Blood CDC42 overexpression is associated with an increased risk of acute exacerbation, inflammation and disease severity in patients with chronic obstructive pulmonary disease

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Abstract. It has been previously reported that cell division control 42 (CDC42) protein can regulate macrophage recruitment, T cell-associated inflammation and lung injury. However, its role in chronic obstructive pulmonary disease (COPD) remain poorly understood. Therefore, the present study aimed to investigate the possible association among CDC42 expression, the risk of acute exacerbation and disease features in patients with COPD. Peripheral blood mononuclear cells (PBMCs) and serum samples were collected from 60 patients with acute exacerbation COPD (AE-COPD), 60 patients with stable COPD (S-COPD) and 60 healthy control (HCs) individuals. The mRNA expression levels of CDC42 in PBMCs were then measured using reverse transcription-quantitative PCR. The serum levels of TNF-α, IL-1β, IL-6 and IL-17 were measured using ELISA. The results showed that the expression of CDC42 was dysregulated among patients with AE-COPD and S-COPD compared with that in HCs. Specifically, the expression level of CDC42 was the highest in patients with AE-COPD, followed by those with S-COPD and the lowest in HCs (P<0.001). Furthermore, receiver operating characteristic curve analysis demonstrated that CDC42 expression was associated with an increased risk of acute exacerbation in COPD with an area under curve of 0.690 (95% confidence interval=0.595-0.785). CDC42 was found to be positively associated with Global Initiative for Chronic Obstructive Lung Disease staging in patients with AE-COPD (P<0.01) and S-COPD (P<0.05). Additionally, CDC42 expression associated positively with the serum levels of TNF-α, IL-1β, IL-6 and IL-17 in patients with AE-COPD (all P<0.05). However, this association was weaker in patients with S-COPD and became negligible in HCs. In conclusion, data from the present study suggest that CDC42 is associated with an increased risk of acute exacerbation, inflammation and disease severity in patients with COPD, implicating its application as a potential biomarker for COPD.

Introduction

Chronic obstructive pulmonary disease (COPD) is a serious lung condition that is characterized by persistent respiratory symptoms and progressive airflow obstruction (1-3). COPD is the third leading cause of mortality worldwide with a global prevalence of 4.2% according to the World Health Organization (1-3). In total, 25% of patients with COPD are diagnosed into the acute exacerbation (AE)-COPD category, which is characterized by more severe respiratory impairment, increased mucus production and worse overall quality of life (4). This in turn worsens the mortality rates among patients with AE-COPD compared with patients with stable COPD (S-COPD) (4). Therefore, early diagnosis and continuous monitoring of disease progression is essential for the timely intervention against the acute exacerbation of COPD (5,6). There is a significant demand to develop novel biomarkers for COPD for predicting the risk of acute exacerbation and monitoring disease severity to implement accurate and personalized management strategies with aims of improving the prognosis of COPD.

Cell division cycle 42 (CDC42) is a key regulator of several cellular processes, including cell proliferation, division, migration, morphogenesis and establishment of epithelial polarity (7-16). It has been previously reported that CDC42 can regulate the physiology of macrophages, recruitment of T cells during inflammation and lung injury (7-16). In addition, CDC42 has been found to facilitate the recruitment, migration, adhesion and retention of macrophages through a number of signaling pathways such as mTOR-CDC42 signaling and PI3Kδ-CDC42 signaling (8-11,17). Previous studies have shown that CDC42 can inhibit the differentiation of naive T cells into Th1 and Th17 cells to further promote cytokine

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secretion and exocytosis (12,13). Furthermore, CDC42 can promote endothelial regeneration and vascular repair after inflammatory lung injury (14-16). Emerging evidence has also suggested that changes in the macrophage phenotype, recruitment of T cells associated with inflammation and lung injury can occur during COPD pathogenesis (1).

Based on these previous observation, it can hypothesized that CDC42 can possibly serve as a novel biomarker for COPD. Therefore, the present study aimed to measure the expression levels of CDC42 in blood samples derived from patients with AE-COPD and S-COPD in addition to healthy control (HCs) individuals. The objective was to evaluate the potential association of CDC42 expression with the risk acute exacerbation of COPD, inflammation and disease severity.

Materials and methods

Patient recruitment. The present study was approved by the Institutional Review Board of the Central Hospital of Wuhan. A total of 60 patients with AE-COPD and 60 with S-COPD were enrolled in the Central Hospital of Wuhan (Wuhan, China) between May 2019 and November 2020. All patients, aged >18 years, were diagnosed with COPD according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria (18). AE-COPD is characterized by the acute worsening of respiratory symptoms, resulting in the need for additional therapy. The most common symptoms of exacerbation include increased dyspnea, airway inflammation, mucus production and marked gas trapping (18). By contrast, patients with S-COPD were clinically stable for ≥3 months without exacerbations. In the present study, patients were classified into the AE-COPD or S-COPD cohorts according to disease status at first admission. The AE-COPD patients denoted those who initially came to the Central Hospital of Wuhan with symptoms of exacerbation including increased dyspnea, airway inflammation, mucus production or marked gas trapping. The S-COPD patients denoted those who initially came to the Central Hospital of Wuhan for reexamination without symptoms of exacerbation in the previous three or more months. This classification was not altered after study enrollment, regardless of any changes in disease status, to avoid overlapping the cases between both cohorts. In both patients with AE-COPD and S-COPD the exclusion criteria were as follows: i) Pregnant or lactating women; ii) patients with COPD with asthma; iii) patients with anaphylactic diseases (e.g., allergic asthma); iv) patients with other respiratory diseases (e.g., active tuberculosis); v) patients with lung cancer; or vi) patients with other malignancies (such as liver cancer, colorectal cancer, gastric carcinoma or hematologic malignancies). Additionally, 60 healthy individuals, who visited the hospital for physical examination were recruited and allocated into the HCs group during the same period. All individuals in the HCs group had no obvious abnormalities in their physical examination and no history of COPD, asthma, respiratory diseases, autoimmune diseases, inflammatory diseases or malignancies. All subjects provided written informed consent.

The mean ages of the HC group, patients with S-COPD and patients with AE-COPD were 66.1±6.4, 67.3±7.1 and 66.7±7.2 years, respectively (Table I). In terms of sex, there were 24 (40%) females and 36 (60%) males in the HC group, 18 (30%) females and 42 (70%) males in the S-COPD group and 16 (26.7%) females and 44 (73.3%) males in the AE-COPD group.

Data and blood sample collection. The demographic features and medical history of patients were recorded after enrollment. Forced expiratory volume in 1 sec (FEV_1) and forced vital capacity (FVC) were recorded following the pulmonary function test (PFT) (19). FEV_1 (% predicted) and FEV_1/FVC ratio were calculated based on the FEV_1 and FVC values. Based on the FEV_1 (% predicted), airflow obstruction severity was classified according to the GOLD criteria (https://goldcopd.org/archived-reports/) as follows: i) GOLD stage 1, FEV_1 (% predicted) ≥80%; ii) GOLD stage 2, FEV_1 (% predicted)=50-79%; iii) GOLD stage 3, FEV_1 (% predicted)=30-49%; and iv) GOLD stage 4, FEV_1 (% predicted) <30%.

In addition, peripheral blood samples were collected from all 180 participants in the present study. The serum samples and peripheral blood mononuclear cells (PBMCs) were collected by centrifugal separation and density gradient separation, respectively. In detail, peripheral blood samples were naturally coagulated for about 20 min at room temperature and centrifuged at 650 x g for 10 min using a refrigerated centrifuge (4°C) (Thermo Fisher Scientific, Inc.). The supernatant serum was collected carefully and stored at -70°C. PBMCs were separated following gradient centrifugation of the blood over Ficoll-Hypaque density gradient (Biochrom, Ltd.). PBMCs were used to measure the expression levels of CDC42 using reverse transcription-quantitative PCR (RT-qPCR). The levels of TNF-α, IL-1β, IL-6a and IL-17 secreted were assessed in the serum samples using the corresponding human ELISA kits.

RT-qPCR. RT-qPCR was performed for the quantitative analysis of CDC42 expression in PBMCs. Total RNA was extracted from PBMCs using PureZOL RNA isolation reagent (Bio-Rad Laboratories, Inc.). Subsequently, total RNA was reverse transcribed into complementary DNA using the iScript™ Reverse Transcription Supermix kit (denaturation at 65°C for 5 min, reverse transcriptase at 37°C for 5 min and inactivation at 85°C for 5 sec) (Bio-Rad Laboratories, Inc.). qPCR was performed using the TB Green Premix DimerEraser™ kit (Takara Bio, Inc.). The primer sequences used for CDC42 detection were the same as those previously reported (20). In detail, the primers for human CDC42 were: Forward, 5'-GGCGATGGTGCTGTT GGTAA-3' and reverse, 5'-GCGGTCGTAATCTGTCATAAT CCT-3'. GAPDH was used as an internal control. Primers for human GAPDH were: Forward, 5'-GAGTCCACTGGCGTC TTCAC-3' and reverse, 5'-ATCTTGAGGCTGTTGTCATAC TTCT-3'. The thermocycling conditions of qPCR were: Initial denaturation at 95°C for 30 sec, followed by 40 cycles of 95°C for 5 sec, 55°C for 30 sec and 72°C for 30 sec, which were performed in an ABI 7500 real-time PCR system. The relative CDC42 expression was calculated using the $2^{-\Delta\Delta Cq}$ method (21).

ELISA assay. Commercial ELISA kits (cat. nos. DTA00D, DLB50, D6050 and D1700; R&D Systems Inc.) were purchased to detect the serum levels of TNF-α, IL-1β, IL-6 and IL-17. All procedures, including sample and reagent preparation, assay procedure and calculation of the results were implemented according to the protocols of the kits.

Table I. Characteristics of patients with COPD and HCs.

Parameter	HCs (N=60)	Stable-COPD (N=60)	Acute exacerbation-COPD (N=60)	Statistic ($F/\chi^2/Z/H$)	P-value
Age, years	66.1±6.4	67.3±7.1	66.7±7.2	0.394	0.675
Sex				2.646	0.266
Female	24 (40.0)	18 (30.0)	16 (26.7)		
Male	36 (60.0)	42 (70.0)	44 (73.3)		
BMI, kg/m^2	22.8±2.6	22.1±2.6	22.5±3.0	0.999	0.370
Family history of COPD	10 (16.7)	20 (33.3)	17 (28.3)	4.550	0.103
History of smoking	17 (28.3)	33 (55.0)	28 (46.7)	9.095	0.011
History of hypertension	24 (40.0)	30 (50.0)	35 (58.3)	4.045	0.132
History of hyperlipidemia	16 (26.7)	14 (23.3)	15 (25.0)	0.178	0.915
History of diabetes mellitus	10 (16.7)	11 (18.3)	14 (23.3)	0.922	0.631
FEV1/forced volume vital capacity, %	82.2 (79.8-84.2)	61.3 (57.6-65.2)	60.7 (54.9-65.8)	119.447	<0.001
FEV1, predicted %	99.2 (95.7-100.6)	74.6 (57.4-82.5)	57.1 (46.6-81.4)	121.708	<0.001
Global Initiative for Chronic Obstructive Lung Disease stage				-1.980	0.048
Stage I	-	27 (45.0)	16 (26.7)		
Stage II	-	22 (36.7)	28 (46.6)		
Stage III	-	11 (18.3)	16 (26.7)		

Data were presented as N (%), the mean ± SD or median (interquartile range). COPD, chronic obstructive pulmonary disease; HCs, health controls; FEV1, forced expiratory volume in 1 second; FVC, forced volume vital capacity.

Statistical analysis. Data were presented as N (%), the mean ± standard deviation (SD) or median (interquartile range); P-value represents the significance of the results; r represents linear coefficient; H and Z represent the statistic of nonparametric rank sum test; F represents the statistic of ANOVA; χ^2 represents the statistic of chi-square test. Data analysis and graph plotting were performed using SPSS 26.0 (IBM Corp.) and GraphPad Prism 7.01 (GraphPad Software Inc.) software, respectively. Wilcoxon rank sum test, χ^2 test, one-way ANOVA, Kruskal-Wallis and Dunn's tests were used to compare the differences amongst groups. Multiple comparisons were corrected by Bonferroni's post hoc test. Spearman's rank correlation test was performed to evaluate the association between the variables. Receiver operating characteristic (ROC) curve analysis was used to evaluate the performance of the variables in distinguishing different subjects. Based on ROC analysis, the best statistical cut-off value of CDC42 expression level was calculated, which corresponded to the point at which the sum of false-positives and false-negatives was less than any other point. Sensitivity and specificity for selected cut-off points were then assessed. P<0.05 was considered to indicate a statistically significant difference.

Results

Characteristics of patients COPD and the HC group. There were no differences in terms of age and sex among the three groups (Table I). Additionally, the proportion of subjects with a history of smoking among the HC, S-COPD and AE-COPD groups varied significantly (P<0.05; Table I). FEV$_1$/FVC ratio and FEV$_1$ (% predicted) also differed significantly among the three groups (both P<0.001 vs. HC; Table I). Furthermore, there was a significant association between the distribution of GOLD stages and the proportion of patients in the S-COPD and AE-COPD groups (P<0.05; Table I). In terms of the secreted levels of inflammatory cytokines TNF-α, IL-1β, IL-6 and IL-17, they were higher in AE-COPD group compared with S-COPD group, higher in AE-COPD group compared with HC group and higher in S-COPD group compared with HC group (all P<0.05; Fig. S1).

Expression levels of CDC42 among the HC, S-COPD and AE-COPD groups. The expression levels of CDC42 varied significantly among the HC, S-COPD and AE-COPD groups (P<0.001; Fig. 1). Specifically, CDC42 expression was the highest in the AE-COPD group, followed by the S-COPD group and those in the HC group exhibited the lowest expression levels of CDC42 (Fig. 1). Comparisons between the sub-groups revealed that the expression of CDC42 was significantly higher in the AE-COPD group compared with that in the HC group (P<0.001) and that in the S-COPD group (P<0.01). Furthermore, the CDC42 expression level was higher in patients with S-COPD compared with that in the HC group (P<0.05; Fig. 1).

Additionally, ROC curve analysis was performed to assess the viability of using CDC42 expression to differentiate patients with AE-COPD to those with S-COPD. According to ROC analysis, CDC42 exhibited potential for distinguishing
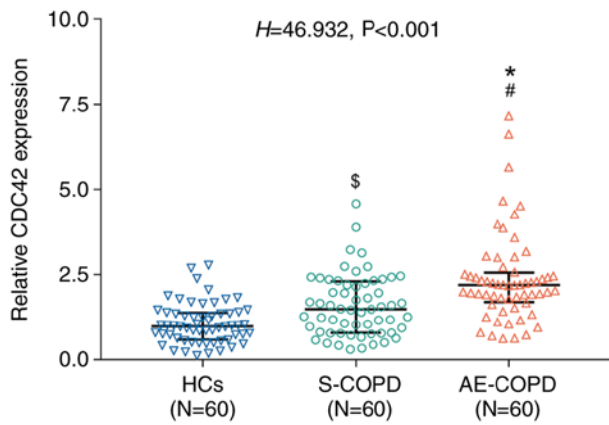
Figure 1. Comparison of CDC42 expression among HCs, patients with AD-COPD and S-COPD. CDC42, cell division cycle 42; COPD, chronic obstructive pulmonary disease; S-COPD, stable COPD; AE-COPD, acute exacerbation COPD; HCs, health controls. *P<0.05 AE-COPD vs. HCs; #P<0.05, AE-COPD vs. S-COPD; $P <0.05, S-COPD vs. HCs.
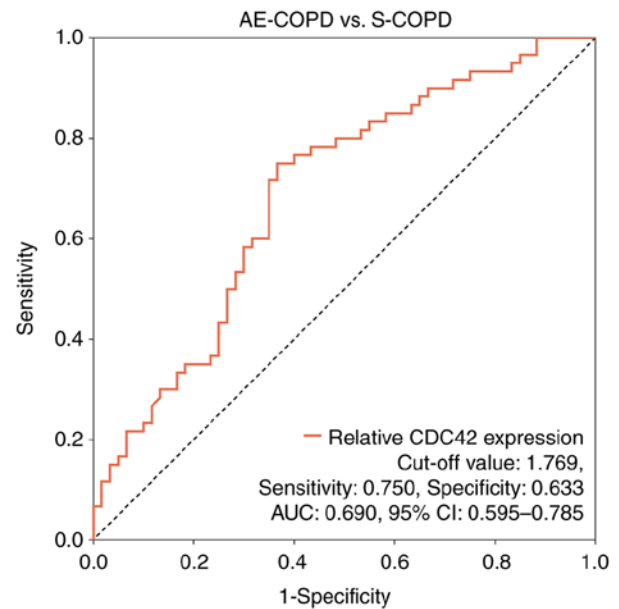

Figure 2. Receiver operating characteristic curve for the ability of differentiating patients with AE-COPD from patients with S-COPD using CDC42 expression. CDC42, cell division cycle 42; COPD, chronic obstructive pulmonary disease; AE-COPD, acute exacerbation COPD; S-COPD, stable COPD; AUC, area under curve; CI, confidence interval.

patients with AE-COPD from S-COPD, yielding an area under the curve (AUC) value of 0.690 (95% confidence interval=0.595-0.785; Fig. 2). At the optimal cut-off point, the AUC for CDC42 expression was 1.769, with a sensitivity and specificity of 0.750 and 0.633, respectively (Fig. 2).

Association of CDC42 expression with disease severity among patients with COPD. Subsequently, the potential association between CDC42 expression and COPD severity was evaluated. The results showed that CDC42 expression was highest in GOLD stage III, followed by GOLD stage II, and lowest in GOLD stage I AE COPD patients (P=0.003; Fig. 3A). Consistently, this trend was also observed in S-COPD patients (P=0.014; Fig. 3B).

Correlation between CDC42 expression and indicators of inflammation. The expression of CDC42 was found to be positively correlated with the serum levels of TNF-α (r=0.331; P<0.05), IL-1β (r=0.259; P<0.05), IL-6 (r=0.397; P<0.01) and IL-17 (r=0.472; P<0.001) in patients with AE-COPD (Fig. 4). Furthermore, CDC42 expression was positively correlated with TNF-α (r=0.377; P<0.01) and IL-17 (r=0.280; P<0.05) levels, but not with those of IL-1β and IL-6, in patients with S-COPD (Fig. S2A-D). Additionally, CDC42 expression was positively correlated with the secreted levels of TNF-α (r=0.308; P<0.05; Fig. S2E), but not with those of IL-1β, IL-6 and IL-17 in the HC group (Fig. S2F-H). Finally, if patients with S-COPD and AE-COPD were combined into one COPD group and compared with the HC group, the levels of TNF-α, IL-1β, IL-6, and IL-17 were all found to be significantly higher in patients with COPD compared with those in the HC group (all P<0.001; Table SI).

Discussion

The present study demonstrated that CDC42 expression recovered phagocytosis of alveolar macrophages and is proliferated in a mouse model of COPD (22). The present study demonstrated that the expression levels of CDC42 in patients with AE-COPD were higher compared with those in patients with S-COPD. Furthermore, CDC42 expression could distinguish

patients with AE-COPD from those with S-COPD. Previous studies demonstrated that CDC42 could serve a protective role in the lungs against inflammation-mediated vascular endothelial injury by inhibiting the p21-activated kinase/Akt pathway, whereby CDC42 expression was upregulated in the lung injury model (14,23). Additionally, lung injury serves a crucial role in the occurrence and development of COPD (1,14,23). Therefore, CDC42 expression may be increased in patients with COPD due to an as yet unknown compensatory mechanism. In addition, patients with AE-COPD typically experienced longer disease durations compared with those in patients with stable COPD, such that prolonged disease may enhance endothelial dysfunction and disease severity (24). Since CDC42 has been demonstrated to exert a protective effect against endothelial injury, CDC42 expression was higher in patients with AE-COPD compared with that in patients with S-COPD.

To date, studies on the possible association between CDC42 expression and COPD severity remain limited. In the present study, CDC42 expression in patients with AE-COPD was found to be associated with increased GOLD staging in both patients with S-COPD and those with AE-COPD. This could be due to the fact that CDC42 upregulation is associated with enhanced inflammation as a result of increased macrophage activation and promotion of Th cell differentiation (8-13).

Emerging evidence has suggested that inflammation can aggravate lung injury and is therefore positively associated with GOLD stage (4). This is may explain why increased CDC42 expression was associated with GOLD stage in patients with COPD in the present study. Based on the aforementioned findings, CDC42 expression may be increased in a compensatory manner in the lung injury model. Furthermore, lung injury could result in reduced FEV$_1$, leading to upgrading to higher GOLD COPD stages (25). Consequently, CDC42
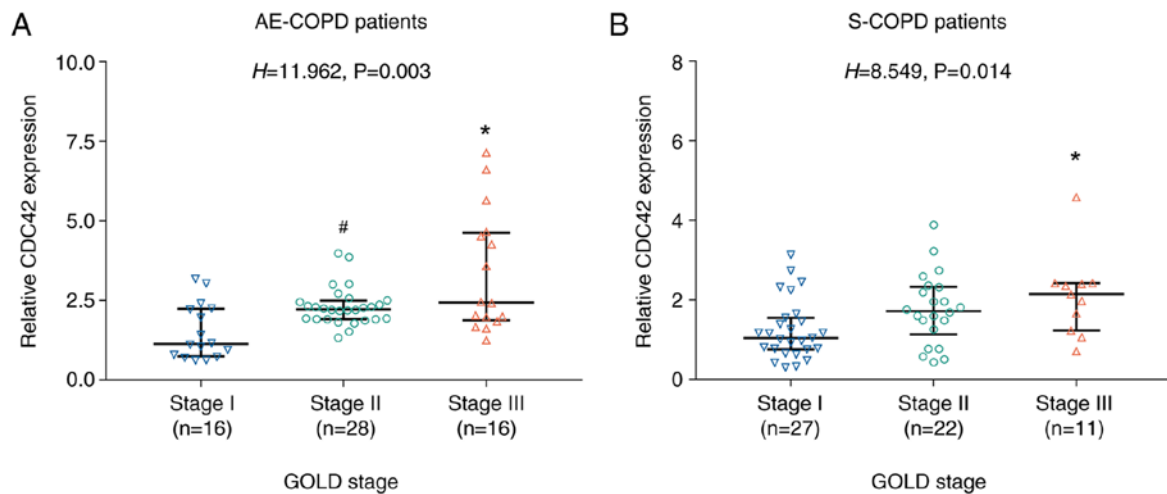
Figure 3. Association between CDC42 expression and GOLD staging. Association of CDC42 expression with GOLD staging in (A) patients with AE-COPD and (B) patients with S-COPD. CDC42, cell division cycle 42; COPD, chronic obstructive pulmonary disease; AE-COPD, acute exacerbation COPD; S-COPD, stable COPD; GOLD, Global Initiative for Chronic Obstructive Lung Disease. *$P<0.05$ Stage III vs. Stage I, #$P<0.05$ Stage II vs. Stage I.
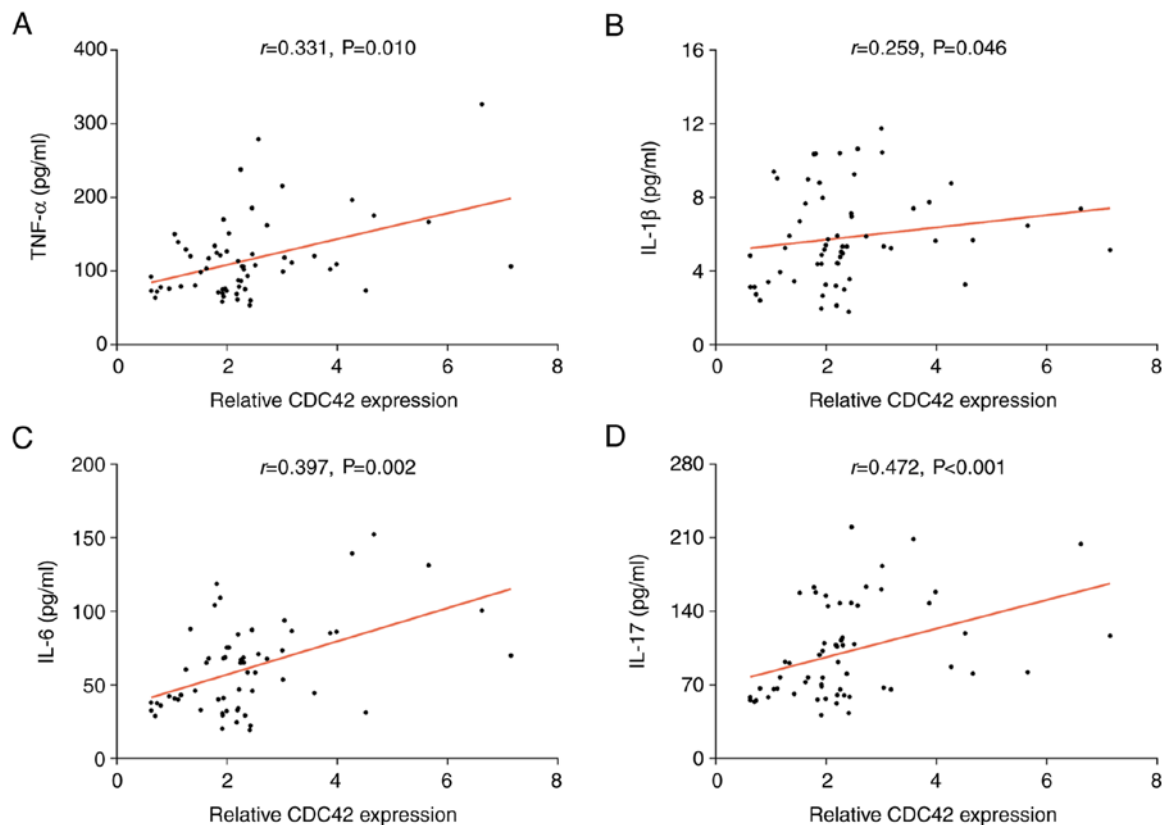

Figure 4. Correlation between CDC42 expression and each of the inflammatory cytokines. Correlation analysis of CDC42 expression with (A) TNF-α, (B) IL-1β, (C) IL-6 and (D) IL-17 in patients with acute exacerbation chronic obstructive pulmonary disease. CDC42: cell division cycle 42.

expression could be positively associated with GOLD stage in this manner.

Previous studies revealed that the expression levels of CDC42 were positively associated with the levels of inflammatory cytokines in lung injury (14,23). The present study showed that CDC42 expression in patients with AE-COPD was positively correlated with the serum levels of inflammatory cytokines TNF-α, IL-1β, IL-6 and IL-17. However, these correlations were relatively weak in patients with S-COPD, possibly owing to the fact that CDC42 could induce the differentiation of T cells into Th1 or Th17 cells to promote the recruitment, migration, adhesion and retention of macrophages (11). Furthermore, it has been previously reported that CDC42 expression is positively associated with IL-6 (primarily secreted by macrophages), TNF-α (which is primarily secreted by Th1 and Th17 cells), IL-1 (primarily secreted by Th1 cells) and IL-17 (primarily secreted by Th17 cells) (8-13,26-29).

However, the present study has several limitations. The present study only included 120 patients with COPD, which is a relatively small sample size. In addition, since the present study was a single-center study, the possibility of selection bias could not be excluded. Furthermore, the molecular mechanism underlying the effects of CDC42 on the regulation of physiological changes in macrophages, recruitment of inflammation-related T cells and lung injury in COPD should be investigated further. In the present study, the expression levels of CDC42 were only assessed at one single time point. Therefore, changes in the expression levels of CDC42 for longer periods in association with COPD progression require further investigation. The present study was designed to explore the preliminary clinical value of CDC42 in patients with COPD. To translate this into clinical practice a series of further studies in the real-world setting are required. The following types of studies should be applied: i) Multicenter studies with larger sample sizes should be performed to verify its potential clinical value; ii) a study aiming to build models for predicting AE-COPD should be performed; and iii) multi-timepoint monitoring should be conducted in future studies to reveal the value of CDC42 in monitoring COPD progression and treatment response. The present study is a case-controlled study, which lacked scheduled follow-up data in the protocol. Therefore, the potential accuracy of CDC42 as a prognostic marker require further investigation.

In conclusion, the present study revealed that CDC42 expression was associated with an increased risk of acute exacerbation, inflammation and COPD severity, which provides a potential novel biomarker for COPD.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by XM, FY and HZ. XM and HZ confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was performed in line with the principles of the Declaration of Helsinki. Ethics approval was granted by the Institutional Review Board of The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China). All individuals in the present study signed the informed consents.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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