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

Yoshihiro Kanemitsu, MD,[a,b]* Hisako Matsumoto, MD, PhD,[a,b]* Kenji Izuhara, MD, PhD,[c] Yuji Tohda, MD, PhD,[b,d] Hideo Kita, MD, PhD,[b,e] Takahiko Horiguchi, MD, PhD,[b,f] Kazunobu Kuwabara, MD, PhD,[b,f] Keisuke Tomii, MD, PhD,[b,g] Kojiro Otsuka, MD, PhD,[a,b,g] Masaki Fujimura, MD, PhD,[b,h] Noriyuki Ohkura, MD, PhD,[b,h] Katsuyuki Tomita, MD, PhD,[b,d] Akihito Yokoyama, MD, PhD,[b,i] Hiroshi Ohnishi, MD, PhD,[b,i] Yasutaka Nakano, MD, PhD,[b,j] Tetsuya Oguma, MD, PhD,[b,j] Soichiro Hozawa, MD, PhD,[b,k] Tadao Nagasaki, MD,[a] Isao Ito, MD, PhD,[a] Tsuyoshi Oguma, MD,[a] Hideki Inoue, MD,[a] Tomoko Tajiri, MD,[a] Toshiyuki Iwata, MD,[a] Yumi Izuhara, MD,[a] Junya Ono, BS,[l] Shoichiro Ohta, MD, PhD,[m] Mayumi Tamari, MD, PhD,[n] Tomomitsu Hirota, DDS, PhD,[n] Tetsuji Yokoyama, MD, PhD,[o] Akio Niimi, MD, PhD,[a,b,p] and Michiaki Mishima, MD, PhD[a,b] *Kyoto, Saga, Osaka, Aichi, Hyogo, Kanazawa, Kochi, Shiga, Hiroshima, Kanagawa, and Saitama, Japan*

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 FEV_1, 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 FEV_1 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 FEV_1 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 FEV_1 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;132:305-12.)

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

Airway inflammation and remodeling are key features of asthma that have been demonstrated by pathologic[1] 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 predisposition[11-13] 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 FEV_1 and develop irreversible airway obstruction despite adequate treatment.[17,18] van Veen et al[18] found that exhaled nitric oxide of 20

From [a]the Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, Kyoto; [b]Kinki Hokuriku Airway disease Conference (KiHAC), Osaka; [c]the Division of Medical Biochemistry, Department of Biomolecular Sciences, Saga Medical School, Saga; [d]the Department of Respiratory Medicine and Allergology, Faculty of Medicine, Kinki University, Osaka; [e]the Department of Respiratory Medicine, Takatsuki Red Cross Hospital, Osaka; [f]the Department of Respiratory Internal Medicine, Fujita Health University Second Educational Hospital, Aichi; [g]the Department of Respiratory Medicine, Kobe City Medical Center General Hospital, Hyogo; [h]the Department of Respiratory Medicine, Cellular Transplantation Biology, Kanazawa University Graduate School of Medicine, Kanazawa; [i]the Department of Hematology and Respiratory Medicine, Kochi University, Kochi; [j]the Division of Respiratory Medicine, Department of Internal Medicine, Shiga University of Medical Science, Shiga; [k]Hiroshima Allergy and Respiratory Clinic, Hiroshima; [l]Shino-Test Corporation, Kanagawa; [m]the Department of Laboratory Medicine, Saga Medical School, Saga; [n]Laboratory for Respiratory Diseases, Center for Genomic Medicine, RIKEN, Yokohama, Kanagawa; [o]the Department of Health Promotion, National Institute of Public Health, Wako, Saitama; and [p]the Department of Medical Oncology and Immunology, Nagoya City University School of Medical Sciences, Aichi.

*These authors contributed equally to this work.

Supported by a project of 2009 KiHAC Respiratory Medicine Group and the Adaptable and Seamless Technology Transfer Program through target-driven R&D, JST.

Disclosure of potential conflict of interest: K. Izuhara has consultant arrangements with Chugai Pharmaceutical Company and has a patent with F. Hoffmann-LaRoche Ltd. A. Yokoyama has received grants from Ono Pharm, Novartis, AstraZeneca, Kyorin, GlaxoSmithKline, and Teijin Pharm and has received payment for lectures from Ono Pharm, Novartis, AstraZeneca, Kyorin, GlaxoSmithKline, Merk Sharp & Dohme, and Boehringer Ingelheim. H. Ohnishi has received payment for lectures from GlaxoSmithKline, Astellas, and AstraZeneca. Y. Nakano has received payment for lectures from Nippon Boehringer Ingelheim. Tsuyoshi Oguma has received payment for lectures from GlaxoSmithKline and AstraZeneca. M. Tamari has received payment for lectures from GlaxoSmithKline, Boehringer Ingelheim, and Sanofi Aventis. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication September 20, 2012; revised April 11, 2013; accepted for publication April 11, 2013.

Available online June 19, 2013.

Corresponding author: Hisako Matsumoto, MD, PhD, Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan. E-mail: hmatsumo@kuhp.kyoto-u.ac.jp.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2013.04.050

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 FEV_1 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 al[21] 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, underwent 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 ± SD, 60.7 ± 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 FEV_1 (ΔFEV_1) 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 ΔFEV_1 were estimated with a generalized linear mixed model with adjustment for sex, height, age at enrollment, and FEV_1 at the first measurement. The institutions were included as random effects in this model. On univariate analysis of ΔFEV_1, 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 software[30] to determine spurious significance in multiple testing. The effects on the dichotomous data for a decline in FEV_1 of 30 mL or greater per year[31] were similarly estimated with a generalized linear mixed model by IBM