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 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

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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

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 ± SDs. P values ≤ .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 FEV_1, follow-up period, and ΔFEV_1 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 ΔFEV_1 in this population is shown in Fig E1 (in the Online Repository available at www.jacionline.org). Within 2 years after

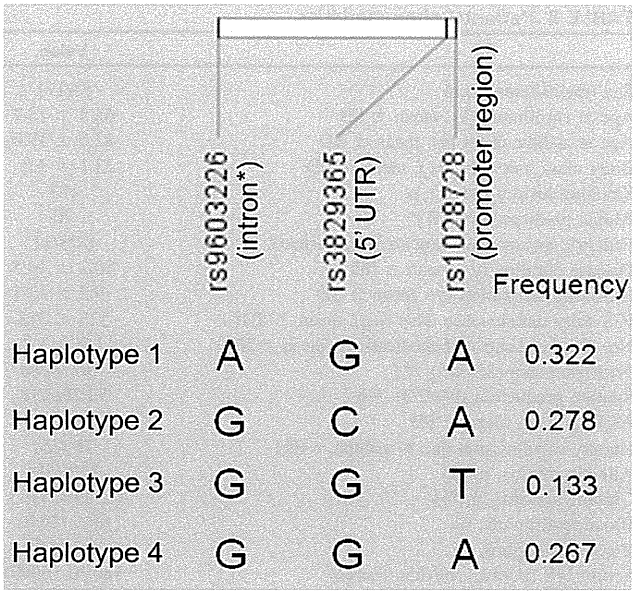

FIG 1. Three tag SNPs that determine 4 major haplotypes of the *POSTN* gene and haplotype frequencies in the Japanese population are presented. *At intron 66 bp upstream of exon 21.

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 FEV_1 and a decline in FEV_1 of 30 mL or greater per year

In an analysis of continuous values of ΔFEV_1, greater decline in FEV_1 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 ± SD	62.3 ± 13.7
Age at asthma onset (y), mean ± SD	42.0 ± 19.0
Body mass index (kg/m^2), mean ± SD	23.1 ± 3.5
Smoking history (never), n	181
Atopic predisposition (%)*	70
Pediatric asthma (none/recurrent/persistent), %	81/8/11
Disease duration (y), mean ± SD	20.2 ± 14.5
ICS-untreated period (y), mean ± SD	9.2 ± 13.1
ICS daily maintenance dose (μg), mean ± SD†	525 ± 318
No. of other controller medications, mean ± SD	1.4 ± 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 ± SD	22.6 ± 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 ± SD	92.8 ± 38.4
hsCRP (mg/L) mean ± SD	1341 ± 3147
ECP (μg/L) mean ± SD	15.1 ± 29.3
FEV_1 at the first measurement (L), mean ± SD‖	2.11 ± 0.69
Predicted FEV_1 at the first measurement (%), mean ± SD	91.9 ± 19.2
FEV_1/FVC at the first measurement (%), mean ± SD	73.9 ± 9.8
FEV_1 at enrollment (L), mean ± SD	2.04 ± 0.73
Predicted FEV_1 at enrollment (%), mean ± SD	97.4 ± 22.2
FEV_1/FVC at enrollment (%), mean ± SD	72.2 ± 10.0
Reversibility at enrollment (%), mean ± SD¶	3.8 ± 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 μg of inhaled salbutamol (n = 206).

decline in FEV_1 (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 FEV_1 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 FEV_1 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 FEV_1 of 30 mL or greater per year. High serum periostin (≥95 ng/mL) was also associated with a decline in FEV_1 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 FEV_1 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 μg or higher doses of ICS equivalent to fluticasone propionate daily). When patients were stratified into the high periostin group, the average ΔFEV_1 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 ΔFEV_1 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 FEV_1 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 μg 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 FEV_1 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 rs9630226 (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 FEV_1 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 FEV_1 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 ΔFEV_1

	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 (μg)	−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 (μg/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 FEV_1 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 ≥ 95 ng/mL, low < 95 ng/mL.

encodes periostin, were associated with serum periostin levels and a decline in FEV_1 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 FEV_1 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 FEV_1 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 FEV_1 decline in patients with difficult-to-treat asthma.[18] Studies on novel therapies for refractory eosinophilic asthma, that is, anti–IL-5 therapy[32] 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 FEV_1. Periostin is secreted by airway epithelial cells[23,24] and lung fibroblasts[21] 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 synthesis[24] and fibrillogenesis[24,37] by binding to collagen[37] 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 FEV_1 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 FEV_1 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.

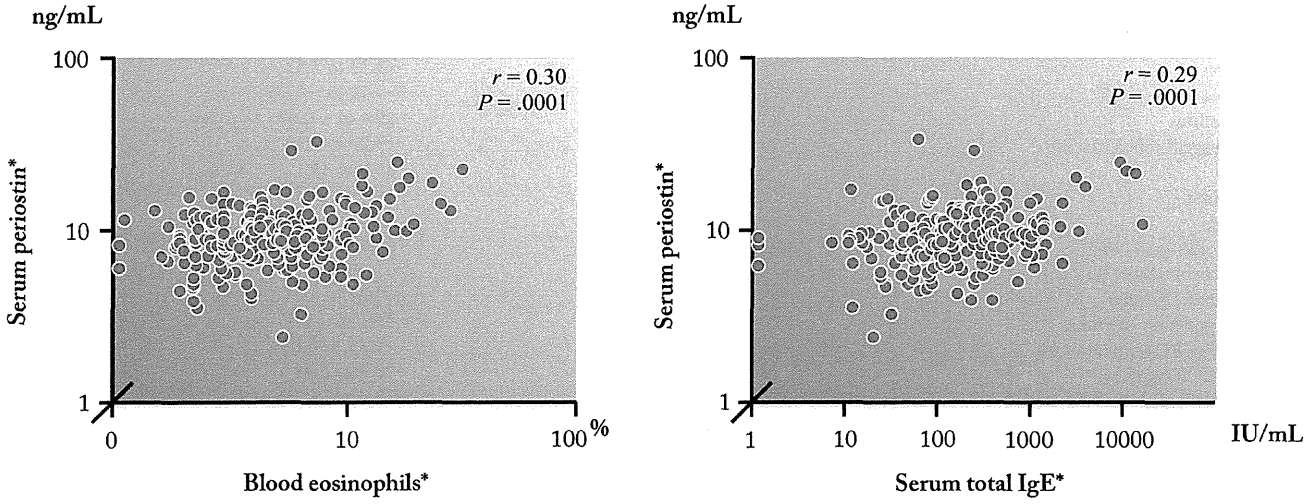

FIG 2. Relationships between serum periostin levels and blood eosinophil counts *(left)* or serum IgE levels *(right)*. *Presented in logarithmic scales on both the x- and y-axes.

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 FEV_1, 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 FEV_1, a functional consequence of airway remodeling.

Needless to say, current smokers with asthma have more accelerated FEV_1 decline[4] than those not smoking, and current smoking impairs the therapeutic response to ICS or oral corticosteroids.[41] Meanwhile, smoking cessation improves FEV_1 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 FEV_1 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

					Serum periostin levels		Frequency of rapid decliners	
					P value		*P* value	
Tag SNP	Genotype	No. (%)	Allelic	No. (%)	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 FEV_1 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 FEV_1 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 FEV_1 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 ΔFEV_1, −7.8 mL per year. Meanwhile, averages of 16.2 measurements of FEV_1 and 8.0 years of follow-up were satisfactory for a longitudinal analysis of pulmonary function,[47] and ΔFEV_1 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 FEV_1 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 FEV_1 decline in asthmatic patients on inhaled corticosteroid treatment. *POSTN* gene polymorphisms may also be helpful for identifying rapid FEV_1 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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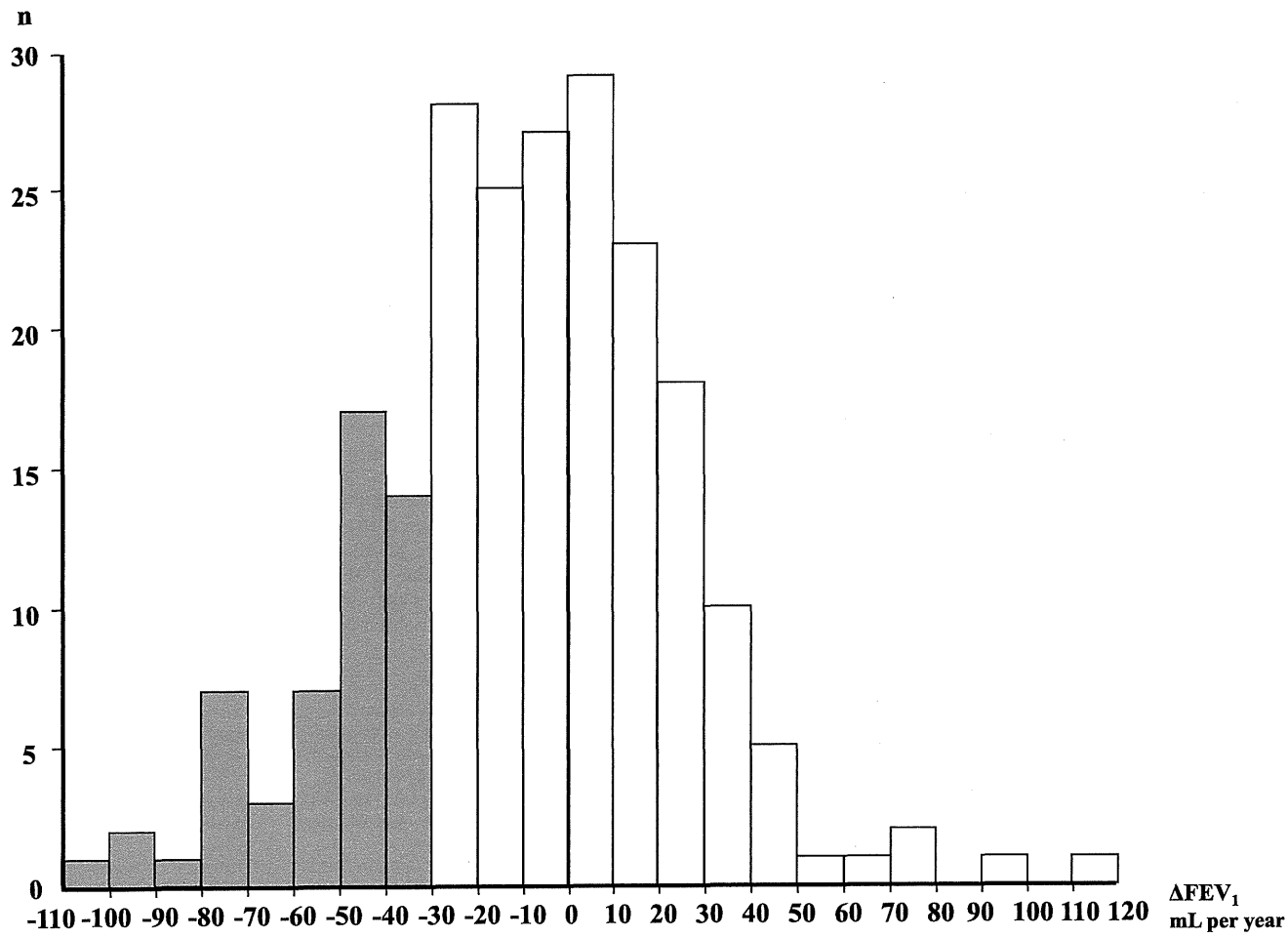

FIG E1. Distribution of ΔFEV_1 in the study population.

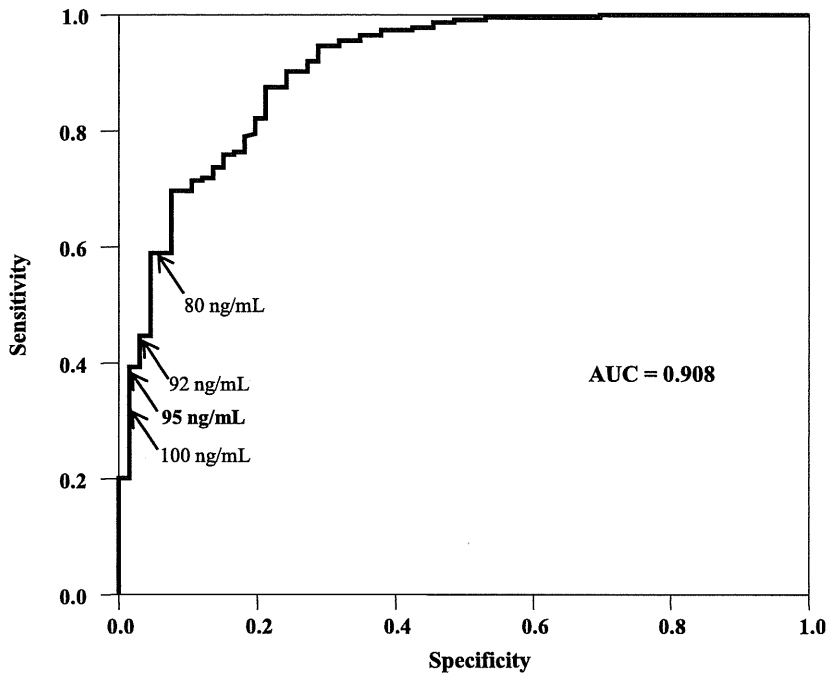

FIG E2. ROC curve analysis of serum periostin levels comparing asthmatic patients with healthy subjects, in which the cutoffs of 95 ng/mL, 80 ng/mL, 92 ng/mL, and 100 ng/mL are presented with *arrows*.

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Quadriceps weakness contributes to exercise capacity in nonspecific interstitial pneumonia

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KEYWORDS
Nonspecific interstitial pneumonia;
Skeletal muscle;
Six-minute walking test

Summary
Background and objective: It has been shown that peripheral muscle dysfunction is a critical factor in determining exercise intolerance in patients with several chronic lung diseases, including idiopathic pulmonary fibrosis. We hypothesized that exercise capacity would be, at least in part, determined by peripheral muscle dysfunction in patients with fibrotic nonspecific interstitial pneumonia (f-NSIP), another major subtype of fibrotic interstitial lung disease. The aim of the current study was to elucidate the relevance of peripheral muscle dysfunction and its contribution to exercise intolerance in f-NSIP.
Methods: The six-minute walk test was evaluated in 30 consecutive patients with f-NSIP along with potential determinants of exercise capacity, including respiratory muscle force and peripheral muscle force.
Results: Among 30 patients, the median age was 61 years, and 21 were female. Sixteen patients showed significantly decreased quadriceps force (QF), and 17 had significant decreases in maximum expiratory pressure. Exercise capacity and muscle power were clearly related to sex. Adjusted for sex, QF showed a significant relation to exercise capacity measured by six-minute walk distance (6MWD), whereas pulmonary function parameters such as vital capacity showed marginal correlations. In stepwise multiple regression analysis, only QF was an independent predictor of 6MWD.

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Conclusions: Quadriceps weakness is often observed in patients with f-NSIP. It seems that QF significantly contributes to exercise capacity in this population.

Introduction

The importance of evaluating exercise capacity is widely recognized in chronic lung diseases. Its clinical relevance has been intensely investigated especially in patients with chronic obstructive lung disease, and its impacts on their prognosis as well as their quality of life have been elucidated.[1] More recently, attention is also being directed to evaluation of exercise capacity in patients with interstitial lung diseases. Several recent studies have reported that six-minute walk distance (6MWD) is an independent and discriminating predictor of mortality among patients classified as having idiopathic pulmonary fibrosis (IPF).[2]

Fibrotic nonspecific interstitial pneumonia (f-NSIP) has been recognized, along with IPF, as one of the major types of chronic idiopathic interstitial pneumonias.[3–5] F-NSIP has come to be recognized as a distinct disease entity with characteristic clinical, radiologic, and pathologic features that differ from other idiopathic interstitial pneumonias.[5] However, the number of previous studies that have conducted exercise testing in f-NSIP patients is very limited.[2,6]

Previously, we reported factors related to exercise capacity in IPF and demonstrated that quadriceps weakness was related to exercise capacity.[7] However, the determinants of exercise tolerance in interstitial pneumonias other than IPF remain uncertain.

In considering the mechanisms of exercise limitation in patients with f-NSIP, we hypothesized that peripheral muscle weakness may exist in patients with f-NSIP and may contribute to exercise intolerance. We therefore assessed exercise tests and respiratory and peripheral muscle function of patients with f-NSIP along with tests of pulmonary function to explore the determinants of exercise capacity.

Materials and methods

Study subjects

Consecutive patients with f-NSIP diagnosed at Tosei General Hospital from April 2003 to March 2011, who consented to the study and underwent subsequent measurements in pulmonary function, exercise capacity and muscle strength, were retrospectively reviewed. During the study period a total of 257 cases of interstitial lung diseases were diagnosed by surgical lung biopsy at this hospital. One hundred and thirty patients were diagnosed as having fibrotic idiopathic interstitial pneumonia, and 38 of them were diagnosed with f-NSIP. The diagnosis of f-NSIP was confirmed by two lung pathologists. F-NSIP was newly diagnosed by a physician using the diagnostic criteria in the ATS/ERS consensus statement.[8] Patients who had received corticosteroids or similar medical treatment prior to the evaluation were excluded. Patients were also excluded if they had (1) clinically evident connective tissue disease (CTD), (2) cardiac disease, (3) obstructive lung disease such as COPD or asthma, and (4) other pathologic conditions (arthritis, malignancy, cerebrovascular disease).

This analysis was approved by our local institutional review board.

Exercise capacity

To evaluate exercise capacity, the current study adopted the six-minute walk test (6-MWT), a reliable, valid, and responsive measure of exercise tolerance in patients with interstitial lung diseases.[9] It also has major advantages over maximal exercise testing in terms of reproducibility in the routine evaluation of fibrotic interstitial pneumonias.[10]

The 6-MWT was conducted in all patients who participated in the study, according to the ATS statement.[11] Briefly, all patients were tested under standardized conditions by trained technicians. Baseline heart rate and oxygen saturation were measured. Patients were instructed to walk as far as possible in 6 min. The distance the patients could walk was recorded. Oxygen saturation was monitored and recorded continuously throughout the test by pulse oximetry. No supplemental oxygen was given during the test.

Pulmonary function tests and arterial blood gas tensions

All patients underwent pulmonary function testing including lung volumes and spirometry (CHESTAC-55V; Chest; Tokyo, Japan), according to the method described in the ATS 1994 update.[12] Single-breath diffusing capacity of carbon monoxide (DLco) was also measured (CHESTACV; Chest). The values for vital capacity (VC) and DLco were also related to predicted values. Arterial blood gas tensions were measured at rest.

Respiratory muscle force

Maximal inspiratory pressure (PI max) and maximal expiratory pressure (PE max) were determined in all patients. PI max was measured at residual volume, and PE max was measured near total lung capacity, according to the well-validated method proposed by Black and Hyatt (Vitalopower KH-101; Chest).[13] The highest value from at least three maneuvers was recorded. Reproducibility of the measurements was fairly good.

To determine patients with significant weakness in respiratory muscle power, the values were related to the predicted values[13] and those less than 80% of the prediction were considered to be significantly weak.[14]

Peripheral muscle force

The measurements of peripheral muscle forces were done with the methodologies well validated in the previous studies.[7,15]

Hand grip force (HF) was measured with a hydraulic hand dynamometer (Smedley's Dynamometer; TTM; Tokyo,

Japan). Peak HF (kg) was assessed with each hand with the shoulder, elbow and wrist in a neutral position.

Quadriceps force (QF) was measured using a dynamometer (Cybex II; Lumex; Bay Shore, NY). Peak torque (Newton-m) was measured in both legs during a maximal isokinetic knee extension maneuver with the hip in 90° flexion. The evaluation was performed in concentric mode with an angular speed of 60 s. After the practice session, each patient performed a series of four knee flexions/extensions on one side of the body and then the other, with a 15-s rest between the series.

The highest values for HF and QF from at least three respective maneuvers were recorded. Reproducibility of both measurements was good.

HF was related to age and sex specific predicted values,[16] and less than 80% of prediction was considered to be significantly weak.[17] QF was also related to normal values derived from healthy Japanese subjects, which were also age and sex specific.[18] QF less than 1.5 SD below norms were considered to be significant weakness, according to previous reports.[18]

Statistics

The data obtained from the 30 patients were analyzed statistically. All statistical analysis was performed using SPSS ver.17 (SPSS Inc., Chicago, IL).

The distribution of numeric data was stratified by sex and examined by Smirnov and Grubbs's test to detect possible significant (two-sided $p < 0.05$) outliers. To determine factors contributing to exercise performance in univariate analysis, linear regression models were assessed with raw 6MWD as the dependent variable and the model was adjusted for sex. Independent variables assessed in the model were age, height, weight, pulmonary function parameters (VC, DLco, PaO_2 and $PaCO_2$ assessed by arterial gas analysis), respiratory and peripheral muscle force (PI max, PE max, HF, and QF), and minimum SpO_2 observed in exercise testing. In the subsequent multivariate model, a stepwise multiple regression analysis was performed using 6MWD as a dependent variable, with adjustment either by sex only or by sex, age, height and weight. Any variable with $p < 0.2$ in univariate analysis were introduced in multivariable models as potential predictors. To avoid multicollinearity, only one of the highly correlated variables (coefficient of correlation ≥ 0.9) was entered in the multivariate model, if present.

In determining the factors contributing to QF, univariate analyses were conducted with all the variables except for 6MWD as independent variables. Subsequent multivariate analyses were conducted in a similar fashion. Comparisons between groups were performed using unpaired t-test.

A p value of <0.05 was considered to be significant. All values are presented as median (range), unless otherwise specified.

Result

Patient characteristics and anthropometric and pulmonary function data

Of 38 patients diagnosed with f-NSIP, six patients could not undergo exercise measurements because of a progressive clinical course, one had severe emphysematous change in lungs, and one had received corticosteroid therapy prior to the exercise measurement. These eight patients were excluded from the analysis. In the end, 30 patients with f-NSIP who fulfilled the full-set measurements were eligible for this study. All patients underwent all the measurements and there were no missing data. A majority of the patients were female and the median age was 61, although the ages were widely distributed. As shown in Table 1. There were significant differences in VC and DLco between males and females. However the prevalence of impaired VC (<80% predicted) or DLco (<70% predicted) did not differed significantly according to sex (male 4/9 versus female 14/21; $p = 0.43$ for VC, male 6/9 versus female 19/21; $p = 0.14$ for DLco). No patients showed resting hypoxemia in arterial blood gas analysis.

Exercise capacity

The exercise capacity of these patients is also shown in Table 1. Median 6-MWT distance was 560 m. Male patients walked longer than female patients ($p = 0.014$ in unpaired t-test). The median of the lowest SpO_2 during 6-MWT was 87.5%, showing hypoxemia on exercise. Of all patients, 50% (15/30 cases) showed exertional desaturation (SpO_2<88%) during 6-MWT. Although some of the patients showed remarkable desaturation measured by pulse oxymetry during exercise, all the patients completed the 6 min walking without any complication and the decreased SpO_2 and elevated pulse rate recovered quickly post test.

Muscle testing

Respiratory and peripheral muscle testing results are also shown in Table 1. There were significant differences between men and women in all muscle strength measurements ($p < 0.01$ for QF, HF, PEmax, and PImax). We defined the thresholds of significant muscle weakness as described in the Methods. According to these definitions, a majority of the patients showed significant weakness in PE max (17 of 30 patients) and QF (16 of 30 patients). Meanwhile, only a minority of the patients had significant weakness in HF (4 patients) and PI max (4 patients).

Explaining factors of exercise capacity

As shown in Table 1, 6MWD as well as muscle strength differed clearly by sex. Each of the possible contributing factors for 6MWD were then assessed by linear regression models adjusted for sex (Table 2). In this univariate analysis only QF showed a significant relation ($\beta = 1.030$, $p = 0.0282$) to 6MWD, whereas the following parameters showed marginal significance: PEmax, PaO_2 and DLco ($p < 0.1$); VC, HF and PImax ($p < 0.2$). A scatter diagram showing the correlation between 6MWD and QF is shown in Fig. 1.

To determine the independent predictors of exercise tolerance, we subsequently conducted a stepwise multiple regression analysis for 6MWD adjusted by gender. In this model, QF was the only independent determinant of 6MWD. The total variance explained by this model was 32% ($p < 0.01$) (Table 3, Model 1). In general, 6MWD is known to

Table 1 Patient characteristics.

	All	Men	Female
No	30	9	21
Age, yrs	61(34–75)	62(36–69)	59(34–75)
Height, cm	155.4(145.4–172.7)	166.5(159.7–172.7)	153.4 (145.4–159.2)
Weight, kg	59.9(41.5–84.4)	65(60.5–75)	54(41.5–84.4)
VC, L	1.96(1.03–4.48)	3.02(1.93–4.48)	1.70(1.03–2.91)
VC, %predicted	75.4(41.7–110.6)	87.5(57.1–110.6)	71.7(41.7–100.0)
DLco, mL/min/mmHg	8.8(2.4–28.8)	11.4(4.8–28.8)	8.4 (2.4–15.3)
DLco, % predicted	52.2(15.5–107.8)	63(25.9–107.8)	49.9 (15.5–87.6)
PaO_2, mmHg	81.4(62.4–96.8)	81.2(62.4–89.6)	81.4 (64.6–96.8)
$PaCO_2$, mmHg	40.8(34.1–49.4)	39.4(34.1–49.4)	41.0(35.9–44.7)
6MWD, m	560(333–710)	610(500–710)	545(333–660)
Lowest SpO_2 during test, %	87.5(58–97)	86.0(58–95)	88.0(70–97)
PI max, cmH_2O	95.1(34.1–181.2)	132.1(76.1–181.2)	82.5(34.1–151.5)
PI max, %predicted	117.0(51.5–194.3)	118.8(69.5–163.1)	115.2(51.5–194.3)
PE max, cmH_2O	123.3(53.4–243.8)	189.4(126.9–243.8)	105.2(53.4–190.2)
PE max, % predicted	75.3(40.8–141.2)	94.2(61.9–107.4)	74.8(40.8–141.2)
HF, kg	24.8(10.0–49.5)	39.3(32.5–49.5)	22.3(10–36.5)
HF, %predicted	96.6(50.5–131.1)	98.9(74.4–131.1)	94.7(50.5–114.2)
QF, Nm	83.3(45–236)	148.5(77.5–236)	74.5(45–110)
QF, %predicted	82.0(49.7–139.5)	106.2(55.4–139.5)	81.8(49.7–114.9)

Values are expressed as median (range) unless otherwise indicated.
SpO_2: percutaneous oxygen saturation. PI max: maximal inspiratory pressure, PE max: maximal expiratory pressure, HF: hand grip force, QF: quadriceps force, Nm: Newton-meter.

be related to age, height and weight. We subsequently assessed the multiple regression models with adjustment for sex, age, height and weight. In the latter model also, QF was proven to be the sole independent determinant of 6MWD among all the parameters. The total variance explained by this model was up to 55% ($p < 0.05$) (Table 3, Model 2).

Discussion

In the present study, we conducted simultaneous assessment of pulmonary function, exercise performance and muscle strength in 30 patients with f-NSIP to elucidate the determining factors for exercise capacity. We found that reduced PEmax and QF were often observed in patients with f-NSIP. Moreover, QF is the sole factor contributing significantly to exercise capacity according to our multivariate analysis. To our knowledge, this is the first report clarifying the relationship between peripheral muscle performance and exercise capacity in patients with f-NSIP.

Peripheral muscle dysfunction in COPD has been well characterized. Previous report by Gosselink et al. revealed

Table 2 Result of univariate analysis for factors relating to 6MWD.

	6MWD, m	
	β	p-Value
Age	−1.589	0.3372
Height	−3.622	0.3348
Weight	−1.680	0.2869
VC	48.188	0.1105
DLco	6.025	0.0975
PaO_2	3.303	0.0725
$PaCO_2$	4.151	0.4451
PI max	0.832	0.1127
PE max	0.704	0.0718
HF	4.452	0.1227
QF	1.030	0.0282
Lowest SpO_2 during test	2.250	0.2525

Models were adjusted for sex.

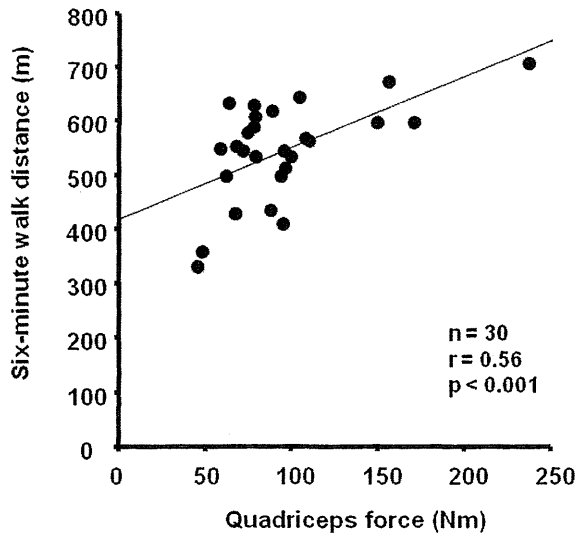

Figure 1 Correlations between six-minute walk distance and QF.

Table 3 Result of stepwise multiple regression analysis for determining factors of 6MWD.

	β	Standard errors	Standardized β	p
Model 1: Adjusted for sex				
QF, Nm	1.030	0.444	0.486	0.028
Sex, male	22.589	41.765	0.113	0.593
Model 2: Adjusted for sex, age, height and weight				
QF, Nm	1.771	0.505	0.835	0.002
Sex, male	118.371	53.124	0.594	0.035
Age	−0.988	1.644	−0.104	0.554
Height	−9.235	4.902	−0.743	0.033
Weight	−2.032	1.607	−0.245	0.218

Model 1: $R^2 = 0.32$, $p = 0.005$.
Model 2: $R^2 = 0.55$, $p = 0.01$.

that lower limb muscles are affected to a greater extent than are upper limb muscles. In their report, QF was correlated significantly with six-minute walking distance.[19] Recently, Mainguy et al. demonstrated that decreased quadriceps strength was correlated with impaired exercise capacity in patients with idiopathic pulmonary arterial hypertension.[20] We have previously reported on the relationship between exercise capacity and peripheral muscle performance of IPF.[7] Our current result in f-NSIP was consistent with these previous reports of other chronic lung diseases, suggesting a generalized process.

Determinants of exercise limitation in patients with interstitial lung diseases (ILD) have been explored. An early study by Burdon et al. reported that decreased exercise capacity was correlated with VC in patients with ILD.[21] Hansen and Wasserman reported that DLco was the best correlate of exercise capacity, from a retrospective review of 43 ILD patients.[22] In these studies, however, patients' diagnoses included IPF, sarcoidosis, hypersensitivity pneumonitis, and some other diseases.

Despite these previous studies, the pathophysiology of the exercise limitation in ILD has not been fully elucidated. Moreover, studies that investigated the mechanisms of exercise limitation in patients with ILD generally included a variety of diseases. It seems likely that the factors determining exercise limitation depend on the etiologies of ILD. In a study that enrolled only IPF patients, we previously revealed that QF is one of the main determinants of exercise capacity. Recently, Caminati et al. also reported moderate correlations between 6MWD and both percent predicted FVC and percent predicted DLco.[23]

To our knowledge, however, there has been no study on exercise capacity in patients with f-NSIP. Additionally, the evaluation of peripheral muscle strength in this study more clearly demonstrated the determinants of exercise capacity in f-NSIP. Thus, our current evaluation seems valuable.

Our current results revealed that quadriceps muscle weakness was frequently observed and related to exercise limitation in f-NSIP patients. This was consistent with previous observation in an IPF cohort. It is assumed that there are several possible factors and mechanisms that cause peripheral muscle dysfunction in ILD.

About half of the patients in this study showed exertional desaturation. Hypoxia is known to increase the oxidative stress that may adversely affect the performance of muscle.[24,25] Thus hypoxia might be a mechanism that adversely affects skeletal muscle and exercise capacity. To test this hypothesis, we also conducted an analysis to explore the determinants of QF (supplemental table). In univariate analysis there were several factors related to QF. In the subsequent stepwise multivariate analysis, DLco appeared to be one of the independent explaining factors for QF. It is supposed that dysfunction in lung parenchyma is relevant to peripheral muscle weakness in patients with f-NSIP; nevertheless, factors more directly related to hypoxia, such as PaO_2 and SpO_2 during exercise, did not show significant relations.

The inflammatory disease process is also thought to be related to quadriceps weakness, as NSIP is characterized by inflammatory cell infiltration in lung interstitium. Deconditioning due to limitation in activities of daily living might be another mechanism of impairment in the quadriceps muscles. Interestingly, aberrant TGF-β signaling was implicated in a model of atrophic muscle caused by hypoxia, inflammation and disuse.[26] TGF-β is known to play a critical role in the pathogenesis of fibrotic lung diseases, and it might be supposed that this molecule also affects peripheral muscles in this population. Unfortunately we did not perform muscle biopsy, but further insights will be obtained if this is done in future study.

In the current study we excluded patients with history of corticosteroid use to avoid possible effects on peripheral muscle weakness. Because CTD is a common complication in NSIP patients and may affect exercise performance as well as peripheral muscle weakness, patients with CTD were also excluded from the analysis. Moreover, patients with a coexisting disorder that might affect QF were excluded from the cohort in order to eliminate possible confounders.

Muscle weakness is known to be related to age, as a 25–30 percent decrease in muscle mass is observed in the seventh decade of life in humans.[24] Therefore we also assessed the multivariate model with the adjustment for age. Thus, we tried to eliminate possible confounding factors that would affect quadriceps weakness. However, the underlying pathophysiology of the weakness of the QF is unknown even in IPF. Clearly, additional research will be necessary to elucidate the pathophysiology of the muscle weakness in NSIP.

Measurement of pulmonary function and exercise indices is integral to the assessment and monitoring of patients with IPF and may also provide useful prognostic information and facilitate treatment decisions.[27,28]

The 6-MWT is a widely used measure of exercise tolerance in patients with various cardiac and pulmonary diseases. A couple of recent controlled trials provided support for the benefits of pulmonary rehabilitation in subjects with IPF and ILD.[29,30] We previously reported significant improvements in 6MWD in subjects with IPF.[29] Holland et al. also found significant improvements in 6MWD following rehabilitation, in a randomized controlled trial of 57 subjects with ILD.[30] The consistent finding that peripheral muscle strength was correlated with exercise capacity in patients with f-NSIP, as well as in IPF patients, raised an intriguing question. Will training of peripheral

muscles, especially in the lower extremities, increase the exercise capacity of patients with f-NSIP? Further study on exercise capacity is warranted in patients with f-NSIP.

Some limitations of this study should be mentioned. First, this was a retrospective and cross-sectional study. Second, the study involved a so small number of patients from one center that we could not make a definitive conclusion. Future study including larger sample size, if possible, may make the conclusion robust. In addition, the patients involved in the study consisted entirely of Japanese. It is unclear whether the present results can be adapted to other ethnic groups. Multiregional prospective studies would be able to eliminate ethnic bias and other possible bias. As there are no available normal values and/or lower limits for HF and respiratory muscle force in Japanese' subjects to our knowledge, it is possible that we did not evaluate the severity of muscle impairment observed in this cohort accurately, except for QF.

In conclusion, we explored the determinants of exercise capacity in 30 consecutive f-NSIP patients. Decreased QF was frequently observed and shown to be an independent predictor of exercise capacity in multivariate analysis. From the current results, it seems that peripheral muscle weakness may be related to exercise intolerance in this population. Further study regarding the therapeutic response to peripheral muscle training is warranted in this population.

Conflict of interest statement

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found in the online version at doi:10.1016/j.rmed.2012.12.013.

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coworkers [6] indicated the efficacy of cisplatin/docetaxel in patients with CUP, with tolerable toxicity. Ohtsubo and colleagues [7] described the effectiveness of tumor marker–oriented chemotherapy. In the present case, tumor markers were not specifically detected at significantly elevated levels. The patient also refused any additional therapy because of her advanced age.

In conclusion, we report a rare case of CUP that developed as isolated, metachronous, mediastinal lymph node metastases. Sequential resection of mediastinal lymph node metastases, without removal of lung parenchyma, may achieve beneficial local control of the disease in selected patients.

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Single-Lobe Lung Transplantation for Rapidly Deteriorating Pulmonary Venoocclusive Disease

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Pulmonary venoocclusive disease, classified as a subgroup of pulmonary arterial hypertension, is known to show poor prognosis and lung transplantation is the only possible treatment. Single living-donor lobar lung transplantation is a unique method of treatment, mostly for small children, and size matching is the most important factor to conduct single living-donor lobar lung transplantation safely. We report a successful single living-donor lobar lung transplantation for a 6-year-old girl with pulmonary venoocclusive disease who received the graft from her mother. Preoperatively, the recipient was intubated under deep sedation because of repeated episodes of pulmonary edema due to rapidly deteriorating pulmonary venoocclusive disease.

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It has been reported that patients with pulmonary venoocclusive disease (PVOD) had worse outcomes than did patients with other subgroups of pulmonary arterial hypertension (PAH) [1]. To date, there are no effective therapies other than lung transplantation for PVOD [1]. Single living-donor lobar lung transplantation (LDLLT) has been performed occasionally when the recipient is a small child or when only one donor is available [2]. Because an adult lower lobe may be too large for a small child, size matching in single LDLLT poses a critical challenge. Herein, we report a successful life-saving single LDLLT for a 6-year-old girl with rapidly deteriorating PVOD who received the graft from her mother.

A 6-year-old girl, who had been healthy, was admitted to a regional hospital owing to severe lung edema associated with pulmonary hypertension. She was intubated and treated for the tentative diagnosis of PAH with epoprostenol, an endothelin receptor antagonist, and a phosphodiesterase-5 inhibitor. She could be extubated because her pulmonary arterial pressure decreased to almost the normal levels, but required reintubation (3 times within 2 months) owing to repeated attacks of severe lung edema and pulmonary hypertension exceeding the systemic arterial pressure. Because of the charac-

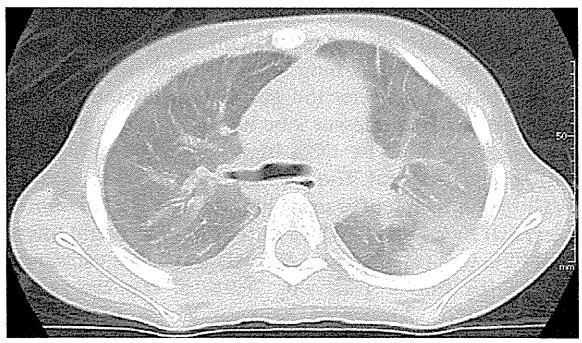

Fig 1. Chest computed tomography of a 6-year-old girl with pulmonary venoocclusive disease before referral to our hospital. Smooth septal thickening and diffuse or mosaic ground-glass opacities were recognized. Areas of alveolar consolidation were also seen in the bilateral posterior lower lobes.

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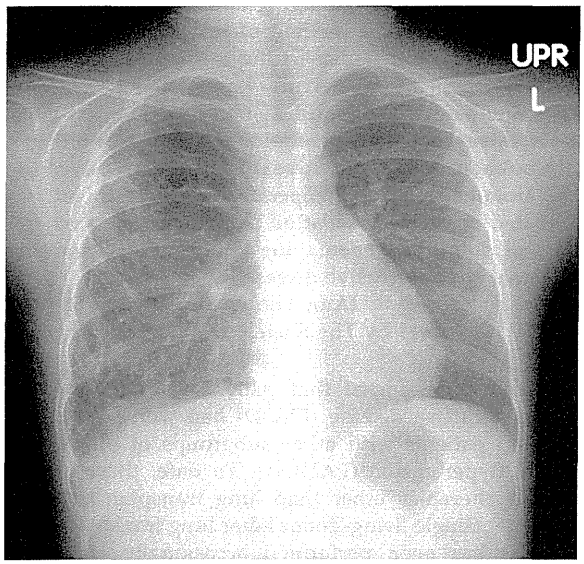

Fig 2. Chest radiography 6 months after living-donor right single-lobe lung transplantation. No severe compression of the implanted lungs was noted.

teristic chest computed tomography (CT) and clinical findings, for example, repetitive pulmonary hypertensive attacks, PVOD was highly suspected, and she was transferred to our hospital for considering possible LDLLT (Fig 1). When she was transferred to our intensive care unit, she was intubated and treated with epoprostenol, an endothelin receptor antagonist, and a phosphodiesterase-5 inhibitor in addition to a small amount of dobutamine, but pulmonary hypertensive attacks occurred several times daily. In a few weeks, her hemodynamic condition seemed stabilized, and pulmonary hypertensive attacks rarely occurred. When she was stabilized, pulmonary arterial pressure was within normal limits. Then, open lung biopsy was performed for the definitive diagnosis, and she was diagnosed pathologically as having PVOD. The girl was 109 cm in height and three-dimensional CT volumetry indicated the recipient's right chest cavity volume to be only 614 mL. Both parents were willing to donate their lungs, but her father's lung was thought to be too big for the girl. The evaluated volume of the mother's right lower lobe was 1,061 mL, indicating that the graft would be 173% bigger than the right chest cavity of the recipient. The predicted forced vital capacity (FVC) of the recipient was 1,130 mL. Because her mother's actual FVC was 3,390 mL, the donor graft (right lower lobe) FVC was calculated as 892 mL. Thus, the estimated FVC of the donor graft was 78.9% of the recipient's predicted FVC. However, because single LDLLT for PVOD has never been reported, our initial strategy was to wait for her to grow until her chest could accept adult double lower lobes. Furthermore, we did not have any answers as to how the contralateral PVOD lung would behave after right single LDLLT. The patient underwent tracheotomy, and we attempted to gradually wean her from the ventilator; however, owing to a series of attacks of pulmonary edema, mechanical ventilation under deep sedation was required again.

One month after the referral to our hospital, she finally underwent right single LDLLT with the graft from her mother. The surgery was performed through a right anterior thoracotomy. The patient was placed on standard cardiopulmonary bypass. After right pneumonectomy, the right lower lobe of the mother was implanted using cardiopulmonary bypass. After bronchial anastomosis, pulmonary vein and artery were anastomosed, respectively. Before the bronchial anastomosis, an endotracheal tube was inserted further to the left main bronchus to ensure the ventilation of the native left lung during the anastomosis to prevent its atelectasis. When the graft was reperfused and reventilated, it became obvious that the graft was too large. The patient was weaned from cardiopulmonary bypass without difficulty. The cardiopulmonary bypass time was 133 minutes, and the ischemic time of the graft was 111 minutes. Systolic pulmonary artery pressure was approximately 45 mm Hg. The graft was manually compressed into the chest cavity, but the pulmonary vein patency was exacerbated by trial closure of the chest, and therefore, we closed the chest loosely only by skin closure. Hemodynamic stability was maintained soon after the reperfusion, and therefore prophylactic postoperative extracorporeal membrane oxygenation was not considered, although reperfusion edema would be a higher known incidence in this type of lobar transplantation. The patient also did not show any hemodynamic instability or desaturation for 24 hours after the reperfusion. Then, she was sent back to the operating room to close the intercostal spaces completely. She was completely weaned from the ventilator after 4 weeks.

Currently, 10 months after lung transplantation, the patient is well without limitations. Her chest radiography demonstrated a well-expanded graft without any apparent atelectasis and severe compression of the implanted lungs (Fig 2). Arterial blood gas analysis on room air revealed a pH of 7.38, arterial oxygen pressure (PaO_2) of 82 mm Hg, and arterial carbon dioxide pressure ($PaCO_2$) of 34 mm Hg. The lung allograft volume measured by CT volumetry was 530 mL, which was 47% of its original size (1,130 mL) in her mother's chest before transplantation. According to cardiac catheterization, the systolic and diastolic pressures of the main pulmonary artery were 41 mm Hg and 20 mm Hg, respectively, while those of the right main pulmonary artery were 37 mm Hg and 14 mm Hg, and those of the left main pulmonary artery were 41 mm Hg and 18 mm Hg. Pulmonary capillary wedge pressure was 7 mm Hg. Pulmonary and systemic vascular resistance was 29.53 and 7.54 Wood's units, respectively. Lung perfusion scintigraphy showed that the right (graft lung)-to-left (native lung) ratio was 62:38.

Comment

This is the first report regarding single LDLLT for a small child with PVOD. There are several points to be