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# Correlation analysis of serum Rac1 level with asthma control, airway inflammatory response and lung function in asthmatic children

Hui Fu<sup>1</sup> and Yun Gao<sup>2\*</sup>

## Abstract

**Objective** To investigate the correlation between serum Rac1 enzyme (Rac1) level with asthma control, airway inflammatory response and lung function in asthmatic children.

**Methods** A retrospective analysis was performed on 79 children with asthma who were diagnosed and treated in our hospital from June 2020 to January 2023. According to the severity of the disease, the children were divided into mild group (25 cases), moderate group (30 cases) and severe group (24 cases). 36 healthy children who underwent physical examination at the same period in our hospital were selected as the control group. The state of an illness, control level, serum mRNA Rac1, inflammatory factors, and lung function of the children in two groups were compared between the control group and the observation group.

**Results** The Rac1 mRNA levels, forced vital capacity (FVC), forced expiratory volume in one second/FVC (FEV1/FVC), peak expiratory flow (PEF), and maximum mid-expiratory flow (MMEF) in the observation group were significantly lower than these in the control group ( $P < 0.05$ ). The tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-5 (IL-5), IL-6, and IL-33 in the observation group were markedly higher than these in the control group ( $P < 0.05$ ). As the state of an illness worsened, the Rac1 mRNA levels, FVC, FEV1/FVC, PEF, and MMEF gradually reduced ( $P < 0.05$ ), while the levels of TNF- $\alpha$ , IL-5, IL-6, and IL-33 increased ( $P < 0.05$ ). As the degree of disease control improved, the Rac1 mRNA levels, FVC, FEV1/FVC, PEF, and MMEF gradually elevated ( $P < 0.05$ ), and the levels of TNF- $\alpha$ , IL-5, IL-6, and IL-33 showed the opposite trend ( $P < 0.05$ ). Rac1 was negatively related to the levels of TNF- $\alpha$ , IL-5, IL-6 and IL-33 ( $P < 0.05$ ), and positively to the levels of FVC, FEV1/FVC, PEF and MMEF ( $P < 0.001$ ). Rac1 mRNA levels, FVC, FEV1/FVC, PEF and MMEF were protective factors, while TNF- $\alpha$ , IL-5, IL-6 and IL-33 were risk factors for the prognosis of children with asthma ( $P < 0.05$ ).

**Conclusion** Children with asthma have obviously lower serum Rac1 mRNA levels, higher inflammatory factor levels and lower lung function. Serum Rac1 mRNA level may be associated with better asthma control, lower airway inflammatory response, better lung function and lower disease severity. It has important reference value for the evaluation of the state of an illness, efficacy and prognosis of children with bronchial asthma.

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**Keywords** Asthma, Rac1, Airway inflammatory response, Lung function, Disease control

## Introduction

Asthma is a heterogeneous and complex disease with the characteristics of wheezing dyspnea, expired congestion, cough, and chest compression. More than 300 million people suffer from asthma worldwide, which seriously affects the life quality of patients [1]. Airway hyper reactivity (AHR), changes in airway wall structure, and chronic inflammation are important features of asthma. When asthma occurs, the lung function and the regulatory T cells will be decreased in patients, and the respiratory function patterns of patients will also be changes, thereby exacerbating the progression of the disease [2]. Chronic airway inflammation, lung function decline, and structural changes in airway wall are main pathological features of bronchial asthma. Airway remodeling is manifested as goblet cell hyperplasia, thickening of basement membrane, angiogenesis and hypertrophy and hyperplasia of airway smooth muscle cell. The degree of remodeling correlates with the severity of asthma and the degree of airflow obstruction. Clinically, it is mainly treated by anti-inflammatory therapies (including inhaled and oral corticosteroids), as well as by inhaled bronchodilators [3, 4]. However, there is currently no cure for asthma.

Ras-related C3 botulinum toxin substrate 1 (Rac1) is a kind of small guanosine triphosphate (GTP) enzyme associated with Rho, which is commonly expressed in various cell types and participates in the cell differentiation and growth regulation [5]. Rac1 has been shown to be a necessary factor for the activation of JAK/STAT pathway mediated by growth factor receptors and G protein coupled receptors. The expression level of Rac1 is abnormally up-regulated in the lung tissue of asthma patients, which promotes the proliferation of human airway smooth muscle cells induced by growth factor. Therefore, Rac1 is defined as the signal network center that controls the proliferation of airway smooth muscle cells [6]. Besides, Rac1 can regulates the epithelial barrier function, thus, plays a key role in the migration, diffusion, and contraction of airway smooth muscle cells [7]. WAN J et al. [8] find that the downregulation of Rac1 in asthma may promote airway epithelial cell apoptosis and affect the clearance of apoptotic cells, which leads to the accumulation of apoptotic cells in the respiratory tract and participation in airway hyperresponsiveness. It is thus speculated that Rac1 plays an integral role in the phagocytic clearance of apoptotic cells by airway epithelial cells and the formation of an anti-inflammatory environment. Rac1 expression can strengthen devouring activity or way of anti-inflammatory function, which can be used as a treatment strategy for allergic airway inflammation. In the process of pulmonary inflammation, the

infiltration of inflammatory cells, such as neutrophils, monocytes, and lymphocytes, is an important reason for the exacerbation of inflammatory response. Rac1 has also been found to inhibit the infiltration of inflammatory cells by regulating intracellular signaling pathways [9], which provides a new idea for the control of pulmonary inflammation. Based on these above research, it can be concluded that Rac1 that has beneficial effects on bronchoconstriction and pulmonary inflammation may be a therapeutic target for asthma. However, the relationship between asthma control level, airway inflammation response, lung function with Rac1 is still unclear.

In this study, children with asthma who were diagnosed and treated in our hospital from June 2020 to January 2023 were selected as the research objects. According to their condition and asthma control, the children were divided into different groups to explore the correlation between serum Rac1 level with asthma control, airway inflammatory response and lung function in asthmatic children, thereby providing a reference for clinical diagnosis and treatment.

## Research subjects

### General material

A total of 79 children with asthma who were diagnosed and treated in our hospital from June 2020 to January 2023 were selected as the observation group. The clinical medical records were retrospectively analyzed, and the inclusion process was shown in Fig. 1.

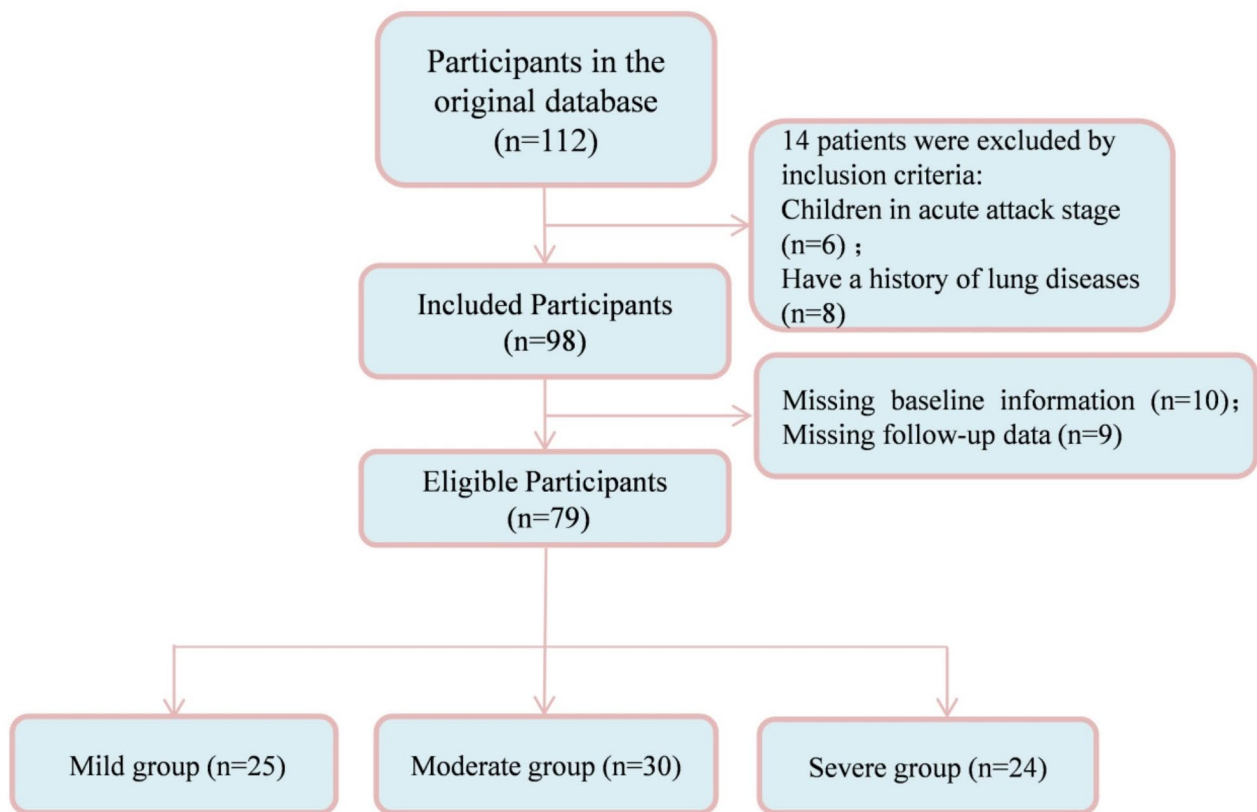
Inclusion criteria: (1) Children met the diagnostic and treatment guidelines for asthma [10]; (2) Children did not receive glucocorticoid treatment 24 h before participating in the study; (3) The family members of the affected child had informed consent regarding the research process and precautions. Exclusion criteria: (1) Children with disease in the acute phase; (2) Children with concomitant respiratory system diseases; (3) Children complicated with autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis; (4) Children with lung function disorders such as tracheal foreign bodies and congenital lung diseases; (5) Children with a history of pulmonary diseases.

36 healthy children received physical examination during the same period in our hospital were taken as the control group. This study was ratified by the hospital Ethics Committee and complied with Medical Ethics.

## Methods

### Grouping method

The severity of asthma [11]: Mild: The frequency of symptom occurrence was more than or equal to once a week,



**Fig. 1** The inclusion process of the children

and activity and sleep might be affected; The frequency of nocturnal asthma symptoms was more than twice/month, and the forced expiratory volume in one second (FEV1)  $\text{pred} \geq 70\%$ , with a peak expiratory flow variability (PEF) or FEV1 variability rate of 20–30%. Moderate: The symptom occurred everyday, and the activity and sleep were obviously affected; The frequency of nocturnal asthma symptoms was more than or equal to twice/week, and the estimated FEV1 value or personal optimal PEF value was  $60\% \sim 79\%$ ,  $60\% \leq \text{FEV1\% pred} \leq 69\%$ . Severe: The symptom occurred everyday; The nocturnal asthma symptoms was frequently appeared, and the physical activity was greatly affected; The personal optimal PEF value was  $< 60\%$ , with a PEF or FEV1 variability rate  $> 30\%$ ,  $35\% \leq \text{FEV1\% pred} \leq 49\%$ . According to the severity of the disease, these children were divided into the mild group (25 cases), the moderate group (30 cases) and the severe group (24 cases).

Therapeutic methods [12]: Mild: Assess the frequency of using SABA to control symptoms for poor symptom control in the past 4 weeks  $\geq$  twice/month; The frequency of holding awake at night  $\geq$  once/month; The risk of acute attacks (At least one acute episode requiring corticosteroids (OCS), emergency or hospitalization in the past year). Moderate: The duration of poor symptom control after the aforementioned treatment lasted for  $\geq 4$  weeks;

The frequency of using SABA to control symptoms was  $\geq 2$  times/week (but not every day); The frequency of acute episodes requiring OCS treatment in the past year was more than twice. Severe: the duration of poor symptom control after the aforementioned treatment lasted for 4–6 weeks; Severe acute attacks occurred when using level 3 treatment, requiring OCS, emergency or hospitalization.

Asthma control level [13]: Asthma Control Test (ACT) was used to evaluate children who had been treated for 3 months (as all subjects included in this study were children, thus the scales were answered by accompanying family members). This scale included 5 questions. 1: How many times in the past 4 weeks had asthma hindered your daily activities during work, study, or home? 2: How many times had you had difficulty breathing in the past 4 weeks? 3: How many times in the past 4 weeks had you woken up at night or woke up earlier than usual due to asthma (wheezing, coughing, difficulty breathing, chest tightness or pain)? 4: How many times had you used emergency medication for treatment in the past 4 weeks? 5: How did you evaluate your asthma control over the past 4 weeks? The above questions were scored on a scale of 1–5 points based on their severity. The total score added up to a maximum of 25 points, indicating

complete control, 20–24 points indicating partial control, and <24 points indicating uncontrolled.

#### Outcome measures and detection methods [14]

The Rac1 mRNA levels of the children were detected using quantitative RT-PCR method: 10 ml elbow vein blood was collected from all subjects on an empty stomach and was kept in a vacuum anticoagulant collection tube. After the supernatant was separated, single nuclear cells were separated to form a cell suspension using Ficoll density gradient separation method. The primer sequences of Rac1 were as follows: sense: 5'-GGACCCG GTGCCTCAGGA-3' and anti-sense: 5'-CAAAGATGG TCACGGTCTGC -3'. *GAPDH* was acted as a reference gene. The amplification program was listed as follows: pre-denaturation at 95 °C for 3 min; 94 °C denaturation for 30 s, annealing for 40 s, 72 °C extension for 1 min, 35 cycles; Extending at 72 °C for 10 min at the end. The amplified products were electrophoretic in 1.5% agarose gel, and the bands were scanned in the gel imaging analysis system. to calculate the test gene was calculated using relative quantitative analysis method ( $2^{-\Delta\Delta ct}$ ). The measurement was repeated triple and the average value was taken.

**Serum indicators:** according to the clinical medical records, the fasting venous blood of each subject was collected on the second day after admission, and centrifuged at 3000 r/min for 10 min. The serum was warily collected, and stored at -40°C to avoid repeated freeze-thaw cycles. Inflammation related indicators including tumor necrosis factor (TNF)- $\alpha$ , Interleukin-5 (IL-5), IL-6 and IL-33 were examined using enzyme-linked immunosorbent assay (ELISA). Result determination: The result could be observed with the naked eye on a white background: The darker color indicated stronger positivity. Colorless or extremely light indicated negative reaction. Results could also be determined by detecting OD values: On the ELISA detector, the OD values of each well were measured at 450 nm after zeroing the blank control well.

**Lung function:** A pulmonary function detector was employed to detect the lung function of children on the second day after admission. The subjects were instructed to maintain an upright position with their heads at a natural level. The AS-507 lung function detector supplied by the Meineng was used to measure the subjects' lung function indicators, including forced vital capacity (FVC), FEV1/FVC, PEF, and MMEF levels. The measurement was repeated triple and the average value was taken. The data were expressed as a percentage of the measured value to the predicted value, i.e., FVC%pred, FEV1/FVC, PEF%pred, MMEF%pred.

#### Statistical analysis

SPSS 24.0 software was used for statistical data analysis. Enumeration data, including the gender, were presented as [cases (%)] and compared using  $\chi^2$  test. The measurement data, including Rac1 mRNA levels, airway inflammation, and lung function indicators, were all presented in the form of ( $\bar{x} \pm s$ ). Comparison between two groups and among multiple groups were proceeded using independent sample t-tests and one-way repeated measures ANOVA, respectively. LSD-t-tests were used for pairwise comparisons. The Pearson correlation method was used for inter group variable correlation analysis. In this study, statistical results  $P < 0.05$  were considered as statistically significant differences.

#### Results

##### General data comparison

There were 16 cases of male and female 9 cases in the mild group with the height of ( $110.65 \pm 6.89$ ) cm and the average age of  $5.64 \pm 1.19$  years old, there were 19 males and 11 females in the moderate group with the height of ( $111.35 \pm 7.02$ ) cm and the average age of  $6.27 \pm 1.80$  years old, and there were 14 males and 10 females in the severe group with the height of ( $109.87 \pm 8.45$ ) cm and the average age of  $5.82 \pm 1.55$  years old. According to the results of the Asthma Test Control (ACT) questionnaire, children were divided into the fully controlled group ( $\geq 25$  points) with 42 cases, the partially controlled group (20–24 points) with 25 cases, and the uncontrolled group ( $< 20$  points) with 12 cases. There were 28 males and 14 females in the fully controlled group with the height of ( $112.06 \pm 5.44$ ) cm and the average age of  $5.72 \pm 1.58$  years old, there were 14 males and 11 females in the partially controlled group with the height of ( $110.97 \pm 7.05$ ) cm and the average age of  $6.01 \pm 1.70$  years old, and there were 7 males and 5 females in the uncontrolled group with the height of ( $111.66 \pm 6.47$ ) cm and the average age of  $5.93 \pm 1.82$  years old. There were 22 males and 14 females in the control group with the height of ( $110.87 \pm 7.92$ ) cm and the average age of  $6.02 \pm 1.73$  years old. There was no significant difference in the general data of each group ( $P > 0.05$ ).

##### Analysis of serum Rac1, airway inflammation, and lung function between two groups

The Rac1 mRNA levels, FVC levels, FEV1/FVC levels, PEF levels and MMEF levels in the observation group were significantly lower than these in the control group ( $P < 0.05$ , Table 1). The levels of TNF- $\alpha$ , IL-5, IL-6, and IL-33 in the observation group were markedly higher than these in the control group ( $P < 0.05$ , Table 1).

**Table 1** Analysis of serum Rac1, airway inflammation, and lung function between two groups ( $\bar{x} \pm s$ )

| Indicators          |               | The control group (n=36) | The observation group (n=79) | t      | P      |
|---------------------|---------------|--------------------------|------------------------------|--------|--------|
| Rac1 mRNA           |               | 0.17±0.05                | 0.08±0.03                    | 11.981 | <0.001 |
| Airway inflammation | TNF-α (μg/L)  | 11.19±3.51               | 24.47±6.36                   | 1.723  | <0.001 |
|                     | IL-5 (ng/mL)  | 6.13±1.20                | 43.73±9.69                   | 23.146 | <0.001 |
|                     | IL-6 (μg/L)   | 7.39±2.86                | 14.25±3.82                   | 9.608  | <0.001 |
|                     | IL-33 (μg/mL) | 1.52±0.30                | 1.94±0.30                    | 6.962  | <0.001 |
| Pulmonary function  | FVC%pred      | 100.35±11.78             | 83.96±10.03                  | 7.687  | <0.001 |
|                     | FEV1/FVC (%)  | 75.47±9.72               | 70.03±9.15                   | 2.900  | 0.005  |
|                     | PEF%pred      | 94.83±6.56               | 71.98±7.78                   | 15.307 | <0.001 |
|                     | MMEF%pred     | 85.03±14.75              | 60.90±14.30                  | 8.310  | <0.001 |

**Table 2** Analysis of serum Rac1, airway inflammation, and lung function in patients with different conditions ( $\bar{x} \pm s$ )

| Indicators          |               | The mild group (n=25) | The moderate group (n=30) | The severe group (n=24) | F     | P      |
|---------------------|---------------|-----------------------|---------------------------|-------------------------|-------|--------|
| Rac1 mRNA           |               | 0.14±0.05             | 0.09±0.03*                | 0.04±0.01**             | 52.64 | <0.001 |
| Airway inflammation | TNF-α (μg/L)  | 20.38±2.18            | 24.63±3.30*               | 26.74±4.84**            | 20.37 | <0.001 |
|                     | IL-5 (ng/mL)  | 39.30±7.46            | 45.41±8.79*               | 50.37±9.42**            | 10.21 | <0.001 |
|                     | IL-6 (μg/L)   | 12.81±2.82            | 15.19±3.96*               | 17.26±4.30**            | 8.63  | <0.001 |
|                     | IL-33 (μg/mL) | 1.70±0.15             | 1.85±0.21*                | 1.98±0.28**             | 10.10 | <0.001 |
| Pulmonary function  | FVC%pred      | 92.56±8.72            | 82.35±11.49*              | 63.26±10.06**           | 51.57 | <0.001 |
|                     | FEV1/FVC (%)  | 72.25±8.34            | 65.06±6.36*               | 59.26±5.51**            | 22.31 | <0.001 |
|                     | PEF%pred      | 75.72±6.74            | 70.35±7.66*               | 66.59±5.80**            | 11.04 | 0.001  |
|                     | MMEF%pred     | 60.88±11.41           | 47.55±13.25*              | 24.90±8.41**            | 62.40 | <0.001 |

Note: \* $P < 0.05$  compared with the mild group; \*\* $P < 0.05$  Compared with the moderate group

**Table 3** Analysis of serum Rac1, airway inflammation, and lung function in patients with different levels of asthma control ( $\bar{x} \pm s$ )

| Indicators          |               | The complete controlled group (n=42) | The partially controlled group (n=25) | The uncontrolled group (n=12) | F     | P      |
|---------------------|---------------|--------------------------------------|---------------------------------------|-------------------------------|-------|--------|
| Rac1 mRNA           |               | 0.15±0.04                            | 0.08±0.05*                            | 0.05±0.02**                   | 38.68 | <0.001 |
| Airway inflammation | TNF-α (μg/L)  | 21.49±3.54                           | 23.55±2.13*                           | 25.99±3.58**                  | 10.36 | <0.001 |
|                     | IL-5 (ng/mL)  | 35.24±5.58                           | 46.63±10.11*                          | 52.90±9.55**                  | 31.02 | <0.001 |
|                     | IL-6 (μg/L)   | 11.84±2.60                           | 14.20±4.85*                           | 16.85±3.21**                  | 10.38 | <0.001 |
|                     | IL-33 (μg/mL) | 1.58±0.27                            | 1.89±0.35*                            | 2.26±0.75**                   | 16.89 | <0.001 |
| Pulmonary function  | FVC%pred      | 91.63±7.55                           | 81.20±8.72*                           | 60.34±8.06**                  | 72.81 | <0.001 |
|                     | FEV1/FVC (%)  | 71.18±9.67                           | 64.56±7.08*                           | 58.35±5.74**                  | 12.52 | <0.001 |
|                     | PEF%pred      | 74.94±4.65                           | 71.31±6.65*                           | 65.52±6.97**                  | 13.30 | <0.001 |
|                     | MMEF%pred     | 63.90±12.30                          | 46.60±12.14*                          | 23.86±9.14**                  | 57.65 | <0.001 |

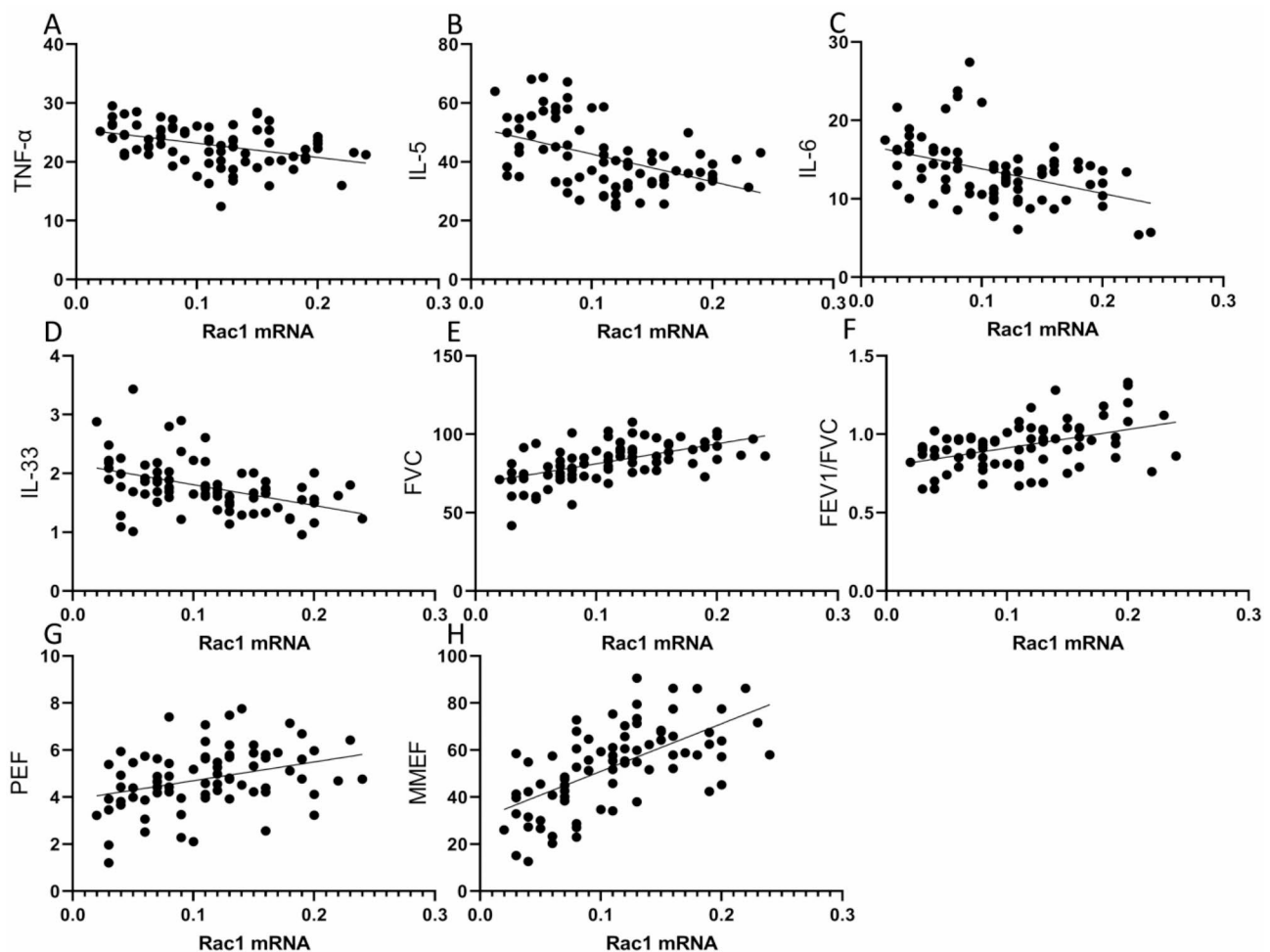
Note: \* $P < 0.05$  compared with the complete control group; \*\* $P < 0.05$  compared with the partially controlled group

### Analysis of serum Rac1, airway inflammation, and lung function in patients with different conditions

Compared with the mild group, the moderate group had significantly lower Rac1 mRNA levels, FVC levels, FEV1/FVC levels, PEF levels and MMEF levels, and markedly higher levels of TNF-α, IL-5, IL-6, and IL-33 ( $P < 0.05$ , Table 2). Compared with the moderate group, the severe group had significantly lower Rac1 mRNA levels, FVC levels, FEV1/FVC levels, PEF levels and MMEF levels, and markedly higher levels of TNF-α, IL-5, IL-6, and IL-33 ( $P < 0.05$ , Table 2).

### Analysis of serum Rac1, airway inflammation, and lung function in patients with different levels of asthma control

Compared with the complete controlled group, the partially controlled group had significantly lower Rac1 mRNA levels, FVC levels, FEV1/FVC levels, PEF levels and MMEF levels, and markedly higher levels of TNF-α, IL-5, IL-6, and IL-33 ( $P < 0.05$ , Table 3). Compared with partially controlled group, the uncontrolled group had significantly lower Rac1 mRNA levels, FVC levels, FEV1/FVC levels, PEF levels and MMEF levels, and markedly higher levels of TNF-α, IL-5, IL-6, and IL-33 ( $P < 0.05$ , Table 3).



**Fig. 2** Pearson analysis of the relevance between airway inflammation response, lung function, and Rac1 mRNA. **A:** The relevance of Rac1 mRNA with TNF- $\alpha$ ; **B:** The correlation between Rac1 mRNA with IL-5; **C:** The correlation between Rac1 mRNA with IL-6; **D:** The correlation between Rac1 mRNA with IL-33; **E:** The correlation between Rac1 mRNA with FVC; **F:** The correlation between Rac1 mRNA with FEV1/FVC; **G:** The correlation between Rac1 mRNA with PEF; **H:** The correlation between Rac1 mRNA with MMEF

**Table 4** Assignment of independent variables

| Variable | Assignments  |
|----------|--|
| X1       | Rac1 mRNA 1 = Rac1 mRNA $\geq 0.10$ , 2 = Rac1 mRNA $< 0.10$   |
| X2       | TNF- $\alpha$ 1 = TNF- $\alpha$ $< 24.05$ $\mu\text{g/L}$ , 2 = TNF- $\alpha$ $\geq 24.05$ $\mu\text{g/L}$ |
| X3       | IL-5 1 = IL-5 $< 47.26$ ng/mL, 2 = IL-5 $\geq 47.26$ ng/mL   |
| X4       | IL-6 1 = IL-6 $< 14.89$ $\mu\text{g/L}$ , 2 = IL-6 $\geq 14.89$ $\mu\text{g/L}$                            |
| X5       | IL-33 1 = IL-33 $< 1.93$ $\mu\text{g/mL}$ , 2 = IL-33 $\geq 1.93$ $\mu\text{g/mL}$                         |
| X6       | FVC 1 = FVC $\geq 80.33$ L, 2 = FVC $< 80.33$ L  |
| X7       | FEV1/FVC 1 = FEV1/FVC $\geq 0.10$ , 2 = FEV1/FVC $< 0.10$  |
| X8       | PEF 1 = PEF $\geq 0.90$ L/s, 2 = PEF $< 0.90$ L/s  |
| X9       | MMEF 1 = MMEF $\geq 50.38\%$ , 2 = MMEF $< 50.38\%$  |
| Y        | Prognosis 1 = complete control, 2 = partial control  |

#### The correlation between airway inflammation response, lung function with Rac1 mRNA

To further explore whether the Rac1 mRNA levels in children with asthma were associated with inflammation, Pearson correlation analysis were performed, and the results showed that the Rac1 mRNA levels were

negatively correlated with levels of TNF- $\alpha$ , IL-5, IL-6 and IL-33 ( $P < 0.05$ ), and positively correlated with the levels of FVC, FEV1/FVC, PEF and MMEF ( $P < 0.001$ , Fig. 2).

#### Multivariate logistic regression analysis of risk factors affecting prognosis in children with asthma

The logistic regression analysis model was established using asthma control as the dependent variable and serological indicators as independent variables. Values were assigned based on the median values of serological indicators (Table 4). Multivariate logistic regression analysis showed that Rac1 mRNA, FVC, FEV1/FVC, PEF and MMEF were protective factors (OR value  $< 1$ ), but TNF- $\alpha$ , IL-5, IL-6 and IL-33 were risk factors (OR value  $> 1$ ) for the prognosis of children with asthma ( $P < 0.05$ , Table 5).

**Table 5** Multivariate logistic regression analysis of risk factors affecting prognosis in children with asthma

| Indicators    | $\beta$ | SE    | Wald   | P     | OR    | 95%CI       |
|---------------|---------|-------|--------|-------|-------|-------------|
| Rac1 mRNA     | -1.062  | 0.501 | 4.499  | 0.034 | 0.346 | 0.125~0.774 |
| TNF- $\alpha$ | 1.122   | 0.450 | 6.202  | 0.013 | 3.071 | 1.254~7.285 |
| IL-5          | 0.220   | 0.057 | 14.704 | 0.001 | 1.246 | 1.118~1.395 |
| IL-6          | 1.281   | 0.326 | 14.461 | 0.001 | 3.602 | 1.902~6.823 |
| IL-33         | 1.237   | 0.316 | 15.336 | 0.001 | 3.446 | 1.855~6.401 |
| FVC           | -1.249  | 0.554 | 5.082  | 0.024 | 0.287 | 0.115~1.725 |
| FEV1/FVC      | -0.552  | 0.160 | 11.886 | 0.001 | 0.576 | 0.421~0.788 |
| PEF           | -1.802  | 0.921 | 3.811  | 0.040 | 0.172 | 0.035~0.972 |
| MMEF          | -0.112  | 0.042 | 5.915  | 0.014 | 0.893 | 0.725~0.968 |

## Discussion

Asthma is a heterogeneous disease, as well as one of the most common chronic diseases in children. Its main manifestations are wheezing, shortness of breath, chest tightness, cough, and expiratory airflow limitations with time and intensity, which significantly limit the daily life, physical exercise, and quality of life of child. Because the organs of children are still in the development stage, the incidence rate is 3–5 times higher than that of adults [15]. At present, clinical diagnosis and treatment of bronchial asthma are mainly based on clinical symptoms, bronchial tests and pulmonary function tests. However, none of the above methods can accurately determine the status and changes in airway inflammation. Therefore, analyzing the pathogenesis of asthma and how to provide effective treatment have become a focus of attention for medical scholars.

Dysfunction of airway smooth muscle cells is one of the key factors in the onset of asthma, and excessive proliferation of airway smooth muscle cells can lead to airflow obstruction in patients with asthma. As the intracellular signaling molecules activated by extracellular stimuli, the Rho family serves as a molecular switch that regulates the signaling cascade and controls the function of airway smooth muscle cells. Rac1 belongs to the Rac subfamily of the Rho superfamily and is widely distributed in various tissues throughout the body. Rac1 can activate the protein kinase system activated by mitogen, which promotes the transfer of extracellular signal regulated kinases to the nucleus. Rac1 can also mediate the occurrence of cellular immunity and inflammatory reaction, participate in the activity of tumor cells, and play a certain role in pterygium, infectious diseases, atrial fibrillation and other diseases [16]. Recent research has found that airway epithelial cells can produce anti-inflammatory cytokines, which are mainly related to their phagocytosis of apoptotic epithelial cells, and this process requires the expression of Rac1 to mediate related signal transduction pathways [17]. Dilaser F et al. [18] report that Rac1 can participate in AHR in mice by mediating the contraction of mouse bronchial smooth muscle (BSM). Kai Y et al. [19] show that Rac1 can regulate the

proliferation, migration, and apoptosis of airway smooth muscle cells (ASMCS) by regulating inflammatory factors such as IL-13. Inflammation is the response of the immune system to damage, which benefits the host under normal circumstances. But, when asthma occurs, the abnormal immune response caused by non-pathogenic stimuli leads to chronic inflammatory reactions related to the pathogenesis of the disease [20]. Abnormal immune activation may lead to the long-term development of asthma. The degree of eosinophilia has been found to may be related to the severity of the disease, and IL-5 plays a prominent role in regulating the differentiation, proliferation and degree of eosinophils [21]. TNF- $\alpha$ , IL-6, IL-33 are inflammation related factors. It has been found in clinical and animal studies [22, 23] that when asthma occurs, the levels of inflammatory indicators such as IL-6 are significantly increased. Similar to the results in this study, the levels of inflammatory factors such as TNF- $\alpha$ , IL-5, IL-6, and IL-33 were significantly elevated, indicating that inflammatory factors might play an important role in the occurrence and development of asthma. Rac1 can regulate cellular transcription factors. Besides, Rac1 may play a vital role in the process of phagocytosis of apoptotic cells by airway epithelial cells, and the production of inflammation-related factors during this process may depend on the regulation of Rac1 [24]. In this study, it was found that serum Rac1 mRNA levels were significantly reduced in children with asthma, and their levels decreased significantly with the aggravation of the state of an illness or the reduction of the control level. It has been reported that Rac1 is positively correlated with bronchial hyperresponsiveness [25], which is contrary to the results of this study. The analysis of the reason may be related to the different sample size and study population included. In addition, the results of our study found a significant negative correlation between inflammatory markers and Rac1. It might be that Rac1 could suppress the proliferation of smooth muscle cells, inhibit airway remodeling, and reduce airway inflammation, thereby improving clinical airflow limitation and respiratory symptoms. Rac1 promotes cytoskeletal rearrangement in cytoskeletal rearrangement by binding to ELMO and

Dock180 to form complexes, a process that is essential for maintaining tissue homeostasis [26]. However, Rac1 also has an anti-apoptotic function and can exert a protective effect by activating anti-apoptosis-related signaling molecules, such as down-regulating caspase molecules, a mechanism that allows Rac1 to inhibit apoptosis of phagocytes in some cases, thereby enhancing their phagocytic ability [27]. In addition, Rac1 also affects the function of phagocytes through other pathways. For instance, it can promote the proliferation and anti-apoptosis of colorectal cancer cells through the WDR12/RAC1 axis [28]. In hepatocellular carcinoma cells, Rac1 may be mediated by TRAF6, while FADD and RIP1 have no significant role [29]. Together, Rac1 enhances phagocytic function in phagocytes by clearing anti-apoptotic signals through a variety of mechanisms, including direct activation of anti-apoptotic signaling molecules, regulation of cytoskeletal rearrangements, and enhancement of cell viability and phagocytosis through specific signaling pathways [30].

Because airflow limitation due to bronchoconstriction and airway inflammation is a hallmark of the pathology underlying asthma symptoms, determination of lung function is a basis for measuring asthma severity, asthma control, and response to treatment [31, 32]. Therefore, physiological evaluation of changes in lung function is crucial for analyzing the phenotype of asthma patients and evaluating the efficacy of treatment interventions. In children with asthma, regular objective evaluation of lung function is a necessary to optimize management and ensure that treatment goals are achieved [33]. Research has shown [34] that persistent bronchial obstruction may occur in asymptomatic children, which is considered a risk factor for severe asthma attacks and is associated with poor prognosis in children with asthma. Therefore, it seems logical to periodically evaluate lung function when monitoring children with asthma. In the results of the present study, the levels of FVC, FEV1/FVC, PEF and MMEF in children with asthma were markedly reduced compared to these in healthy children. Besides, with the state of an illness and the control level decreased, the levels of FVC, FEV1/FVC, PEF and MMEF were further strongly reduced. The detection of pulmonary function plays an important role in evaluating the severity and control level of the state of an illness of patients. In addition, the results of this study found a significant positive correlation between lung function and the Rac1 mRNA levels in children with asthma. Logistic regression analysis showed that the Rac1 mRNA was an independent protective factor affecting the prognosis of children with asthma, suggesting that Rac1 had an indicative role in evaluating the progression of asthma in children, that Rac1 could be used as a reference for the formulation of treatment plans in clinical practice, and that may become

a new target for the treatment of bronchial asthma. It may be because the endothelium forms the walls of blood vessels, and the pulmonary endothelium is a target that affects its important barrier function. Rac1 plays a significant role in the regulation of endothelial barrier function, which can increase the contractility of endothelial cell division proteins and the formation of intercellular spaces, thereby affecting lung function.

In general, children with asthma have obviously lower serum Rac1 mRNA levels, higher inflammatory factor levels and lower lung function. Serum Rac1 mRNA level may be associated with better asthma control, lower airway inflammatory response, better lung function and lower disease severity. It has important reference value for the evaluation of the state of an illness, efficacy and prognosis of children with bronchial asthma.

Despite the results of this study, there are still some limitations. Firstly, the sample size of this study is small, and it is necessary to expand the sample size in the future to further verify the results of this study. Secondly, this study only analyzes the correlation between serum Rac1 mRNA level and asthma control level, airway inflammation, and lung function, but did not explore the specific mechanism of action. Future studies can investigate the cells primarily express Rac1 and further explore the mechanism of Rac1 in the pathogenesis of asthma at the molecular level, to provide a more powerful theoretical basis for the clinical treatment of children with asthma. In addition, the differences in serum Rac1 levels among children with different age, gender and asthma severity are not involved in this study, which is also worthy of attention in future research. Furthermore, this study detected the mRNA level of Rac 1 by RT-PCR but detect content of other inflammatory factors by ELISA. The content of Rac 1 measured by ELISA is also needed in the following research. In conclusion, this study provides the treatment and prevention of asthma in children with a new train of thought, and is expected to contribute to children improve their quality of life.

### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12890-024-03266-5>.

Supplementary Material 1

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### Author contributions

Hui Fu confirmed the authenticity of all the raw data and edited the manuscript, Yun Gao collected data and processed the data. Hui Fu and Yun Gao conducted the statistics. Yun Gao reviewed and revised the article. All authors read and approved the final manuscript.

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### Data availability

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

### Declarations

#### Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of Wujin Affiliated Hospital, Nanjing University of Chinese Medicine (2020-YLJSA027). Written informed consent was obtained from all subjects and/or their legal guardian(s).

#### Consent for publication

Written informed consent was obtained from all subjects and/or their legal guardian(s). The patients participating in the study all agree to publish the research results.

#### Competing interests

The authors declare no competing interests.

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