



Serum progranulin as an indicator of neutrophilic airway inflammation and asthma severity



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ABSTRACT

Background: Progranulin, a protein secreted from the airway epithelium, is known to attenuate the downstream cascade of neutrophilic inflammation in particular. We hypothesized that progranulin may have a role in inflammatory regulation in asthma.

Objective: To investigate the association between serum progranulin levels and various clinical features in patients with asthma.

Methods: Serum samples and clinical data of 475 patients with asthma and 35 healthy controls at a tertiary referral hospital and its affiliated health promotion center were collected. Serum progranulin levels were compared between patients with asthma and healthy controls and then were compared within the patients with asthma in terms of pulmonary function and measures of inflammatory status. Univariate and multivariate analyses were performed to identify factors associated with severity of asthma.

Results: Serum progranulin levels were significantly lower in the asthma group than in healthy group and were positively correlated with prebronchodilator forced expiratory volume in 1 second predicted within patients with asthma. We found a negative correlation between serum progranulin levels and blood neutrophil counts. Multivariate analysis revealed that higher serum progranulin levels were associated with a lower risk of severe asthma (odds ratio, 0.888; 95% confidence interval, 0.846–0.932; $P < .001$) after adjustment for other variables, such as age, sex, smoking status, blood neutrophil count, and current use of systemic corticosteroids.

Conclusion: Although the exact mechanism of the anti-inflammatory action of progranulin remains unknown, we suggest that serum progranulin may be an indicator of severe asthma with airflow limitation. Future studies with comprehensive airway sampling strategies are warranted to clarify its role, particularly in neutrophilic asthma.

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Introduction

Asthma is a common chronic inflammatory airway disease considered a heterogeneous clinical syndrome with variable treatment responses.¹ The heterogeneity has necessitated a need for establishing different therapeutic strategies for different

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subtypes of asthma. Much effort in clinical research has been expended in the categorization of patients with asthma into different phenotypes to improve asthma management, which many researchers believe corresponds with various pathophysiologic pathways that lead to airway inflammation.^{2,3} Currently, inflammatory phenotyping of asthma by the presence of sputum neutrophils and eosinophils has been effectively used for guiding treatment strategy, especially in severe asthma.^{1,4}

Mechanistic studies have been conducted for eosinophilic asthma and have led to the development of targeted therapy, such as anti-interleukin (IL) 5⁵ and anti-IL-5 receptor⁶ biologic agents. However, little is known regarding neutrophilic asthma, which has been identified in both stable and severe asthma.^{7,8} In particular, neutrophilic airway inflammation receiving conventional

treatment, together with progressive airway remodeling, remains an unsolved problem. However, the categorization of asthma into well-characterized subgroups remains an incomplete task because gold standard biomarkers that can direct all patients with asthma to target therapies have not been established.

Progranulin is a multifunctional protein expressed in various tissues, especially in epithelial and myeloid cells.⁹ Studies using murine models of inflammatory dermatitis,¹⁰ arthritis,¹¹ inflammatory bowel disease,¹² and acute lung injury¹³ have identified progranulin to be a potent anti-inflammatory molecule whose anti-inflammatory action is mediated by inhibition of neutrophil degranulation by binding to the tumor necrosis factor receptor α receptor (TNFR- α) and disturbing the TNF- α –TNFR interaction.¹¹ In support of these findings, a recent article on human sputum samples of chronic obstructive lung disease (COPD), which is associated with dysregulated neutrophilic inflammation, has identified a higher serum progranulin level as an indicator of less severe neutrophilic inflammation.¹⁴

The present study tests the hypothesis that a defect in progranulin synthesis or function may play a role in the regulation of airway inflammation in asthma, particularly in severe asthma or neutrophilic asthma. As a first step, we planned to investigate serum progranulin levels in patients with asthma.

Methods

Study Participants

The study participants consisted of 475 randomly selected patients with asthma from outpatient clinic of a tertiary referral hospital, as a participating hospital of Cohort for Reality and Evolution of Adult Asthma in Korea (COREA)¹⁵ and 35 age-matched healthy controls from the health promotion center in the same hospital. Asthma was diagnosed by allergy specialists as either airway hyperresponsiveness (provocative concentration of methacholine causing a 20% decrease in forced expiratory volume in 1 second [FEV₁] <25 mg/mL) or reversible airway obstruction (bronchodilator response to 400 μ g of salbutamol >12% and >200 mL). We did not exclude current smokers or anyone who shared features with COPD (secondary diagnosis of asthma–COPD overlap syndrome [ACOS]).¹⁶ All participants provided written informed consent, and the study was approved by the institutional review board.

Study Flow

Patient demographic data and clinical information were collected from the COREA registry database. Serum samples were obtained from all study participants, and serum progranulin level was measured using a quantitative sandwich enzyme-linked immunosorbent assay kit for human progranulin (R&D Systems, Minneapolis, Minnesota).

First, serum progranulin levels were compared between patients with asthma and the healthy control group. Next, serum progranulin levels were compared among patients with asthma, according to pulmonary function variables (percentage of predicted FEV₁, and ratio of FEV₁ to forced vital capacity [FVC]), age, sex, and various clinical variables associated with inflammatory status, including smoking status, body mass index, atopic status, current use of systemic corticosteroid, total IgE level, and both sputum and blood inflammatory cell counts.

Second, we investigated whether serum progranulin can function as a biomarker of asthma severity. Asthma severity was graded for every patient according to the Global Initiative for Asthma (GINA) 2002 guidelines,¹ which integrates the number of day and night symptoms with the degree of pulmonary function. Patients were then divided into 2 groups: severe asthma, comprising patients with severe persistent asthma graded by the guideline, and

nonsevere asthma, comprising patients with mild intermittent, mild persistent, and moderate persistent asthma. We evaluated the factors associated with a higher risk of severe asthma, including serum progranulin levels.

Statistical Analysis

All statistical analyses were conducted using SPSS statistical software, version 22.0 (IBM Corp, Armonk, New York). Normality of distribution was assessed using the Kolmogorov–Smirnov test. Continuous variables were analyzed using the *t* test and 1-way analysis of variance. Binary data were analyzed using the χ^2 test. Correlations were evaluated using the Pearson test.

Univariate and multivariate logistic regression analyses were also performed to identify clinical factors associated with a higher risk of severe asthma, including the progranulin level. Multivariate analysis with backward elimination was performed after selection of clinical variables, satisfying a confidence level of 10%. Moreover, receiver operating characteristics curve analysis was conducted to determine the best cut-off value of progranulin to separate patients with asthma into severe and nonsevere groups.

Results

Serum progranulin levels negatively correlated with the degree of airway obstruction

Serum progranulin levels were significantly lower in the asthma group than in the healthy group (mean [SD], 25.51 [6.72] vs 33.70 [10.12] ng/mL; *P* < .001) (Fig 1).

Subsequent analyses were performed in asthma patients, and we identified a positive correlation between serum progranulin levels and prebronchodilator FEV₁ (*R* = 0.371; 95% confidence interval [CI], 0.291–0.446; *P* < .001) (Fig 2A). Serum progranulin levels were negatively correlated with age (*R* = –0.152; 95% CI, –0.238 to –0.0624; *P* = .001; data not shown) but did not differ between sexes (data not shown). The positive correlation between the percentage of predicted FEV₁ and progranulin was not affected by the adjustment for age. We also determined that progranulin levels were higher in patients with FEV₁/FVC ratios greater than 0.7 (Fig 2B).

Serum progranulin levels negatively correlated with blood neutrophil counts

Next, to investigate the association between serum progranulin levels and sputum or blood inflammatory cell counts, we executed simple correlation analyses for the following variables: sputum neutrophil percentage, eosinophil percentage, blood neutrophil

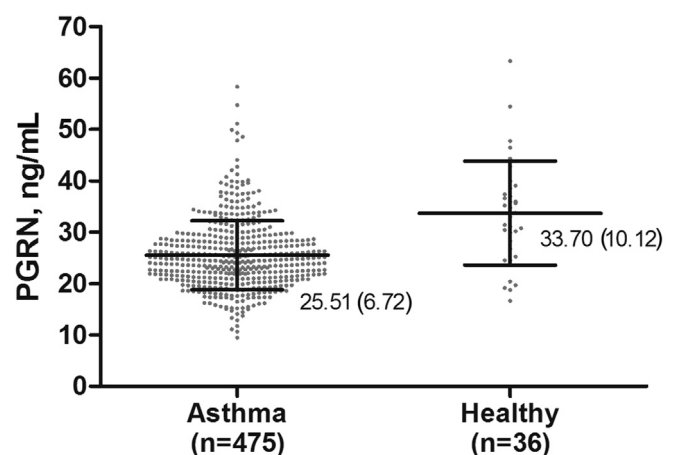


Figure 1. Comparison of serum progranulin levels in patients with asthma and healthy controls. Mean (SD) expressed in horizontal lines and error bars. *P* < .001 (*t* test).

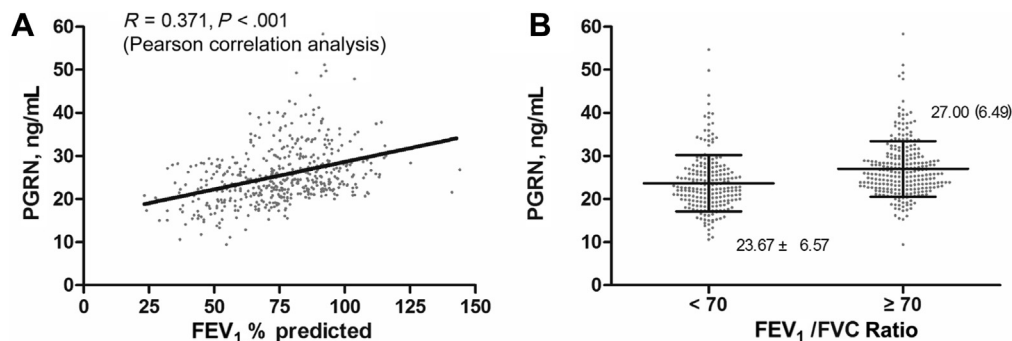


Figure 2. A, Correlation between serum progranulin levels and prebronchodilator percentage of predicted forced expiratory volume in 1 second (FEV₁). B, Comparison of serum progranulin levels in patients with asthma according to ratio of FEV₁ to forced vital capacity (FVC). Mean (SD) expressed in horizontal lines and error bars. $P < .001$ (t test).

counts, and blood eosinophil counts. As shown in Figure 3, we found no correlation between serum progranulin levels and sputum neutrophil, eosinophil percentages, and blood eosinophil counts. However, there was a negative correlation between serum progranulin levels and blood neutrophil counts ($R = -0.207$; 95% CI, -0.292 to -0.119 ; $P < .001$) (Fig 3C).

Lower serum progranulin levels were associated with severe asthma

Next, we investigated whether serum progranulin can function as a biomarker of asthma severity and whether a cut-off value of progranulin level discriminating severe asthma can be established. As indicated in Table 1, the results of the univariate analysis revealed that a serum progranulin level of 1 ng/mL had a risk ratio of 0.877 (95% CI, 0.841–0.915; $P < .001$) for severe asthma. Older age, male sex, ever-smokers, nonatopic status, use of a systemic corticosteroid within 1 month, a higher blood neutrophil count,

and higher total IgE levels were all also associated with severe asthma.

In multivariate analysis, 2 variables, namely, total IgE level and atopy status, were excluded from the analysis because the number of missing values exceeded 20%. Nevertheless, higher serum progranulin levels were associated with a lower risk of severe asthma (OR, 0.888; 95% CI, 0.846–0.932; $P < .001$) after adjustment for other variables, such as age, sex, smoking status, blood neutrophil count, and current use of systemic corticosteroids. In other words, lower serum progranulin levels were associated with severe asthma. The association remained significant with older age, use of a systemic corticosteroid within 1 month, and higher blood neutrophil counts for severe asthma.

Finally, received operating characteristic curve analysis found the optimal cut-off value of severe asthma to be 19.89 ng/mL of serum progranulin, with a sensitivity of 38.0% and a specificity of

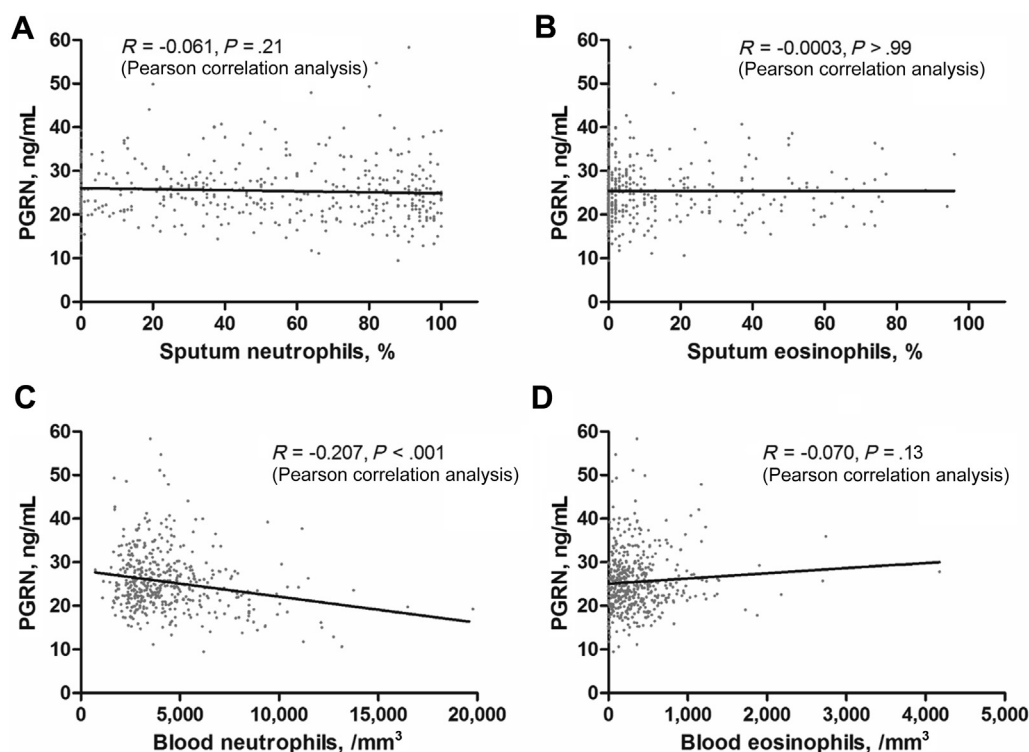


Figure 3. Correlation of serum progranulin (PGRN) level with sputum neutrophil counts (A), sputum eosinophil percentages (B), blood neutrophil counts (C), and blood eosinophils counts (D).

Table 1
Univariate and Multivariate Logistic Regression Analysis for Factors Associated With Severe Asthma

Factor	Mild or moderate asthma (n = 338)	Severe asthma (n = 137)	Univariate analysis		Multivariate analysis	
			OR (95% CI)	P value	OR (95% CI)	P value
Progranulin, ng/mL ^a	26.77 (6.71)	22.42 (5.70)	0.877 (0.841–0.915)	<.001	0.888 (0.846–0.932)	<.001
Age, y ^a	47.5 (14.9)	54.5 (14.5)	1.033 (1.019–1.048)	<.001	1.022 (1.003–1.040)	.02
Sex						
Male	132	78	1 [Reference]		1 [Reference]	
Female	206	59	0.485 (0.324–0.725)	<.001	0.654 (0.353–1.214)	.18
Smoking status						
Ever smoker	146	76	1 [Reference]		1 [Reference]	
Never smoker	184	59	0.608 (0.406–0.910)	.02	1.060 (0.574–1.957)	.85
BMI ^a	23.70 (3.16)	24.20 (3.40)	1.049 (0.986–1.115)	.13	1.019 (0.941–1.103)	.65
Atopy status						
Atopic	125	26	1 [Reference]			
Nonatopic	112	64	2.747 (1.630–4.632)	<.001		
Use of systemic steroid (within 1 month)						
Yes	42	28	1 [Reference]	.02	1 [Reference]	
No	296	109	0.552 (0.326–0.935)	.03	0.438 (0.227–0.844)	.01
Sputum neutrophils, % ^b	50 (18.75–82)	62.5 (29.25–88)	1.003 (0.998–1.009)	.22	1.003 (0.99–1.010)	.40
Sputum eosinophils, % ^b	2 (0, 13)	2 (0–19.25)	1.003 (0.992–1.013)	.61	1.007 (0.994–1.021)	.27
Blood neutrophils, /mm ^{3,b}	3,478.5 (2726.6–4,631.3)	4,462.5 (3,051.2–7,137.3)	1.030 (1.020–1.040)	<.001	1.025 (1.013–1.038)	<.001
Blood eosinophils, mm ^{3,b}	269.3 (132.9–452.8)	230.3 (92.8–510.3)	1.001 (0.997–1.004)	.74	1.003 (0.999–1.007)	.14
Total IgE, kU/L ^b	193 (74.1–469.3)	238 (56.0–737.8)	1.003 (1.000–1.006)	.04		

Abbreviations: BMI, body mass index (calculated as the weight in kilograms divided by square of height in meters); CI, confidence interval; OR, odds ratio.

^aExpressed as mean (SD).

^bExpressed as median (interquartile range).

91.4% (area under the curve, 0.681; 95% CI, 0.626–0.735; $P < .001$) (Fig 4).

Discussion

We found that serum progranulin levels were significantly lower in patients with asthma compared with healthy controls and that serum progranulin levels were positively correlated with pulmonary function in patients with asthma. Lower serum

progranulin levels were associated with a higher risk of severe asthma. In addition, the blood neutrophil count had a negative correlation with serum progranulin levels. These findings suggest that progranulin is linked to severe asthma with airflow obstruction and possibly plays an inhibitory role within neutrophilic inflammation of the airways.

It appears that uncontrolled asthma, even under optimal treatment, is driven by an inflammatory process that is not suppressed by any of the currently available therapeutics. During the past 2 decades, research in asthma has focused on elucidation of the pathobiology of asthma—in other words, the pathway detailing how eosinophils, mast cells, and neutrophils are dysregulated to produce the airway inflammation and result in a progressive decline in lung function and frequent exacerbations.¹⁷ Indeed, this has been linked directly to searching for target molecules to downregulate disease activity, and we have witnessed meteoric growth in this area.^{5,6,18,19}

For severe eosinophilic asthma, overproduction of type 2 immunity cytokines, such as IL-4, IL-5, and IL-13, has been suggested as a key pathomechanism. Development of biologic agents that block these molecules or their receptors and clinical trials confirming their effectiveness and safety are continually being performed.²⁰ Anti-TNF- α agents and anti-IL-17 monoclonal antibodies have been tested as therapeutic agents to target neutrophilic inflammation in severe asthma, and a number of clinical trials are currently under way; however, most have not documented significant improvement so far.¹⁷

Progranulin is a growth factor expressed in many different organs and is known to serve various physiologic functions, one of which is inhibition of neutrophilic inflammation. Previous animal studies have found that progranulin is a potent anti-inflammatory molecule in murine models of inflammatory dermatitis, rheumatoid arthritis, and lipopolysaccharide-induced acute lung injury.^{10,11,13} Moreover, the administration of recombinant progranulin in disease models has resulted in improvement of their pathologic signs.¹¹

Specifically, in a murine model of rheumatoid arthritis, anti-inflammatory action was found to be mediated by directly binding to TNFRs, thereby interfering with the TNF- α –TNFR interaction,

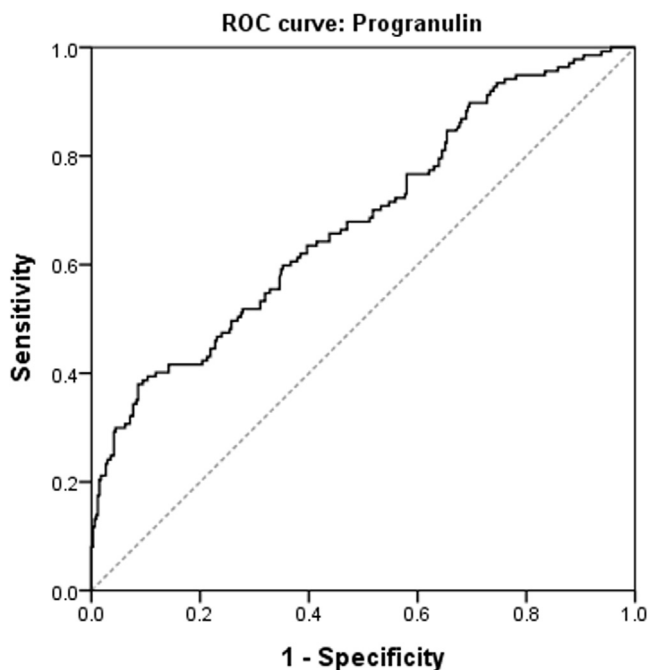


Figure 4. Receiver operating characteristics (ROC) curve for the best cut-off value of serum progranulin to discriminate between severe asthma sensitivity (38.0%) and specificity (91.4%) (area under the curve, 0.681; 95% confidence interval, 0.626–0.735; $P < .001$).

and subsequently antagonized the downstream cascade of inflammatory process.¹¹ Moreover, treatment of progranulin on human regulatory T (T_{reg}) cells protected these cells from being negatively regulated by TNF- α and promoted differentiation of naive T cells to T_{reg} cells.¹¹ It is known that development of T_H2 and T_H17 cells in the adaptive immune response is facilitated by impairment of T_{reg} cells, and defective functioning in T_{reg} cells can occur in all forms of asthma^{21,22} but predominantly in severe subtypes.²³

A recent study using human sputum samples evaluated the possible influence of progranulin on COPD and found that progranulin correlated negatively with markers of neutrophilic inflammation, including neutrophil elastase and proteinase 3.¹⁴

Although the exact mechanism(s) behind the functions of progranulin in the development of neutrophilic inflammation remains unclear, with increasing evidence, we could presume that progranulin would have a certain beneficial role in the regulation of airway inflammation. We hypothesized that defect in either progranulin synthesis or function would influence the regulation of airway inflammation in asthma, particularly in cases of severe or neutrophilic asthma. Thus, we planned the present study as a preliminary concept to measure the presence of serum progranulin levels in patients with asthma compared with healthy controls and possibly to predict its potential role in the disease by comparing its levels according to the clinical characteristics of patients.

The results were partly in agreement with what we had expected because lower serum progranulin levels were associated with severe asthma with airflow limitation, and the blood neutrophil counts were higher in patients with lower serum progranulin levels. We did not observe any association between the percentage of sputum neutrophil and serum progranulin levels. Unfortunately, we could not measure progranulin levels with comprehensive airway sampling strategies in the present study. Further studies are warranted to clarify the association between progranulin and neutrophilic infiltration.

In univariate analysis, older age, male sex, history of smoking, nonatopic status, and higher blood neutrophil counts were associated with a higher risk of severe asthma. Clinically, these factors appear to fit for the recently emphasized disease concept ACOS, and our data may suggest that patients with ACOS are partly included in the severe asthma group.

As a major hurdle to the treatment of bronchial asthma, a respiratory disease that is increasing worldwide, severe asthma is a problem awaiting a solution. The lack of overwhelmingly positive outcomes from the precedent research on pathomechanisms and target therapies for severe asthma indicates that asthma is truly a complex clinical syndrome, which cannot be explained by one mechanism alone. As a preliminary step, in our study, progranulin was found to be one of the possible markers of severe asthma and neutrophilic airway inflammation. Future studies are warranted, starting from mechanistic studies in conjunction with previously known cytokines and chemokines to relate animal and clinical studies and confirm the role of progranulin in the inflammatory regulation of the asthmatic airways.

There are several limitations to the present study. We were unable to subclassify severe asthma by several heterogeneous phenotypes and thereby identify different expression levels of progranulin in each phenotype (eg, ACOS). Further studies on progranulin in well-defined subgroups of severe asthma will be needed in this regard. Furthermore, we could not exclude non-adherent patients because we were unable to evaluate the adherence factor. The many updated versions of definitions of severe asthma established by different workgroups^{24,25} emphasize the need for continuous follow-up on patients for at least 1 year for the determination of severe asthma to see whether the patient requires high-dose inhaled corticosteroids or frequent bursts of systemic

corticosteroids. Moreover, asthma severity was determined with an outdated version of the GINA guidelines because many of our patients were registered in the cohort before the recent update. Therefore, asthma severity for some patients could have been misclassified if the current guidelines had been applied.

In conclusion, serum progranulin levels had a positive correlation with pulmonary function in patients with asthma and a negative correlation with blood neutrophil counts. A lower serum progranulin level was associated with severe asthma. Although the exact mechanism of the anti-inflammatory action of progranulin remains unclear, we suggest that serum progranulin may be used as an indicator of severe asthma with airflow obstruction and that it possibly has an inhibitory role on neutrophilic airway inflammation.

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