondence:**
Liu Yingying
liuyy312@sina.com
Dong Jingcheng
jcdong2004@126.com

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1 INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a common, preventable and treatable disease, characterized by persistent airflow limitation that is mainly progressive and is associated with an enhanced inflammatory response in the airways and lung to noxious particles or gases (GOLD, 2021). The pathology of COPD encompasses a variety of structural alterations, involving airways, lung parenchyma, and pulmonary vasculature (Decramer et al., 2012). An epidemiological survey showed that the prevalence of COPD among Chinese adults and smokers who aged >40 and >60 years old was 13.7% and 40%, respectively (Wang et al., 2018). A survey performed in South Korea revealed that the prevalence of COPD among adult non-smokers who aged ≥40 years old was 6.67% (Kim et al., 2014). COPD has gradually become a global public health crisis (Patel et al., 2019). The World Health Organization (WHO) announced that the prevalence of COPD will continue to increase in the upcoming 40 years, exceeding 5.4 million COPD and other related diseases patients who will annually die by 2060 (Chronic Obstructive Pulmonary Disease Group of Chinese, 2021). COPD generates substantial costs for the health system, mainly related to moderate to severe stages and the exacerbations and complications entailed, (Gutiérrez Villegas et al., 2021) and the biggest driver of these healthcare costs is hospitalization (Khakban et al., 2017). Thus, how to prevent or delay the progression of COPD and reduce the frequency of acute exacerbations not only improves patients' quality of life and reduces mortality, but also saves medical costs. At present, traditional Chinese medicine, such as Tai Chi and Qigong, may be significant for the treatment of COPD patients in terms of enhancing lung function, relieving dyspnea, and improving patients' quality of life (Ding et al., 2014). However, the most important treatment for chronic obstructive pulmonary disease is still to give patients the correct drug treatment through various channels. The current treatment of COPD is mainly based on different combinations of bronchodilators and inhaled corticosteroids, and the amount of drug have increased during the exacerbation of COPD. However, the therapeutic effect is sometimes not ideal, and the long-term use of some drugs will produce side effects with different manifestations. In recent years, with the gradual deepening of the etiology, pathogenesis and clinical research of COPD, the development of new drugs for the treatment of COPD has become possible. Bibliometrics is a statistical method which could quantitative analysis the research papers concerned about one special topic via mathematical ways (Chen et al., 2014). It could also access the quality of the studies, analysis the key areas of researches and predict the direction of future studies (Yu et al., 2020).

In this study, we employed bibliometric analysis to analyze all COPD drugs-related articles that were indexed in the Web of Science Core Collection (WOSCC) database until August 28, 2021, in order to provide a reliable reference for the treatment of COPD.

2 METHODS

2.1 Search Strategy

A comprehensive search was conducted to analyze all COPD drugs-related articles using WOSCC database from inception to August 28, 2021. Abstracts and potentially eligible articles, which were retrieved during literature search, were screened by two reviewers. Any discrepancies between reviewers in the study selection were resolved via consultation with a third reviewer. Those articles that entitled the terms "COPD" or "chronic obstructive pulmonary disease" or "chronic obstructive pulmonary diseases" were included in our search. The search indices included Science Citation Index Expanded (SCI-Expanded), the Conference Proceedings Citation Index–Science (CPCI-S), and Current Chemical Reactions Expanded (CCR-Expanded). WOS Core Collection (formerly Institute for Scientific Information Web of Knowledge) is the most used and authoritative research literature search engine, providing comprehensive coverage of key research outputs from around the world. It is a multidisciplinary database with more than 100 subjects, including the major sciences, arts, humanities, and social sciences (e.g., political science, architecture, and philosophy) (Shao et al., 2021).

2.2 Data Extraction and Quality Assessment

Data extraction and quality evaluation were performed independently. After searching in the WOSCC database, the number of publications and the total and average citations for the authors and journals were recorded. For authors actively publishing on COPD-related drugs, the following indicators were measured: the h-index, which is the number of publications and the number of times the publication is cited; the R-index, which is the square-root of the total citation frequency in the h-core, defined by the h-index; the $h^{(2)}$-index, or the number of publications $h^{(2)}$ that are cited at least $[h^{(2)}]^2$ times; and the i10-index, or number of publications cited at least 10 times. The higher the values of these bibliometric indicators, the greater the influence of the authors and their publications. Besides, the CiteSpace (Chen, 2006) (5.8.R1) software and VOSviewer (van Eck and Waltman, 2010) (1.6.17) was utilized to analyze the overall structure of the network, the network clusters, the links between clusters, the key nodes or pivot points, and the pathways. A node in the map represented the type of study being analyzed, and links between the nodes represented relationships or collaborations, co-occurrence, or co-citations. For literature analysis, the time slice was 1 year, and the correlation strength was cosine. The threshold for each time slice selected Top N = 50.

2.3 Number of Clinical Trials

Using the name of the country as the search term, the COPD clinical trials carried out in various countries since the establishment of the database were searched in the American clinical trials database (https://clinicaltrials.gov).

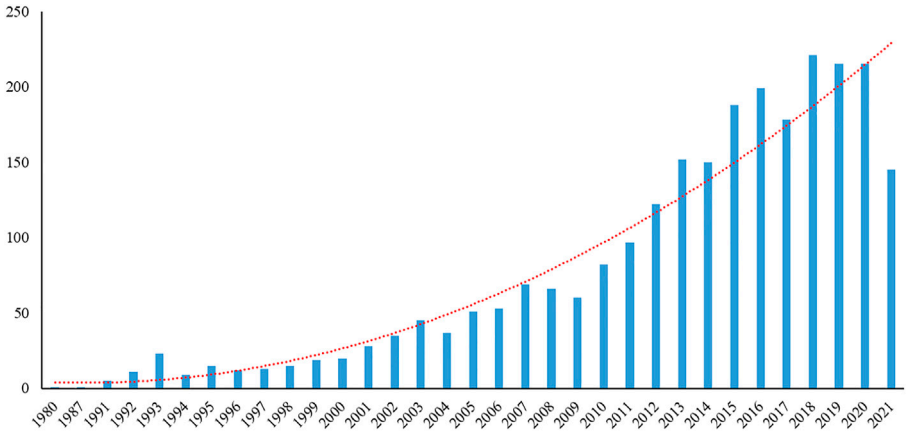

FIGURE 1 | The statistics of COPD drugs-related articles from 1980 to 2021.

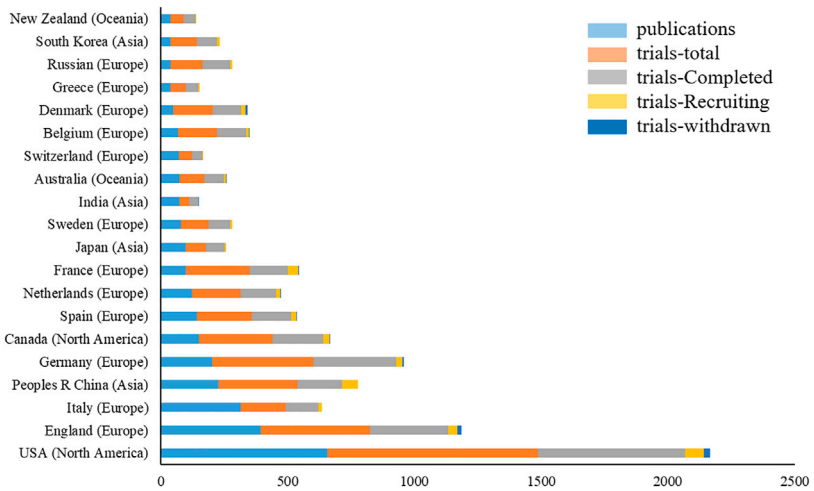

FIGURE 2 | Top 20 countries that published COPD drugs-related studies and the relevant clinical trials from 1980 to 2021.

3 RESULTS

3.1 General Data

From 1980 to August 28, 2021, 2552 articles were published. From 1980 to 2004, the number of published articles was not noticeable, with an average of (18.06 ± 12.82) articles per year, and it rapidly increased in 2012. From 2012 to 2020, the average number of published articles was (182.22 ± 34.58), accounting for 66.76% of the total publications. The majority of articles were published in 2018 (n = 221) (**Figure 1**).

3.2 Categorization of Published Articles Based on Country, Region, and Institution

Authors were from 83 countries. Among the top 20 countries, including 12 from Europe, 4 from Asia, 2 from North America, and 2 from Oceania. In terms of proportion of each continent, 53.99%, 24.05%, and 15.83% of COPD drugs-related studies were published in Europe, North America, and Asia, respectively (**Figure 2**). The United States (n = 657, centrality = 0.27) and the UK (centrality = 0.41, n = 396) accounted for the highest number of articles published. The greatest number of completed clinical trials was recorded in the USA (n = 582), followed by Germany (n = 327), and the UK (n = 308). China (20.13%) has the highest proportion of ongoing clinical trials, followed by France (16.27%), and Greece (13.56%). Compared with Asian countries, a closer cooperation was found among European countries (**Figure 3**). Among the top 20 research institutions, 13 were located in Europe and 7 in North America. The majority of published articles were from Tor Vergata University of Rome (Italy). Of the top 20 pharmacological companies, GlaxoSmithKline, AstraZeneca, and Boehringer Ingelheim had the highest rates of contribution in the research projects (**Figure 4**). In terms of the annual number of articles published, among the top 6 countries, the USA ranked the first, and China was the country with a steady increase in the number of published articles (**Figures 5**, **6**). A sudden increase in publications

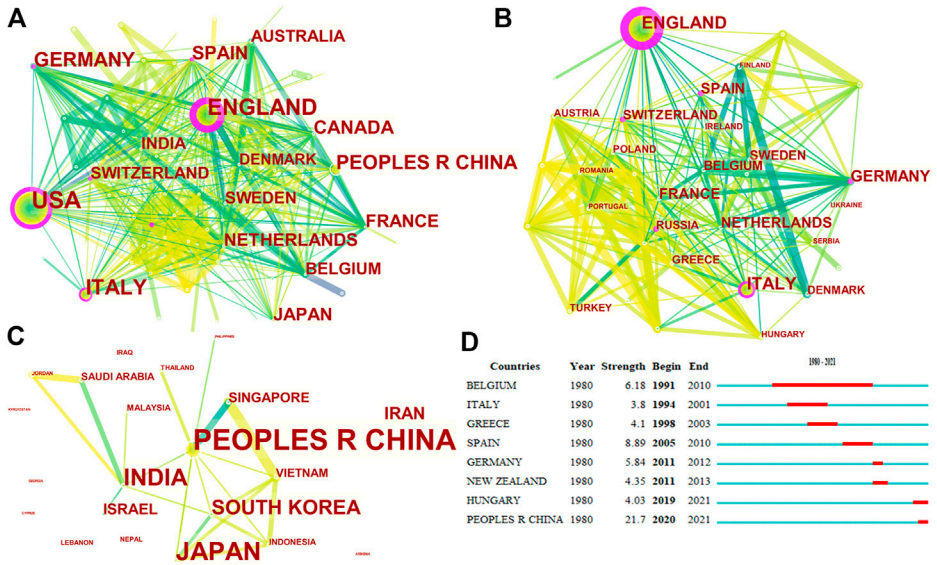

FIGURE 3 | Cooperation among countries that published COPD drugs-related studies from 1980 to 2021. Note: **(A)**: countries around the world; **(B)**: European countries; **(C)**: Asian countries; **(D)**: top 8 countries with the strongest citation bursts.

was seen from Univ Roma Tor Vergate, Univ Toronto, Kings Coll London, Johns Hopkins Univ, Univ Manchester, Univ Groningen between 2015 and 2021, Imperial Coll London, Karolinska Inst, Univ Tennessee between 2016 and 2021, and Astrazeneca, Univ Campania Luigi Vanvitelli, German Ctr Lung Res DZL between 2020 and 2021 (**Table.1**).

3.3 Distribution of Fields of Study

The respiratory system (n = 1,004), Pharmacology and pharmacy (n = 604), and general medicine (n = 518) totally accounted for 46.54% of the fields of study. Critical care medicine, Cardiovascular system and cardiology, Biochemistry and molecular biology, Public, environmental and occupational health, Chemistry, medicinal, Immunology, Geriatrics and gerontology, Health policy and services and Toxicology were also included (**Figure 7**).

3.4 Authors' Collaborations

As displayed in **Figure 8**, the size of each circle indicates the number of articles produced by the author. The distance between any two circles demonstrates the relatedness of their co-

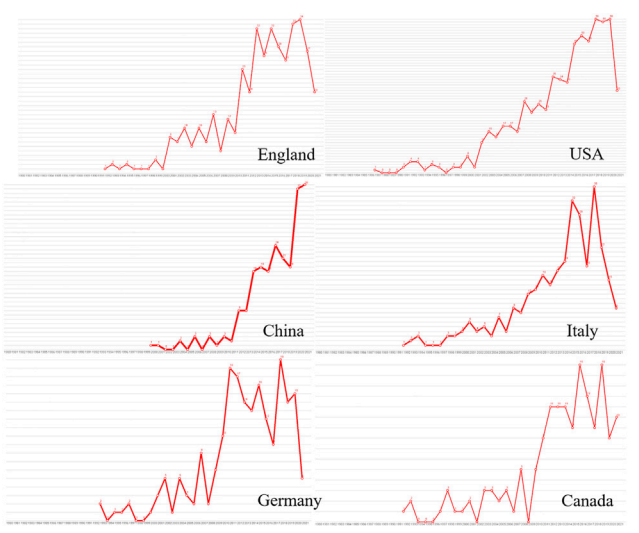

FIGURE 4 | Annual number of COPD drugs-related studies in each country from 1980 to 2021.

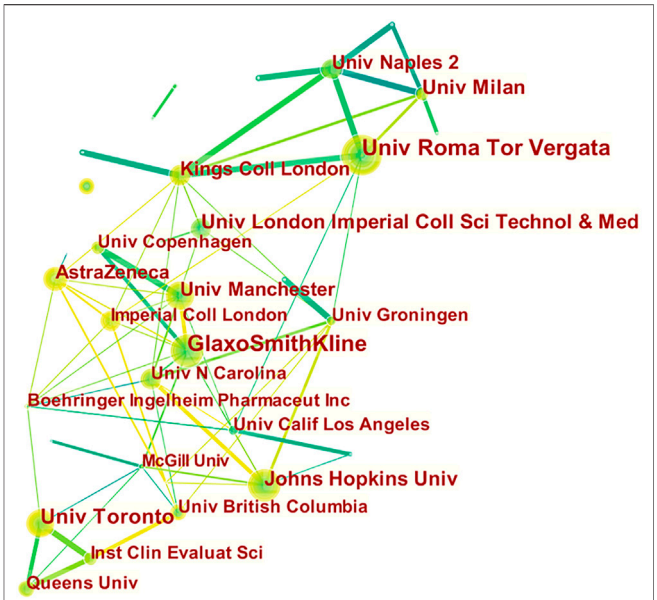

FIGURE 5 | Cooperation among institutions that published COPD drugs-related studies from 1980 to 2021.

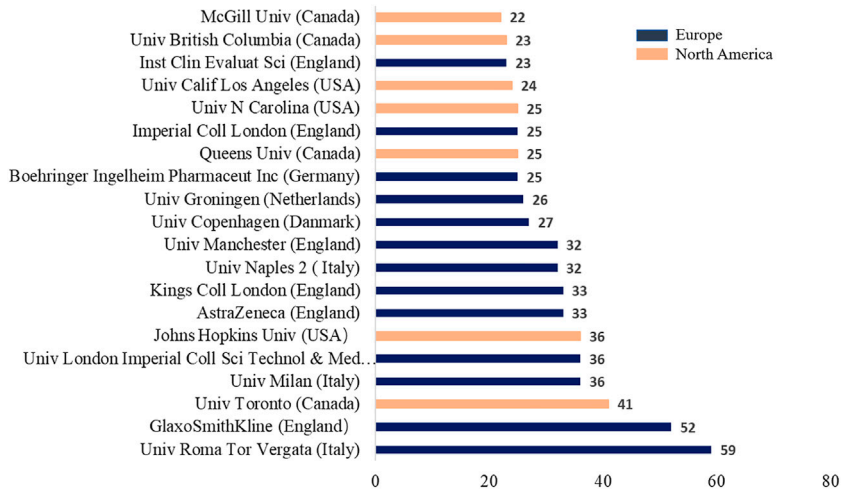

FIGURE 6 | Top 20 institutions that cooperated in publishing COPD drugs-related studies from 1980 to 2021.

authorship link, and the thickness of the connecting line indicates the strength of the link. We found that authors who published a large number of papers generally had fixed partners, and they accordingly created their own research team.

3.5 Citations

3.5.1 Author Co-Citation Analysis

Among the top 10 cited authors (**Table 2**), Barnes PJ and Celli BR had the highest participation in finalizing global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017–2019 Report (GOLD).

TABLE 1 | Top 27 institutions with the strongest citation bursts that published COPD drugs-related studies from 1980 to 2021.

Begin	End	Strength	Year	Entity
1998	2006	5.3941	1980	A Cardarelli Hosp
2005	2010	4.1669	1980	Univ Calif Los Angeles
2011	2013	4.1035	1980	Univ Auckland
2013	2015	5.8454	1980	Keio Univ
2015	2021	9.4482	1980	Univ Roma Tor Vergata
2015	2019	9.4252	1980	GlaxoSmithKline
2015	2021	9.1131	1980	Univ Toronto
2015	2018	7.3131	1980	Inst Clin Evaluat Sci
2015	2021	6.2404	1980	Kings Coll London
2015	2021	6.1765	1980	Johns Hopkins Univ
2015	2018	5.882	1980	St Michaels Hosp
2015	2021	5.7948	1980	Univ Manchester
2015	2016	5.6786	1980	Univ Naples 2
2015	2019	5.5957	1980	Univ N Carolina
2015	2021	5.2239	1980	Univ Groningen
2015	2016	4.0515	1980	Univ Ferrara
2015	2019	4.0359	1980	Univ Southern Denmark
2016	2021	9.4024	1980	Imperial Coll London
2016	2019	6.5962	1980	Queens Univ
2016	2017	5.3541	1980	GSK
2016	2019	4.4053	1980	Univ Alabama Birmingham
2016	2021	4.0044	1980	Karolinska Inst
2016	2021	3.8956	1980	Univ Tennessee
2017	2021	10.4528	1980	AstraZeneca
2017	2021	9.1418	1980	Univ Campania Luigi Vanvitelli
2017	2019	7.5462	1980	Harvard Med Sch
2017	2021	3.8611	1980	German Ctr Lung Res DZL

3.5.2 Journal Co-Citation Analysis

The top 10 cited journals and article published journals are listed in **Table 3**. The top 3 cited journals were European Respiratory Journal, American Journal of Respiratory and Critical Care Medicine, and Chest. The top 3 journals were International Journal Of Chronic Obstructive Pulmonary Disease, Respiratory Medicine and European Respiratory Journal. The top 10 and 3 cited journals accounted for 42.07% and 17.72% of the total number of COPD-related journals, The top 10 and 3 journals accounted for 25.45% and 12.45% of the total number of COPD-related journals, respectively, indicating that the top journals in the field of respiratory diseases were further concentrated on COPD-related medication.

3.5.3 Co-Citation Analysis

The co-citation analysis showed that, among 2552 articles, 53154 citations were recorded, and the co-citation network is shown in **Figure 9**. It can be seen that 24 clusters could be achieved, with a Q-value and a silhouette value of 0.902 and 0.954, respectively. The size of the circle represents the size of the surge index. The clusters in the co-citation network are presented in **Table 4**, including tiotropium, glycopyrronium, salmeterol, neltenexine, and other drugs. The five most frequently cited articles and the most frequently cited researches published in 2021 are listed in **Table 5**. Research involves Tiotropium, QVA149 [indacaterol/glycopyrronium (Matera et al., 2015)], single-inhaler combination of an extra fine formulation of beclometasone dipropionate, formoterol fumarate, and glycopyrronium bromide and triple therapy (corticosteroid, fluticasone furoate, and vilanterol). The most frequently cited article in 2021 is a real-world clinical evidence.

3.6 Analysis of Keywords

When the keywords with the same meaning were combined, the frequency of the published keywords was statistically analyzed,

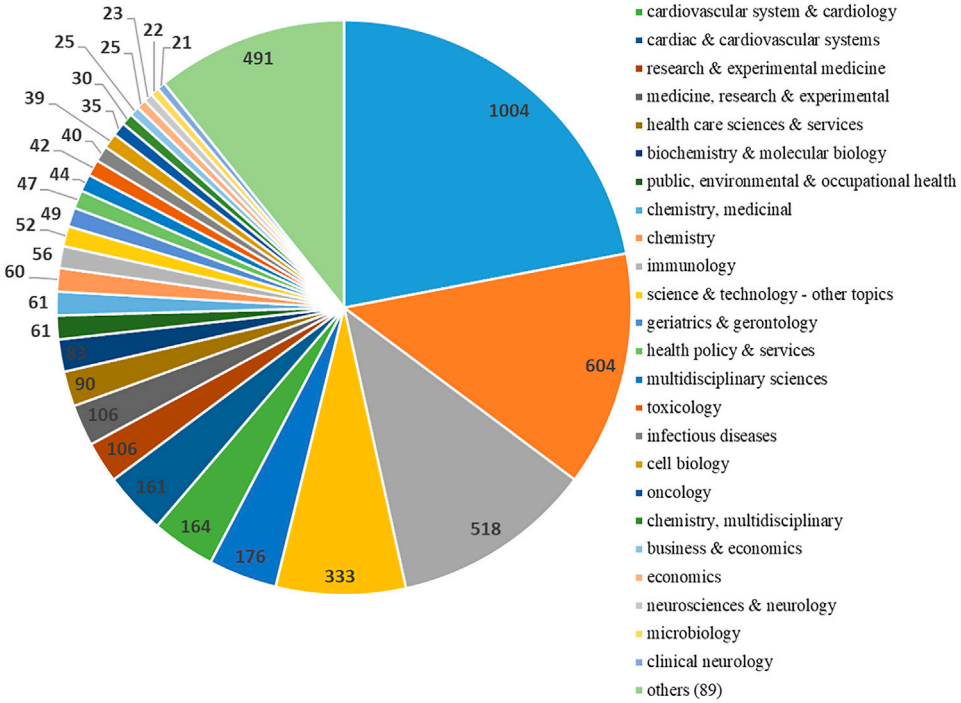

FIGURE 7 | Research areas of COPD drugs-related studies that were published from 1980 to 2021.

and the top 20 keywords are summarized in **Table 6**. A total of 26 items could be achieved by cluster analysis (**Table 7**), including interventions, pathogenesis of the disease, complications, etc. Interventions for COPD included exercise, Oxitropium bromide, antibiotic therapy, theophylline, ipratropium. The pathogenesis of the disease included metabolic pathways, hypoxia, recurrent airway obstruction, airway resistance, respiratory muscles, alveolar development and other factors. Heart failure was the major complication. Susceptible groups of people (e.g., elderly) and different risk factors (e.g., living in industrial areas) were recorded as well. In addition to the disease name, the most frequently cited keywords were related to exacerbation, tiotropium, mortality, management, therapy, double blind and efficacy, reflecting the importance of medications in the management of COPD, prevention of acute attacks, and reduction of mortality (**Figures 10**, **11**).

So-called "burst words" represent words that are cited frequently over a period of time, (Liang et al., 2017) Burst keywords show the frontier topics and key areas of research. This article focuses on the keywords with the strongest citation bursts that continue to 2021, keywords suddenly increased: "Safety" suddenly increase between 2014 and 2021. "risk," "oxidative stress," "risk factor" suddenly increase between 2015 and 2021. "Drug delivery" suddenly increase between 2016 and 2021. "impact," "device" suddenly increase between 2017 and 2021. "Prevalence," "triple therapy," "prevention," "parallel group," "health," "comorbidity" suddenly increase between 2018 and 2021. "adherence," "*in vitro*," "association," "resistance," "nf kappa b," "COPD exacerbation," "drug," "inhaler," "tuberculosis," "metered dose inhaler" suddenly increase between 2019 and 2021. "COPD," "depression," "burden," "COVID-19," "intervention," "diagnosis," "older adult" suddenly increase between 2020 and 2021 (**Table 8**).

4 DISCUSSION

In recent years, significant progress has been made in the development of new pharmacological and surgical tools to treat COPD, while the rates of prevalence and mortality owing to COPD are still noticeable. Through the visual bibliometric analysis by the CiteSpace software, we could understand the progression of research on COPD drugs, enabling us to more accurately predict the study direction in the future. The number of publications can reflect the overall development trend. The number of COPD drugs-related articles has been elevated from 1980 to 2021, especially an explosive growth was recorded in 2012, indicating that the treatment of COPD has markedly attracted clinicians' attention in the recent decades.

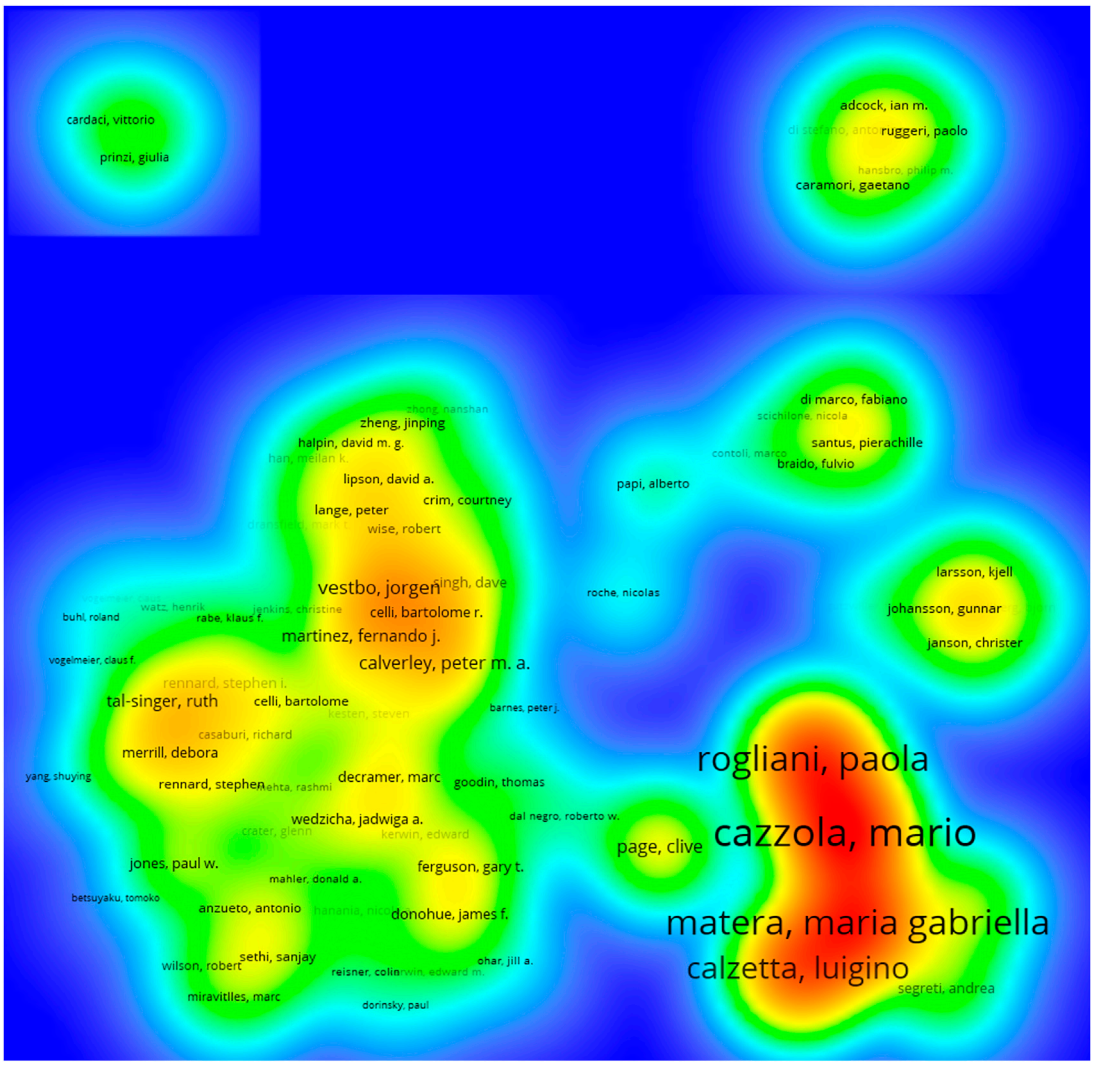

FIGURE 8 | Cooperation among authors that published COPD drugs-related studies from 1980 to 2021.

From the perspective of categorization of published articles based on country, the USA is the country with the largest number of published articles and completed clinical trials, highlighting the important role of this country in the treatment of COPD. However, in terms of the proportion of ongoing clinical trials, China has the highest proportion, suggesting that China will play

TABLE 2 | The top 10 authors with the highest citations of COPD drugs-related studies from 1980 to 2021.

No	Cited author	Frequency	Author	N
1	Barnes PJ	423	Cazzola M	53
2	Calverley PMA	392	Matera MG	38
3	Celli BR	372	Rogliani P	33
4	Tashkn DP	351	Calzetta L	28
5	Cazzola M	349	Cazzola M	20
6	Vestbo J	342	Vestbo J	16
7	Jones PW	269	Singh D	16
8	Rabe KF	258	Vozoris NT	15
9	Mahler DA	251	Miravitlles M	14
10	Wedzicha JA	244	Donohue JF	14

TABLE 3 | The list of top 10 cited journals that published COPD drugs-related studies from 1980 to 2021.

Cited-journal	N	Journal	N
Eur Respir J	1,619	Int J Chronic Obstr	111
Am J Resp Crit Care	1,582	Resp Med	108
CHEST	1,562	Eur Respir J	99
Thorax	1,322	CHEST	83
New Engl J Med	1,193	COPD	56
Resp Med	1,103	Pulm Pharmacol Ther	56
Lancet	1,076	Am J Resp Crit Care	38
Resp Res	673	Resp Res	36
Int J Chronic Obstr	621	Cochrane Db Syst Rev	32
COPD	559	Thorax	31

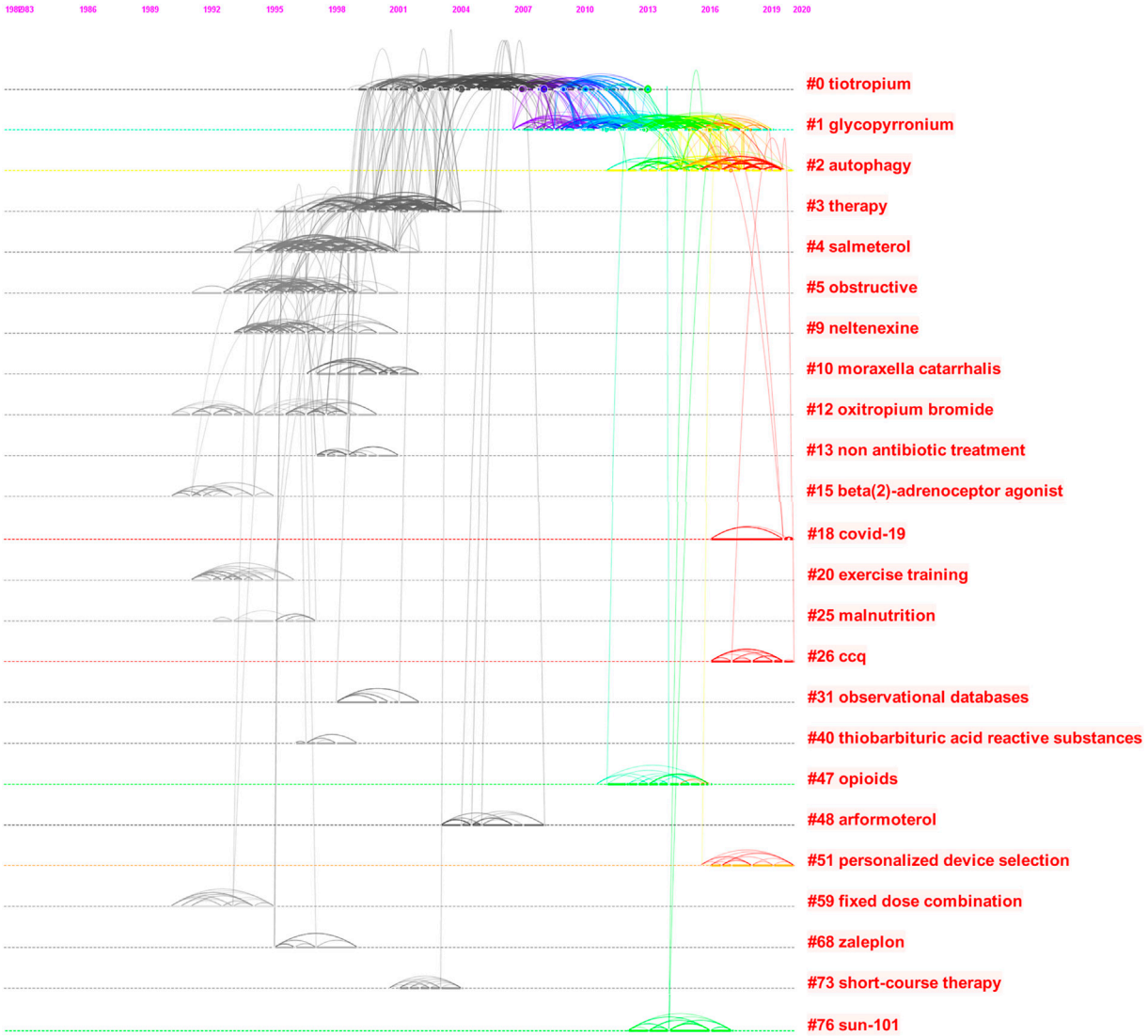

FIGURE 9 | Co-citation analysis of COPD drugs-related studies that were published from 1980 to 2021.

a more pivotal role in the medication of COPD in the future. A sudden increase in publications was seen from Hungary between 2019 and 2021, and China between 2020 and 2021 also proved this. From the perspective of cooperation among countries, the cooperation among European countries was closer than that among Asian countries. In the recent three decades, the top 20 institutions, with a particular concentration on the treatment of COPD, were from North America (n = 7) and Europe (n = 13). The identification of core journals with high publication and co-citation counts provide important information for authors to select high-quality journals (Wang et al., 2020). The present study revealed that a variety of journals have published COPD-drugs related articles. High cited journals were relatively concentrated, and the top 10 journals accounted for 42.07% of the total number of journals related to the medication of COPD, suggesting that the importance of COPD was acknowledged by these journals.

The co-citation analysis showed that, among 2552 articles, 53154 citations were recorded, and the co-citation network indicated that 24 clusters could be achieved, with a Q-value and a silhouette value of 0.902 and 0.954, respectively. Bronchodilators play a pivotal role in the treatment of symptomatic patients with COPD. Inhaled short-acting bronchodilators are currently recommended for relieving symptoms of patients with mild COPD, whereas inhaled long-acting bronchodilators are recommended as first-line agents for maintenance therapy of patients with moderate and severe COPD (Steiropoulos et al., 2012). Tiotropium resulted in a higher FEV1 than placebo at 24 months and ameliorated the annual decline in the FEV1 after bronchodilator use in patients with COPD of GOLD stage 1 or 2 (Zhou et al., 2017). This also reveals the significant role of drug delivery systems, for example, SUN-101 is a combination of glycopyrrolate delivered through an innovative,

TABLE 4 | Co-citation clustering of COPD drugs-related studies that were published from 1980 to 2021.

Cluster	Size	Silhouette	Mean (Year)	Label (LLR)
1	278	0.947	2005	Tiotropium
2	252	0.934	2013	Glycopyrronium
3	173	0.915	2016	Autophagy
4	165	0.945	2000	Therapy
5	138	0.967	1997	Salmeterol
6	126	0.931	1996	Obstructive
7	65	0.988	1996	Neltenexine
8	45	0.995	2000	*Moraxella catarrhalis*
9	40	0.985	1995	Oxitropium bromide
10	34	0.997	1998	Non antibiotic treatment
11	30	1	1991	Beta (2)-adrenoceptor agonist
12	28	1	2019	COVID-19
13	27	0.999	1992	Exercise training
14	22	1	1994	Malnutrition
15	21	1	2018	CCQ
16	20	0.996	2000	Observational databases
17	15	1	1997	Thiobarbituric acid reactive substances
18	14	0.998	2014	Opioids
19	13	0.997	2005	Arformoterol
20	11	1	2017	Personalized device selection
21	10	0.998	1996	Fixed dose combination
22	8	1	1996	Zaleplon
23	7	1	2002	Short-course therapy
24	1	1	2014	Sun-101

TABLE 5 | The top 5 cited COPD drugs-related studies that were published from 1980 to 2021 and the most cited papers in 2021.

No	Author	Cited frequency	Drug	Condition or disease	Conclusion
1	(Vogelmeier et al., 2011)	455	Tiotropium	Moderate-to-very-severe COPD	In patients with moderate-to-very-severe COPD, tiotropium is more effective than salmeterol in preventing exacerbations
2	(Wedzicha et al., 2013)	364	QVA149	COPD stages III-IV, and one or more moderate COPD exacerbation in the past year	The dual bronchodilator QVA149 was superior in preventing moderate to severe COPD exacerbations compared with glycopyrronium, with concomitant improvements in lung function and health status
3	(Vogelmeier et al., 2013)	238	QVA149	COPD stages II-III, without exacerbations in the previous year	Once-daily QVA149 provides significant, sustained, and clinically meaningful improvements in lung function versus twice-daily salmeterol-fluticasone, with significant symptomatic benefit
4	(Vestbo et al., 2016)	231	Corticosteroid, fluticasone furoate, and vilanterol	Moderate COPD and heightened cardiovascular risk	In patients with moderate COPD and heightened cardiovascular risk, treatment with fluticasone furoate and vilanterol did not affect mortality or cardiovascular outcomes, reduced exacerbations, and was well tolerated
5	(Singh et al., 2016)	216	Single-inhaler combination of an extra fine formulation of beclometasone dipropionate, formoterol fumarate, and glycopyrronium bromide (BDP/FF/GB)	COPD had post-bronchodilator FEV1 of lower than 50%, one or more moderate-to-severe COPD exacerbation in the previous 12 months, CAT ≥10, and a Baseline Dyspnea Index focal score of 10 or less	This paper provide evidence for the clinical benefits of stepping up patients with COPD from an inhaled corticosteroid/long-acting β2-agonist combination treatment to triple therapy using a single inhaler
6	(Izquierdo et al., 2021)	13	Clinical Management of COPD in a Real-World Setting	COPD	This study identifies the main features of an unselected COPD population and the major errors made in the management of the disease

TABLE 6 | The top 20 keywords of COPD drugs-related studies that were published from 1980 to 2021.

Rank	Frequency	Centrality	Key words
1	1,312	0.04	COPD
2	717	0.03	Obstructive pulmonary disease
3	286	0.02	Exacerbation
4	244	0.03	Tiotropium
5	206	0.06	Mortality
6	192	0.05	Management
7	176	0.03	Therapy
8	173	0.05	Double blind
9	170	0.12	Efficacy
10	169	0.01	Lung function
11	155	0.02	Inflammation
12	147	0.02	Risk
13	143	0.01	Salmeterol
14	136	0.01	Safety
15	131	0.11	Disease
16	131	0.05	Prevalence
17	115	0.03	Bronchodilator
18	105	0.03	Quality of life
19	102	0.04	Chronic bronchiti
20	101	0.03	Acute exacerbation

electronic nebulizer (Kerwin et al., 2017). Meanwhile, fixed-dose combination and personalized medicine are also important topics. One study (Hu et al., 2020) showed that acute exacerbation and hospitalization of COPD patients were infrequent during the coronavirus disease 2019 (COVID-19) pandemic. However, COVID-19 patients with pre-existing COPD had a higher risk of all-cause mortality.

Based on the keyword analysis of COPD-drugs related articles, we summarize the following four keywords: 1) Management: the target of COPD management is to improve a patient's functional status and quality of life by preserving optimal lung function, improving symptoms, and preventing the recurrence of exacerbations. 2) Anticholinergics, such as ipratropium bromide and tiotropium bromide, are the most effective group of bronchodilators in the treatment of COPD. Tiotropium bromide was the first long-acting muscarinic antagonist (LAMA) available for COPD in clinical practice. There are two pulmonary drug delivery modes: delivery of inhalation powder via a dry powder inhaler (DPI) and drug delivery via a soft mist inhaler (SMI). Tiotropium was found comparable to inhaled corticosteroid (ICS)/long-acting β2-agonist (LABA) in improving lung function and reducing exacerbations and had a greater influence on exacerbation rates than LABAs. Hence, fixed-dose LAMA/LABA combinations have also been developed. Studies showed that tiotropium and olodaterol dual bronchodilator therapy may improve lung function and quality of life and reduce exacerbations in patients with COPD in early stages (Criner and Duffy, 2021). The co-formulation of indacaterol and glycopyrronium can be usefully utilized to optimize and maximize bronchodilation in many COPD patients, who do not experience an adequate airflow increase by using a single bronchodilator (Pelaia et al., 2014). Oxitropium bromide can improve the exercise capacity of patients with stable COPD (Ikeda et al., 1994). Our results showed that, tiotropium has not only attracted clinicians' attention in the treatment of COPD previously, but also it plays a significant role in the development of further effective therapies for COPD. 3) Pulmonary drug delivery is a compelling noninvasive technique to deliver systemic drugs into circulation. Nowadays, most inhaled drugs are delivered by pressurized metered dose inhaler, (Newman and Dhand, 2015) dry powder inhaler (Hickey and Dhand, 2015) or nebulizer (Fink et al., 2015). The advantage of COPD pulmonary drug delivery is to use a relatively low dose, a low incidence of systemic side effects and a rapid onset of action. And pulmonary drug delivery is a very complicated process. First, the respiratory tract has evolved defense mechanisms that are intended to keep inhaled materials out of the lungs, as well as removing or inactivating them once they have been deposited (Labiris and Dolovich, 2003). Second, it is necessary for a patient to use an inhaler device, and to use it correctly (Newman, 2014). Handling errors of inhaler devices are underestimated in real life and are associated with an increased rate of severe COPD exacerbation (Molimard et al., 2017). Meeting the challenge of delivering drugs to the lungs requires selection of an appropriate inhaler and formulation (Newman, 2017). A review article on tiotropium/olodaterol in the treatment of COPD showed that once-daily delivery of fixed-dose combinations of tiotropium and olodaterol via a very efficient and simple-to-use inhaler device such as Respimat significantly contributes to enhance the therapeutic efficacy of dual bronchodilation, as well as to increase patient adherence to inhaled treatment (Pelaia et al., 2015). Therefore, it is highly essential to further concentrate on the development of COPD drugs in terms of components of drug, drug delivery systems, as well as training patients to take medications safely. 4) COPD in elderly patients: Khakban A predict, the total number of patients

TABLE 7 | Clustering of keywords in COPD drugs-related studies that were published from 1980 to 2021.

Cluster	Size	Silhouette	Mean (Year)	Label (LLR)
0	203	0.564	2002	COPD
1	94	0.824	1997	Exercise
2	85	0.902	2000	Emphysema
3	76	0.901	1998	Metabolism
4	71	0.921	1996	Hypoxia
5	62	0.939	1998	Recurrent airway obstruction
6	58	0.861	1996	Oxitropium bromide
7	58	0.879	2000	Airway resistance
8	58	0.938	1997	Disease
9	56	0.896	2000	Antibiotic therapy
10	55	0.866	2000	Theophylline
11	54	0.937	1998	Respiratory muscles
12	46	0.939	1999	Expression
13	43	0.934	1996	Pharmacokinetics
14	33	0.907	2000	Trial
15	31	0.962	2000	Heart failure
16	20	0.958	1997	Elderly
17	16	0.999	1999	Candidate gene
18	14	0.986	2000	Ipratropium
19	10	0.999	2002	Pulmonary drug delivery
21	10	0.992	2001	Alveolar development
22	9	0.998	2003	COPD
23	8	0.996	1998	Xylazine
26	7	1	1991	Screening test
30	4	0.995	2004	Logistic models
35	3	1	2000	Industrial area

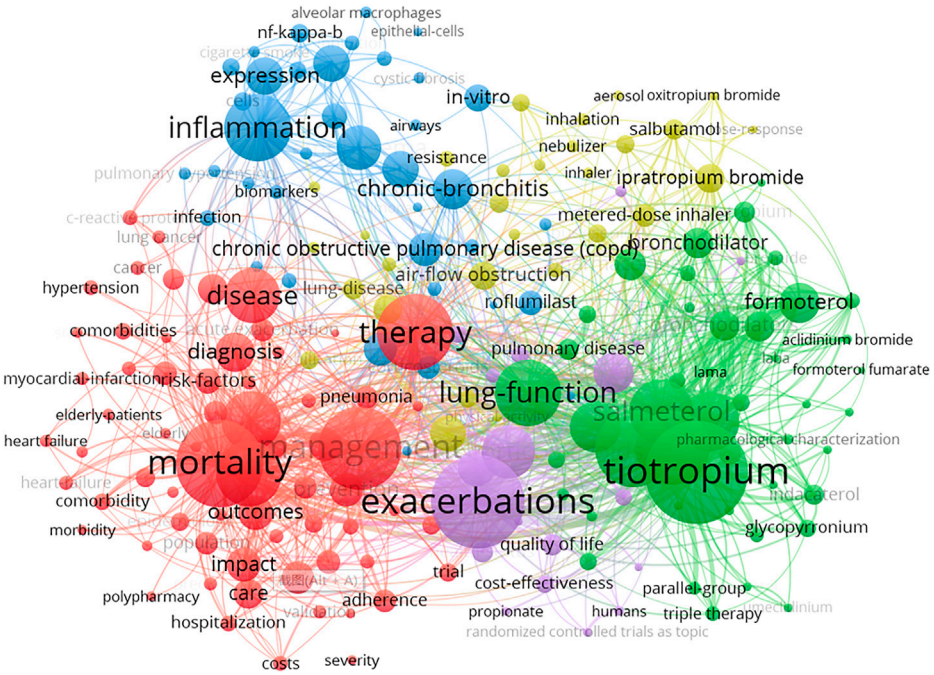

FIGURE 10 | Keywords analysis of COPD drugs-related studies that were published from 1980 to 2021.

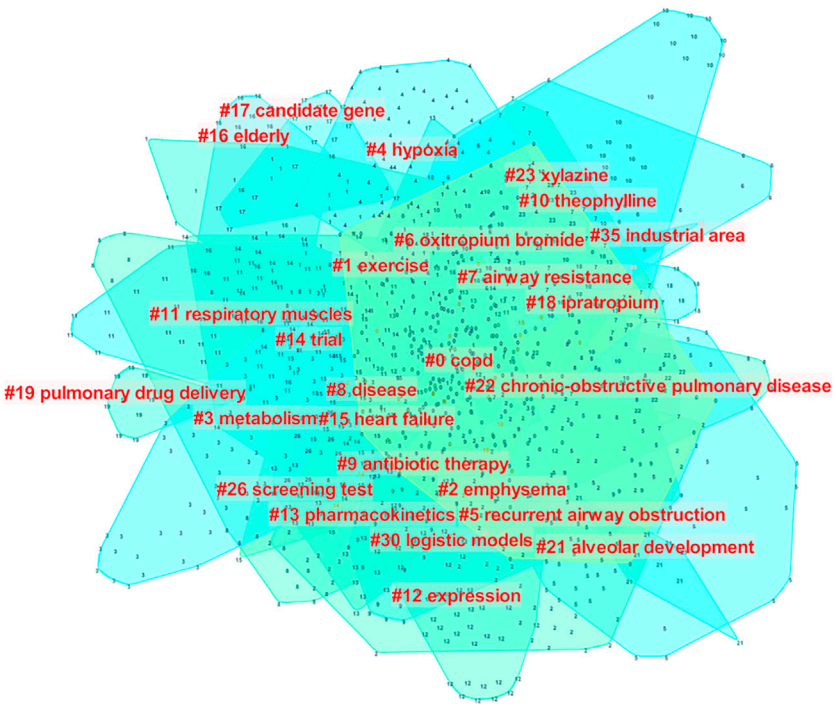

FIGURE 11 | Keywords co-occurrence analysis of COPD drugs-related studies that were published from 1980 to 2021.

TABLE 8 | Keywords with the strongest citation bursts of COPD drugs-related studies from 1980 to 2021.

Begin	End	Strength	Year	Entity
2014	2021	8.30	1980	Safety
2015	2021	12.16	1980	Risk
2015	2021	7.98	1980	Oxidative stress
2015	2021	5.36	1980	Risk factor
2016	2021	10.72	1980	Drug delivery
2017	2021	10.65	1980	Impact
2017	2021	5.31	1980	Device
2018	2021	14.09	1980	Prevalence
2018	2021	10.64	1980	Triple therapy
2018	2021	9.60	1980	Prevention
2018	2021	8.40	1980	parallel group
2018	2021	8.08	1980	Health
2018	2021	6.50	1980	Comorbidity
2019	2021	8.98	1980	Adherence
2019	2021	8.58	1980	*In vitro*
2019	2021	6.96	1980	Association
2019	2021	6.55	1980	Resistance
2019	2021	5.92	1980	nf kappa b
2019	2021	5.12	1980	Copd exacerbation
2019	2021	4.92	1980	Drug
2019	2021	4.82	1980	Inhaler
2019	2021	4.18	1980	Tuberculosis
2019	2021	4.01	1980	Metered dose inhaler
2020	2021	7.93	1980	COPD
2020	2021	6.18	1980	Depression
2020	2021	5.67	1980	Burden
2020	2021	5.38	1980	COVID-19
2020	2021	5.28	1980	Intervention
2020	2021	5.12	1980	Diagnosis
2020	2021	3.77	1980	Older adult

diagnosed with COPD will increase by 155%, and COPD-related hospitalization will increase by 210% in 2010–2030. By 2030, 55% of the patients with COPD will be 75 years and older. (Khakban et al., 2017) Frontier studies on the treatment of COPD should particularly involve elderly populations. COPD is a common disease among elderly patients, in which treatment of elderly patients with COPD is highly challenging, and randomized controlled trials may underestimate the risk of adverse effects of interventions. Although age is an important factor in the incidence of COPD, there is a lack of age-based research on COPD drugs. This may be one of the next efforts in COPD drug research.

Diagnosis and treatment of COPD are complicated because COPD may manifest as multiple phenotypes. Research showed that the phenotypical approach is crucial in the management of COPD as it allows to individualize the therapeutic strategy and to obtain more effective clinical outcomes (Dal Negro et al., 2021). According to the analysis of keywords, we summarize five research directions in the following. 1) Identification of the risk factors of COPD: exposure to cigarette smoke worsens lung edema and inflammation, (Hou et al., 2019) and smoking is the main risk factor for COPD (Mannino and Buist, 2007). However, there are still numerous unknown risk factors for COPD, which should be explored through epidemiological assessment. 2) Triple therapy: research showed that addition of fluticasone-salmeterol to tiotropium therapy did not statistically influence rates of COPD exacerbation but did improve lung function, quality of life, and hospitalization rates in patients with moderate to severe COPD (Aaron et al., 2007). Subsequent studies of this kind have gradually become the hotspot and frontier of COPD drug research. The route of administration should be mainly pulmonary inhalation, and attention should be paid to the development and utilization of drug delivery devices. Study showed that, once-daily fluticasone furoate/umeclidinium/vilanterol (FF/UMEC/VI) was non-inferior to twice-daily budesonide/formoterol via metered-dose inhaler plus once-daily tiotropium via HandiHaler (BUD/FOR + TIO) for weighted mean change from baseline in 0–24 h FEV1 at Week 12 in patients with COPD. Greater improvements in trough and serial FEV1 measurements at Week 12 with FF/UMEC/VI versus BUD/FOR + TIO, together with similar health status improvements and safety outcomes including the incidence of pneumonia (Ferguson et al., 2020). And for patients with frequent and/or severe acute exacerbations in the past, although these patients have received triple, LABA/ICS, single bronchodilator or double bronchodilator, they used closed triple inhalation therapy compared with fixed-dose double bronchodilator therapy, can still benefit from mortality (GOLD, 2021). The main administration route should be pulmonary inhalation, and further attention should be paid to the research and development of drug delivery systems. 3) COPD and comorbidity: the Global Initiative for GOLD guidelines (2011) has recommended that, in general, the presence of co-morbidities should not alter COPD treatment, and comorbidities should be treated urgently, and a new version has emphasized the role of acute exacerbation and complications of COPD in the disease assessment. The most common comorbidities are ischaemic heart disease, diabetes, skeletal muscle wasting, cachexia, osteoporosis, depression, and lung cancer (Corlateanu et al., 2016). It was found that, the relationship between complications of COPD and treatment is still in infancy, and depression and COVID-19 are worthy of consideration to better explore the mentioned relationship. 4) Elderly patients with COPD: further research should be conducted to improve medications more effectively for elderly patients with COPD. 5) Pharmacoeconomics analysis: pharmacoeconomics analysis of COPD better clarifies the economic benefits of the proposed medications. Revealing the appropriate initial and maintenance drug regimens for patients with different phenotypes or subtypes of COPD, which is expected to control medical costs. Study showed that promptly initiating triple therapy after two moderate or one severe exacerbation is associated with decreased morbidity and economic burden in COPD, (Tkacz et al., 2022) for example.

The two important limitations of the current study should be pointed out. Firstly, we only analyzed studies indexed in the WOSCC database. Secondly, the Matthew effect, which might influence the results of bibliometric analysis, was not considered (Jiang et al., 2021).

In summary, the administration of bronchodilators and pulmonary drug delivery systems, as well as consideration of elderly COPD patients require further attention in frontier

studies on medications for COPD, and exact mechanisms underlying the pathogenesis of COPD should be explored as well.

AUTHOR CONTRIBUTIONS

GZ designed the study, DJC directed the design of this study. GZ and LYY collected and verified the data. GZ performed software analysis. GZ and LYY drafted the first vision. GZ, LYY, and DJC revised and approved the final version of the manuscript.

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REVIEW
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Unravelling the Therapeutic Potential of Botanicals Against Chronic Obstructive Pulmonary Disease (COPD): Molecular Insights and Future Perspectives

Sicon Mitra[1†], Uttpal Anand[2†], Mimosa Ghorai[3], Balachandar Vellingiri[4], Niraj Kumar Jha[1], Tapan Behl[5], Manoj Kumar[6], Radha[7], Mahipal S. Shekhawat[8], Jarosław Proćków[9] and Abhijit Dey[3]**

[1]Department of Biotechnology, School of Engineering and Technology, Sharda University, Greater Noida, India, [2]Department of Life Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel, [3]Department of Life Sciences, Presidency University, Kolkata, India, [4]Human Molecular Cytogenetics and Stem Cell Laboratory, Department of Human Genetics and Molecular Biology, Bharathiar University, Coimbatore, India, [5]Department of Pharmacology, Chitkara College of Pharmacy, Chitkara University, Chandigarh, India, [6]Chemical and Biochemical Processing Division, ICAR-Central Institute for Research on Cotton Technology, Mumbai, India, [7]School of Biological and Environmental Sciences, Shoolini University of Biotechnology and Management Sciences, Solan, India, [8]Department of Plant Biology and Biotechnology, Kanchi Mamunivar Government Institute for Postgraduate Studies and Research, Puducherry, India, [9]Department of Plant Biology, Institute of Environmental Biology, Wrocław University of Environmental and Life Sciences, Wrocław, Poland

Background: COPD (chronic obstructive pulmonary disease) is a serious health problem worldwide. Present treatments are insufficient and have severe side effects. There is a critical shortage of possible alternative treatments. Medicinal herbs are the most traditional and widely used therapy for treating a wide range of human illnesses around the world. In several countries, different plants are used to treat COPD.

Purpose: In this review, we have discussed several known cellular and molecular components implicated in COPD and how plant-derived chemicals might modulate them.

Methods: We have discussed how COVID-19 is associated with COPD mortality and severity along with the phytochemical roles of a few plants in the treatment of COPD. In addition, two tables have been included; the first summarizes different plants used for the treatment of COPD, and the second table consists of different kinds of phytochemicals extracted from plants, which are used to inhibit inflammation in the lungs.

Conclusion: Various plants have been found to have medicinal properties against COPD. Many plant extracts and components may be used as novel disease-modifying drugs for lung inflammatory diseases.

Keywords: lungs, inflammation, alternative therapy, medicinal plants, COVID-19, COPD, clinical efficacy, plant-based formulation

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Ting-Yu Lin,
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George Hsiao,
Taipei Medical University, Taiwan

****Correspondence:***
Jarosław Proćków
jaroslaw.prockow@upwr.edu.pl
Abhijit Dey
abhijit.dbs@presiuniv.ac.in

[†]These authors have contributed equally to this work

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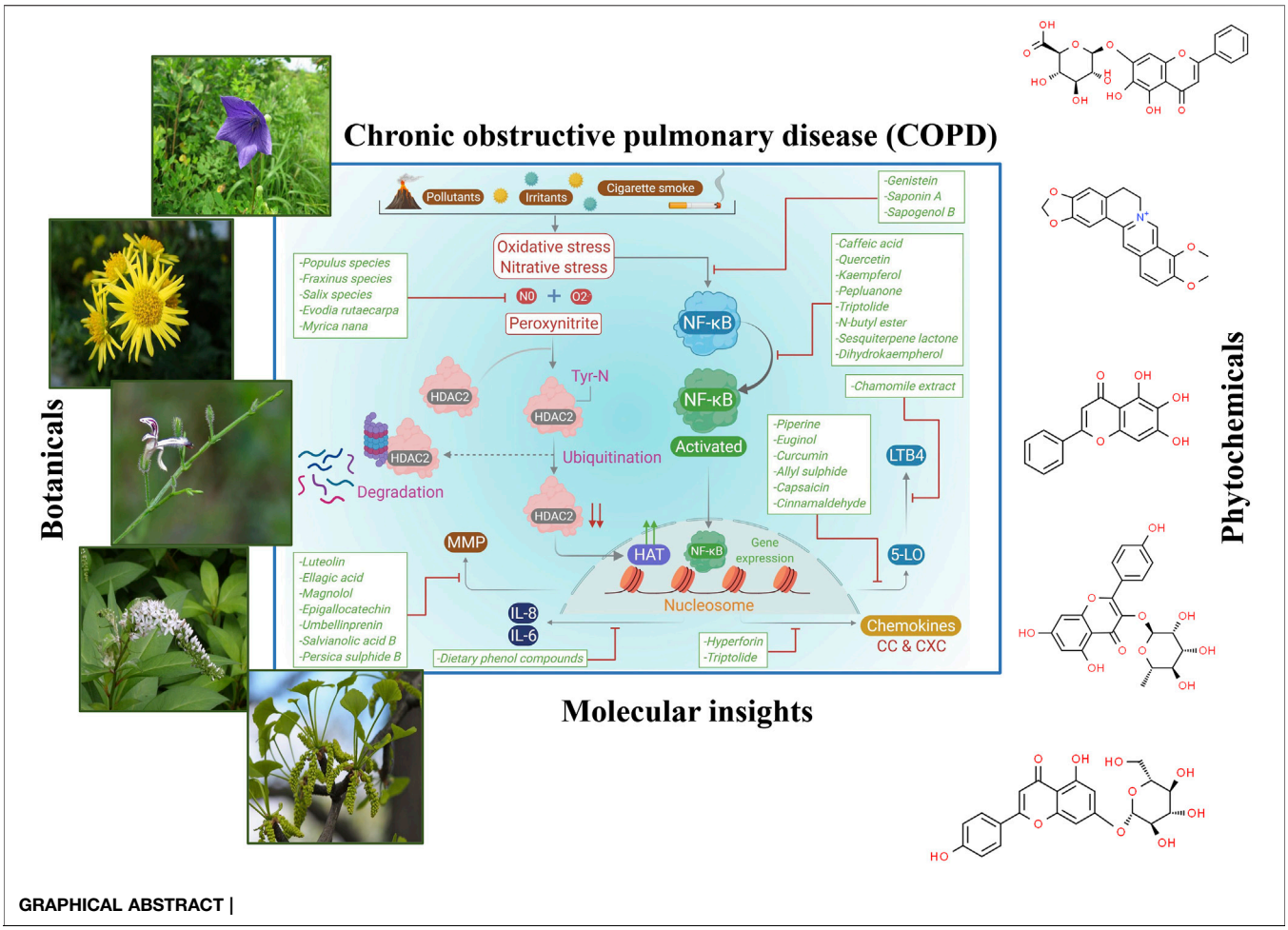

GRAPHICAL ABSTRACT |

1 INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterized by airflow restriction that does not completely reverse and is a significant cause of morbidity and death globally (GBD 2015 Chronic Respiratory Disease Collaborators, 2017; Vogelmeier et al., 2017; Anzueto and Miravitlles, 2018). Both asthma and COPD are the most common respiratory illnesses worldwide that are characterized by airway blockage and persistent respiratory inflammation. However, the pattern is noticeably different from one another. In the case of asthma, inflammation begins with $CD4^+$ T helper 2 (Th2) cells, as well as dendritic cells. This further proceeds by eosinophilic infiltration along with sensitization of the mast cell. This results in the release of many inflammatory mediators. However, COPD is marked by an increase in the number of neutrophils and T lymphocytes in the lungs, leading to a significant increase in activated macrophages (Barnes, 2008).

Tobacco use is a key risk factor for COPD, yet despite decades of lowering smoking rates in many countries, associated reductions in disease burden have been modest (Adeloye et al., 2015). In population-based observational samples from 1987 to 1988 and 2005 to 2009, only a small percentage of lifetime smokers were found to have spirometry-defined COPD with up to 30% occurring among people who had never smoked (Bakke et al., 1991). Standard medicines are ineffective and have a slew of negative side effects. As a result, there has been a strong push for safer and potentially effective alternative treatments. Medicinal plants are the oldest and most widely used for treating a variety of human diseases (Anand et al., 2019; Anand et al., 2020; Anand et al., 2021). Traditional medicine and ethnobotany had always played crucial roles in reducing human morbidity and mortality (Biswas et al., 2021; Dutta et al., 2021; Paul et al., 2021). Crude plant extracts and preparations have been recommended against a variety of human ailments (Das et al., 2021; Mohammed et al., 2021; Tandon et al., 2021). A number of phytoconstituents have also been reported as promising disease modifying agents (Bandopadhyay et al., 2021; Banerjee et al., 2021; Datta et al., 2021). Many investigations have also carried out to explore the pre-clinical and clinical efficacy of botanical-derived products (Khare et al., 2021; Mitra et al., 2021; Mitra et al., 2022b). However, COPD could be treatable if exposure to risk factors can be avoided (Vogelmeier et al., 2020). In terms of COPD, several plants have been suggested in many nations that may be effective (Zhou et al., 2016; Hwang and Ho, 2018; Sun et al., 2020). However, only the bare minimum of solid scientific evidence is accessible in the literature. Except for

a few early research works where detailed examinations of any plant or its derived compounds have not been conducted specifically for COPD patients. In this review, we have included several plants that have been highlighted for their effectiveness in patients with COPD.

2 DATABASES AND SEARCH STRINGS USED TO RETRIEVE LITERATURE

Google Scholar (https://scholar.google.com/) search engine was given the most attention in this review article since it provides a straightforward approach to search for various scholarly publications. As a result, this was utilized as an index to a wide range of scientific publications. Furthermore, additional journal articles available on the internet helped to find this literature review study. This includes databases used in particular for retrieving published research, such as ScienceDirect, Elsevier, PubMed and Scopus. The relevant literature was recovered using search -strings like "COPD," "COVID-19," "coronaviruses," "inflammation," "medicinal plants," and "conventional therapeutics" in various combinations. The retrieved liretaure and cross-referencing among them included the references describing the potential of plant and plant-derived phytochemicals against COPD, are dicussed in the present artcle.

3 EPIDEMIOLOGY

Due to the paucity of data representative of the worldwide population and the lack of agreement on case definitions, studying the global prevalence of COPD was previously challenging. However, the scope and start of international COPD research have increased our awareness of the disease's worldwide impact and shown the prevalence of varying diseases across nations. Global burden of disease (GBD) research employed national surveys, census data, and a central database of registries from more than 100 nations, stratified by sociodemographic index (SDI), a composite measure of fertility, income, and education (GBD Chronic Respiratory Disease Collaborators, 2017). According to a comprehensive review of population-based research conducted in 52 countries in 2015, the Americas had the highest prevalence of COPD (15% in 2010), while Southeast Asia had the lowest (10%). The study predicted a global prevalence of 12%, equivalent to 384 million cases in 2010, and a figure far higher than the GBD study estimate (Adeloye et al., 2015).

4 MOLECULAR EVENTS INVOLVED IN COPD

From the study by Osoata et al. (2009), it was found that HDAC2 expression was decreased by nitration of certain tyrosine residues under nitrative/oxidative stress. *In vitro*, hydrogen peroxide, peroxynitrite, and cigarette smoke-conditioned media decreased HDAC2 expression in A549 epithelial cells. This decrease was caused by enhanced proteasomal degradation followed by ubiquitination and did not decrease mRNA production or stability. HDAC2 was nitrated in the peripheral lung tissues of smokers and patients with COPD, as well as under nitrative/oxidative stress (Osoata et al., 2009). Furthermore, oxidative stress has been implicated in the decrease in sirtuin-1 (SIRT1), which is a crucial anti-aging molecule that is both a protein deacetylase and plays a key role in controlling MMP-9 (Stockley et al., 2009). Reactive oxygen species (ROS) are also important in the development of COPD. Tobacco smoke includes significant levels of oxidants and generates a wide range of free radicals, including ROS. Excess ROS production causes oxidative stress, increasing inflammatory responses and leading to the severe stage of COPD. Nuclear factor erythroid 2-related factor 2 (Nrf2) is induced via a Keap1-dependent signaling mechanism in which Nrf2 is inhibited at the basal level via Keap1-controlled ubiquitination-proteasomal degradation. It is induced by oxidants and electrophiles via alteration of critical cysteine thiols in Keap1 and Nrf2. Activated Nrf2 regulates drug metabolism, antioxidant defense, and oxidant signaling by mediating the increased production of a slew of enzymes and signaling proteins, regulating oxidant physiology and disease. Many plants constituents such as eriodictyol, baicalein etc. are therefore found to regulate the Nrf2 pathway against COPD (Ma, 2013). Myeloperoxidase is most known for its capacity to catalyze reactive oxidants, which aid in the elimination of infections. Oxidants generated by myeloperoxidase leads to tissue damage as well as the development and spread of acute and chronic vascular inflammation. Myeloperoxidase from neutrophils also plays a crucial role in cancer growth and progression (Valadez-Cosmes et al., 2021). Plant constituents like fisetin, morin, etc. tend to regulate myeloperoxidase. As a result, numerous oxidative stress-related molecules, such as NADPH oxidase, Nrf2, superoxide dismutase, and myeloperoxidase may be considered as the potential targets for COPD treatment. PI3K-mediated signaling in neutrophils and macrophages is involved in inflammation and immunological responses, and its activity is increased in the lungs of COPD. In a mouse smoke model, inhibition of certain PI3K isoforms decreased lung neutrophilia (Doukas et al., 2009).

Several literary works have so far identified that PI3K inhibitors are indispensable for potentialand effective COPD treatments. Furthermore, inhibitors targeting nuclear transcription factor-B (NF-κB), which plays a key role in the encoding of numerous inflammatory genes and related kinases such as IκB kinase, have been explored (Schuliga, 2015). From the studies of Renda et al. (2008), it has been reported that the presence of active p38 MAPK in alveolar spaces and alveolar walls of smokers with COPD suggests that activation of the MAPK pathway is a critical stage in the disease aetiology. Western blot examination verified the enhanced expression of phosphorylated p38 in COPD patient' alveolar macrophages. Moreover, the

expression of phospho-p38 was linked to deterioration of lung function and the amount of CD8 T-lymphocytes invading the walls of alveoli. Therefore, p38-MAPK can be used as a potential molecular target for the synthesis of novel and more effective drugs for the treatment of COPD. In the smoke-induced mouse model system, NF-κB can be inhibited by intratracheal administration of NF-κB decoy oligodeoxynucleotides (ODNs), and decoy ODN-mediated NF-κB inhibition can suppress smoke-induced lung inflammation, respiratory dysfunction, and improve pathological changes in the lung parenchyma (Renda et al., 2008).

Pro-inflammatory cytokines including interleukin-1 (IL-1), tumor necrosis factor- α (TNF-α), and IL-6, as well as chemokines such as IL-8 are vital in the pathogenic system because they can induce and recruit circulating cells. The transforming growth factor-β (TGF-β) has been linked to airway fibrosis, which can result in airway damage (Rovina et al., 2009). Therefore, in therapeutic trials treating COPD, many techniques for inhibiting such cytokines or their receptors were explored. Hesperidin inhibited the synthesis of pro-inflammatory cytokines such as IL-6 and TNF-α while increasing the synthesis of anti-inflammatory cytokines like IL-10 and IL-4. Hesperidin activity may be regulated via the disruption of the AP-1 and NF-κB pathways (Yeh et al., 2007).

5 COVID-19 IS ASSOCIATED WITH MORTALITY AND THE SEVERITY OF COPD

According to the studies by Alqahtani et al. (2020), the frequency of COPD in patients with COVID-19 was low. However, mortality (60%) and the risk of severity (63%) were high, implying that COPD patients with positive COVID-19 infection are at increased risk of major complications and even death. Furthermore, the proportion of current smokers with COVID-19 infection was 9% [95% confidence intervals (CI), 4–14%] and this was associated with higher severity (22.30%) and death (38.5%) (Alqahtani et al., 2020). Although the incidence of COPD with the verified COVID-19 cases was not great, however, COVID-19 imposes a significant burden on patients with COPD with increasing disease severity (Zhang et al., 2020a; Guan et al., 2020). Furthermore, data from two investigations on COPD patients suffering from COVID-19 infection reveal a mortality rate of 60% (Zhang et al., 2020b; Yang et al., 2020). Despite the fact that COPD is not very common in reported cases of COVID-19, COVID-19 infection is associated with substantial severity and death in COPD. Current smokers were also at increased risk of serious illness and death. To minimize the risk of COVID-19 in COPD patients and current smokers, effective preventive interventions are urgently needed.

TABLE 1 | Different plant extracts inhibiting lung inflammation.

Sl no.	Plants	Inflammagen used	Extracts	Reference
1.	Aconitum tanguticum (Maxim.) Stapf [Ranunculaceae]	LPS (rat)	Alkaloid fraction	Wu et al. (2014a)
2.	Alisma plantago-aquatica subsp. orientale (Sam.) Sam. (= Alisma orientale (Sam.) Juz.) [Alismataceae]	LPS	80% ethanol	Kim et al. (2013)
3.	Alstonia scholaris (L.) R. Br. [Apocynaceae]	LPS (i.t.) (rat)	Alkaloid fraction	Zhao et al. (2016)
4.	Angelica decursiva (Miq.) Franch. and Sav. [Apiaceae]	LPS	70% ethanol	Lim et al. (2014)
5.	Asparagus cochinchinensis (Lour.) Merr. [Asparagaceae]	LPS	70% ethanol	Lee et al. (2015a)
6.	Azadirachta indica A. Juss. [Meliaceae]	Cigarette smoke	Water	Koul et al. (2012)
7.	Callicarpa japonica Thunb. [Lamiaceae]	Cigarette smoke	Methanol	Lee et al. (2015b)
8.	Canarium lyi C.D. Dai & Yakovlev [Burseraceae]	LPS	Methanol	Hong et al. (2015)
9.	Chrysanthemum indicum L. [Asteraceae]	LPS (i.t.)	Supercritical CO2 extract	Wu et al. (2014b)
10.	Cnidium monnieri (L.) Cusson [Apiaceae]	Cigarette smoke extract/ LPS (i.t.)	Water	Kwak and Lim (2014)
11.	Eleutherococcus senticosus (Rupr. and Maxim.) Maxim. (= Acanthopanax senticosus (Rupr. and Maxim.) Harms) [Araliaceae]	LPS (i.t.)	-	Fei et al. (2014)
12.	Ginkgo biloba L. [Ginkgoaceae]	LPS (i.t.)	Egb761	Huang et al. (2013)
13.	Houttuynia cordata Thunb. [Saururaceae]	LPS	70% ethanol	Lee et al. (2015c)
14.	Isodon japonicus var. glaucocalyx (Maxim.) H.W.Li (= Rabdosia japonica var. glaucocalyx (Maxim.) H.Hara) [Lamiaceae]	LPS (i.t.)	Flavonoid fraction	Chu et al. (2014)
15.	Lonicera japonica Thunb. [Caprifoliaceae]	LPS (i.t.)	50% ethanol	Kao et al. (2015)
16.	Lysimachia clethroides Duby [Primulaceae]	LPS	Methanol	Shim et al. (2013)
17.	Morus alba L. [Moraceae]	LPS	70% ethanol	Lim et al. (2013)
18.	Schisandra chinensis (Turcz.) Baill. [Schisandraceae]	LPS	Water	Bae et al. (2012)
19.		Cigarette smoke-induced	Aqueous ethanol	Zhong et al. (2015)
20.	Stemona tuberosa Lour. [Stemonaceae]	Cigarette smoke	Water	Lee et al. (2014)
21.	Taraxacum mongolicum Hand.-Mazz. [Asteraceae]	LPS	Water	Ma et al. (2014)
22.	Tripterygium wilfordii Hook. f. [Celastraceae]	LPS	Ethanol	Brinker et al. (2007)

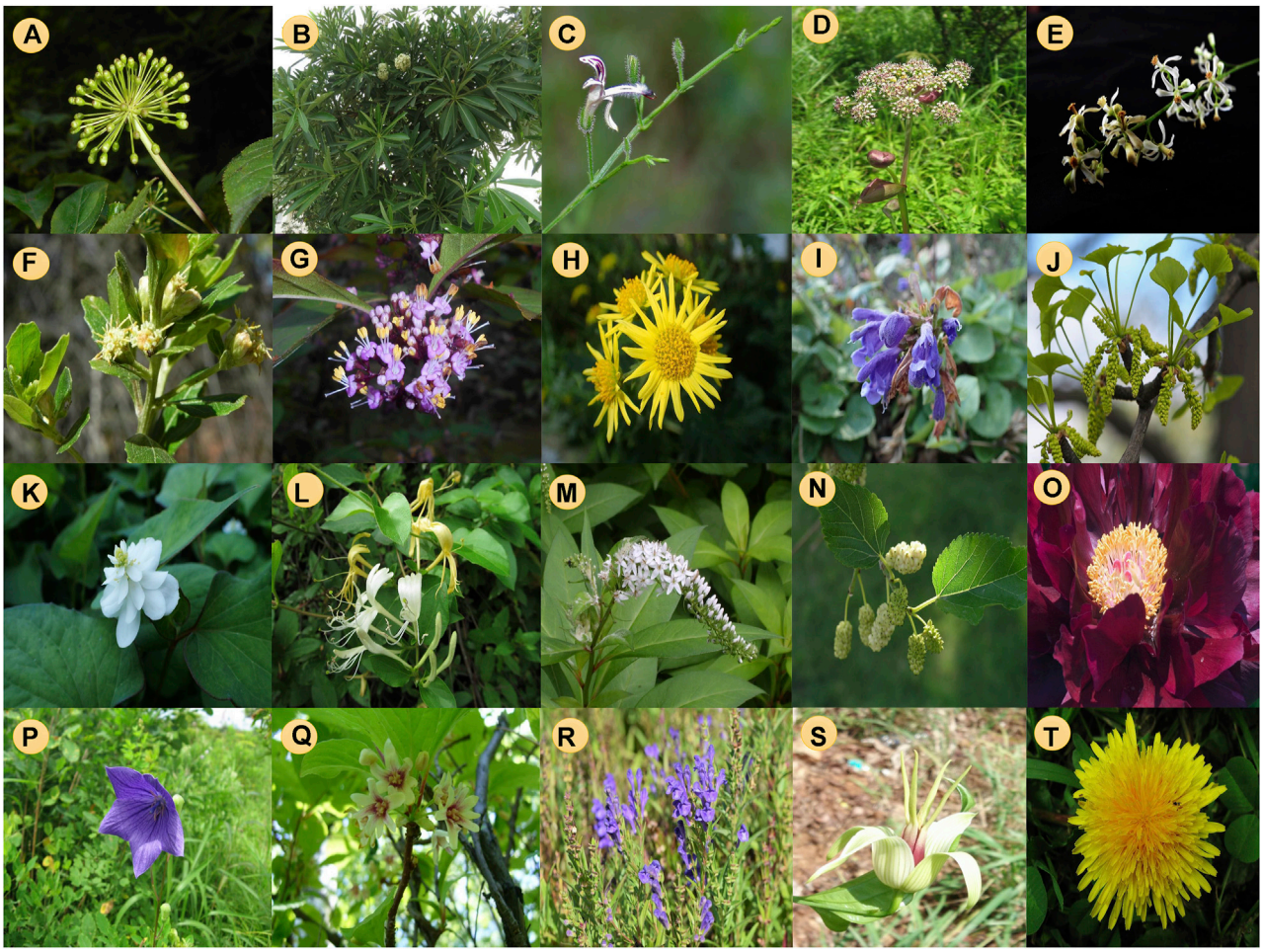

FIGURE 1 | Medicinal plants investigated against COPD. **(A)** Eleutherococcus senticosus (= Acanthopanax senticosus), **(B)** Alstonia scholaris, **(C)** Andrographis paniculata, **(D)** Angelica decursiva, **(E)** Azadirachta indica, **(F)** Baccharis retusa, **(G)** Callicarpa japonica, **(H)** Chrysanthemum indicum, **(I)** Dracocephalum rupestre, **(J)** Ginkgo biloba, **(K)** Houttuynia cordata, **(L)** Lonicera japonica, **(M)** Lysimachia clethroides, **(N)** Morus alba, **(O)** Paeonia × suffruticosa, **(P)** Platycodon grandiflorum, **(Q)** Schisandra chinensis, **(R)** Scutellaria baicalensis, **(S)** Stemona tuberosa, **(T)** Taraxacum campylodes (= T. officinale).

6 ADVERSE EFFECTS OF CONVENTIONAL THERAPIES FOR THE TREATMENT OF COPD

Several adverse effects are associated with conventional therapy of COPD. There exists one type of treatment for COPD, which is symptomatic pharmacological and based on bronchodilators, i.e., glucocorticoid, β2-adrenoreceptor agonists, theophylline, anticholinergics, and a combination of such drugs (Montuschi, 2006). However, in the case of β2-adrenoreceptor agonists, there are several adverse effects, such as myocardial ischemia, electrolyte imbalance, tachycardia, hypertension, osteoporosis etc. Due to such adverse effects, this group of drugs is not recommended for the treatment of COPD. Anticholinergic drugs impart many adverse effects such as blurred vision, cognitive disorders, constipation, urinary complications, and dryness of mouth when used for COPD treatment. Similarly, glucocorticoids are avoided because of their high cost, side effects, and high-risk factor. Alongside, theophylline also has many notable side effects, *viz.* headache, vomiting, diarrhoea, myocardial infraction, nausea, arrhythmias, and restlessness (Gupta et al., 2008). Side effects of steroids include blurred vision, hypertension, increased appetite, glaucoma, and weight gain (Hubbard and Tattersfield, 2004). Furthermore, the use of steroids for the treatment of COPD can negatively affect innate immunity and leads to susceptibility to many other diseases. Therefore, the development of new alternative therapeutics with safer pharmacological approaches must be introduced for the treatment of COPD (Sing and Loke, 2010).

7 MEDICINAL PLANTS USED FOR THE TREATMENT OF COPD

Over the past few years, many medicinal plants have been extensively studied for their properties against COPD. After a thorough investigation of such medicinal plants, they have been considered as an alternative treatment source to the systemic

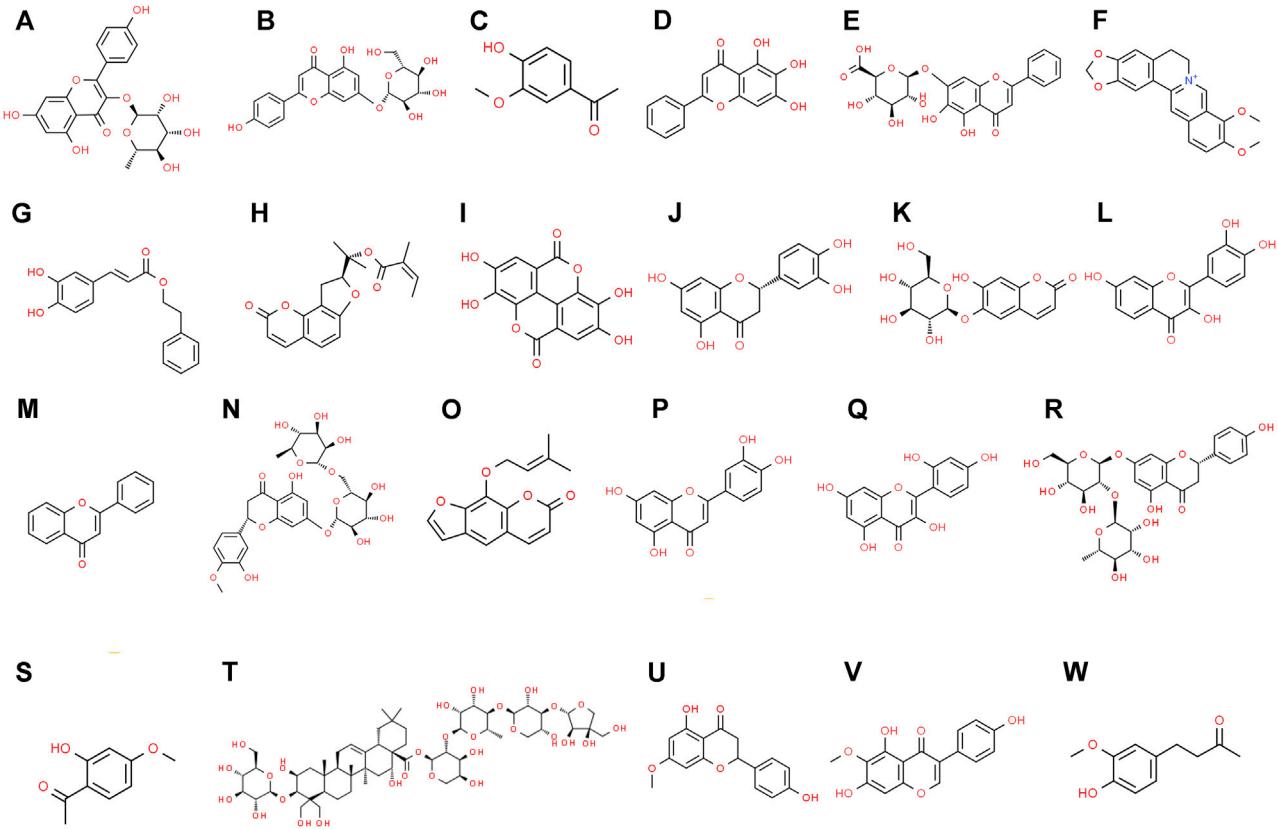

FIGURE 2 | Chemical structures of natural plant products reported against COPD. **(A)**. Afzelin, **(B)**. Apigenin-7-glucoside, **(C)**. Apocynin, **(D)**. Baicalein, **(E)**. Baicalin, **(F)**. Berberine, **(G)**. Caffeic acid phenethyl ester, **(H)**. Columbianadin, **(I)**. Ellagic acid, **(J)**. Eriodictyol, **(K)**. Esculin, **(L)** Fisetin, **(M)** Flavone, **(N)** Hesperidin, **(O)** Imperatorin, **(P)** Luteolin, **(Q)** Morin, **(R)** Naringin, **(S)** Paeonol, **(T)** Platycodin D, **(U)** Sakuranetin, **(V)** Tectorigenin, **(W)** Zingerone.

treatment of different types of lung-related diseases. In this review, the different plants conferring such medicinal properties are listed in **Table 1**. Some of the brief reports regarding those medicinal plants are also described below. **Figure 1** presents the medicinal plants investigated against COPD. **Figure 2** represents the natural chemical structures of the plant natural products reported against COPD.

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[The chemical structures are obtained from the free chemical structure database (www.chemspider.com)].

7.1 *Aconitum tanguticum*

Wu et al. (2014a) reported that total alkaloids of *Aconitum tanguticum* (TAA) substantially decreased the lung W/D ratio and increased the value of PaO2 or PaO2/FiO2 in ALI rats at 6, 12, and 24 h after lipopolysaccharide (LPS) challenge. TAA also decreased the overall protein content, as well as the total number of cells, neutrophils, and lymphocytes. Furthermore, TAA reduced MPO activity and reduced histological alterations in the lung. Furthermore, TAA also reduced the concentrations of TNF-α, IL-6, and IL-1b concentrations in bronchoalveolar lavage fluid (BALF) at 6, 12, and 24 h after LPS administration. TAA substantially reduced NF-κB activation in lung tissue (Wu et al., 2014a).

7.2 *Alstonia scholaris*

This plant belongs to the family of Apocynaceae. Over hundreds of years, it has been traditionally used for the treatment of respiratory diseases such as asthma, cough, COPD, phlegm, etc. Total alkaloids (TA) isolated from *Alstonia scholaris* leaves were tested for their ability to protect rats against lipopolysaccharide (LPS)-induced airway inflammation (AI), and TA was found to reduce the proportion of WBC, AKP, LDH, and ALB levels, and neutrophils in the BALF while increasing the ALB content in the blood. It also raised nitric oxide (NO) levels in the lungs, serum, and BALF while decreasing MDA concentrations in the lungs. TNF-α and IL-8 production in BALF and lung were also inhibited by total alkaloids. Finally, histological analysis revealed that total alkaloids reduced lung tissue damage in LPS-induced airway inflammation (Zhou et al., 2016).

TABLE 2 | Different plant constituents inhibiting lung inflammation.

Sl. no.	Constituent	Plant origin	Inflammagen used	Class	Reference
1.	Apigenin-7-glucoside	*Andrographis paniculata* (Burm.f.) Nees [Acanthaceae]	LPS (i.t.)	Flavonoid	Li et al. (2015)
2.	Apocynin	*Picrorhiza kurroa* Royle ex Benth. [Plantaginaceae]	LPS (hamster)	Phenol	Stolk et al. (1994)
3.	Baicalein	*Scutellaria baicalensis* Georgi [Lamiaceae]	LPS (i.t.) (rat)	Flavonoid	Tsai et al. (2014)
4.	Baicalin		Cigarette smoke	Flavonoid	Li et al. (2012)
5.	Berberine	-	Cigarette smoke	Alkaloid	Xu et al. (2015)
6.	Caffeic acid phenethyl ester	Honey-bee propolis	Cigarette smoke (rabbit)	Phenol	Sezer et al. (2007)
7.	Ellagic Acid	-	Acid	Phenol	Cornélio Favarin et al. (2013)
8.	Eriodictyol	*Dracocephalum rupestre* Hance [Lamiaceae]	LPS	Flavonoid	Zhu et al. (2015)
9.	Esculin	-	LPS (i.t.)	Coumarin	Tianzhu and Shumin, (2015)
10.	Flavone, Fisetin	-	LPS (i.t.)	Flavonoid	Geraets et al. (2009)
11.	Hesperidin	-	LPS (i.t.)	Flavonoid	Yeh et al. (2007)
12.	Imperatorin	-	LPS	Coumarin	Sun et al. (2012)
13.	Luteolin	*Lonicera japonica* Thunb. [Caprifoliaceae]	LPS (i.t.)	Flavonoid	Lee et al. (2010)
14.	Morin	-	LPS	Flavonoid	Tianzhu et al. (2014)
15.	Naringin	Prunus persica (L.) Batsch [Rosaceae]	Cigarette smoke (rat)	Flavonoid	Nie et al. (2012)
16.	Paeonol	*Paeonia* × *suffruticosa* Andrews [Paeoniaceae]	Cigarette smoke	Phenol	Liu et al. (2014)
17.	Platycodin D	*Platycodon grandiflorum* (Jacq.) A.DC. [Campanulaceae]	LPS (i.t.)	Triterpenoid saponin	Tao et al. (2015)
18.	Sakuranetin	*Baccharis retusa* DC. [Asteraceae]	Elastase-induced emphysema	Flavonoid	Taguchi et al. (2015)
19.	Tectorigenin	*Taraxacum campylodes* G.E.Haglund (= *T. officinale* (L.) Weber ex F.H.Wigg.) [Asteraceae]	LPS	Flavonoid	Huang et al. (2013)
20.	Zingerone	Lichen species	LPS	Phenol	Xie et al. (2014)

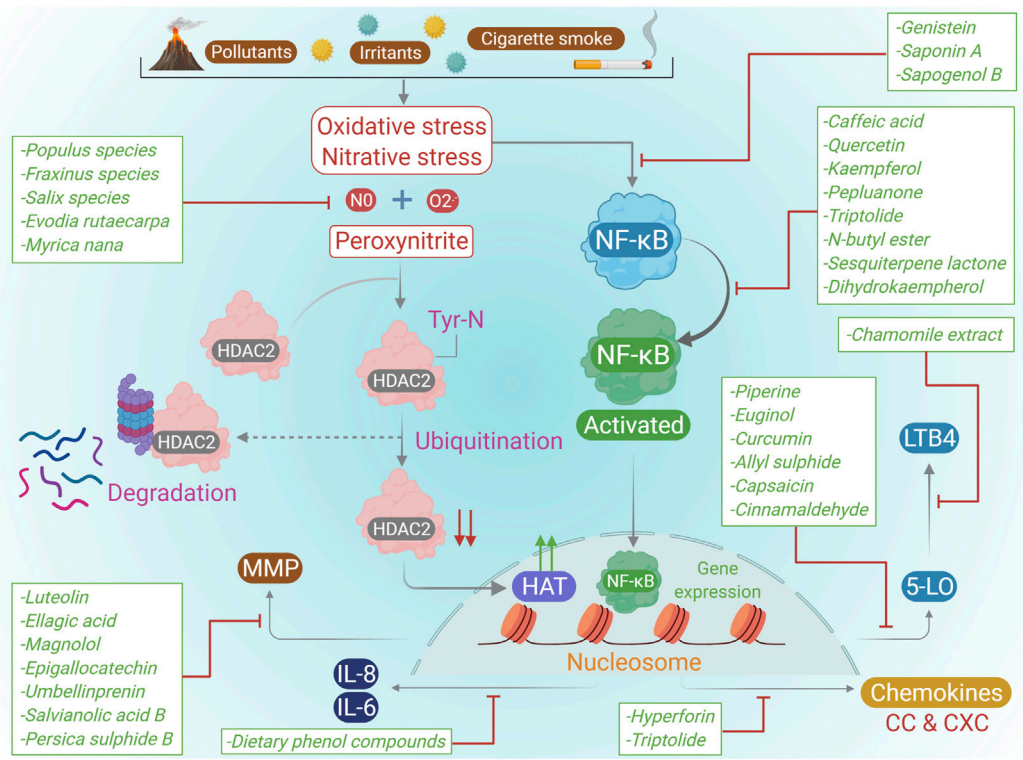

FIGURE 3 | Molecular mechanisms of COPD pathogenesis and the effects of natural plant products.

7.3 *Alisma orientale*

LPS-treated mice when treated with ethanol extracts of *Alisma orientale* (EEAO) leads to the suppression of pulmonary inflammation significantly. Septic mice post-treated with EEAO enhanced the survival rate in mice. Therefore, these findings indicate that EEAO has a therapeutic impact on acute lung injury caused by sepsis, implying that EEAO might be used as a therapeutic approach to treat acute lung disorders such as acute lung injury (Kim et al., 2013).

7.4 *Angelica decursiva*

Extracts from roots of *Angelica decursiva* demonstrated significant inhibitory action towards LPS-induced lung inflammation in mice. Few coumarin derivatives were identified from the extracts, including columbianadin, umbelliferone, umbelliferone 6-carboxylic acid, nodakenin, and nodakenetin. Among the identified compounds, columbianadin was shown to have potent anti-inflammatory action against IL-1-treated A549 cells and LPS-treated MH-S cells. Columbianadin was discovered to reduce NO synthesis by inhibiting inducible NO synthase. Furthermore, columbianadin was shown to have strong inhibitory action against LPS-induced lung inflammation following oral treatment (Lim et al., 2014).

7.5 *Asparagus cochinchinensis*

The ethanol extract of roots from *Asparagus cochinchinensis* (ACE) was reported to prevent IL-6 synthesis from IL-1-treated lung epithelial cells (A549), as well as the primary component, methyl protodioscin (MP), furthermore heavily suppressed synthesis of IL-6, IL-8, and TNF-α from A549 cells. The suppression of c-Jun N-terminal kinase (JNK) and the c-Jun activation pathway was shown to be involved in the downregulation of the synthesis of pro-inflammatory cytokine. In LPS-induced acute lung damage, oral treatment with ACE and MP effectively decreased cell invasion in BALF. In lung parenchyma, methyl protodioscin (MP) also decreased the synthesis of pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1 (Lee et al., 2015a).

7.6 *Azadirachta indica*

The modulatory and preventive properties of aqueous *Azadirachtaindica* leaf extract (AAILE) against cigarette smoke-induced pulmonary oxidative stress have been examined. Regular smoking and smoking disrupted the enzymatic and non-enzymatic defense systems of pulmonary tissue, as evidenced by higher levels of MDA, alterations in FTIR spectra, and an increase in the 3H-B [a] P-DNA adduct. AAILE cotreatment, on the other hand, was shown to be protective in terms of these characteristics. As a result, AAILE administration may be useful in combating pro-oxidant conditions caused by cigarette smoke (CS) in the lungs (Koul et al., 2012).

7.7 *Callicarpa japonica*

In H292 cells stimulated with cigarette smoke condensate (CSC), *Callicarpa japonica* therapy substantially reduced ERK phosphorylation. There was no discernible reduction in JNK and p38 phosphorylation in response to CSC stimulation. As a result, their data suggest that CJT suppression of MUC5AC synthesis was strongly related to ERK phosphorylation inhibition. CJT reduced neutrophil infiltration and mucus generation in a mouse model with COPD and decreased MUC5AC expression in a CSC stimulated H292 human lung mucoepidermoid cell line (Lee et al., 2015b).

7.8 *Gingko biloba*

When administered intraperitoneally, *Ginkgo biloba* leaves extract can significantly inhibited lung inflammation in LPS-induced ALI in modest doses. As a result, this plant material can cure inflammatory or allergic disorders of the lungs. Ginkgolides and flavonoids are the main components present in this plant. Additionally, flavonoid derivatives have anti-inflammatory properties in the lungs (Huang et al., 2013).

7.9 *Houttuynia cordata*

In the LPS induced ALI mouse model, *Houttuynia cordata* markedly reduced the synthesis of pro-inflammatory mediators such as IL-6 and NO in lung epithelial cells (A549) and alveolar macrophages (MH-S). Significant flavonoids such as hyperoside, afzelin, and quercitrin were effectively separated from the extract, and they also reduced LPS-induced lung inflammation in mice (Lee et al., 2015c).

7.10 *Rabdosia japonica*

The flavonoids fraction of *Rabdosia japonica* var. *glaucocalyx* (RJFs) reduced LPS-induced lung damage by decreasing lung wet-to-dry weight ratio, inhibited protein levels, and increased the synthesis of NO in the BALF. Furthermore, in ALI mice RJF helps in the reduction of TNF-α, IL-6, and IL-1 levels in BALF. Pretreatment of ALI mice by RJF leads to enhancement in the activity of SOD and suppression in the activity of MPO. RJF also leads to dramatically reduced lung damage by lowering complement deposition. Meanwhile, in the serum of ALI mice, RJFs lowered the amount of complement 3. RJF' anti-ALI actions were linked to suppression of synthesis of pro-inflammatory mediators and a reduction in complement levels (Chu et al., 2014).

7.11 *Tripterygium wilfordii*

Triptolide, possibly followed by tripdiolide, is among the most bioactive molecules of *Tripterygium* extract. On the molecular level, a few pharmacological effects of triptolide could be described by the observation that it heavily suppresses transcription of TNF-α and prevents the activation of NF-κB as well as other transcription factors. This further results in the inhibition of transcription of inflammation- and immune-related genes. Triptolide has also been demonstrated to interact with the glucocorticoid receptor. Glucocorticoid-responsive genes cannot be activated by the glucocorticoid receptor-1 complex and may downregulate the expressional activity of NF-κB and AP-1, resulting in a steroid-sparing, and anti-inflammatory effect (Brinker et al., 2007).

8 ROLES OF OTHER PLANTS IN THE TREATMENT OF COPD

In LPS-regulated RAW 264.7 cells, the expression of several pro-inflammatory mediators has been suppressed by *Canarium lyi* and it also inhibited activation of NF-κB and MAPKs in ALI mice (Hong et al., 2015). The extracts of *Chrysanthemum indicum* can successfully reduce LPS-stimulated acute lung injury in mice. The therapeutic efficacy of *C. indicum* was correlated with changes in TLR4 signaling pathways (Wu et al., 2014b). In lung tissues of an ALI mouse AS reduced the levels of IL-6 and TNF-α *via* suppressing the NF-κB pathway (Fei et al., 2014). *Lonicera japonica* has exhibited protective activity against LPS-induced lung inflammatory cytokine release (Kao et al., 2015). When Raw 264.7 cells are pre-treated with Lysimachia clethroides extract, it reduced release of LPS-stimulated NO, and synthesis of interleukin (IL)-1, and IL-6 cells in a dose-dependent manner. LPS-mediated IRF3 and STAT1 phosphorylation was also reduced by the extract (Shim et al., 2013). The ethanol extract of *Morus alba* root barks suppressed bronchitis-like symptoms when examined against LPS-mediated inflammation, as measured by TNF-α production. *M. alba* and its principal flavonoid components, including kuwanone G, norartocarpanone, and kuwanone E reduced synthesis of IL-6 in epithelial cells (A549) of lungs and biosynthesis of NO in lung macrophages (MH-S) (Lim et al., 2013). *Schisandra chinensis* extracts suppressed cytokine mixture-mediated synthesis of NO and lowered secretions of IL-8 and MCP-1 in A549 cells. In LPS-induced BALB/c mice. In addition, the extracts effectively inhibited infiltrations of neutrophil and macrophage infiltrations within lung tissues. Meanwhile, it increased the levels of IL-6 and TNF-α in BALF (Bae et al., 2012). In cough hypersensitive Guinea pigs which are induced by cigarette smoke, *S. chinensis* lowered cough intensity and lung inflammation (Zhong et al., 2015). *Stemona tuberosa* dramatically reduced the number of total cells, lymphocytes, neutrophils, and macrophages in the BALF of mice that are exposed to cigarette smoke. Furthermore, it lowered the levels of cytokines (TNF-α, IL-6) and the tested chemokine (KC) in BALF. Also, it prevented the expansion of the alveolar airways caused by cigarette smoke exposure (Lee et al., 2014). Water extract of *Taraxacum mongolicum* reduced inflammatory cell counts in the BALF, lowered protein levels of PI3K/Akt/mTOR in the lung, enhanced activity of SOD, inhibited the activity of myeloperoxidase, and significantly suppressed LPS-induced neutrophils (Ma et al., 2015).

9 PLANT CONSTITUENTS USED IN THE TREATMENT OF COPD

Some phenolics were also shown to be helpful against pulmonary inflammation when administered orally. Caffeic acid derivative, apocynin, ellagic acid, zingerone, and paeonol are among them. At 10 mg/kg/day, paeonol, the main component of *Paeonia* × *suffruticosa*, reduced "cigarette smoke-induced lung inflammation" in a mouse model of COPD (Liu et al., 2014). This observation is consistent with the ability of the *P.* × *suffruticosa* extract to prevent LPS-induced ALI in rats (Fu et al., 2012). Berberine, one of the active chemicals of *Argemone ochroleuca* [Papaveraceae], was discovered to have a relaxing impact on the tracheal muscle, which might be due to its antagonistic action on muscarinic acetylcholine receptors. Therefore, berberine has been found to be quite effective in the treatment of COPD (Sánchez-Mendoza et al., 2008). Hesperidin, naringin, and sakuranetin were found to function as anti-inflammatory agents in the lungs. In a smoke-induced COPD model, quercetin reduced lung inflammation, and mucus production (Yang et al., 2012). This suppressing effect can be achieved by reducing oxidative stress, decreasing NF-κB activation, and further inhibiting EGFR phosphorylation as some diterpenoids and triterpenoids have been shown to reduce lung inflammation. For example, triterpenoid saponins are important components of the *Hedera helix* [Araliaceae] that often play a key role in the treatment of lung inflammation (Hocaoglu et al., 2012). Platycodin D, which is a triterpenoid saponin, is derived from *Platycodon grandiflorum*, which further inhibited ALI (Tao et al., 2015). Furthermore, in human airway smooth muscle cells (HASMC), salvianolic acid B isolated from *Salvia miltiorrhiza* [Lamiaceae] substantially reduced H_2O_2-induced MMP-2 mRNA levels, along with gelatinolytic activity (Zhang and Wang, 2006). MMP activity and MMP-1 expression have been inhibited by umbelliprenin extracted from *Ferula persica* var. *persica* [Apiaceae] and luteolin, which is extracted from *Zostera marina* [Zosteraceae] (Kim et al., 2004; Shahverdi et al., 2006). Polyphenolic substances extracted from the bark of *Tristaniopsis calobuxus* [Myrtaceae], such as epigallocatechin and ellagic acid, reduced the levels of MMP-9 mRNA in mice peritoneal macrophages (Bellosta et al., 2003).

Apigenin-7-glycoside extracted from *Andrographis paniculata* dramatically suppressed LPS-mediated inflammation in the lung, and it also had an anti-inflammatory action *via* the MAPK and NF-κB (IκB) pathways (Li et al., 2015). In hamsters with LPS-enhanced lung injury, treatment with apocynin extracted from *Picrorhiza kurroa*, inhibited the secretion of oxidants from inflammatory cells, as well as apocynin increased the efficacy of recombinant human secretory leukocyte protease inhibitor (rSLPI) (Stolk et al., 1994). Baicalein and baicalin are extracted from *Scutellaria baicalensis*, and baicalein confers protection in rats against LPS-induced ALI. It inhibits NF-κB-regulated inflammatory responses and upregulates the Nrf2/HO-1 pathway (Tsai et al., 2014). Whereas, in cigarette smoke-induced inflammatory models in mice and A549 cells, baicalin possesses anti-inflammatory properties, which may be mediated *via* decreasing phosphorylation of histone deacetylase 2 (HDAC2) (Li et al., 2012). Moreover, by modulating the Nrf2 pathway and reducing the production of inflammatory cytokines in macrophages, eriodictyol extracted from *Dracocephalum rupestre* was able to reduce LPS-mediated ALI in mice (Zhu

et al., 2015). Fisetin, a flavonoid, dramatically lowered lung myeloperoxidase levels as well as the expression of several inflammatory mediator genes such as IL-6, TNF-alpha, IL-1beta, MIP-1, and MIP-2. Furthermore, fisetin substantially decreased LPS-regulated gene expression of HO-1 and SOD2 (Geraets et al., 2009).

Some coumarin compounds such as columbianadin and imperatorin also have anti-inflammatory properties in the lungs (Sun et al., 2012; Lim et al., 2014). Esculin reduced LPS-induced ALI by blocking the activation of MyD88 (myeloid differentiation primary response gene-88). This molecule has been identified to function upstream of NF-κB and NF-κB p65 activation (Tianzhu and Shumin, 2015). Treatment with morin and tectorigenin significantly reduced the number of inflammatory cells in the BALF, and in the lungs, morin lowered the amount of the NLRP3 which is an inflammasome protein. These two constituents also enhanced SOD activity, and downregulated activity of myeloperoxidase (Huang et al., 2013; Tianzhu et al., 2014). Furthermore, zingerone inhibited NF-κB and MAPK signaling pathways by suppressing phosphorylation of p38/MAPK, NF-κB/p65, IκBα, and ERK (Xie et al., 2014).

As stated above, data on various plant components that exhibit inhibitory effects on lung inflammation are constantly growing and some have shown encouraging results. The clinical efficacy of some compounds may be demonstrated in human studies in the near future. Few plant constituents found in different medicinal plants which inhibited lung inflammation are summarized in **Table 2**. **Figure 3** shows the molecular mechanisms of COPD pathogenesis and the effects of plant natural products.

10 CONCLUSION

COPD is a serious disease and the conventional treatments are either ineffective or insufficient. Medicinal plants are an important resource for alternative medicine, and numerous powerful medicines have been developed from plants for a variety of human diseases, including respiratory infections. Several plant extracts have the potential to be therapeutically helpful against lung inflammatory diseases such as COPD. Furthermore, several other types of plant components have been shown to suppress inflammatory reactions in the lung. Other plants with relaxing, bronchodilatory, antitussive, anticholinergic, mucociliary clearance, and antispasmodic characteristics might be explored in addition to these. In the future, other cellular pathways will need to be studied to determine the efficacy of natural compounds. Sirtuins, for example, have recently been identified as target molecules in COPD diseases. MMPs are also involved in the regulation of lung elasticity. With continued research, several plant extracts and components may be produced as new disease-modifying drugs for lung inflammatory diseases. In addition to the aforementioned points of view, several safety concerns should be thoroughly explored and investigated. Furthermore, critical studies must first be conducted in animal models to examine the functioning of essential organs and diagnostic markers.

AUTHOR CONTRIBUTIONS

All authors listed have contributed to the concept, literature mining, writing, and methodology of the review, provided critical feedback, and critically revised the manuscript. All authors contributed to the writing or revision of the final manuscript. SM: Conceptualization, writing—original draft, prepared tables, and arranged references. UA: Contributed to the study idea, planned and designed the review structure, writing-review and editing, prepared the tables, and arranged the references. MG: Writing-review and editing, arranged the references, revised the tables. BV: Overall reading and reviewing, responded to reviewer comments. NJ: Revised the manuscript, prepared the figures and response. TB: Completed the critical revision of the manuscript, data validation. MK: Completed the critical revision of the manuscript, data validation, and suggestions. R: Writing-review and editing. MS: Review and editing, suggestions. JP: Completed the critical revision of the entire manuscript, supervised the drafting process of the review, suggestions, editing, nomenclature, formal interpretation, response, final draft, resources, project administration, and funding acquisition. AD: Conceptualization, revised the review structure, suggestions, completed the critical revision of the manuscript, formal interpretation, supervised the drafting process of the review, resources, and final draft. All authors have read and approved the final version of the manuscript for submission to this journal.

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GLOSSARY

AAIL azadirachta indica leaf extract
AI airway inflammation
AKP alkaline phosphatase
ALB albumen
A549 cells basal adenocarcinoma human alveolar epithelial cells
BALF bronchoalveolar lavage fluid
CJT Callicarpa japonica Thunb.
COPD chronic obstructive pulmonary disease
CS cigarette smoking
CSC cigarette smoke condensate
EGFR epidermal growth factor receptor
ERK extracellular signal-regulated kinases
FTIR fourier transform infrared spectroscopy
GBD global burden of disease
HASMC human airway smooth muscle cells
HDAC2 histone deacetylase 2
IL-6 interleukin 6
JNK c-Jun N-terminal kinases
LDH lactate dehydrogenase
LPS lipopolysaccharide
MDA malondialdehyde
MMP-9 matrix metallopeptidase 9
MPO myeloperoxidase
mRNA messenger RNA
NF-κB nuclear factor-kappa B
NO nitric oxide
ROS reactive oxygen species
SDI sociodemographic index
SIRT1 sirtuin 1
SOD superoxide dismutase
TA total alkaloids
TAA total alkaloids of aconitum tanguticum
TNF-alpha tumor necrosis factor-alpha
WBC white blood cell

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Effect of Smoking on Lung Function Decline in a Retrospective Study of a Health Examination Population in Chinese Males

Ting Tian[1†], Xueqin Jiang[2†], Rujie Qin[3,4†], Yuqing Ding[5†], Chengxiao Yu[6,7], Xin Xu[6,7] and Ci Song[6,7]*

[1] School of Public Health, Medical College of Yangzhou University, Yangzhou University, Yangzhou, China, [2] Department of Geriatric Medicine, Huadong Sanatorium, Wuxi, China, [3] Joyfulway Clinic, Fosun Health Co., Ltd., Shanghai, China, [4] Department of Health Management, Huadong Sanatorium, Wuxi, China, [5] Department of Medical Record Statistics, The Cancer Hospital of the University of Chinese Academy of Sciences (Zhejiang Cancer Hospital), Institute of Basic Medicine and Cancer (IBMC), Chinese Academy of Sciences, Hangzhou, China, [6] Department of Epidemiology, Center for Global Health, School of Public Health, Nanjing Medical University, Nanjing, China, [7] Jiangsu Key Lab of Cancer Biomarkers, Prevention and Treatment, Collaborative Innovation Center for Cancer Personalized Medicine, Nanjing Medical University, Nanjing, China

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***Correspondence:**
Xueqin Jiang
906921532@qq.com

†These authors have contributed equally to this work and share first authorship

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Objective: China has established a goal of reducing adult smoking prevalence from 27.7% to 20% by 2030. Understanding the possible ongoing impairment in lung function in smokers, is critically important to encourage the populations to change their smoking behavior.

Methods: A total of 14,273 males joined the health examination at Huadong Sanatorium from Jan 2012 to Dec 2019 were included. In cross-sectional analysis, we used multiple linear regression to evaluate the association between baseline lung function and smoking status. Then, 3,558 males who received ≥2 spirometry exams were analyzed in longitudinal study. Annual lung function decline was compared using mixed linear models adjusted for confounders.

Results: In cross-sectional analysis, compared with never-smokers, decreases of −133.56 mL (95% CI: −167.27, −99.85) and −51.44 mL (−69.62, −33.26) in FEV_1, −1.48% (−1.94, −1.02) and −1.29% (−1.53, −1.04) in FEV_1/FVC were observed in former and current smokers. In longitudinal analysis, significant declines were observed in FEV_1 [5.04 (2.30, 7.78) mL] and FEV_1/FVC [0.09 (0.05, 0.13) %] in current smokers but not observed in former smokers after adjustment. Participants with long duration of smoking cessation had decelerate lung function than short duration. The annual decline rate of current smokers with high smoking intensity (≥30 cigarettes per day) was 13.80 and 14.17 times greater than that of never-smokers in FEV_1 and FVC. Thus, early smoking cessation can slow down lung function decline trend for current smokers.

Conclusions: The harms of current smoking on lung function emphasize the necessity of smoking cessation, especially for those with comorbidities.

Keywords: smoking cessation, FEV_1, FVC, FEV_1/FVC, health examination

INTRODUCTION

Smoking is the major cause of premature death worldwide (1, 2). As the Global Adult Tobacco Survey (GATS) approximated in 2010, China is the largest producer and consumer of tobacco products in the world, with an estimation of 301 million current smokers (3–6). The current smoking prevalence among men was 52.9% and that among women was 2.4% in China (5). Such a high smoking rate of Chinese males shifts the negative effects on pulmonary health and accounts for nearly 20% of all-cause mortality during the past decades (7). The magnitude of tobacco related pulmonary disease has created a healthcare crisis in China (3).

Lung function is a critical measurement and early severity predictor for indicating cardio-pulmonary health (8). Decline of FEV_1 indicates a higher risk of COPD (9); while the ratio of forced expiratory volume in 1 s (FEV_1) to forced vital capacity (FVC), also known as FEV_1/FVC, is the primary index of airflow limitation or airway obstruction (10). Current smoking was found associated with accelerated age-related FEV_1 decline (11, 12). While one meta-analysis showed a homogeneity effect of current and former smoking on FEV_1 decline (9). However, former smokers having changed the smoking habits for part of the period during which the betas were estimated may lead to the non-significant estimates in this meta-analysis (9). Furthermore, prior studies always focused on the association between smoking status and FEV_1 decline among COPD or asthma population (13–15). Besides, the mentioned studies were mainly conducted in the developed countries, e.g., the United States, Swedish, UK et.al. In these countries, workplace smoking cessation (SC) intervention is effective in increasing quit rate and more cases were voluntary SC promotion (16). In contrast, Chinese populations were less likely to promote voluntary SC, most of them quit smoking due to smoking-related diseases. Thus, it is essential to evaluate the association between different smoking status and lung function decline in the Chinese population.

As of the Health China 2030 strategy, the government has established a goal of reducing adult smoking prevalence from 27.7 to 20% by 2030 (17). Challenges remain in accomplishing the goal. Understanding the possible ongoing impairment of smoking in lung function, is increasingly important, to encourage the voluntary SC promotion. Hence, we conducted this retrospective study to evaluate the association between smoking exposure and changes of lung function (i.e., FEV_1, FVC and FEV_1/FVC) among Chinese males with repeated measure of the indicators.

MATERIALS AND METHODS

Data Source

We used data from Huadong Sanatorium health examination database (HSHED) between Jan 1, 2012 and Dec 31, 2019. Huadong Sanatorium (HS) is a municipal medical institution integrating convalescence, rehabilitation and health care, which providing personalized health management services for the entire society. Most participants taking health examination in HS are employees of various employers from Shanghai aged 15–95 years old. HSHED was established based on hospital information system (HIS) in 2003. All the results of examination were recorded in the HSHED.

We extracted data from participants who volunteered to receive basic health examination and additional spirometry exams in HS. A total of 22,051 participants took spirometry exams were included. Date when participants first underwent a spirometry exam in HS was set as baseline. Female participants, with low smoking rate (<1%), were excluded, left 14,273 males to evaluate the association between lung function and smoking status in the cross-sectional analysis phase (Substudy 1, **Supplementary Figure 1**). In order to examine the longitudinal association of lung function annual changes with smoking status, we restricted to the participants with valid spirometry at two or more exams. Then, 3,558 males were included in the longitudinal analysis (Substudy 2, **Supplementary Figure 1**).

The approval of this study was obtained from ethics committees at Huadong Sanatorium (No. 2020-01). Anonymized and de-identified information were used for analyses, and therefore informed consent was not required.

Measurements

Smoking status was self-reported as "never" "former" and "current" cigarettes smoking at each spirometry exam. Ever-smokers were defined as former and current smokers. In the cross-sectional analysis phase, all the 14,273 participants were divided into three groups according to baseline smoking status: never-smokers (N = 5,468), former smokers (N = 1,111), and current smokers (N = 7,694) (**Table 1**). In the longitudinal analysis phase, 3,268 participants reported smoking status unchanged across the follow-up period. These participants were classified as sustained never-smokers (N = 1,305), former smokers (N = 245), and current smokers (N = 1,718). Other 290 participants were classified as having variable smoking status (**Supplementary Table 1**).

Spirometry was performed using a MiniSpir spirometer at baseline and follow-up visits. A bronchodilator was not administered prior to spirometry. Lung function was measured with standardized protocols by the same equipment and acquired by the same investigators. To harmonize these data, we retrospectively did quality control checks according to the American Thoracic Society/European Respiratory Society 2005 standards, which define valid exams as two or more acceptable curves reproducible within 150 mL (18). Lung function outcomes were forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), and their ratio (FEV_1/FVC). The Global Lung Function Initiative equations (19) were used to define lower limit of normal (LLN).

Diagnosed clinical lung disease was defined as self-reported physician diagnosis of COPD, asthma, chronic bronchitis, bronchiectasis, emphysema, bullae and postoperative lung cancer. Airflow limitation was defined as FEV_1/FVC lower than the LLN, defined by the NHANES III reference equations (20). Restrictive pattern was defined as $FEV_1/FVC \geq LLN$ and $FVC < LLN$ (21).

TABLE 1 | Baseline characteristics of 14,273 male participants according to smoking status in the cross-section analysis.

	Overall (*N* = 14,273)	Never-smokers (*N* = 5,468)	Former smokers (*N* = 1,111)	Current smokers (*N* = 7,694)	*P*[c]
Age, years	48.52 (11.30)	46.66 (12.84)	54.41 (10.22)	49.00 (9.86)	<0.001[b]
<50	7,551 (52.90%)	3,236 (59.18%)	360 (32.40%)	3,955 (51.40%)	<0.001
≥50	6,722 (47.10%)	2,232 (40.82%)	751 (67.60%)	3,739 (48.60%)	
Height, cm	171.17 (5.95)	171.28 (6.05)	170.59 (5.93)	171.18 (5.87)	0.001[b]
weight, kg	73.91 (10.29)	73.64 (10.31)	74.24 (9.44)	74.06 (10.39)	0.006[b]
Body-mass index, kg/m^2	25.20 (3.06)	25.08 (3.07)	25.50 (2.86)	25.24 (3.07)	<0.001[b]
Normal weight (18.5–24.9)	6,656 (46.63%)	2,649 (48.45%)	465 (41.85%)	3,542 (46.04%)	<0.001
Underweight (<18.5)	171 (1.20%)	70 (1.28%)	7 (0.63%)	94 (1.22%)	
Overweight (25–29.9)	6,565 (46.00%)	2,432 (44.48%)	571 (51.40%)	3,562 (46.30%)	
Obesity (≥30)	881 (6.17%)	317 (5.80%)	68 (6.12%)	496 (6.45%)	
Alcohol consumption					
Never	3,759 (26.34%)	1,867 (34.14%)	233 (20.97%)	1,659 (21.56%)	<0.001
Former	214 (1.50%)	49 (0.90%)	70 (6.30%)	95 (1.23%)	
Current	10,173 (71.27%)	3,494 (63.90%)	782 (70.39%)	5,897 (76.64%)	
Unknown	127 (0.89%)	58 (1.06%)	26 (2.34%)	43 (0.56%)	
Smoking behavior					
Pack-years	24.54 (18.61)	-	23.02 (18.41)	24.69 (18.63)	0.181[a]
Cigarettes per day	17.99 (9.92)	-	18.58 (11.23)	17.94 (9.78)	0.903[b]
Hypertension	5,093 (35.68%)	1,805 (33.01%)	516 (46.44%)	2,772 (36.03%)	<0.001
Diabetes	5,365 (37.59%)	2,002 (36.61%)	455 (40.95%)	2,908 (37.80%)	0.021
Elevated TC (>5.2 mmol/L)	5,576 (39.24%)	2,007 (36.89%)	446 (40.40%)	3,123 (40.74%)	<0.001
Elevated TG (>1.7 mmol/L)	5,932 (41.75%)	1,835 (33.73%)	424 (38.41%)	3,673 (47.92%)	<0.001
Elevated total bilirubin (>24.20 umol/L)	664 (4.67%)	334 (6.14%)	63 (5.71%)	267 (3.48%)	<0.001
Diagnosed clinical lung disease					
COPD	5 (0.04%)	0 (0.00%)	2 (0.18%)	3 (0.04%)	0.013
Asthma	76 (0.53%)	41 (0.75%)	14 (1.26%)	21 (0.27%)	<0.001
Chronic bronchitis	158 (1.11%)	29 (0.53%)	20 (1.80%)	109 (1.42%)	<0.001
Bronchiectasis	50 (0.35%)	31 (0.57%)	5 (0.45%)	14 (0.18%)	0.001
Emphysema	243 (1.70%)	19 (0.35%)	38 (3.42%)	186 (2.42%)	<0.001
Bullae	184 (1.29%)	42 (0.77%)	21 (1.89%)	121 (1.57%)	<0.001
Postoperative lung cancer	22 (0.15%)	9 (0.16%)	12 (1.08%)	1 (0.01%)	<0.001

Data are n (%), mean (SD).
TC, total cholesterol; TG, triglycerides; COPD, chronic obstructive lung disease.
[a]*One-way ANOVA test for the equal variances.*
[b]*Kruskal-Wallis test for the unequal variances.*
[c]*Chi-square test.*
In this analysis, 65 participants missing detailed information of TC, TG, total bilirubin. Among ever-smokers, only 2,781 ever-smokers had detailed information of pack-years and 2,936 current smokers had detailed information of cigerattes consumptions.

Statistical Analysis

Baseline Characteristics of the Participants

Demographic characteristics of the study participants according to baseline smoking status were calculated and compared among groups. Baseline characteristics were assessed by one-way ANOVA, Chi-squared and Kruskal-Wallis test. Analyses were performed separately for Substudy 1 and Substudy 2.

Relationship Between Smoking Status and Lung Function at Baseline and Follow-Up

Firstly, we examined the relationship between smoking status and lung function at participants' first visit using cross-sectional analysis. We evaluated the mean differences in the lung function across different smoking exposure by multiple linear regression analysis.

To further evaluate the decline rate of lung function among different smoking status, longitudinal analysis was then performed. In this analysis, linear mixed models were used to test associations with repeated measures of lung function.

Sensitivity Analysis

Analyses were repeated in the participants without prevalent lung disease, with variable smoking status or aged older than 30 years to minimize the potential confounding effect.

Methods of smoking details, clinical and laboratory assessments, multiple linear regression, and linear mixed models were provided in the **Supplementary Material**. Data were analyzed using STATA software version 13 (STATA Corp, College Station, TX, USA). Statistical significance was defined as a two-tailed $P < 0.05$.

RESULTS

Substudy 1 Cross-Sectional Associations of Lung Function With Smoking Exposures Among 14,273 Male Participants at Baseline

In the cross-sectional phase, baseline characteristics are shown in **Table 1**. Most of them (90.31%) were aged 30–70 years old. Former smokers and currents smokers were older than never-smokers ($P < 0.001$). Mean cumulative cigarette exposure of former smokers and current smokers were 23.02 ± 18.41 and 24.69 ± 18.63 pack-years (PYs), respectively. Current smokers consumed an average of 17.94 ± 9.78 cigarettes per day. Former smokers were more likely to have an underlying disease at the first visit, i.e., hypertension (46.44%), diabetes (40.95%), elevated total bilirubin (5.71%) and lung diseases (10.08%), when compared with never smokers and current smokers ($P < 0.001$).

Multiple linear regression was used to evaluate the associations between smoking exposures with lung function (**Table 2**). After adjustment, ever-smoking was significantly related with lower FEV_1 and FEV_1/FVC at the first visit. Compared with never-smokers, current smokers had a −51.44 mL (95% CI: −69.62, −33.26, $P < 0.001$) decrease in FEV_1 and a −1.29% (95% CI: −1.53, −1.04, $P < 0.001$) decrease in FEV_1/FVC; and former smokers had an even lower level of lung function, with a −133.56 mL (95% CI: −167.27, −99.85, $P < 0.001$) decrease in FEV_1 and a −1.48% (95% CI: −1.94, −1.02, $P < 0.001$) decrease in FEV_1/FVC. For ever-smokers, greater cumulative cigarettes consumptions were associated with lower lung function, significantly when the cumulative pack-years exceeded to 20–30 or ≥30 PYs [Mean difference for FEV_1: −101.70(−145.84, −57.57) and −143.22(−180.24, −106.20); FVC: −66.22(−120.95, −11.50) and −80.75(−126.66, −34.85); FEV_1/FVC: −1.15(−1.75, −0.55) and −2.06(−2.57, −1.55) for 20–30 PYs and ≥30 PYs, when compared to never-smoking, **Table 2**]. For former smokers, longer durations of smoking cessation had a lower lung function [Mean difference for FEV_1: −214.28 (−336.45, −92.12) vs. −135.75 (−192.29, −79.20); FVC: −213.93 (−365.52, −62.34) vs. −131.52 (−201.68, −61.36); FEV_1/FVC: −1.42 (−3.08, 0.25) vs. −1.15 (−1.92, −0.38) for ≥10 and <10 years cessation duration]. For current smokers, those with ≥10 cigarettes/day had significant FEV_1 and FEV_1/FVC decline compared with never-smokers [Mean difference for FEV_1: −46.70 (−85.14, −8.26), −92.73 (−124.65, −60.82), −80.71 (−138.31, −23.12); FEV_1/FVC: −0.61 (−1.13, −0.08), −0.94 (−1.37, −0.51), −1.73 (−2.51, −0.94) for 10–20, 20–30, and ≥30 cigarettes/day, respectively].

Substudy 2 Longitudinal Associations Between Smoking Exposures and Lung Function Among 3,558 Male Participants

In the previous step, former smokers were observed with a lower level of lung function than that of never or current smokers. We further explored whether persistent smoking would accelerate the declines of lung function along with age. In the current longitudinal analyses, 3,558 male participants with ≥2 valid spirometry exams contributed 8,935 spirometry exams during follow-up. The baseline characteristics of 3,558 males in this longitudinal analysis was similar with those of participants in the cross-sectional analysis (**Table 1** and **Supplementary Table 2**). The mean pack-years of current smokers was 25.18 ± 18.73, which was greater than that of former smokers (21.97 ± 15.44) before baseline. The mean values of FEV_1, FVC, and FEV_1/FVC for former smokers at baseline were lower than that of other participants. Compared with never-smokers and current smokers, former smokers were older, had a higher proportion of drinkers and more likely to have underlying diseases.

Current smokers showed accelerated lung function decline compared with never-smokers. The unadjusted FEV_1, FVC and FEV_1/FVC decline among former smokers was 42.04 (36.77, 47.31), 47.36 (40.70, 54.03) mL and 0.16 (0.08, 0.24) % per year, compared to 33.99 (32.02, 35.97), 38.38 (35.90, 40.87) mL, and 0.09 (0.06, 0.11) % per year among never-smokers, and 39.26 (37.12, 41.40), 41.36 (38.55,44.17) mL, and 0.16 (0.14, 0.19)% per year among current smokers (**Table 3**). After adjusted for covariates, current smokers had an accelerated FEV_1 decline of 5.04 ml (95% CI: 2.30, 7.78, $P < 0.001$) per year and FEV_1/FVC decline of 0.09% (95% CI: 0.05, 0.13, $P < 0.001$) per year, when compared with never-smokers (**Figure 1**). Effect estimates were observed in participants with variable smoking status, with an accelerated FEV_1 decline of 7.61 ml (95% CI: 3.04, 12.18, $P = 0.001$) per year and FVC decline of 6.25 ml (95%CI: 0.59, 11.90, $P = 0.030$) per year, compared to never-smokers. However, no significant estimates were analyzed for former smokers [FEV_1, 3.90(−1.65, 9.44), $P = 0.168$; FVC, 3.38(−3.46, 10.22), $P = 0.332$; FEV_1/FVC, 0.05(−0.03, 0.13), $P = 0.224$, **Figure 1**].

For the former smokers, shorter durations of smoking cessation (<10 years) were associated with more accelerated FEV_1 decline than longer durations (≥10 years), when compared to never-smokers (3.68 vs. 7.52 mL, **Figure 1**). Compared with never-smokers, FEV_1/FVC decline was accelerated by 0.18% per year (95% CI: 0.06, 0.29, $P = 0.002$) in former smokers with <10 years of cessation, while the estimate was not obvious in former smokers with ≥10 years of cessation ($P = 0.438$) (**Figure 1**). Compared to never-smokers, decline was accelerated by 8.68 mL per year ($P = 0.004$) in FEV_1 and by 0.10% per year ($P = 0.029$) in FEV_1/FVC among 177 observed quitters (**Supplementary Table 4**).

For ever-smokers, the unadjusted estimates of declines in FEV_1 accelerated with the increase of cumulative smoking pack-years (estimates for exposure with <10, 10–20, 20–30, and ≥30 PYs were 31.99, 38.93, 44.09, and 47.48, respectively, **Table 3**). In the adjusted model, adjusted mean FEV_1 decline accelerated with increased pack-years (estimates for exposure with <10,

TABLE 2 | Cross-sectional associations of lung function and different smoking status at baseline.

	Number of participants	FEV_1 (mL)		FVC (mL)		FEV_1/FVC (%)	
		Mean Difference (95% CI)*	*P**	Mean Difference (95% CI)*	*P**	Mean Difference (95% CI)*	*P**
Smoking status							
Never-smokers	5,468	Ref		Ref		Ref	
Former smokers	1,111	−133.56 (−167.27, −99.85)	<0.001	−102.10 (−143.94, −60.26)	<0.001	−1.48 (−1.94, −1.02)	<0.001
Current smokers	7,694	−51.44 (−69.62, −33.26)	<0.001	1.75 (−20.81, 24.32)	0.879	−1.29 (−1.53, −1.04)	<0.001
Duration of smoking cessation							
Never-smokers	5,468	Ref		Ref		Ref	
Former smokers, by duration of cessation							
≥10 years	68	−214.28 (−336.45, −92.12)	0.001	−213.93 (−365.52, −62.34)	0.006	−1.42 (−3.08, 0.25)	0.095
<10 years	336	−135.75 (−192.29, −79.20)	<0.001	−131.52 (−201.68, −61.36)	<0.001	−1.15 (−1.92, −0.38)	0.003
Cumulative cigarette consumption							
Never-smokers	5,468	Ref		Ref		Ref	
Ever smokers to by pack-years							
<10 pack-year	577	5.02 (−39.29, 49.32)	0.824	3.43 (−51.51, 58.37)	0.903	−0.03 (−0.63, 0.58)	0.933
10 to <20 pack-years	672	−62.56 (−103.84, −21.27)	0.003	−75.88 (−127.08, −24.69)	0.004	−0.06 (−0.63, 0.50)	0.827
20 to <30 pack-years	585	−101.70 (−145.84, −57.57)	<0.001	−66.22 (−120.95, −11.50)	0.018	−1.15 (−1.75, −0.55)	<0.001
≥30 pack-years	947	−143.22 (−180.24, −106.20)	<0.001	−80.75 (−126.66, −34.85)	0.001	−2.06 (−2.57, −1.55)	<0.001
Current cigarette consumption							
Never-smokers	5,468	Ref		Ref		Ref	
Current smokers to by cigarette per day							
<10 cigarettes per day	326	−41.52 (−98.41, 15.36)	0.152	−41.99 (−112.87, 28.89)	0.246	−0.17 (−0.94, 0.60)	0.670
10 to <20 cigarette per day	775	−46.70 (−85.14, −8.26)	0.017	−30.23 (−78.12, 17.67)	0.216	−0.61 (−1.13, −0.08)	0.023
20 to <30 cigarette per day	1,251	−92.73 (−124.65, −60.82)	<0.001	−66.51 (−106.28, −26.74)	0.001	−0.94 (−1.37, −0.51)	<0.001
≥30 cigarette per day	327	−80.71 (−138.31, −23.12)	0.006	−14.84 (−86.61, 56.93)	0.685	−1.73 (−2.51, −0.94)	<0.001

FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; CI, confidence interval.

Mean difference in spirometry measures in each category of smoking exposure and the reference category (never-smokers).

**Multivariable cross-sectional analyses were adjusted for baseline covariates: age (<50/≥50), height, weight, BMI (normal/underweight/overweight/obesity), alcohol intake (never/former/current/unknown), hypertension (yes/no), diabetes (yes/no), elevated triglycerides (yes/no), elevated total cholesterol (yes/no), elevated total bilirubin (yes/no).*

TABLE 3 | Association between smoking status, duration of smoking cessation, cumulative and current cigarette consumption, and lung function decline in the longitudinal analysis.

	Number of participants (observations)	Unadjusted FEV_1 decline in mL per year (95% CI)	Unadjusted FVC decline in mL per year (95% CI)	Unadjusted FEV_1/FVC decline in % per year (95% CI)
Smoking status				
Never-smokers	1,305 (3,270)	33.99 (32.02, 35.97)	38.38 (35.90, 40.87)	0.09 (0.06, 0.11)
Former smokers	245 (593)	42.04 (36.77, 47.31)	47.36 (40.70, 54.03)	0.16 (0.08, 0.24)
Current smokers	1,718 (4,264)	39.26 (37.12, 41.40)	41.36 (38.55, 44.17)	0.16 (0.14, 0.19)
Variable smoking status	290 (808)	43.82 (39.39, 48.26)	48.34 (42.46, 54.22)	0.13 (0.06, 0.19)
Former smokers, by duration of cessation				
≥10 years	42 (113)	42.49 (29.42, 55.57)	47.31 (29.67, 64.95)	0.09 (-0.09, 0.26)
<10 years	114 (286)	42.25 (34.55, 49.96)	43.53 (33.44, 53.61)	0.24 (0.12, 0.36)
Ever-smokers, by baseline pack-years				
<10 pack-year	269 (679)	31.99 (27.29, 36.69)	36.18 (30.34, 42.02)	0.06 (0.001, 0.12)
10 to <20 pack-years	317 (843)	38.93 (33.91, 43.94)	39.43 (32.70, 46.17)	0.20 (0.13, 0.27)
20 to <30 pack-years	307 (796)	44.09 (38.51, 49.67)	51.84 (44.65, 59.04)	0.13 (0.05, 0.21)
≥30 pack-years	432 (1,142)	47.48 (41.88, 53.09)	51.62 (44.35, 58.59)	0.20 (0.12, 0.29)
Current smokers, by cigarettes per day				
<10 cigarettes per day	130 (335)	30.52 (23.84, 37.19)	30.95 (23.11, 38.79)	0.12 (0.03, 0.21)
10 to <20 cigarettes per day	305 (805)	36.78 (32.12, 41.44)	37.25 (31.32, 43.18)	0.19 (0.13, 0.25)
20 to <30 cigarettes per day	593 (1,539)	40.70 (36.93, 44.46)	43.64 (38.74, 48.55)	0.17 (0.12, 0.22)
≥30 cigarettes per day	149 (388)	49.37 (40.64, 58.09)	55.45 (43.74, 67.16)	0.15 (0.03, 0.27)

FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; CI, confidence interval.
Unadjusted mean lung function decline was estimated from a model including only age as predictors.
Unadjusted models were created separately for each stratum of the primary exposures: smoking status, duration of smoking cessation for former smokers, baseline pack-years for ever smokers and cigarette exposure for current smokers.

10–20, 20–30 and ≥30 PYs were −1.24, 4.52, 8.31, and 9.86, respectively, **Figure 1**). The adjusted effect estimate of FVC decline was significant but attenuated among participants with ≥20 PYs (estimates for exposure with 20–30 and ≥30 PYs were 11.10 and 8.36, respectively, **Figure 1**).

At the levels of current smoking intensity, current smokers with greater smoking intensity had more accelerate in FEV_1 and FVC decline (**Table 3** and **Figure 1**). The adjusted effect estimates of FEV_1 decline for those smoking ≥30 cigarettes per day (13.80, 95% CI: 6.02, 21.58, $P < 0.001$) was 2.92 times greater than that for those smoking 20–30 cigarettes per day (4.73, 95% CI: 0.94, 8.52, $P = 0.015$). Compared with never-smokers, FVC decline was accelerated by 14.17 mL per year (95% CI: 4.22, 24.12) in current smokers with ≥30 cigarettes per day (**Figure 1**).

Although there was statistical evidence of effect modification by age, height and weight ($P < 0.05$), effect sizes did not change considerably across strata of age, height and weight (**Supplementary Figure 2**). As former smokers had a higher prevalence of hypertension and diabetes, we further explored the associations stratifying by baseline diagnosis of hypertension and diabetes. For never-smokers, participants with baseline hypertension and diabetes had more accelerated FEV_1 decline than those without underlying diseases ($P = 0.008$, data not shown in **Supplementary Figure 3**). Among participants without hypertension and diabetes, current smokers had accelerated FEV_1 decline compared to never-smokers after adjustment ($P = 0.004$, data not shown in **Supplementary Figure 3**), which can also show that current smoking was an independent risk factor for lung function. Compared with never-smokers without underlying hypertension and diabetes, FEV_1 decreased more rapidly in heavy smokers (≥20 pack-years for ever-smokers and ≥20 cigarettes per day for current smokers) with underlying hypertension and diabetes ($P = 0.032$ and < 0.001, data not shown in **Supplementary Figure 3**).

Sensitivity Analysis

After excluding the participants with prevalent lung diseases, the cross-sectional associations were slightly attenuated (**Supplementary Table 3**). The same longitudinal analyses were repeated among the participants without prevalent lung diseases (**Supplementary Figure 4**). The similar mean estimates were observed in these sensitivity analyses.

DISCUSSION

Our study has documented the cross-sectional and longitudinal associations of smoking exposure and lung function in a general male population in China. Compared with never-smokers, we found that ever-smokers had a worse lung function. Current smokers, if not quit, would have an accelerated decline of lung function than former and never smokers. Furthermore, smokers with comorbid conditions, such as hypertension,

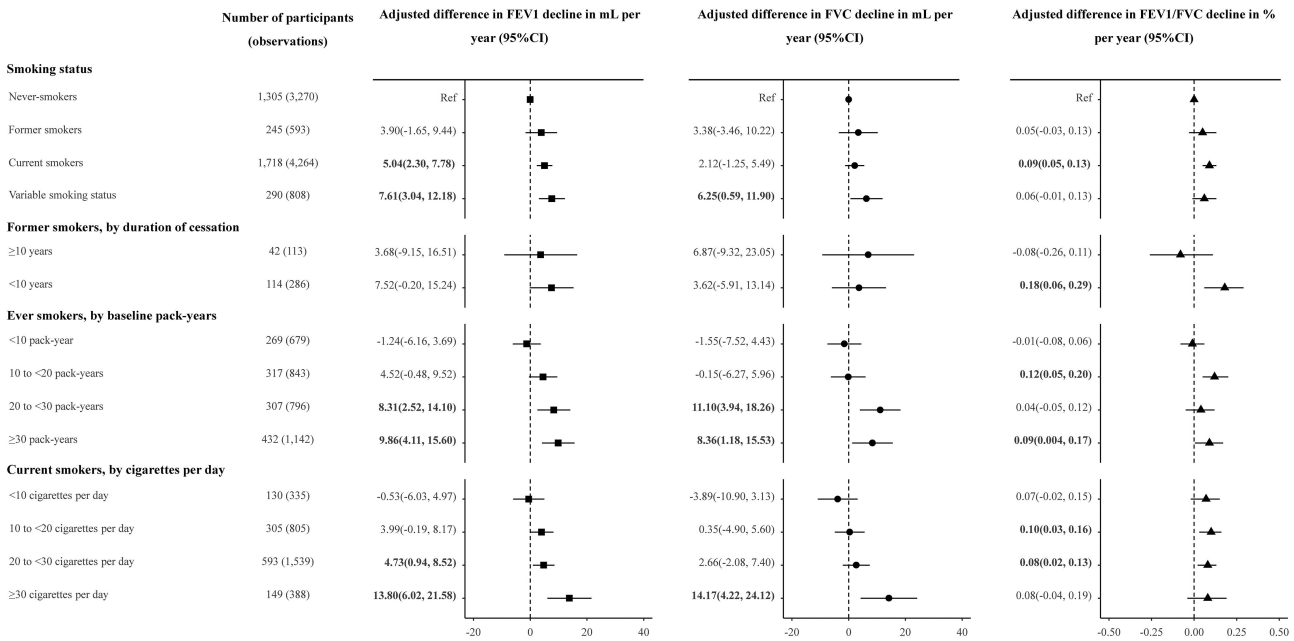

FIGURE 1 | Adjusted association between smoking status, duration of smoking cessation, cumulative and current cigarette consumption, and lung function decline in the longitudinal analysis. FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; CI, confidence interval. Linear mixed models were used to test associations with repeated measures of FEV_1, FVC and FEV_1/FVC. Participants with variable smoking status were excluded from analyses of duration of smoking cessation and of cumulative and current cigarette consumption. Adjusted effect estimates for smoking exposures, relative to never-smoking, were generated with models adjusted for the smoking parameter, age, age^2, height, weight, BMI, and alcohol consumption, hypertension, diabetes, TG, TC, total bilirubin at baseline. Multiplicative interactions with age were modeled for covariates. The effect estimate for smoking-exposure multiplied by age was interpreted as the association of the smoking exposure with annualized lung function decline.

elevated triglycerides and elevated total cholesterol, should raise more concerns about their lung health.

Evidence of significant decline of lung function in smokers has been present (21, 22). The unadjusted mean decline in FEV_1 in healthy male never-smokers in our study were similar to that in the European Community Respiratory Health Survey (22). In our cross-sectional study, former smokers had a worse lung function than never smokers, even than current smokers. An older age, with more comorbid conditions including hypertension, diabetes, and lung diseases in those former smokers may account for this phenomenon. In the longitudinal assessment, current cigarette smokers showed a more rapid decline in lung function than never-smokers and former smokers. Greater pack-years and cigarette consumptions have been associated with accelerated lung function decline in our study, which was consistent with previous study (23). Several previous studies have indicated that smoking cessation has a beneficial effect on FEV_1 decline (12, 21, 24). Our findings showed that although the former smokers had worse lung function at baseline, their annual declines in lung function were approximately identical to those of never-smokers during the follow-up. All the results above reinforce the importance of smoking cessation.

Cigarette smoking leads to numerous pulmonary and systemic immunological changes (25). Previous studies have indicated that smoking increases the number of macrophages, neutrophils, eosinophils, and mast cells in the lung, and decreases the number of airway dendritic cells, and alters macrophage and neutrophil function (26, 27). These pathways of inflammation and immunity making the lung dysregulation have been observed to be associated with smoking-related lung function decline. Additionally, smoking can decelerate the lung function along with epigenetic alterations (28), airway hyper-responsiveness (29), mucous hypersecretion (30), and altered airway dimensions (31).

One strength of the present study was that we conducted two sub-studies to evaluate the association of smoking exposure and lung function among healthy subjects in China. In addition, the dynamic data of smoking status and lung function can be collected during the follow-up. However, several limitations of this study should not be ignored. Firstly, the sample size of participant received two or more spirometry exams was relatively short. Due to lack of standard questionnaire, part of the participants did not report the detailed information of smoking exposure such as duration of cessation, cigarette consumptions. Secondly, China is both the world's largest producer and consumer of tobacco products, with 52.9% of men and 2.4% of women being current smokers in 2010 (3, 4, 6). Regarding this situation, we only restricted male subjects to samples in this study. The number of former smokers was significantly less than never-smokers and current smokers. Thirdly, despite the large sample size, the included participants were limited in the single center. Further robust epidemiological evidence and functional

study is urgently needed to better understand the biological mechanism of smoking exposure on lung function. Moreover, some potential confounders, such as physical activities, exercises and second-hand smoke exposure, cannot be collected.

In 2016, President Xi Jinping announced the Healthy China (HC2030) blueprint. According to the blueprint, a target to reduce the smoking rate among people ≥15 years of age to 20% by 2030 from the current 27.7% has been set (17, 32). To achieve this goal, more and more current smokers should participate in quitting smoking. Our data suggest that smoking cessation can slow the lung function decline even if the initial state of lung function is poor. It is essential for current smokers to quit smoking as soon as possible, especially for those with comorbidities.

CONCLUSION

Our results therefore reinforce the view that acceleration of decline in lung function must be added to the long list of negative health consequences of smoking and that smoking cessation is the most effective means of harm reduction. Our findings about the harms of current smoking also raise concerns about lung health, especially for those with comorbid conditions, which can further encourage people to quit smoking.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Data of the present research is available from the corresponding author on reasonable request. Requests to access these datasets should be directed to 906921532@qq.com.

ETHICS STATEMENT

The approval of this study was obtained from Ethics Committees at Huadong Sanatorium (No. 2020-01). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

XJ: full access to all of the data in the study, takes responsibility for the integrity of the data, and the accuracy of the data analysis. TT and CS: concept and design and obtain funding. XJ and RQ: acquisition of data. TT and YD: drafting of the manuscript and statistical analysis. CS: critical revision of the manuscript for important intellectual content. CY and XX: administrative, technical, or material support. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.843162/full#supplementary-material

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