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RESEARCH ARTICLE

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PBMC CDC42 reveals the disease activity and treatment efficacy of TNF inhibitor in patients with ankylosing spondylitis

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Abstract

Objective: Cell division cycle 42 (CDC42) regulates the polarization of M2 macrophage and maintains the T cell homeostasis, to participate in multiple autoimmune diseases, while its clinical involvement in ankylosing spondylitis (AS) remains unclear. Hence, the current study aimed to investigate the correlation of CDC42 with clinical characteristics and treatment outcome in AS patients receiving tumor necrosis factor (TNF) inhibitor therapy.

Methods: Peripheral blood mononuclear cell (PBMC) CDC42 expression was detected at baseline, week (W) 4, W8, and W12 after TNF inhibitor treatment in 91 AS patients and in 50 HCs after enrollment. Furthermore, serum TNF-α, interferon-γ (IFN-γ), interleukin-10 (IL-10), and interleukin-17A (IL-17A) from AS patients were detected at baseline.

Results: Blood CDC42 was lower in AS patients compared with HCs ($p < 0.001$). Additionally, blood CDC42 was negatively linked with CRP ($r = -0.349$, $p = 0.001$), BASDAI score ($r = -0.243$, $p = 0.020$), and $ASDAS_{CRP}$ score ($r = -0.238$, $p = 0.023$) in AS patients; however, blood CDC42 was not correlated with other clinical characteristics. Besides, CDC42 was negatively correlated with TNF-α ($r = -0.237$, $p = 0.024$) and IL-17A ($r = -0.339$, $p = 0.001$) but not with IFN-γ ($p = 0.083$) or IL-10 ($p = 0.280$). Moreover, blood CDC42 was elevated after TNF inhibitor treatment ($p < 0.001$). Meanwhile, blood CDC42 was not varied at baseline and W4 between response patients and non-response patients, while it was higher at W8 ($p = 0.019$) and W12 ($p = 0.002$) in response patients than in non-response patients after treatment.

Conclusion: Blood CDC42 deficiency links with elevated pro-inflammatory cytokines, disease activity and unsatisfying response to TNF inhibitor in AS patients.

KEYWORDS

Ankylosing spondylitis, Cell division control protein 42, Disease activity, Inflammatory cytokines, Tumor necrosis factor inhibitor

Qian Zhang and Du Jin contributed equally to this work.

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1 | INTRODUCTION

Ankylosing spondylitis (AS), characterized by inflammation, pathological osteophyte formation, and ankylosis of the axial skeleton, is a chronic inflammatory arthritis that affects 0.09%–0.3% of the population globally.[1-4] Currently, therapy includes physicotherapeutics, non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, and biological agents.[5] Interestingly, the biological agents (such as TNF inhibitor, secukinumab, and ixekizumab) exhibit to be the most effective treatment for AS patients.[5,6] However, a non-negligible subset of patients cannot response to biological agents such as TNF inhibitor.[7,8] Correspondingly, it is urgent to find molecular biomarkers to provide guidance for determining alternative treatment in the context of stratified or precision medicine; furthermore, it helps to reduce unnecessary side effects or costs as well as improve the therapeutic success for AS patients.

Cell division cycle 42 (CDC42), a member of the small Rho GTPase family, mediates actin polymerization and epithelial polarity establishment.[9,10] More importantly, CDC42 has been reported to be involved in modulating the inflammation via regulating the cellular phenotypic change of macrophage and maintaining the T cell homeostasis.[11-18] In detail, CDC42 facilitates the polarization of macrophage to M2 phenotype and decrease the release of TNF-α in multiple inflammation-related diseases.[11-14] Besides, CDC42 not only suppresses T cell differentiation into T helper 1 (Th1) and Th17 cells but also inhibits the development of autoimmunity and further modulates the corresponding cytokines' production and their exocytosis.[15-18] Additionally, it is supposed that the activation of macrophage, Th1, Th17 cells, and the recruitment of inflammation are closely involved in AS etiology.[1,2] Considering the accumulating evidence above, we hypothesized that CDC42 could probably serve as a hallmark in monitoring disease progression in AS. Therefore, the current study detected blood CDC42 expression not only at baseline, at week 4 (W4), week 8 (W8), and week 12 (W12) after treatment in AS patients but also in healthy controls (HCs) after enrollment, aiming to explore the correlation of blood CDC42 with disease characteristics and treatment response to TNF inhibitor in AS patients.

2 | MATERIALS AND METHODS

2.1 | Subjects

After being permitted by the Institutional Review Board, this prospective study consecutively enrolled 91 AS patients who were about to receive etanercept treatment between May 2018 and July 2020. The criteria for enrollment were as follows: (a) confirmed as AS according to AS classification criteria proposed by the Assessment of SpondyloArthritis international Society[19]; (b) age more than 18 years; (c) Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) >4 (based on 10 cm visual analog scale (VAS)) and Ankylosing Spondylitis Disease Activity Score with C-reactive protein ($ASDAS_{CRP}$) >2.1; (d) about to receive etanercept treatment for at least 12 weeks. The exclusion criteria were as follows: (a) with lung, kidney, liver, or heart dysfunction; (b) with contraindications to etanercept (such as combining the following conditions: infection, tuberculosis, active hepatitis B, human immunodeficiency virus (HIV), etc.); (c) concomitant with cancers or malignancies; (d) pregnant or lactating women. In addition, this study also enrolled 50 healthy subjects with matched age (18 – 50 years old) and gender (male-female ratio as 4:1) to the AS patients as healthy controls (HCs). The exclusion criteria for the AS patients were suitable for the HCs as well. All subjects provided the written informed consent.

2.2 | Collection of clinical data

After enrollment, clinical characteristics of all subjects were collected. Subsequently, disease characteristics of AS patients were evaluated using BASDAI score, Bath Ankylosing Spondylitis Functional Index (BASFI) score, total back pain score, Patient's Global Assessment of Disease Activity (PGADA) score, and $ASDAS_{CRP}$ score.

2.3 | Collection of samples

Peripheral blood (PB) samples were collected from AS patients before initiation of the treatment as well as from HCs after enrollment. Then, the PB samples of all subjects were processed by gradient density centrifugation using Ficoll Hypaque method for isolation of peripheral blood mononuclear cell (PBMC). For AS patients, PB samples were also centrifuged in a refrigerated centrifuge for separation of serum. In addition, at W4, W8, and W12 after starting the treatment, PB samples of AS patients were also collected to isolate PBMC for the further analysis. The isolated samples were stored in an ultra-low temperature freezer at −80°C until determination.

2.4 | Determination of inflammatory cytokines

The serum separated from AS patients' PB samples was used to determine inflammatory cytokines level by enzyme linked-immunosorbent assay (ELISA). The inflammatory cytokines included tumor necrosis factor-α (TNF-α) which was mainly secreted by activated monocytes/macrophages, interferon-γ (IFN-γ) which was T helper 1 (Th1) cytokine, interleukin-10 (IL-10) which was T helper 2 (Th2) cytokine, and interleukin-17A (IL-17A) which was T helper 17 (Th17) cytokine. ELISA was carried out using commercial human ELISA kits (Bio-Techne China Co. Ltd, Shanghai, China), and the procedures were performed strictly referring to complete kit instructions. Additionally, each detection was performed with three replicates in the current study.

2.5 | Determination of CDC42

The PBMC isolated from all subjects' PB samples was used to determine the expression of CDC42 by reverse transcription quantitative

polymerase chain reaction (RT-qPCR) assay. In detail, the total RNA was extracted with RNeasy Protect Mini Kit (Qiagen, Duesseldorf, Nordrhein-Westfalen, Germany). Besides, complementary DNA was synthesized using iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, California, USA). Meanwhile, qPCR was performed applying KOD SYBR® qPCR Mix (Toyobo, Osaka, Kansai, Japan). Additionally, Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal reference for CDC42; meanwhile, the procedure of qPCR was referred to previous research.[20] Then, $2^{-\Delta\Delta Ct}$ method was applied to calculate CDC42 relative expression. The primers for human CDC42 were forward, 5'- GGCGATGGTGCTGTTGGTAA-3' and reverse, 5'- GCGGTCGTAATCTGTCATAATCCT-3'. Meanwhile, primers for human GAPDH were forward, 5'- GAGTCCACTGGCGTCTTCAC-3' and reverse, 5'- ATCTTGAGGCTGTTGTCATACTTCT-3'. Additionally, each detection was performed with three replicates in the current study.

2.6 | Treatment and assessment

All patients were administered with etanercept (Enbrel, Pfizer, Brooklyn, New York, USA) at a dose of 25 mg through subcutaneous injection twice a week for 12 weeks. In the course of treatment, the efficacy was evaluated at W2, W4, W8, and W12 with the Assessment SpondyloArthritis international Society (ASAS) 40 response which was defined in accordance with a previous study.[19] Based on the assessment of ASAS40 response at W12, the AS patients were classified as response patients and non-response patients.

2.7 | Statistical analysis

Statistical analysis and graph plotting were respectively completed using SPSS 24.0 (IBM Corp., Armonk, New York, USA) and GraphPad

TABLE 1 Clinical characteristics

Items	HCs (N = 50)	AS (N = 91)	*P* value
Age (years), mean±SD	36.2±9.0	35.7±9.0	0.752
Gender, No. (%)			0.207
Male	40 (80.0)	80 (87.9)	
Female	10 (20.0)	11 (12.1)	
CRP (mg/L), median (IQR)	3.8 (2.4–7.5)	25.5 (18.8–40.1)	<0.001
ESR (mm/H), median (IQR)	6.0 (2.5–11.6)	30.3 (18.2–42.2)	<0.001
HLA-B27, No. (%)			
Negative	-	11 (12.1)	-
Positive	-	80 (87.9)	-
Disease characteristics, mean±SD			
Disease duration (years)	-	5.8±3.3	-
BASDAI score	-	6.2±1.0	-
BASFI score	-	5.0±1.1	-
Total back pain score	-	5.8±1.4	-
PGADA score	-	6.1±1.4	-
$ASDAS_{CRP}$ score	-	3.9±0.9	-
Inflammatory cytokines, median (IQR)			
TNF-α (pg/mL)	-	58.9 (45.0–88.2)	-
IFN-γ (pg/mL)	-	80.9 (63.0–108.1)	-
IL–10 (pg/mL)	-	70.6 (53.4–98.0)	-
IL–17A (pg/mL)	-	111.9 (71.2–139.0)	-
History of TNF inhibitor, No. (%)			
No	-	62 (68.1)	-
Yes	-	29 (31.9)	-

Note: HCs, healthy controls; AS, ankylosing spondylitis; SD, standard deviation; CRP, C-reactive protein; IQR, interquartile range; ESR, erythrocyte sedimentation rate; HLA-B27, human leukocyte antigen-B27; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; PGADA, Patient Global Assessment of Disease Activity; $ASDAS_{CRP}$, Ankylosing Spondylitis Disease Activity Score with C-reactive protein; TNF-α, tumor necrosis factor-α; IFN-γ, Interferon-γ; IL-10, interleukin-10; IL-17A, interleukin-17A; TNF, tumor necrosis factor.

Prism 6.01 (GraphPad Software Inc., San Diego, California, USA). Comparison between groups was examined by Student's t test, Wilcoxon rank sum test, and chi-square test. Correlation between CDC42 expression and clinical characteristics was determined by Spearman's rank correlation test and Wilcoxon rank sum test. Changes in CDC42 expression over time were analyzed by the Friedman test. A *P* value less than 0.05 was considered statistically significant.

3 | RESULTS

3.1 | Clinical characteristics

There was no difference between the HCs and AS patients regarding age and gender (both $p > 0.05$, Table 1). In detail, the mean ages were 36.2 ± 9.0 years and 35.7 ± 9.0 years in HCs and AS patients, respectively; meanwhile, there were 40 (80.0%) males and 10 (20.0%) females in HCs, as well as 80 (87.9%) males and 11 (12.1%) females in AS patients. In addition, median CRP were 3.8 (inter quartile range (IQR) 2.4 – 7.5) mg/L and 25.5 (IQR 18.8 – 40.1) mg/L in HCs and AS patients, respectively ($p < 0.001$); meanwhile, median ESR were 6.0 (IQR 2.5 – 11.6) mm/H and 30.3 (IQR 18.2 – 42.2) mm/H in HCs and AS patients, separately ($p < 0.001$). In regards to human leukocyte antigen-B27 (HLA-B27), 80 (87.9%) patients were positive, meanwhile, 11 (12.1%) patients were negative. As for disease characteristics, disease duration, BASDAI score, BASFI score, total back pain score, PGADA score, and $ASDAS_{CRP}$ score were 5.8 ± 3.3 years, 6.2 ± 1.0, 5.0 ± 1.1, 5.8 ± 1.4, 6.1 ± 1.4, and 3.9 ± 0.9, respectively. More detailed information on AS disease characteristics is also listed in Table 1.

3.2 | Comparison of blood CDC42 expression between HCs and AS patients

The median blood CDC42 expressions in HCs and AS patients were 0.998 (IQR 0.731 – 1.428) and 0.365 (IQR 0.260 – 0.731), separately, which showed that blood CDC42 expression was lower in AS patients compared with HCs ($p < 0.001$, Figure 1).

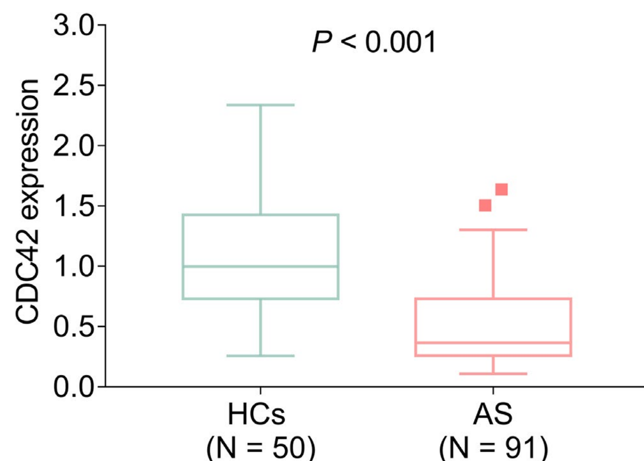

FIGURE 1 CDC42 expression was declined in AS patients compared with controls. HCs, healthy controls; AS, ankylosing spondylitis; CDC42, cell division cycle 42.

3.3 | Correlation of blood CDC42 expression with clinical features and inflammatory cytokines in AS patients

Blood CDC42 expression was negatively linked with CRP ($r = -0.349$, $p = 0.001$), BASDAI score ($r = -0.243$, $p = 0.020$) and $ASDAS_{CRP}$ score ($r = -0.238$, $p = 0.023$) in AS patients; however, blood CDC42 expression was not correlated with other clinical characteristics such as age, ESR, disease duration, BASFI score, total back pain score, PGADA score, $ASDAS_{CRP}$ score, gender, HLA-B27, and history of TNF inhibitor (all $p > 0.05$) (Table 2 and Table 3).

TABLE 2 Correlation of CDC42 expression with clinical characteristics (continuous variables) in AS patients

Items	Spearman *r* value	*P* value
Age	0.064	0.547
CRP	−0.349	**0.001**
ESR	−0.175	0.098
Disease duration	−0.117	0.269
BASDAI score	−0.243	**0.020**
BASFI score	−0.152	0.151
Total back pain score	−0.168	0.111
PGADA score	−0.160	0.129
$ASDAS_{CRP}$ score	−0.238	**0.023**

Note: CDC42, cell division control protein 42; AS, ankylosing spondylitis; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; PGADA, Patient Global Assessment of Disease Activity; $ASDAS_{CRP}$, Ankylosing Spondylitis Disease Activity Score with C-reactive protein.

TABLE 3 Correlation of CDC42 expression with clinical characteristics (categorical variables) in AS patients

Items	CDC42 expression, median (IQR)	*P* value
Gender		0.149
Male	0.429 (0.268–0.742)	
Female	0.275 (0.229–0.520)	
HLA-B27		0.327
Negative	0.432 (0.308–0.715)	
Positive	0.331 (0.250–0.742)	
History of TNF inhibitor		0.711
No	0.409 (0.270–0.599)	
Yes	0.307 (0.254–0.861)	

Note: CDC42, cell division control protein 42; AS, ankylosing spondylitis; IQR, interquartile range; HLA-B27, human leukocyte antigen-B27; TNF, tumor necrosis factor.

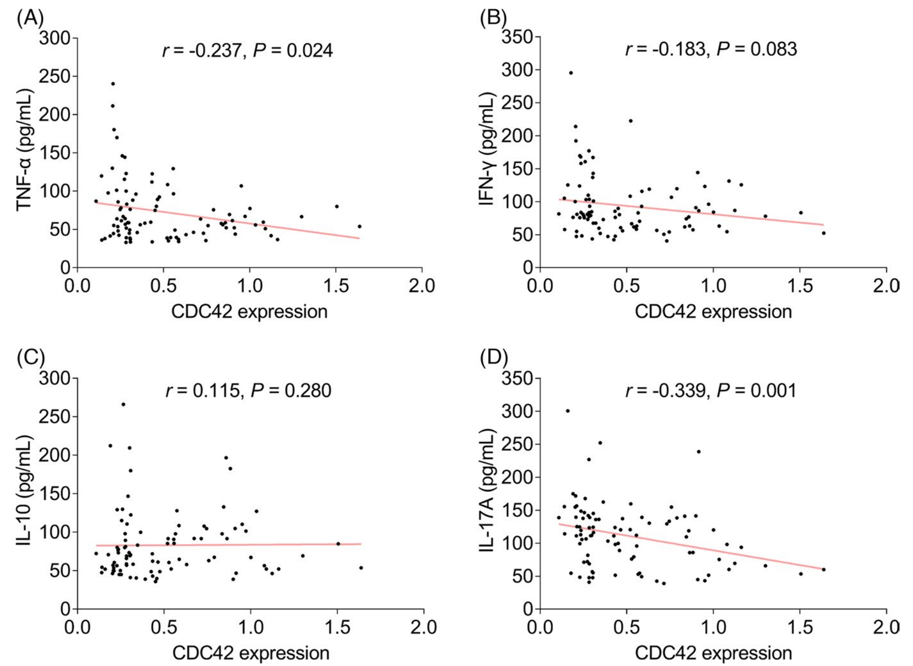

FIGURE 2 Correlation between blood CDC42 level and inflammatory cytokines in AS patients. Correlation of CDC42 level with TNF-α (A), IFN-γ (B), IL-10 (C) and IL-17A (D). TNF-α, tumor necrosis factor-α; IFN-γ, Interferon-γ; IL-10, interleukin-10; IL-17A, interleukin-17A

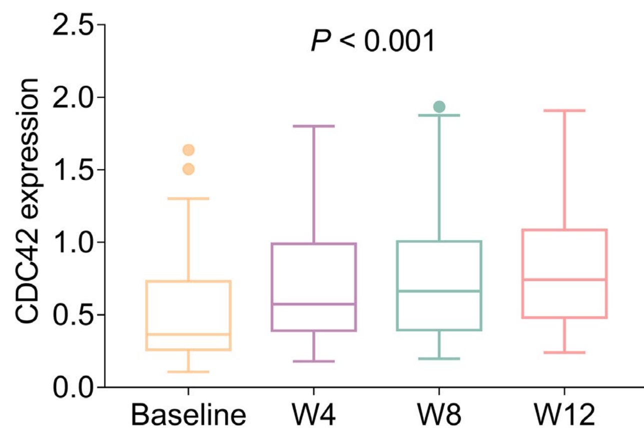

FIGURE 3 Blood CDC42 expression at baseline, W4, W8, and W12 in AS patients. W, week; CDC42, cell division cycle 42.

In terms of inflammatory cytokines, blood CDC42 expression was negatively correlated with TNF-α ($r = -0.237$, $p = 0.024$) and IL-17A ($r = -0.339$, $p = 0.001$); whereas, blood CDC42 expression was not associated with IFN-γ ($r = -0.183$, $p = 0.083$) or IL-10 ($r = 0.115$, $p = 0.280$) (Figure 2A-D).

3.4 | Blood CDC42 expression after treatment and its association with ASAS40 response rate

Blood CDC42 expression was measured at baseline, W4, W8, and W12 after the initiation of TNF inhibitor treatment, which disclosed that blood CDC42 expression was elevated gradually during the TNF inhibitor treatment ($p < 0.001$, Figure 3). Moreover, ASAS40 response rate at W2, W4, W8, and W12 after the initiation of TNF inhibitor treatment were 17 (18.7%), 30 (33.0%), 43 (47.3%), and 52 (57.1%), separately (Figure 4A). In addition, blood CDC42 was not varied at baseline ($p = 0.532$) between response patients and non-response patients, while blood CDC42 expression was higher at W8 ($p = 0.019$) and W12 ($p = 0.002$) in response patients compared with non-response patients after the initiation of treatment, respectively (Figure 4B). Whereas, there was no difference of blood CDC42 expression at baseline and W4 after the initiation of treatment between non-response patients and response patients.

4 | DISCUSSION

CDC42 is dysregulated in several immune- and inflammation-related diseases such as inflammatory bowel diseases (IBD).[21-24] Interestingly, IBD shares part of similar pathogenesis of AS meanwhile it is known that around 40% of AS patients have subclinical bowel inflammation.[25-27] Whereas, no evidence reveals the blood CDC42 expression in AS patients. The present study exhibited that blood CDC42 expression was lower in AS patients compared with HCs. Possible explanations could be that (1) CDC42 suppresses the immune response via regulating T cell receptor, therefore, reduces the systemic inflammation. More importantly, abnormal recruitment of inflammation is linked with the initiation of the AS; thus, CDC42 downregulation reveals the initiation of AS.[28,29] (2) CDC42 regulates the differentiation of the osteoclasts and osteoblasts, further maintain the function of bone modeling and remodeling. Thus, the downregulation of CDC42 might be correlated with the initiation of the AS.[30-33]

Apart from the aberrant expression of CDC42 in inflammation-mediated diseases, its correlation with disease activity and inflammation is also of great interest. To be specific, previous studies reveal that elevated CDC42 is negatively correlated with the Crohn's disease Activity Index and recruitment of inflammatory cytokines

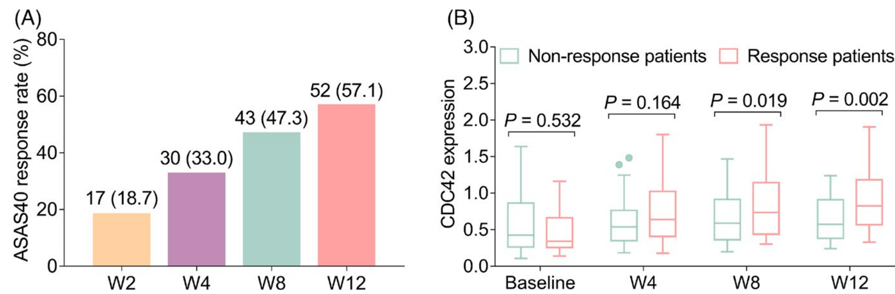

FIGURE 4 ASAS40 response rate and the correlation of blood CDC42 expression with treatment response in AS patients. ASAS40 response rate at W2, W4, W8, and W12 after the initiation of TNF inhibitor treatment (A). Comparison of CDC42 expression at baseline, W4, W8, and W12 after the initiation of TNF inhibitor treatment between non-response patients and response patients (B). W, week; CDC42, cell division cycle 42; ASAS40, at least a 40% improvement and an absolute improvement from baseline of at least 2 units (range 0–10) in three or more of four domains (patient global, spinal pain, function, and inflammation) without any worsening in the remaining domain; ASAS, Assessment of SpondyloArthritis international Society

in inflammatory bowel disease.[28,29] Whereas, no study explores the clinical relevance of CDC42 with clinical features and inflammatory cytokines in AS patients. In the current study, blood CDC42 expression was negatively correlated with CRP, BASDAI score, $ASDAS_{CRP}$ score, TNF-α, and IL-17, whereas blood CDC42 expression was not associated with other clinical features or cytokines. Possible explanations could be that (1) CDC42 facilitates macrophage polarized to M2 phenotype and inhibits the differentiation of $CD4^+$ T cells into Th17 cells, which leads to a negative correlation of CDC42 with their secreted cytokines (in detail: IL-17A was mainly secreted by Th17 cells and TNF-α was mainly secreted by macrophage) in AS patients.[11-18] (2) Based on the evidence above, CDC42 inhibits immune response through multiple mechanisms; therefore, CDC42 leads to reduced disease activity in AS patients.[22-24,28,29]

It is vital to identify the potential indicators for monitoring disease progression and treatment response to biological agents such as TNF inhibitor in AS patients. Whereas, no previous study investigates the correlation of blood CDC42 with the treatment response to TNF inhibitor in AS patients. In the current study, we observed that blood CDC42 expression was elevated during TNF inhibitor treatment in AS patients. Besides, elevated blood CDC42 was correlated with more satisfying treatment efficacy of TNF inhibitor in AS patients. The possible explanation is as follows: Based on the evidence above, increased CDC42 is correlated with reduced inflammation status. What is more, inflammation status is alleviated in patients who had treatment response to TNF inhibitor. Subsequently, CDC42 is elevated during treatment in response patients.

Some limitations existed in the present study. (1) The sample size was relatively small, which might decrease the statistical power. (2) The underlying role of CDC42 in pathogenesis of AS needed further exploration. (3) The age- and gender-matched disease control such as patients with non-inflammatory arthritic diseases should be enrolled in the forthcoming study. (4) The forthcoming study should evaluate the CDC42 expression in synovium or fluid from articular cavity. (5) The normal range of CDC42 also needed to be further investigated. (6) The IL-4 level is not detected in the current study. (7) What kind of events regulated the level of CDC42 remained unexplored in the current study? (8) The correlation of CDC42 level during anti-IL-17 therapy also needed further exploration. (9) The correlation of radiological change with CDC42 expression was not assessed due to the relatively short follow-up.

Collectively, blood CDC42 deficiency links with elevated pro-inflammatory cytokines, disease activity, and unsatisfying response to TNF inhibitor in AS patients.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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