

Increased periostin associates with greater airflow limitation in patients receiving inhaled corticosteroids

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Background: Periostin, an extracellular matrix protein, contributes to subepithelial thickening in asthmatic airways, and its serum levels reflect airway eosinophilic inflammation. However, the relationship between periostin and the development of airflow limitation, a functional consequence of airway remodeling, remains unknown.

Objective: We aimed to determine the relationship between serum periostin levels and pulmonary function decline in asthmatic patients on inhaled corticosteroid (ICS) treatment.

Methods: Two hundred twenty-four asthmatic patients (average age, 62.3 years) treated with ICS for at least 4 years were enrolled. Annual changes in FEV₁, from at least 1 year after the initiation of ICS treatment to the time of enrollment or later (average, 16.2 measurements over 8 years per individual), were assessed. At enrollment, clinical indices, biomarkers that included serum periostin, and periostin gene polymorphisms were examined. Associations between clinical indices or biomarkers and a decline in FEV₁ of 30 mL or greater per year were analyzed.

Results: High serum periostin levels (≥ 95 ng/mL) at enrollment, the highest treatment step, higher ICS daily doses, a history of admission due to asthma exacerbation, comorbid or a history of sinusitis, and ex-smoking were associated with a decline in FEV₁ of 30 mL or greater per year. Multivariate analysis showed that high serum periostin, the highest treatment step, and ex-smoking were independent risk factors for the decline. Polymorphisms of periostin gene were related to higher serum periostin levels (rs3829365) and a decline in FEV₁ of 30 mL or greater per year (rs9603226).

Conclusions: Serum periostin appears to be a useful biomarker for the development of airflow limitation in asthmatic patients on ICS. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: Asthma, inhaled corticosteroids, lung function decline, periostin, POSTN gene polymorphism, sinusitis, treatment step

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Airway inflammation and remodeling are key features of asthma that have been demonstrated by pathologic¹ and radiologic^{2,3} findings. Physiologically, patients with asthma show a greater decline in pulmonary function than subjects without asthma.⁴ Studies that were mostly conducted in the era before inhaled corticosteroids (ICS) reported that more severe symptoms or severe exacerbations,⁵⁻⁷ long-standing asthma,⁸ and smoking history^{4,8} were moderate-to-strong risk factors for greater decline in pulmonary function.⁵ Blood and sputum eosinophilia^{9,10} and genetic predisposition¹¹⁻¹³ were also potential risk factors. Because of early intervention with ICS, however, airway inflammation and the degree of annual decline in pulmonary function have been attenuated in most asthmatic patients.¹⁴⁻¹⁶ Meanwhile, a subset of patients still show accelerated decline in FEV₁ and develop irreversible airway obstruction despite adequate treatment.^{17,18} van Veen et al¹⁸ found that exhaled nitric oxide of 20

Abbreviations used

ACT: Asthma control test
 ECP: Eosinophil cationic protein
 FAS I: Fasciclin I
 hsCRP: High-sensitivity C-reactive protein
 ICS: Inhaled corticosteroids
 ROC: Receiver operating characteristic
 SNP: Single-nucleotide polymorphism

ppb or higher is a predictor of accelerated decline in pulmonary function in patients with difficult-to-treat asthma. However, other biomarkers for greater decline in FEV₁ despite treatment with ICS remain unknown.

The airway inflammation of asthma is classically characterized by infiltration and activation of eosinophils, mast cells, and T_H2 cells with several mediators and T_H2 cytokines, such as IL-4, IL-5, and IL-13.^{19,20} Periostin, a secreted, 90-kDa, extracellular matrix protein that is induced by IL-4 and IL-13, was originally isolated as an osteoblast-specific factor; it shares structural homology to the insect cell adhesion molecule fasciclin I (FAS I) and binds to fibronectin, tenascin-C, and collagen.^{21,22} In airway epithelial cells collected from patients with asthma, periostin is one of the upregulated genes,²³ and its expression is correlated with thickness of the airway basement membrane.²⁴ Takayama et al²¹ clearly demonstrated that periostin is deposited in the airway subepithelial layer in asthmatic patients. Moreover, serum periostin is identified as the single best predictor of airway eosinophilia in patients with severe asthma who remain symptomatic despite maximal ICS treatment.²⁵ Therefore, we hypothesized that periostin would be a novel biomarker of T_H2/eosinophil-driven airway inflammation and greater decline in pulmonary function, a functional consequence of airway remodeling in patients with asthma.

In this study, the effects of biomarkers and clinical indices on greater annual decline in pulmonary function in asthmatic patients on ICS treatment were examined, with the specific aim of determining the association between serum periostin levels and pulmonary function decline. Polymorphisms of the *POSTN* gene, which encodes periostin, were also examined on the hypothesis that *POSTN* gene polymorphisms may affect serum periostin levels.

METHODS**Patients**

Patients with asthma were recruited from 9 institutions belonging to the Kinki Hokuriku Airway disease Conference where asthma specialists manage patients. Asthma was diagnosed according to the American Thoracic Society criteria (see the Methods section in this article's Online Repository at www.jacionline.org).²⁶ From September 2009 to December 2011, patients were enrolled if they had received ICS treatment for 4 years or more, undergone 3 or more pulmonary function tests when they were stable, and were free from exacerbations for at least 1 month. The first pulmonary function test was performed at least 1 year after the commencement of ICS treatment and at 25 years of age or older. Patients who had smoked >10 pack years, smoked in the past 1 year, or had other pulmonary diseases were excluded.

This study was approved by the ethics committee of each participant institution and was registered in the UMIN Clinical Trials Registry (Registry ID UMIN000002414). Written informed consent was obtained from all participants.

Measurements

At enrollment, patients underwent a workup that included answering a self-completed questionnaire, spirometry, and blood tests. After enrollment, spirometry was repeated at least 6 months later for up to 12 months.

Self-completed questionnaire and clinical indices

The self-completed questionnaire was composed of 4 major items, as presented in Table I. (More detail is included in the Methods section in this article's Online Repository.) The Asthma Control Test (ACT) was also scored. The treatment step at enrollment was determined according to the Global Initiative for Asthma 2010 guideline.²⁷

Pulmonary function

Spirometry was performed with an electrical spirometer, which was calibrated once a week, at each institution. Spirometry data were obtained only when patients were stable. To determine pulmonary function on daily medications, ICS, and other controllers, including long-acting β_2 agonists, leukotriene receptor antagonists, or slow-release theophylline, were not withdrawn before spirometry.

Measurement of systemic biomarkers

Blood eosinophil and neutrophil counts, and serum levels of total IgE, specific IgE against common inhaled allergens, eosinophil cationic protein (ECP), high-sensitivity C-reactive protein (hsCRP), and periostin were determined.

Serum periostin levels were measured with an enzyme-linked immunosorbent assay at Shino-test (Kanagawa, Japan), as described previously (for additional information, see the Methods section in this article's Online Repository).²⁸ Pooled serum periostin level data from 66 healthy subjects (age, mean $\pm$ SD, 60.7 $\pm$ 16.7 years; 40 males)^{28,29} were used for comparison with those of asthmatic patients.

Haplotype analysis, DNA extraction, and genotyping of the *POSTN* gene

A total of 47 single-nucleotide polymorphisms (SNPs) in the region of the *POSTN* gene and its upstream, total 39 kb, was captured in the HapMap Japanese data set. Haplotype analysis identified 4 major haplotypes and 2 minor haplotypes. Two minor haplotypes were grouped into the closest major haplotype, and 3 tag SNPs that determined the 4 haplotypes were identified (Fig 1). (More detail is included in the Methods section in this article's Online Repository.)

Genomic DNA was isolated from blood cells with the use of a QIAamp DNA Blood Mini Kit (Qiagen, Tokyo, Japan). SNPs were genotyped with a Taqman genotyping assay according to the manufacturer's instructions (Applied Biosystems, Tokyo, Japan) and analyzed with an Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems).

Statistical analysis

Statistical analyses were performed with JMP version 9.0 (SAS Institute Inc, Tokyo, Japan). Annual changes in FEV₁ (Δ FEV₁) were estimated for each subject by fitting a least-square regression line to all of the subject's available data points. Receiver operating characteristic (ROC) curve analysis was performed to determine a serum periostin cutoff value for asthmatic patients. The effects of serum biomarkers or other indices on Δ FEV₁ were estimated with a generalized linear mixed model with adjustment for sex, height, age at enrollment, and FEV₁ at the first measurement. The institutions were included as random effects in this model. On univariate analysis of Δ FEV₁, the adjusted *P* value, that is, *q* value, which was a measure of significance in terms of the false discovery rate, was obtained with R and QVALUE software³⁰ to determine spurious significance in multiple testing. The effects on the dichotomous data for a decline in FEV₁ of 30 mL or greater per year³¹ were similarly estimated with a generalized linear mixed model by IBM

TABLE I. Contents of the self-completed questionnaire

Asthma-related history
Family history of asthma
Age of asthma onset
History of pediatric asthma
History of admission due to asthma worsening or exacerbation
Aspirin hypersensitivity
Asthma deterioration at the working place
Comorbidity or a history of the following diseases:
Allergic dermatitis
Allergic rhinitis
Seasonal rhinitis
Allergic conjunctivitis
Chronic sinusitis
Cardiovascular diseases, including ischemic heart disease
Gastrointestinal diseases, including GERD
Collagen vascular diseases, including rheumatoid arthritis
Diabetes mellitus
Pulmonary diseases other than asthma
Other diseases including malignancy
Lifestyle and environment
Smoking history
Pet breeding
Type of occupation
A highway near the home
Age at menopause
Adherence to medication, sputum production, and exacerbations
How often do you forget to take inhaled corticosteroids or other medications?
0, never; 1, seldom; 2, sometimes; 3, often; 4, always
How often do you produce sputum?
0, never; 1, once in a few days; 2, every morning; 3, every morning and daytime
How often did you receive systemic steroids due to asthma exacerbations during the recent 6 months?
0, never; 1, once; 2, twice or more

GERD, Gastroesophageal reflux disease.

SPSS Advanced Statistics 19 (SPSS Inc, Tokyo, Japan). Multivariate analysis was performed with variables with $P < .10$ on univariate analysis, except for ICS daily maintenance dose because of its strong correlation with treatment step. On multivariate analysis, the periostin level was considered as a dichotomous variable (high or low) instead of a continuous variable. Correlation coefficients between serum periostin levels and clinical indices were estimated by fitting least-square regression lines to data, in which institutions were included as random effects. Unpaired t and χ^2 tests were performed for comparisons of continuous and dichotomous variables, respectively. When data were not normally distributed, they were log-transformed. Data are presented as means $\pm$ SDs. P values $\leq .05$ were considered significant.

RESULTS

Patients' characteristics

Initially, 233 patients were enrolled in this study, but 9 patients were excluded: 5 with a smoking history of >10 pack years and 4 who did not have enough pulmonary function data available. The demographic data of the remaining 224 patients are presented in Table II. The mean age at enrollment was 62.3 ± 13.7 years. Overall, 130 (58%) had onset of asthma at 40 years of age or older. The average number of measurements of FEV₁, follow-up period, and Δ FEV₁ of 224 patients were 16.2 ± 13.9 times, 8.0 ± 4.5 years, and -7.8 ± 34.6 mL per year, respectively. The distribution of Δ FEV₁ in this population is shown in Fig E1 (in the Online Repository available at www.jacionline.org). Within 2 years after

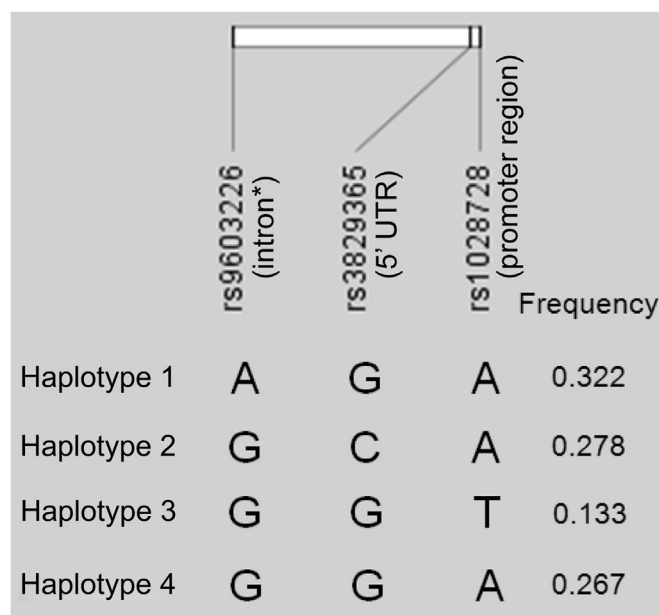


FIG 1. Three tag SNPs that determine 4 major haplotypes of the *POSTN* gene and haplotype frequencies in the Japanese population are presented. *At intron 66 bp upstream of exon 21.

diagnosis, 46% of patients started ICS treatment. At enrollment, 82% of patients took controllers such as long-acting β_2 agonists, leukotriene receptor antagonists, or sustained release theophylline to achieve adequate asthma control. On the basis of a questionnaire, adherence to medication was satisfactory; 49% of the participants never and 38% seldom forgot to take ICS or other medications. On the basis of ACT scores, 50% was totally controlled, and 38% scored from 20 to 24, indicating that they were well controlled at enrollment.

Serum periostin levels of asthmatic patients (92.8 ± 38.4 ng/mL) were significantly higher than those of healthy subjects (39.1 ± 24.5 ng/mL; $P < .001$). The ROC curve analysis was performed to discriminate patients with asthma who were thought to have refractory T_H2 inflammation despite long-term ICS treatment from healthy subjects. The highest specificity among the 4 cutoff values tested was achieved at 95 ng/mL (0.985) in the comparison study of 224 asthmatic patients and 66 healthy subjects. Therefore, a cutoff value of 95 ng/mL was used to define a high serum periostin group, although it had relatively lesser sensitivity (0.379) (see Fig E2 in this article's Online Repository at www.jacionline.org). In asthmatic patients, 85 patients (38%) had high serum periostin levels (≥ 95 ng/mL). Of the 85 patients, 40 patients (47%) were on treatment step 4, according to the treatment step classification,²⁷ and 9 patients (11%) were on treatment step 5.

Associations between serum periostin levels and greater annual decline in FEV₁ and a decline in FEV₁ of 30 mL or greater per year

In an analysis of continuous values of Δ FEV₁, greater decline in FEV₁ was associated with higher serum periostin levels at enrollment, treatment step 5, lower ACT scores, incomplete adherence to medications, comorbid or a history of sinusitis, and comorbid diabetes mellitus (Table III). When patients were stratified into 2 groups according to their serum periostin levels, high serum periostin (≥ 95 ng/mL) was also associated with greater

TABLE II. Patients' characteristics

	Value
Sex (males/females), n	53/171
Age at enrollment (y), mean $\pm$ SD	62.3 $\pm$ 13.7
Age at asthma onset (y), mean $\pm$ SD	42.0 $\pm$ 19.0
Body mass index (kg/m ²), mean $\pm$ SD	23.1 $\pm$ 3.5
Smoking history (never), n	181
Atopic predisposition (%)*	70
Pediatric asthma (none/recurrent/persistent), %	81/8/11
Disease duration (y), mean $\pm$ SD	20.2 $\pm$ 14.5
ICS-untreated period (y), mean $\pm$ SD	9.2 $\pm$ 13.1
ICS daily maintenance dose (μ g), mean $\pm$ SD†	525 $\pm$ 318
No. of other controller medications, mean $\pm$ SD	1.4 $\pm$ 1.2
Treatment step (2/3/4/5), %‡	16/27/49/8
Sputum production (0/1/2/3), %§	54/20/8/18
ACT (points), mean $\pm$ SD	22.6 $\pm$ 3.5
History of admission due to asthma, n (%)	78 (35)
Allergic rhinitis, n (%)	129 (58)
Chronic sinusitis, n (%)	65 (29)
Blood neutrophils, %	60.1 (10.0)
Blood eosinophils, %	5.2 (4.9)
Serum IgE (IU/mL), median (range)	180 (0-16000)
Serum periostin (ng/mL), mean $\pm$ SD	92.8 $\pm$ 38.4
hsCRP (mg/L), mean $\pm$ SD	1341 $\pm$ 3147
ECP (μ g/L), mean $\pm$ SD	15.1 $\pm$ 29.3
FEV ₁ at the first measurement (L), mean $\pm$ SD	2.11 $\pm$ 0.69
Predicted FEV ₁ at the first measurement (%), mean $\pm$ SD	91.9 $\pm$ 19.2
FEV ₁ /FVC at the first measurement (%), mean $\pm$ SD	73.9 $\pm$ 9.8
FEV ₁ at enrollment (L), mean $\pm$ SD	2.04 $\pm$ 0.73
Predicted FEV ₁ at enrollment (%), mean $\pm$ SD	97.4 $\pm$ 22.2
FEV ₁ /FVC at enrollment (%), mean $\pm$ SD	72.2 $\pm$ 10.0
Reversibility at enrollment (%), mean $\pm$ SD¶	3.8 $\pm$ 6.0

Data at enrollment are presented unless otherwise stated.

FVC, Forced vital capacity.

*Considered atopic when 1 or more specific IgE antibodies against cat or dog dander, weed, grass, or Japanese cedar pollens, molds, or house dust mite were positive.

†Equivalent to fluticasone propionate.

‡According to the Global Initiative for Asthma 2010 guideline.²⁷

§0 = never; the details are shown in Table I.

||The first pulmonary function test was performed at least 1 year after the commencement of ICS treatment and at 25 years of age or older.

¶Airway reversibility to 200 μ g of inhaled salbutamol (n = 206).

decline in FEV₁ (Table III). Of these, high serum periostin was significant after controlling for multiple testing with the use of the false discovery rate ($q = 0.03$; data not shown in Table III).³⁰ Multivariate analysis showed that greater decline of FEV₁ was solely associated with high serum periostin (≥ 95 ng/mL) (estimated effect, -5.39 ; 95% CI, -10.0 to -0.77 ; $P = .02$).

Fifty-two patients (23%) showed a decline in FEV₁ of 30 mL or greater per year (mean, -51.8 ± 18.4 mL per year) and were considered rapid decliners.³¹ When adjusted by confounders, higher serum periostin levels at enrollment, treatment step 5, a history of admission due to asthma exacerbation, higher ICS daily doses, comorbid or a history of sinusitis, and ex-smoking were associated with a decline in FEV₁ of 30 mL or greater per year. High serum periostin (≥ 95 ng/mL) was also associated with a decline in FEV₁ of 30 mL or greater per year (Table IV). On multivariate analysis, high serum periostin (≥ 95 ng/mL), treatment step 5, and ex-smoking were independent risk factors for a decline in FEV₁ of 30 mL or greater per year (Table IV).

Of the 224 patients, 19 patients were on treatment step 5, and 36 patients took high-dose ICS (1000 μ g or higher doses of ICS equivalent to fluticasone propionate daily). When patients were stratified into the high periostin group, the average Δ FEV₁ of patients on treatment step 5 (n = 9) was -41.0 ± 49.3 mL per year, and 7 of them (78%) had excess decline; the average Δ FEV₁ of patients on high-dose ICS (n = 18) was -34.3 ± 39.4 mL per year, and 11 of them (61%) had a decline in FEV₁ of 30 mL or greater per year.

Serum periostin levels and clinical indices

In 224 patients, serum periostin levels were weakly associated with blood eosinophil counts (Fig 2), serum IgE (Fig 2) and ECP levels ($r = 0.25$, $P = .0005$), ICS-untreated period, that is period between onset of asthma and the initiation of ICS therapy ($r = 0.16$, $P = .01$), daily maintenance doses of ICS at enrollment ($r = 0.13$, $P = .05$), and a history of admission due to asthma exacerbation ($r = 0.15$, $P = .03$). Serum periostin levels were significantly higher in patients on high-dose ICS (≥ 1000 μ g daily) than in the remaining patients (110.3 ng/mL vs 89.5 ng/mL; $P = .003$). Finally, serum periostin levels were higher in patients with sinusitis than in patients without sinusitis (103.9 ng/mL vs 88.3 ng/mL; $P = .007$). Serum periostin levels did not show any seasonal variability or association with age at onset of asthma (data not shown).

POSTN gene polymorphisms

Associations between polymorphisms of the *POSTN* gene, which encodes periostin, and both serum periostin levels and pulmonary function decline were then investigated. In one patient, DNA quality was insufficient for genotyping; thus, 3 tag SNPs of the *POSTN* gene were analyzed in 223 patients. All genotyped data were in Hardy-Weinberg equilibrium. The frequencies of the 3 tag SNPs and analysis results with the use of dominant and recessive models for serum periostin levels and a decline in FEV₁ of 30 mL or greater per year are presented in Table V.

Serum periostin levels were higher in patients with the GG genotype of rs3829365 than in patients with the GC/CC genotype (GG 98.7 ng/mL vs GC/CC 86.1 ng/mL; $P = .003$). rs1028728 was not associated with serum periostin levels or with the frequency of rapid decliners, but patients with the TT genotype of rs1028728, 4 patients only, showed no significant decline compared with the AA/AT genotype (AA/AT, -8.6 mL per year vs TT, 29.3 mL per year; $P = .03$). Rapid decliners were more frequently observed in patients with the minor A allele of rs9603226 than in the GG genotype (GG 16% vs AG/AA 30%; $P = .02$). A marked difference in the frequency of rapid decliners was observed when patients were stratified into the high periostin group [GG of rs9603226 (n = 37) 19% vs AG/AA (n = 47) 45%; $P = .01$].

DISCUSSION

To the best of our knowledge, this is the first study to identify a relationship between greater decline in FEV₁ and higher serum periostin levels, particularly if they were 95 ng/mL or more, in asthmatic patients on ICS treatment. It was also shown that high serum periostin, together with treatment step 5 and light ex-smoking (ie, ex-smoking with 10 pack-years or less), was an independent risk factor for a decline in FEV₁ of 30 mL or greater per year. In addition, polymorphisms of the *POSTN* gene, which

TABLE III. Estimated effects of clinical indices and biomarkers on Δ FEV₁

	Estimates	95% CI	P value
Smoking history, ex vs never	−8.48	−20.2 to 3.27	.16
Atopic predisposition	−1.10	−6.29 to 4.09	.68
Disease duration (y)	−4.79	−18.4 to 8.86	.56
ICS-untreated period (y)	0.10	−0.24 to 0.45	.65
ICS daily maintenance dose (μ g)	−0.01	−0.03 to 0.001	.07
No. of other controller medications	−0.36	−4.21 to 3.49	.86
Adherence to medication, incomplete vs complete*	−4.56	−9.08 to −0.04	.05
Treatment step, 5 vs 2 to 4†	−7.77	−15.7 to 0.13	.05
Sputum production, never vs others‡	0.99	−3.53 to 5.51	.67
ACT (points)	1.53	0.29 to 2.77	.02
History of admission due to asthma	−4.49	−9.45 to 0.46	.08
Aspirin hypersensitivity	−6.52	−20.0 to 6.98	.34
Asthma deterioration at the working place	−12.2	−54.4 to 30.0	.57
Allergic rhinitis	−1.21	−5.88 to 3.45	.61
Allergic dermatitis	4.51	−1.51 to 10.5	.14
Chronic sinusitis	−10.1	−19.8 to −0.27	.04
Ischemic heart disease	3.41	−16.6 to 23.4	.74
Hypertension	−3.79	−9.12 to 1.53	.16
Dyslipidemia	−3.67	−9.42 to −2.06	.21
Diabetes mellitus	−8.03	−15.4 to −0.67	.03
Gastroesophageal reflux disease	−3.85	−9.89 to 2.19	.21
Malignancy	−3.44	−26.0 to 19.1	.76
Postmenopause	5.05	−14.2 to 24.3	.60
Pet breeding	−0.28	−12.6 to 12.0	.96
Log blood neutrophils (%)	−7.40	−69.1 to 54.3	.81
Log blood eosinophils (%)	−0.67	−1.60 to 0.27	.16
Log serum IgE (IU/mL)	−2.85	−9.74 to 4.04	.42
Log serum periostin (ng/mL)	−29.1	−56.2 to −1.97	.04
Log serum hsCRP (mg/L)	−1.88	−9.85 to 6.10	.64
Log serum ECP (μ g/L)	−4.47	−15.7 to 6.81	.44
Periostin group, high vs low§	−6.96	−11.4 to −2.51	.002

Estimated effects were adjusted by sex, height, age at enrollment, and FEV₁ at the first measurement.

*Complete, when patients answered that they never forgot to take ICS or other medications; incomplete, the remaining cases.

†According to the Global Initiative for Asthma 2010 guideline.²⁷

‡The details are shown in Table I.

§Patients were stratified into 2 groups according to their serum periostin levels: high $\geq$ 95 ng/mL, low < 95 ng/mL.

encodes periostin, were associated with serum periostin levels and a decline in FEV₁ of 30 mL or greater per year in asthmatic patients. These findings suggest that serum periostin may be a useful biomarker for the development of airflow limitation in asthmatic patients on ICS.

In this study, despite long-term treatment with ICS with or without other controllers, 23% of asthmatic patients were rapid decliners who showed a decline in FEV₁ of 30 mL or greater per year, for which treatment step 5 was an independent risk factor. Adherence to ICS treatment and the frequency of early intervention with ICS did not differ between rapid decliners and nondecliners, although long-term adherence to ICS was undetermined in the present study. In previous studies of patients who were not treated with ICS, severe exacerbation of asthma contributed to greater annual decline of pulmonary function,^{6,7} but the exacerbation-related greater annual decline disappeared in an early intervention group with ICS treatment in the START study,⁶ which might be interpreted to mean that asthmatic patients on ICS treatment have little risk of accelerated FEV₁ decline. However, because the START study originally recruited patients with mild persistent asthma, its results cannot simply be applied to patients with severe asthma. As observed in the present study, there would be a subset of asthmatic patients still at risk of greater

annual decline of pulmonary function despite intensive treatment for asthma.

Persistent eosinophilic airway inflammation is a key process in irreversible airway obstruction.¹⁰ Indeed, exhaled nitric oxide of 20 ppb or higher is a risk factor for accelerated FEV₁ decline in patients with difficult-to-treat asthma.¹⁸ Studies on novel therapies for refractory eosinophilic asthma, that is, anti-IL-5 therapy³² and anti-IL-13 therapy,³³ reported that these treatments may reverse airway remodeling when patients are adequately targeted, suggesting the necessity of establishing “companion diagnostics” for this population. According to the most recent study, serum periostin is the single best biomarker to reflect sputum and tissue eosinophilia among several biomarkers, including blood eosinophils and exhaled nitric oxide.²⁵ In the present study, the serum periostin level, which was associated with the blood eosinophil count, was the sole biomarker that reflected greater decline in FEV₁. Periostin is secreted by airway epithelial cells^{23,24} and lung fibroblasts²¹ in response to IL-4 and IL-13 and is thought to be secreted into the capillary vessels. Downstream of IL-13, which plays a pivotal role in subepithelial airway fibrosis,³⁴ airway remodeling,³⁵ and steroid insensitivity,³⁶ periostin mediates collagen synthesis²⁴ and fibrillogenesis^{24,37} by binding to collagen³⁷ and activates TGF- β .²⁴ In the asthmatic airway, periostin is

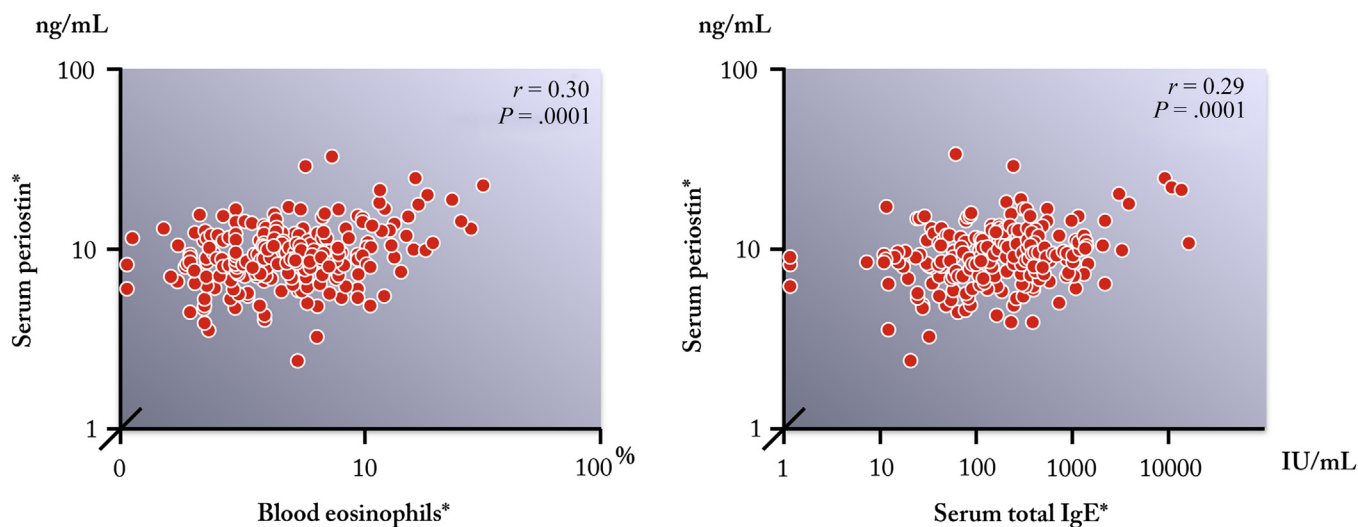
TABLE IV. Estimated effects of clinical indices and serum periostin on a decline in FEV₁ of 30 mL or greater per year

	Univariate analysis			Multivariate analysis		
	Estimates	95% CI	P value	Estimates	95% CI	P value
Treatment step, 5 vs 2 to 4*	1.63	0.51 to 2.60	.004	1.24	0.078 to 2.30	.04
History of admission due to asthma	1.09	0.37 to 1.90	.003	0.70	−0.11 to 1.50	.09
ICS daily maintenance dose (μg)	0.001	0.00 to 0.002	.01	—		
Chronic sinusitis	0.82	0.11 to 1.53	.03	0.61	−0.15 to 1.37	.12
Smoking history, ex vs never	0.87	−0.002 to 1.74	.05	0.98	0.030 to 1.93	.04
Log serum periostin (ng/mL)	2.96	0.78 to 5.13	.008	—		
Periostin group, high vs low†	1.03	0.33 to 1.72	.004	0.87	0.11 to 1.63	.03

Estimated effects were adjusted by sex, height, age at enrollment, and FEV₁ at the first measurement. ICS daily maintenance dose was excluded from multivariate analysis because of its strong correlation with treatment step.

*According to the Global Initiative for Asthma 2010 guideline.²⁷

†Patients were stratified into 2 groups according to their serum periostin levels: high ≥ 95 ng/mL, low < 95 ng/mL.

**FIG 2.** Relationships between serum periostin levels and blood eosinophil counts (*left*) or serum IgE levels (*right*). *Presented in logarithmic scales on both the x- and y-axes.

deposited in the subepithelial layer, colocalizing with collagens I, III, and V; fibronectin; tenascin-C; and periostin itself,²¹ which indicates involvement of periostin in airway remodeling in asthma. Collectively, periostin may be a key molecule that links eosinophilic inflammation and remodeling via IL-13 in asthmatic airways. Further roles of periostin in allergic inflammation and remodeling in the airways remain undetermined because studies that used periostin-deficient mice with acute allergen exposure have yielded conflicting findings^{38–40}; one study showed that periostin facilitates eosinophil infiltration into the lung,³⁸ whereas 2 other studies suggested protective roles of periostin.^{39,40} Meanwhile, a recent study of a chronic mouse model of atopic dermatitis reported periostin's role in the chronicity of T_H2 inflammation.²⁹

In the present study, patients on high-dose ICS showed higher serum periostin levels than the other patients. Although a longitudinal study is needed to determine responses of serum periostin levels to ICS treatment, we do not think that the high serum periostin levels in patients on high-dose ICS were induced by ICS treatment, because periostin expression in the airway epithelium was decreased with ICS treatment.²³ Rather, the elevation of serum periostin in this population may reflect IL-13-mediated inflammation that is partly refractory to ICS, as was reported in a recent study by Jia et al.²⁵ They showed that, in

patients with severe asthma who were treated with high doses of ICS (>1000 μg daily), elevation of serum periostin levels was associated with persistent airway tissue eosinophilia, concluding that serum periostin is a systemic biomarker of airway eosinophilia refractory to high-dose ICS.²⁵ Providing further support, among patients with moderate-to-severe asthma who are inadequately controlled despite ICS treatment, patients with high serum periostin levels are likely to benefit from anti-IL-13 antibody, lebrikizumab, treatment.³³ The novelty of the present finding is that high serum periostin is an independent risk factor for greater decline in FEV₁, providing the first evidence for the potential association between persistent T_H2- or IL-13-driven inflammation refractory to ICS treatment and greater decline in FEV₁, a functional consequence of airway remodeling.

Needless to say, current smokers with asthma have more accelerated FEV₁ decline⁴ than those not smoking, and current smoking impairs the therapeutic response to ICS or oral corticosteroids.⁴¹ Meanwhile, smoking cessation improves FEV₁ levels,⁴² and ex-smokers with asthma with 10 pack years or more show an intermediate response to short-term oral corticosteroid treatment, between current smokers and never-smokers.⁴¹ In the present study, rather unexpectedly, ex-smoking with 10 pack years or less was still an independent risk factor for a decline in FEV₁ of 30 mL or greater per year. It should be recognized that

TABLE V. Frequencies of 3 tag SNPs and analysis results with the use of dominant and recessive models for serum periostin levels and frequency of rapid decliners

Tag SNP	Genotype	No. (%)	Allelic	No. (%)	Serum periostin levels		Frequency of rapid decliners	
					P value		P value	
					Dominant*	Recessive†	Dominant*	Recessive†
rs1028728	AA	164 (74)	A	383 (86)	.40	.46	.17	.14
	AT	55 (25)	T	63 (14)				
	TT	4 (2)						
rs3829365	GG	113 (51)	G	316 (71)	.003	.70	.40	.33
	GC	90 (40)	C	130 (29)				
	CC	20 (9)						
rs9603226	GG	107 (48)	G	311 (70)	.80	.33	.01	.81
	AG	97 (44)	A	135 (30)				
	AA	19 (9)						

Rapid decliners are defined as patients who showed a decline in FEV₁ of 30 mL or greater per year.

*Assuming that heterozygotes have the same increased risk as minor homozygous genotypes.

†Assuming that heterozygotes have no increased risk.

even light ex-smoking increases the risk of airway remodeling in asthmatic patients on ICS, and its underlying mechanisms should be clarified.

Chronic sinusitis is a well-known comorbidity with severe asthma.^{43,44} In the present study, rapid decliners were more frequently observed in asthmatic patients with sinusitis than patients without sinusitis on univariate analysis, and their periostin levels were higher than in patients without sinusitis. In the present study, polypoid lesions in the sinuses were not evaluated by otolaryngologists at enrollment. However, considering that periostin is upregulated in nasal polyp tissue in patients with chronic rhinosinusitis,⁴⁵ asthmatic patients with sinusitis may have had severe upper and lower airway inflammation with persistent increases in periostin expression, which may have resulted in a decline in FEV₁ of 30 mL or greater per year. Periostin is a potential molecule that unifies sinusitis and severe asthma.

Periostin is encoded on the *POSTN* gene, which is located on chromosome 13q13.3. rs3829365, which is located at the 5' untranslated region that may contain sequences to regulate translation efficiency or mRNA stability, was associated with serum periostin levels. This finding suggests that, besides IL-13, a master regulator of periostin, genetic background partly determines periostin levels, although a replication study would be necessary to confirm this. The minor A allele of rs9603226, located 66 bp upstream of exon 21 in the C-terminal region, was associated with a decline in FEV₁ of 30 mL or greater per year. In periostin, FAS I domains are thought to be primary binding sites to fibronectin, tenascin-C, and collagen V,²¹ whereas the C-terminal region in its intact form may down-regulate the binding activity of periostin to these extracellular matrix proteins.²¹ We therefore speculate that the minor A allele of rs9603226 might modify the binding activity at the C-terminal region and might facilitate airway remodeling, particularly if the airway is in a periostin-enriched milieu. Further studies are needed to clarify if these SNPs are functional variants.

The age of patients in this study appears to be older than in other Euro-American studies.^{6,7,14,18,20,23,25} One reason for the age distribution would be the entry criteria of this study. Another reason would be explained by population aging, including population with asthma in Japan. According to a patient survey by the Japanese Ministry of Health, Labour and Welfare in 2008, patients aged 70 to 74 years were the most frequent age group of

adult patients with asthma,⁴⁶ which is still older than the average age of patients in this study.

There are several limitations to the present study. First, because this study was observational in nature, ICS doses and numbers or types of controllers were not fixed during the follow-up period. Controllers such as long-acting β_2 agonists were not withdrawn at pulmonary function testing to evaluate function on daily medications, which may have resulted in the small average Δ FEV₁, -7.8 mL per year. Meanwhile, averages of 16.2 measurements of FEV₁ and 8.0 years of follow-up were satisfactory for a longitudinal analysis of pulmonary function,⁴⁷ and Δ FEV₁ was normally distributed. Second, serum biomarkers were measured only once at enrollment, but the significant associations between *POSTN* gene polymorphisms and serum periostin levels or a decline in FEV₁ of 30 mL or greater per year may circumvent the inherent insufficiency of single measurement of serum periostin. Third, most of the clinical information, including smoking history and chronic sinusitis, was based on a self-completed questionnaire, which might be biased by recall memory. Despite these limitations, the present findings may provide directions for future research.

In conclusion, serum periostin appears to be a useful biomarker that reflects the development of airflow limitation in patients on prolonged treatment with ICS. *POSTN* gene polymorphisms may also be helpful for identification of rapid decliners.

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Clinical implications: Serum periostin levels reflect greater FEV₁ decline in asthmatic patients on inhaled corticosteroid treatment. *POSTN* gene polymorphisms may also be helpful for identifying rapid FEV₁ decliners.

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METHODS

Patients

Patients with asthma were recruited from 9 institutions belonging to the Kinki Hokuriku Airway disease Conference where asthma specialists manage patients, including 6 university hospitals, 2 satellite general hospitals, and 1 satellite clinic. Asthma was diagnosed according to the American Thoracic Society criteria^{E1} on the basis of a history of recurrent episodes of wheezing and chest tightness with or without cough and documented airway reversibility to a bronchodilator or hyper-responsiveness to inhaled methacholine. From September 2009 to December 2011, patients were enrolled if they had received ICS treatment for 4 years or more, undergone 3 or more pulmonary function tests when they were stable, and were free from exacerbations for at least 1 month. The first pulmonary function test was performed at least 1 year after the commencement of ICS treatment and at 25 years of age or older. Patients who had smoked >10 pack years, smoked in the past 1 year, or had other pulmonary diseases were excluded.

Self-completed questionnaire and clinical indices

The self-completed questionnaire was composed of 4 major items, as presented in Table I.

Adherence to ICS or other medications, frequency of sputum production, and requirement for systemic corticosteroids during the past 6 months were graded as shown in Table I. The Asthma Control Test (ACT) was also scored. Duration of ICS treatment and details on medication at enrollment were recorded from medical charts by patients' physicians. The treatment step at enrollment was determined according to the Global Initiative for Asthma 2010 guideline.^{E2}

Measurement of systemic biomarkers

Blood eosinophil and neutrophil counts and serum levels of total IgE (ImmunoCAP total IgE; Phadia K.K., Tokyo, Japan), specific IgE against common inhaled allergens (ImmunoCAP specific IgE), eosinophil cationic protein (ECP; ImmunoCAP ECP), high-sensitivity C-reactive protein (hsCRP; CardioPhase hsCRP; Siemens Healthcare Diagnostics K.K., Tokyo, Japan), and periostin were determined.

Serum periostin levels were measured with an enzyme-linked immunosorbent assay at Shino-test (Kanagawa, Japan), as described previously.^{E3} Briefly, 2 rat anti-human periostin monoclonal antibodies (SS18A and SS17B) were used. SS18A and SS17B are antibodies against the first and fourth FAS I domains, respectively. Intra-assay and interassay coefficients of variation ranged from 1.31% to 2.54% and 1.49% to 2.01%, respectively.

Haplotype analysis, DNA extraction, and genotyping of the *POSTN* gene

A total of 47 single-nucleotide polymorphisms (SNPs) in the region of the *POSTN* gene and its upstream, total 39 kb, was captured in the HapMap Japanese data set with minor allele frequencies of >0.10. Pairwise tagging was performed at $r^2 > 0.8$ with the use of a tagger in Haploview 4.2 software. Haplotype analysis identified 4 major haplotypes and 2 minor haplotypes. Two minor haplotypes were grouped into the closest major haplotype, and 3 tag SNPs that determined the 4 haplotypes were identified (Fig 1). These 3 tag SNPs were located at promoter region (rs1028728), 5' untranslated region (rs3829365), and at intron 66 bp upstream of exon 21 (rs9603226). The frequencies of the minor alleles in the Japanese population were 0.136 (rs1028728), 0.278 (rs3829365), and 0.330 (rs9603226).

Genomic DNA was isolated from blood cells using a QIAamp DNA Blood Mini Kit (Qiagen, Tokyo, Japan). SNPs were genotyped with a Taqman genotyping assay according to the manufacturer's instructions (Applied Biosystems, Tokyo, Japan) and analyzed with an Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems).

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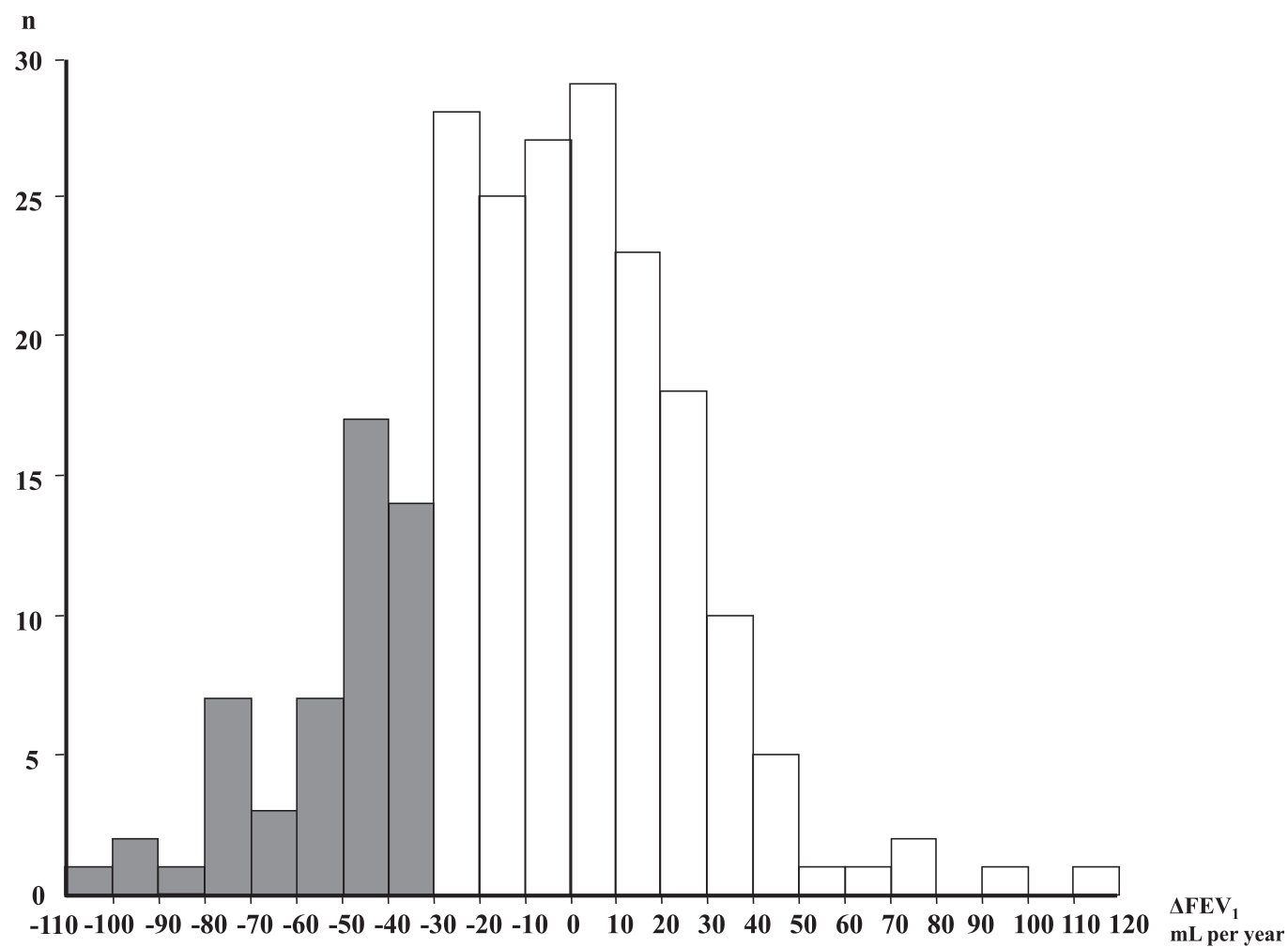


FIG E1. Distribution of ΔFEV_1 in the study population.

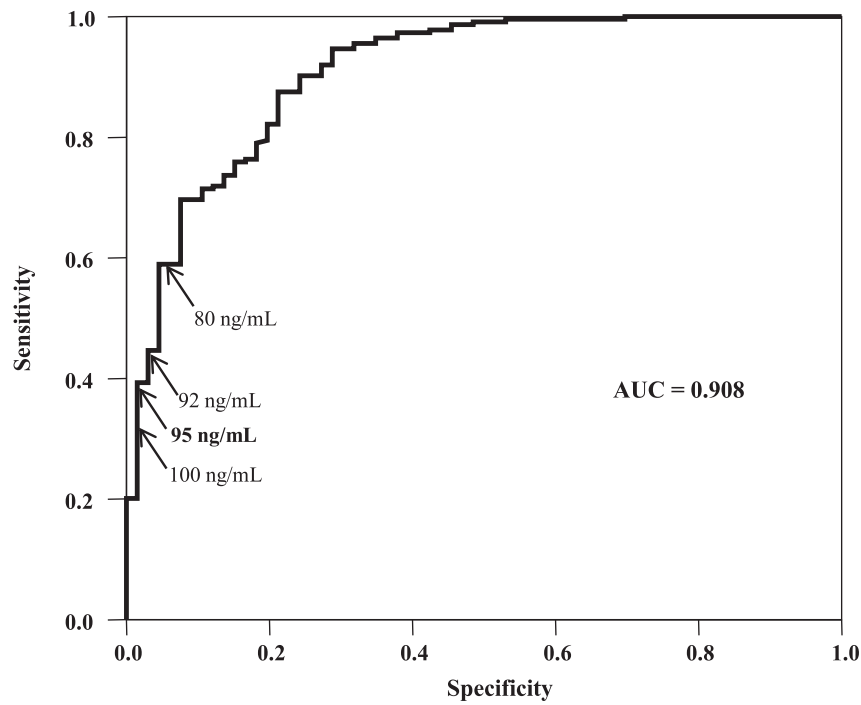


FIG E2. ROC curve analysis of serum periostin levels comparing asthmatic patients with healthy subjects, in which the cutoffs of 95 ng/mL, 80 ng/mL, 92 ng/mL, and 100 ng/mL are presented with *arrows*.