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.

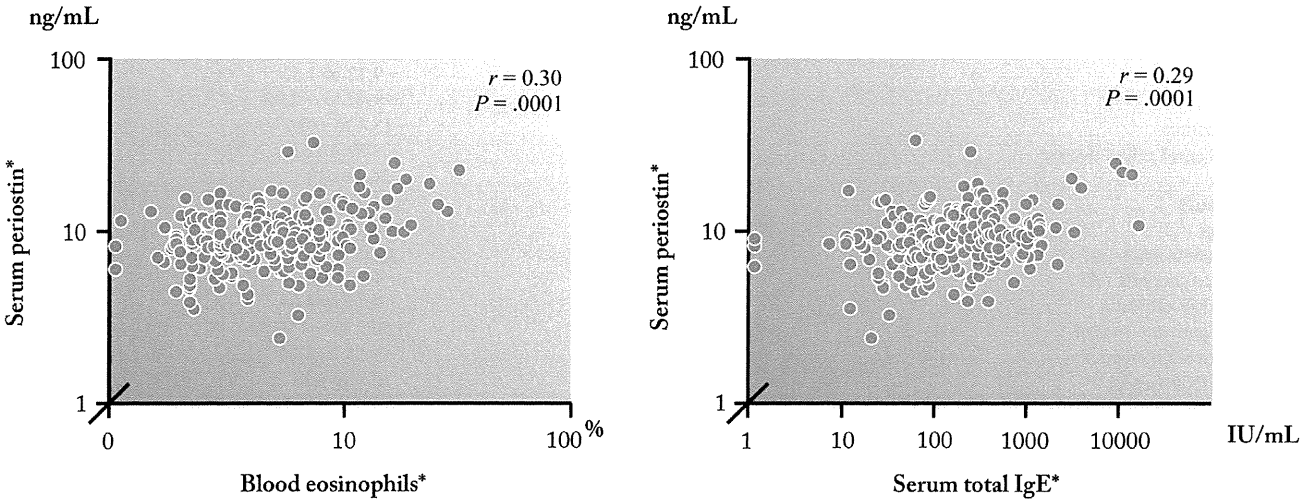

FIG 2. Relationships between serum periostin levels and blood eosinophil counts *(left)* or serum IgE levels *(right)*. *Presented in logarithmic scales on both the x- and y-axes.

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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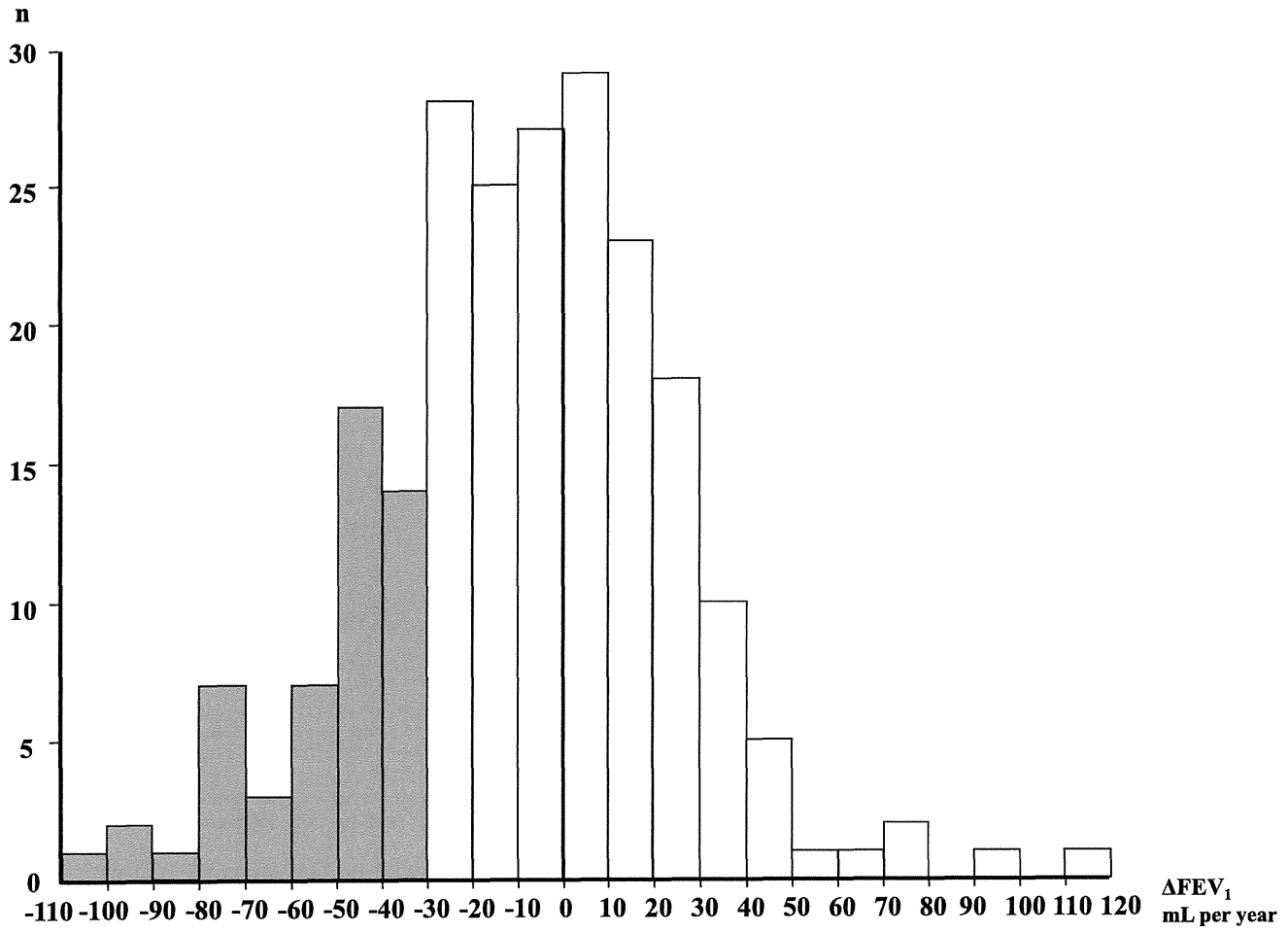

FIG E1. Distribution of ΔFEV_1 in the study population.

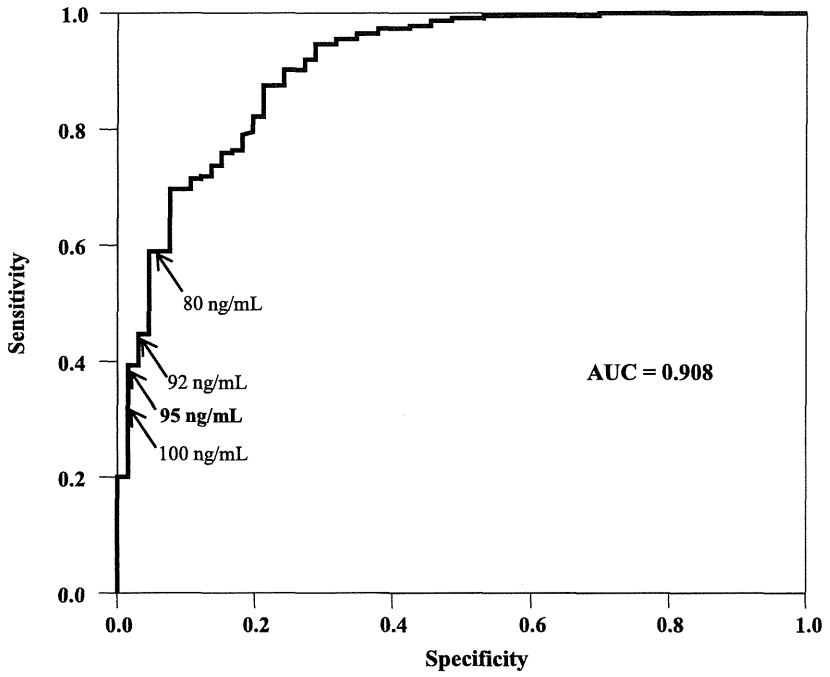

FIG E2. ROC curve analysis of serum periostin levels comparing asthmatic patients with healthy subjects, in which the cutoffs of 95 ng/mL, 80 ng/mL, 92 ng/mL, and 100 ng/mL are presented with *arrows*.

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.

We thank Dr Ito and Dr Misumi for their clinical assistance.

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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-

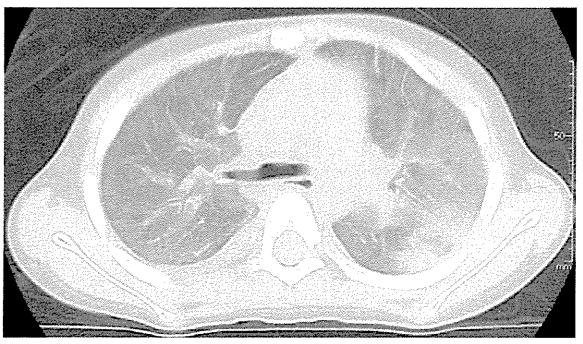

Fig 1. Chest computed tomography of a 6-year-old girl with pulmonary venoocclusive disease before referral to our hospital. Smooth septal thickening and diffuse or mosaic ground-glass opacities were recognized. Areas of alveolar consolidation were also seen in the bilateral posterior lower lobes.

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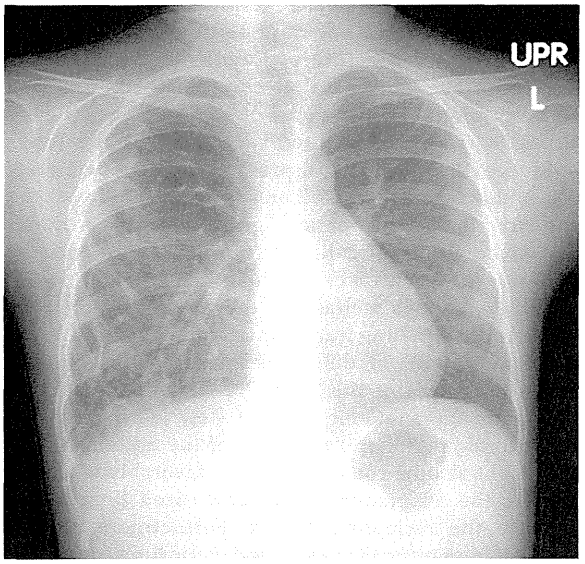

Fig 2. Chest radiography 6 months after living-donor right single-lobe lung transplantation. No severe compression of the implanted lungs was noted.

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

discussed with regard to this rare experience. Firstly, when the patient was transferred to our hospital, PVOD was clinically highly suspected; however, treatment for the other types of PAH is entirely different from that for PVOD. Because the obstructive vasculopathy in PVOD is predominantly located on the venous aspect of the pulmonary circulation, treatment with PAH-specific vasodilator therapies may cause augmentation of the pulmonary arteriolar blood flow against the fixed resistance of occluded pulmonary venules and veins. Therefore, we performed open lung biopsy before LDLLT, although PVOD was most likely from a clinical point of view.

Secondly, as cadaveric donors for pediatric patients are not practically expected in Japan, a single LDLLT from her mother was the last and only practical option. If the patient's condition is stable, physicians could consider medical therapy to wait for the patient to grow. However, in the current case, the patient was intubated again and under deep sedation to prevent attacks due to pulmonary edema and pulmonary hypertension. According to the recent guidelines for the treatment of pulmonary hypertension [1], there is no established medical therapy for PVOD. Most importantly, vasodilators and especially prostanoids must be used with great caution because of the high risk of pulmonary edema. Epoprostenol can make the patient's condition better and could be a bridge to lung transplantation [1], but there is a report showing epoprostenol-induced pulmonary edema in a patient with PVOD [3].

Thirdly, we have accepted size mismatches between recipients and donor lobar grafts only if the predicted FVC of the graft is at least more than 45% of the expected recipient FVC. In the present case, the predicted FVC of the graft was 78.9% of the expected recipient FVC. Furthermore, to assess the use of oversized grafts, we also have used three-dimensional CT volumetry [2, 4, 5]. In single LDLLT, size matching is crucial because the use of oversized grafts can cause high airway resistance, atelectasis, and hemodynamic instability at the time of chest closure. However, because of our experiences [2, 6], size discrepancy would probably be acceptable if the graft is less than 200% bigger than the right chest cavity of the recipient. In the current case, the graft was calculated to be 173% bigger than the right chest cavity of the recipient and was thus adjustable in size for the recipient's chest. However, delayed chest closure was required after LDLLT. Conversely, the evaluated volume of the right lobe of the patient's father was 1,495 mL, indicating that the graft would be 243% bigger than the right chest cavity of the recipient. This calculation confirmed that the father's graft was too large for the recipient. Furthermore, the recipient's growth should be taken into consideration when performing LDLLT for a child. At 10 months after LDLLT, the grafted lung was still compressed to less than 50% of its original size, indicating that the grafted lung has a potential ability to adjust to some extent to the recipient's growth. In the present case, we did not want to downsize the adult lobe because of the future growth of the recipient. Despite no available data on how much compression can be tolerated by the donor lungs in the recipient's thorax [4], the present case revealed that the lungs could function when compressed to approximately 50% of their original size. Long-term durability of the lung function as the patient grows remains a major unresolved issue, and life-long follow-up should be mandatory in such cases of single LDLLT.

Fourthly, various methods of downsizing donor lungs can be applied in lung transplantation [7]. In LDLLT, if a moderate amount of differences need to be adjusted, superior segment of the lower lobe can be performed easily from the anatomic point of view [4]. By contrast, nonanatomic peripheral resection of the anterior donor lung might also be considered more favorably when we consider the adaptation of the downsized lung to the shape of the recipient's thorax. Furthermore, in this setting of lung transplantation, cadaveric lung transplantation using the upper lobe only or the lower lobe only from an adult cadaveric donor might provide an even better size match, resulting in no requirement of living donation.

Lastly, only 1 case report documented that no attacks of pulmonary edema occurred after single lung transplantation for PVOD [8]; however, it is necessary to perform a close follow-up with regard to how the graft and native lung behave.

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A urine biomarker for severe obstructive sleep apnoea patients: lipocalin-type prostaglandin D synthase

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ABSTRACT Lipocalin-type prostaglandin D synthase (L-PGDS), which is responsible for the biosynthesis of prostaglandin D_2, has been reported to have a close connection with cardiovascular disease and sleep regulation. This study aimed to test the hypothesis that the L-PGDS level is a useful marker to identify patients with obstructive sleep apnoea.

64 subjects were enrolled in this prospective study. Urinary concentrations of L-PGDS were measured in the morning. Measurements were made every 4 h in 25 of the 64 patients. Endothelial function was assessed by the reactive hyperaemia peripheral arterial tone index.

Circadian variations in L-PGDS concentrations had a significant time-dependent fluctuation ($p=0.0002$). L-PGDS was higher in the subjects with severe obstructive sleep apnoea (median 784.7 ng per mg of creatinine, $n=23$) than in control subjects (262.1 ng per mg of creatinine, $n=16$; $p=0.004$) and in those with moderate obstructive sleep apnoea (371.7 ng per mg of creatinine, $n=25$; $p=0.0008$). After 2 days of continuous positive airway pressure treatment, L-PGDS concentrations in severe obstructive sleep apnoea subjects ($n=12$) decreased significantly ($p=0.02$) to levels present in control subjects whereas endothelial function did not change significantly. Morning urinary L-PGDS concentrations had significant correlations with the apnoea/hypopnoea index ($R^2=13.9\%$) and serum high-density lipoprotein cholesterol ($R^2=6.2\%$), but not with sleepiness.

Urinary L-PGDS might be a moderately useful marker to identify patients with severe obstructive sleep apnoea.

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Introduction

Obstructive sleep apnoea (OSA) is one of the most important medical conditions identified in the last 50 years and is a major cause of morbidity and mortality throughout the world [1].

Most patients with OSA that should be treated are undiagnosed. One reason is that the diagnostic methods for OSA, such as polysomnography (PSG), are difficult to perform. Therefore, a reasonable biomarker for OSA would be extremely helpful, especially in identifying patients who have OSA with a degree of severity that would put them at risk for cardiovascular disease (CVD).

Prostaglandin (PG)D_2 is formed by the action of PGD synthases on the cyclooxygenase (COX) product PGH_2. PGD_2 is widely distributed in rat and human brain [2]. In peripheral tissues, PGD_2 executes a wide range of functions, including vasodilatation, inhibition of platelet aggregation, glycogenolysis, vasoconstriction, allergic reaction mediation and intraocular pressure reduction [3–8]. In the brain, PGD_2 has been shown to contribute to sleep induction, modulation of body temperature, olfactory function, hormone release, nociception and neuromodulation [9–12]. Thus, because PGD_2 has significant effects on platelet aggregation, vasodilation and vasoconstriction, it has been supposed that PGD_2 is relevant to the occurrence of CVD. Although the prostanoids, including PGD_2, are released from cells immediately after synthesis, it is believed that prostanoids work only locally, near their site of production, because they are either chemically or metabolically unstable [13].

One of the enzymes characterised as a PGD synthase, which catalyses the isomerisation of PGH_2 to PGD_2, is the lipocalin-type prostaglandin D synthase (L-PGDS) [14], and is responsible for the biosynthesis of PGD_2 in the brain and heart (cardiovascular system). L-PGDS is a unique protein with enzyme activity and ligand-binding properties. L-PGDS binds various lipophilic compounds, such as retinoids, bilirubin, biliverdin, gangliosides and amyloid-β peptides, with high affinity, acting as an extracellular transporter of these compounds and serving as an endogenous amyloid-β chaperone to prevent amyloid deposition *in vivo* [15]. The half-life of L-PGDS in canines was reported to be 0.77 h [16].

L-PGDS was confirmed to be secreted into blood and urine [14], and we have established a system to measure its urine, serum or plasma levels by an ELISA system [14, 17–19]. L-PGDS is a very stable enzyme and is highly resistant to heat treatment [14] and protease digestion [20], whereas PGD_2 is an unstable substance, as mentioned earlier [13]. L-PGDS is localised where PGD_2 would have its effect, that is, in the central nervous system, male genital organs and the human heart. In the human heart, L-PGDS is localised in myocardial and atrial endocardial cells, smooth muscle cells in the arteriosclerotic intima, and in the atherosclerotic plaques of severely stenotic coronary arteries. In addition, the chemical properties of L-PGDS are similar to those of serum albumin; however, its molecular weight is much smaller than that of serum albumin (26 000 *versus* 66 000 Da). Thus, L-PGDS more easily passes through glomerular capillary walls of the kidney than serum albumin. Indeed, it has been reported that urinary L-PGDS excretion increased in the microalbuminuric stage in patients with type 2 diabetes mellitus (DM) and in hypertensive patients who were apparently free from overt proteinuria [21, 22]. Although the enzymatic activity of L-PGDS cannot be determined, the amount of serum or urinary L-PGDS increased when conditions such as coronary heart disease, hypertension or type 2 DM worsened [21–23].

As OSA induces multi-organ damage and diseases such as hypertension, DM, renal insufficiency, coronary disease and cerebral-cardiovascular diseases, the degree of severity of OSA in individuals with OSA would have significant associations with morbidity and mortality from these conditions [24]. In addition, serum L-PGDS levels were slightly elevated in individuals with OSA with excessive daytime sleepiness [25]. Thus, we hypothesised that L-PGDS could be a biomarker for OSA because of its close relationships with sleep and CVD. In consideration of this hypothesis, we tested whether plasma or urine L-PGDS would be a powerful biomarker for OSA.

Methods

Study subjects

64 clinically stable adults (age >20 years) with suspected OSA were consecutively enrolled in the present prospective study. This study is registered at www.clinicaltrials.gov with identifier number NCT01096433.

Major exclusion criteria were history of CVD, DM under treatment with hypoglycaemic agents or insulin, use of glucocorticoid or nonsteroidal anti-inflammatory drugs, and being a current smoker. This study was approved by the Ethics Committee of Kyoto University (Kyoto, Japan). All patients gave written informed consent to participate. The other exclusion criteria are shown, in detail, in the online supplementary material.

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Study design

At baseline, the subjects' medical history was recorded and a physical examination was performed. In the medical history, hypertension was defined as a systolic blood pressure $\geqslant$140 mmHg or diastolic blood pressure $\geqslant$90 mmHg, or use of an antihypertensive medication. Dyslipidaemia was defined as serum low-density lipoprotein cholesterol $\geqslant$140 mg·dL^{-1} or high-density lipoprotein cholesterol (HDL-C) $<$40 mg·dL^{-1}, triglycerides $\geqslant$150 mg·dL^{-1} [26], or use of an antilipidaemic medication.

All patients underwent attended diagnostic overnight PSG. Before the patient slept, urine samples were collected at 22:00 h. In addition, the first urine voided in the morning following the overnight PSG was collected at 06:00 h. Peripheral venous blood samples were collected from 06:00 h to 7:00 h following a 12-h overnight fast and PSG. Morning endothelial dysfunction measured by reactive hyperaemia peripheral arterial tone (RH-PAT) [27] was measured. After the overnight PSG, five blood pressure measurements, each 1 min apart, were taken in the morning after the patient had rested for at least 5 min in the supine position. The average of the latter two readings was calculated.

After 2 days of continuous positive airway pressure (CPAP) treatment, urine and blood samples were collected at the same time as during and after PSG. In all of the patients, adequate CPAP pressure was determined to have overcome obstruction and all flow limitations by a full night's titration.

During the PSG day, we investigated the circadian variations in urinary L-PGDS concentrations in 25 patients who were randomly selected from all 64 subjects at 4-h intervals except for midnight (14:00, 18:00, 22:00, 06:00 and 10:00 h). Urinary sampling was not performed at midnight in order not to disturb physiological sleep.

Polysomnography

PSG was performed according to recommendations in American Academy of Sleep Medicine manual (online supplementary material). Apnoea/hypopnoea index (AHI) values were expressed as the number of episodes of apnoea and hypopnoea per hour over the total sleep time. OSA severity was defined by the AHI as follows: control (AHI $<$15 events·h^{-1}), moderate OSA (15$\leqslant$AHI$<$30 events·h^{-1}) and severe OSA (AHI $\geqslant$30 events·h^{-1}).

Measurements of plasma and urinary L-PGDS concentrations

In the present study, plasma samples were centrifuged immediately at 1470 $\times$ *g* at 4°C for 15 min and urine samples were pooled (not centrifuged), as described previously [15, 17–19]. The separated samples were stored at -80°C until assay. Concentrations of urinary or plasma L-PGDS were measured by an ELISA using two monoclonal antibodies, Mab-7F5 and Mab-1B7, as described previously (online supplementary material) [15, 17–19].

With this ELISA system, it has been demonstrated that intra- and interassay coefficients of variation in urine samples ranged from 3.2% to 5.8% and 7.6% to 8.3%, respectively. The intra- and interassay coefficients of variation in serum samples were 3.6% and 5.8%, respectively. The ELISA showed no significant interference by a variety of urinary constituents [18]. In addition, it was shown that serum and plasma L-PGDS values in individual subjects were almost the same [15]. All the samples were measured in duplicate and the results were averaged.

Other parameters

Venous blood samples were taken in the fasting state in the morning after one night of PSG and examined for markers of glucose and lipid metabolism and C-reactive protein. As it was difficult to measure urinary catecholamine and L-PGDS levels at the same time, we measured plasma catecholamine levels.

Measurements of the RH-PAT

The RH-PAT is a newly established method to measure endothelial function [27]. Endothelial dysfunction as measured by RH-PAT has been reported in patients with OSA [28]. Morning endothelial function assessed by a finger plethysmographic device (Itamar Medical Ltd., Caesarea, Israel) that allows the isolated detection of pulsatile arterial volume changes [27] was measured after an overnight PSG and after 2 days of CPAP.

Statistical analysis

Data were analysed using JMP 9.0 (SAS Institute, Inc., Cary, NC, USA). Continuous variables were expressed as mean$\pm$SE or median values and ranges because the sample size of each group was small. The associations between patients' characteristics, PSG data, biomarkers (blood and urine) and OSA severity were assessed by the Kruskal–Wallis test. When a significant difference was observed, we used the Bonferroni-corrected t-test to identify where differences were significant. We evaluated the sensitivity and

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specificity of the cut-off value of L-PGDS for predicting severe OSA with the use of receiver operating characteristic curve analysis, estimating the area under the curve (AUC). The relationship between L-PGDS concentrations (urine and plasma), the RH-PAT index and other parameters were analysed by Pearson's correlation coefficient test. Multiple regression analysis was performed to adjust for confounders such as age, sex, body mass index (BMI), and morning systolic and diastolic blood pressure. Next, multiple regression analyses, with a p-value <0.10 required for entry into the models, were performed to identify those variables that could best predict morning urinary L-PGDS.

To investigate changes in L-PGDS concentrations, the RH-PAT index, and other parameters before and after 2 days of CPAP, comparisons of data between those two time-points were tested by a paired t-test. Multiple ANOVA with repeated measures was performed to analyse urinary L-PGDS concentrations across the 24-h period. In all analyses, p-value <0.05 was considered statistically significant.

Results

Clinical characteristics of study subjects, L-PGDS concentration and RH-PAT index according to OSA severity

Patient characteristics, PSG data and laboratory data are shown in table 1. There were significant differences among the groups in morning urinary L-PGDS concentrations ($p=0.0009$) but not in night urinary L-PGDS concentrations ($p=0.19$) and plasma L-PGDS levels ($p=0.09$) (table 1 and fig. 1a). After adjustment for age and BMI, subjects with severe OSA had significantly higher morning urinary L-PGDS values than control subjects ($p=0.007$) and subjects with moderate OSA ($p=0.002$). There were significant differences among the groups in the RH-PAT index (fig. 1b).

The cut-off value for predicting severe OSA with minimal false-negative and false-positive errors was 621.8 $ng \cdot mg^{-1}$ of creatinine (sensitivity 65.2%, specificity, 85.4%). This cut-off value had moderate accuracy for predicting severe OSA (AUC 0.78) (fig. 2).

Relationship between urinary L-PGDS concentrations, RH-PAT index and clinical indices

Morning urinary L-PGDS concentrations were positively correlated with several parameters, including the AHI (table 2 and fig. 3). Morning urinary L-PGDS concentrations were positively correlated with AHI after adjustment for age, sex, BMI and morning systolic and diastolic blood pressure (fig. 3). There was a strongly positive correlation between morning and night urinary L-PGDS concentrations ($p<0.0001$). The Epworth Sleepiness Scale did not correlate significantly with morning urinary L-PGDS (table 2).

After adjustment for age, sex, BMI, and morning systolic and diastolic blood pressure, morning urinary L-PGDS concentrations were still positively correlated with the AHI (β coefficient=0.373; $p=0.006$), 3% oxygen desaturation index (ODI) ($\beta=0.322$; $p=0.02$), arousal index ($\beta=0.370$; $p=0.007$) and plasma noradrenaline ($\beta=0.258$; $p=0.04$). The RH-PAT index was negatively correlated with the AHI ($\beta= -0.305$; $p=0.04$) and arousal index ($\beta= -0.359$; $p=0.01$) after adjustment.

Relationship between plasma L-PGDS concentrations and clinical indices

Plasma L-PGDS levels were positively correlated with several parameters (table 2). Plasma L-PGDS also tended to have a positive correlation with morning urinary L-PGDS ($p=0.07$). After adjustment for age, sex, BMI, and morning systolic and diastolic blood pressure, plasma L-PGDS levels were only positively correlated with serum creatinine levels ($\beta=0.361$; $p=0.03$).

Clinical determinants of morning urinary L-PGDS concentrations

Table 3 shows results of multiple regression analyses to identify those variables (morning systolic and diastolic blood pressure, arousal index, AHI, 3% ODI, serum HDL-C, plasma adrenalin, plasma noradrenalin and plasma L-PGDS) that could predict morning urinary L-PGDS concentrations. Then, one of the variables that had very strong co-linearity ($r>0.70$), such as the arousal index, AHI and 3% ODI, was selected.

In these three models, morning urinary L-PGDS had a significant and independent correlation with the AHI or the 3% ODI, or the arousal index and serum HDL-C (table 3).

Effects of CPAP treatment on urinary L-PGDS concentrations

In Japan, patients whose AHI is $\geqslant 20$ can use CPAP under the health insurance system. In this study, 20 permitted patients were investigated (table 4). 12 of the 20 patients had severe OSA.

After 2 days of CPAP treatment, morning urinary L-PGDS concentrations were significantly decreased compared with baseline values (fig. 4a). In contrast, the plasma L-PGDS level, the RH-PAT index (fig. 4b) and the other biomarkers were not significantly changed (table 4). In the 12 patients with severe OSA, morning urinary L-PGDS concentrations were significantly decreased compared with baseline values (591.2

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TABLE 1 Patients' characteristics, polysomnography (PSG) data and laboratory data according to severity of obstructive sleep apnoea (OSA)

	Control	Moderate OSA	Severe OSA	p-value
Subjects	16	25	23	
Age years	47.5 (21–76)	55 (31–74)	55 (27–78)	0.12
Male	12 (75.0)	21 (84.0)	21 (91.3)	0.39
BMI kg·m^{-2}	26.0 (20.4–35.8)	24.5 (20.2–34.8)	26.6 (21.6–39.9)	0.10
Waist circumference cm	87 (76–118)	90 (78–113)	94 (82–120)	0.13
Morning systolic BP mmHg	114 (95–136)	118 (99–139)	127 (96–150)$^{\#,\P}$	0.003
Morning diastolic BP mmHg	70 (58–91)	74 (57–96)	80 (69–97)$^{\#,\P}$	0.0004
Ex-smoker	9 (56.3)	12(48.0)	17 (73.9)	0.18
ESS score	14 (1–24)	12 (2–19)	12 (4–20)	0.13
Comorbidity				
Hypertension	3 (18.8)	10 (40.0)	10 (47.8)	0.25
Dyslipidaemia	9 (56.3)	17 (68.0)	15(65.2)	0.74
Diabetes mellitus	2 (12.5)	2 (8.0)	2 (8.7)	0.88
PSG data				
TST min	408.5 (256.5–510)	389.0 (205.5–515.5)	378.0 (240.5–499)	0.56
Sleep efficiency %	81.1 (63.3–94.4)	76.6 (42.9–94.2)	72.9 (50.4–96.4)	0.50
Arousal index events·h^{-1}	17.8 (9.5–26.7)	22.2 (10.8–46.5)	44.3 (12.6–61.2)$^{\#,\P}$	<0.0001
AHI events·h^{-1}	7.3 (1.2–14.8)	22.7 (15.2–29.8)$^{\#}$	47.2 (31.9–85.4)$^{\#,\P}$	<0.0001
3% ODI events·h^{-1}	5.3 (0.5–14.1)	17.4 (10.1–27.2)$^{\#}$	48.0 (26.9–86.4)$^{\#,\P}$	<0.0001
Minimum Sp_{O_2} %	90.5 (81–97)	81.5 (73–90)$^{\#}$	75.0 (61–86)$^{\#,\P}$	<0.0001
Sp_{O_2} <90% % TST	0 (0–5.0)	2.5 (0–8.1)	11.9 (1.2–87.7)$^{\#,\P}$	<0.0001
RH-PAT index	1.91 (1.30–2.87)	2.00 (1.32–3.91)	1.65 (1.42–3.23)¶	0.02
Blood				
Creatinine mg·dL^{-1}	0.8 (0.4–1.0)	0.8 (0.6–1.1)	0.8 (0.6–1.1)	0.49
TC mg·dL^{-1}	204 (125–241)	197 (130–255)	199 (141–299)	0.58
HDL-C mg·dL^{-1}	51 (35–86)	53 (40–93)	49 (30–82)	0.30
TG mg·dL^{-1}	103 (54–245)	100 (44–334)	132 (70–286)	0.21
CRP mg·dL^{-1}	0.1 (0.0–0.2)	0.1 (0.0–0.2)	0.1 (0.0–0.5)$^{\#,\P}$	0.004
Glucose mg·dL^{-1}	87 (81–114)	95 (75–146)	96 (85–121)	0.07
Adrenaline pg·mL^{-1}	14 (5–31)	16 (5–30)	16 (5–45)	0.65
Noradrenaline pg·mL^{-1}	241 (67–465)	252 (91–398)	261 (82–569)	0.52
L-PGDS ng·mL^{-1}	422.0 (291.7–588.6)	469.3 (270.3–657.0)	491.4 (343.3–726.0)	0.09
Urine				
Morning L-PGDS ng·mg^{-1} of creatinine	262.1 (21.3–1178.6)	371.7 (92.3–2378.2)	784.7 (124.4–3274.1)$^{\#,\P}$	0.0009
Night L-PGDS ng·mg^{-1} of creatinine	745.8 (30.5–1754.0)	659.5 (78.9–2937.9)	958.3 (221.0–5621.8)	0.19

Data are presented as n, median (range) or n (%), unless otherwise stated. BMI: body mass index; BP: blood pressure; ESS: Epworth Sleepiness Scale; TST: total sleep time; AHI: apnoea/hypopnoea index; ODI: oxygen desaturation index; Sp_{O_2}: arterial oxygen saturation measured by pulse oximetry; RH-PAT: reactive hyperaemia peripheral arterial tone; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides; CRP: C-reactive protein; L-PGDS: lipocalin-type prostaglandin D synthase. $^{\#}$: p<0.0167 *versus* control; ¶: p<0.0167 *versus* moderate OSA.

at baseline *versus* 317.8 ng·mg^{-1} of creatinine after 2 days of CPAP; p=0.02). Those decreased L-PGDS concentrations reached the levels present in control subjects (p=0.65).

Circadian variations in urinary L-PGDS concentrations

Circadian variations in urinary L-PGDS concentrations are shown in figure 5a. In 25 patients, a multiple ANOVA with repeated measures revealed a significant time-dependent fluctuation (p=0.0002), with the highest levels at 14:00 h and lowest levels at 06:00 h. Compared with the 06:00 h values, urinary L-PGDS concentrations significantly increased at 14:00 h (p<0.0001) and 18:00 h (p=0.02). Regarding the associations between the AHI and urinary L-PGDS concentrations at each time-point, the 06:00 h values were only significantly positively correlated with AHI (r=0.566; p=0.004). In the patients with AHI ⩾30, 06:00 h values for urinary L-PGDS were significantly increased compared with patients with AHI <30 (AHI <30 (n=13) 227 ng·mg^{-1} of creatinine *versus* AHI ⩾30 (n=12) 780.3 ng·mg^{-1} of creatinine; p=0.003) (fig. 5b).

Discussion

The major findings of our study are that morning urinary L-PGDS concentrations were positively correlated with the severity of OSA as indicated by AHI, 3% ODI and the arousal index after adjustment for several

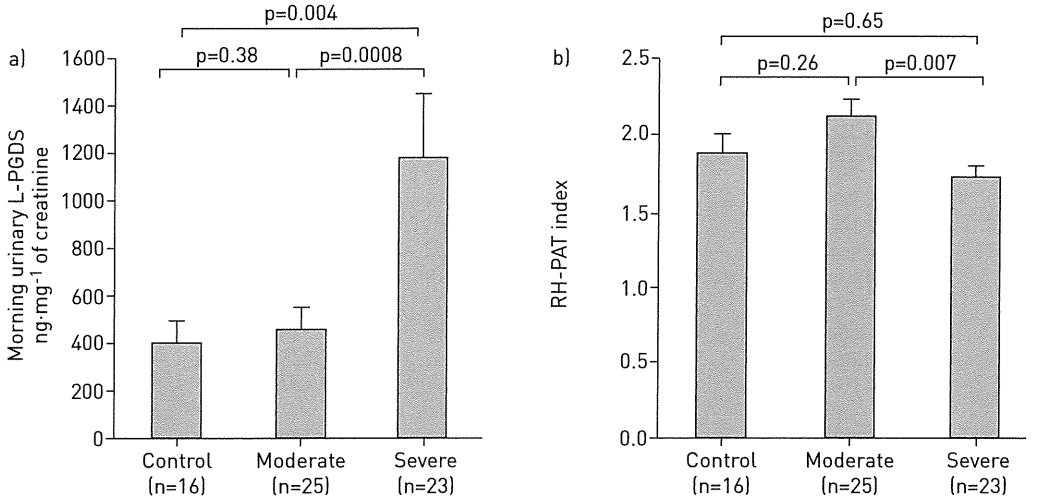

FIGURE 1 Comparison of a) morning urinary lipocalin-type prostaglandin D synthase (L-PGDS) concentrations and b) reactive hyperaemia peripheral arterial tone (RH-PAT) index between controls and moderate and severe obstructive sleep apnoea patients. Data are presented as mean±SE.

confounding factors, and that urinary L-PGDS was significantly elevated in patients with severe OSA in comparison with control subjects and those with moderate OSA. Multivariate modelling of L-PGDS determinants revealed that HDL-C and the AHI, 3% ODI or the arousal index were significantly and independently associated with the morning urinary L-PGDS (table 3). However, it is of interest that urinary L-PGDS levels at 14:00 h were the same in the non-to-moderate and severe OSA patients. Our results also showed that morning urinary L-PGDS concentrations were decreased to control values after only 2 days of CPAP treatment, although the RH-PAT did not change. These results suggest that the urinary L-PGDS concentration might be a moderately useful marker to identify patients with severe OSA.

In the current study, subjects with severe OSA had elevated urinary L-PGDS values and decreased endothelial function determined by the RH-PAT index whereas the relationship between urinary L-PGDS and the RH-PAT index was not significant. Therefore, the combined measurement of endothelial function and L-PGDS may be a useful tool for finding and managing patients with severe OSA.

Prostaglandin systems, including PGD_2, are very important in health maintenance and disease prevention, but they are either chemically or metabolically unstable. Therefore, substitutions such as L-PGDS are used as parameters for identifying the functions and effects of prostaglandin systems. Previous studies have demonstrated that L-PGDS levels in serum, cerebrospinal fluid or urine were elevated in patients with cardiovascular, neurological and renal diseases [21, 23, 29, 30]. The elevation in L-PGDS concentration occurs at an early stage of CVD, and the concentrations of serum or urinary L-PGDS were shown to

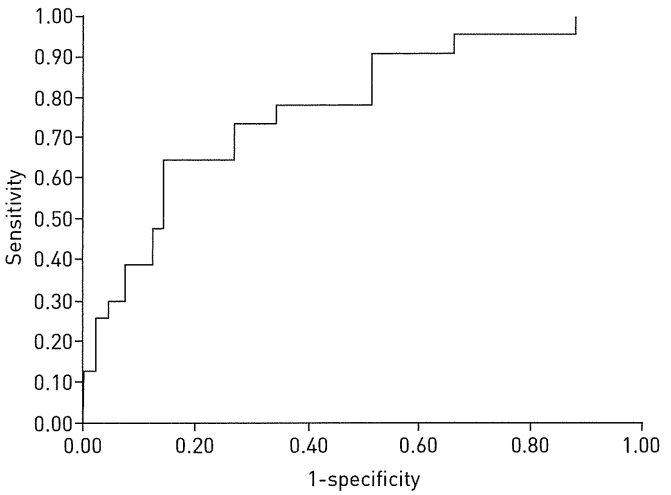

FIGURE 2 Receiver operating characteristic curve analysis to assess the diagnostic validity of morning urinary lipocalin-type prostaglandin D synthase concentrations to detect severe obstructive sleep apnoea.

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TABLE 2 Associations of lipocalin-type prostaglandin D synthase (L-PGDS) level and reactive hyperaemia peripheral arterial tone (RH-PAT) index with patients' characteristics, polysomnography data and biomarkers

	Morning urinary L-PGDS ng·mg^{-1} of creatinine		Plasma L-PGDS ng·mL^{-1}		RH-PAT index	
	r	p-value	r	p-value	r	p-value
Age years	0.164	0.20	0.362	0.003	0.074	0.56
Male	-0.013	0.92	0.048	0.71	-0.160	0.21
BMI kg·m^{-2}	-0.060	0.64	-0.132	0.31	-0.135	0.29
Waist circumference cm	-0.062	0.63	-0.076	0.55	-0.081	0.53
Morning systolic BP mmHg	0.394	0.001*	0.279	0.03*	-0.150	0.24
Morning diastolic BP mmHg	0.323	0.009*	0.274	0.03*	0.001	0.99
ESS	-0.139	0.27	-0.035	0.78	0.075	0.56
Arousal events·h^{-1}	0.472	<0.0001*	0.220	0.08	-0.268	0.03*
AHI events·h^{-1}	0.426	0.0005*	0.180	0.16	-0.241	0.06
3% ODI events·h^{-1}	0.384	0.002*	0.173	0.18	-0.244	0.054
Minimum Sp_{O_2} %	-0.112	0.38	0.099	0.44	0.046	0.72
Sp_{O_2} <90% % TST	0.128	0.31	0.017	0.90	-0.190	0.14
RH-PAT index	-0.068	0.59	0.030	0.82		
Creatinine mg·dL^{-1}	0.052	0.68	0.256	0.04	-0.067	0.60
TC mg·dL^{-1}	0.189	0.14	0.233	0.07	-0.003	0.98
HDL-C mg·dL^{-1}	0.214	0.09	0.045	0.73	0.079	0.54
TG mg·dL^{-1}	-0.067	0.60	0.170	0.18	0.134	0.30
CRP mg·dL^{-1}	0.071	0.58	0.083	0.52	-0.132	0.30
Glucose mg·dL^{-1}	0.158	0.22	-0.161	0.21	-0.143	0.27
Adrenaline pg·mL^{-1}	0.310	0.02*	0.220	0.10	0.018	0.89
Noradrenaline pg·mL^{-1}	0.329	0.008*	0.149	0.25	0.128	0.32
Plasma L-PGDS ng·mL^{-1}	0.228	0.07			0.030	0.82
Morning urinary L-PGDS ng·mg^{-1} of creatinine			0.228	0.07	-0.068	0.59
Night urinary L-PGDS ng·mg^{-1} of creatinine	0.868	<0.0001*	0.090	0.51	0.050	0.71

BMI: body mass index; BP: blood pressure; ESS: Epworth Sleepiness Scale; AHI: apnoea/hypopnoea index; ODI: oxygen desaturation index; Sp_{O_2}: arterial oxygen saturation measured by pulse oximetry; TST: total sleep time; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides; CRP: C-reactive protein. *: $p<0.05$.

increase when diseases such as coronary heart disease or type 2 DM worsen [21–23], although the activity of L-PGDS is not well known. OSA induces multi-organ damage such as hypersomnolence and CVD. Therefore, it is supposed that L-PGDS could have significant associations with the pathophysiology and severity of OSA. In addition, a urinary biomarker is attractive because urine is easily obtained and the results of urine testing are easy to follow. Although our present data might not be definitive because of the small sample size, the information gained from this study appears to be of interest and the topic warrants further study.

It has been said that serum L-PGDS levels increase with age and are higher in males than in females [19], while urinary L-PGDS was also shown to be higher in males than in females and is weakly correlated with age in female subjects [22]. In that study [22], control subjects were slightly younger, but not with significance, and those in the moderate and severe OSA groups were almost the same age as those in the present study (table 1). In addition, morning urinary L-PGDS values were still correlated with the severity of OSA after adjustment for BMI, age and sex. Therefore, BMI, sex and age could not be considered to have significant effects on our results. Although L-PGDS concentrations were shown to be increased in essential hypertension [21], morning urinary L-PGDS values were still correlated with the severity of OSA after adjustment for blood pressure. The BMI in individuals with severe OSA was slightly but not significantly higher than in the other groups. It was reported that the BMI [21] was not associated with elevations in blood or urinary L-PGDS. In addition to the fact that morning urinary L-PGDS levels were significantly higher after adjustment for BMI, morning urinary L-PGDS concentrations were also significantly decreased after 2 days of CPAP, which is a specific treatment for OSA, although other factors remained the same. Furthermore, from the viewpoint of circadian measurements, morning urine L-PGDS levels were higher in

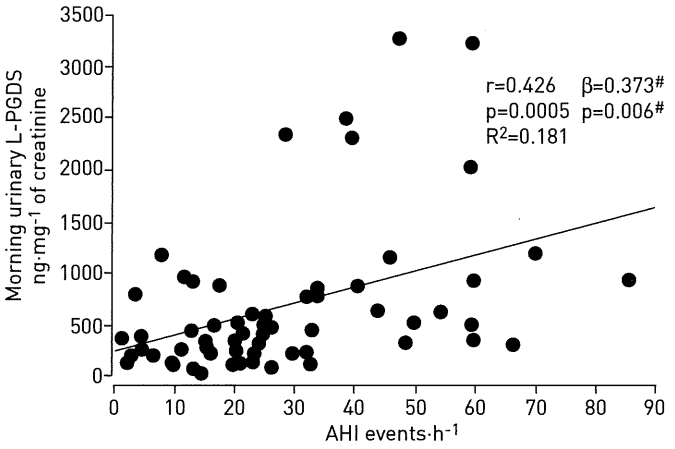

FIGURE 3 Relationship between apnoea/hypopnoea index (AHI) and morning urinary lipocalin-type prostaglandin D synthase (L-PGDS) concentrations. #: values after adjustment for age, sex, body mass index, and morning systolic and diastolic blood pressure.

patients with severe OSA than in the other subjects, while values at 14:00 h were the same. These findings indicate that L-PGDS is related to OSA independently, and is especially related to severe OSA.

Several causes for elevated L-PGDS concentrations in OSA have been considered, especially in severe OSA. First, intermittent hypoxia induces COX2 expression and activity, which in turn accelerate arachnoid acid metabolism to PGH_2 and PGE_2 [31, 32]. HAN *et al.* [33] noted that the expression levels of L-PGDS mRNA and protein in the heart of C57BL/6 mice were significantly increased after 14 days of hypoxia (10% oxygen) compared with mice kept under normoxia. OSA is characterised by repeated episodes of oxygen desaturation; however, in OSA there is also sustained hypoxaemia, which is expressed by arterial oxygen saturation measured by pulse oximetry <90% (% of total sleep time). Thus, both sustained and intermittent hypoxaemia could possibly increase urinary L-PGDS. Secondly, hypoxia causes pulmonary vasoconstriction and increases pulmonary artery pressure [34]. It has been reported that fluid shear stress increases the expression of L-PGDS in vascular endothelial cells [35, 36] and that urinary PGD_2 metabolites were increased in primary pulmonary hypertension [37]. SEMENZA [38] reported that intermittent hypoxaemia induced systemic hypertension and that sustained hypoxaemia induced pulmonary hypertension. Both systemic and pulmonary hypertension induced by OSA might increase L-PGDS values [21, 37].

It has been shown that sleep fragmentation and arousals caused by sleep apnoea induce an elevation of sympathetic activation [39]. If the severity of OSA might be associated with urinary or plasma L-PGDS concentrations, we thought that it would be important to determine whether there is a significant relationship between sympathetic activation and urinary or plasma L-PGDS. Therefore, we wanted to measure urinary catecholamine concentrations as indicators of sympathetic activity. However, it was

TABLE 3 Multivariate linear regression analysis for morning urinary lipocalin-type prostaglandin D synthase

	β	r	p-value	R^2 %
Model 1				
AHI events·h^{-1}	0.326	0.426	0.02	13.9
HDL-C mg·dL^{-1}	0.297	0.214	0.02	6.4
Cumulative R^2				20.3
Model 2				
Arousal, events·h^{-1}	0.411	0.472	0.001	19.4
HDL-C mg·dL^{-1}	0.290	0.214	0.01	6.2
Cumulative R^2				25.6
Model 3				
3% ODI events·h^{-1}	0.291	0.384	0.03	11.2
HDL-C mg·dL^{-1}	0.280	0.214	0.02	6.0
Cumulative R^2				17.2

β: standard regression coefficient; r: correlation coefficient; R^2: contribution rate; AHI: apnoea/hypopnoea index; HDL-C: high-density lipoprotein cholesterol; ODI: oxygen desaturation index.

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TABLE 4 Effects of continuous positive airway pressure (CPAP) on the reactive hyperaemia peripheral arterial tone (RH-PAT) index, various parameters and lipocalin-type prostaglandin D synthase (L-PGDS)

	Before CPAP	After 2 days of CPAP	p-value
BMI	25.2 (21.0–39.9)	24.8 (20.8–39.9)	0.11
Morning systolic BP mmHg	126 (102–138)	121 (103–138)	0.15
Morning diastolic BP mmHg	80 (69–94)	79 (63–94)	0.44
Arousal index events·h^{-1}	31.6 (10.8–54.8)	16.8 (8.6–42.8)	0.0005
AHI events·h^{-1}	33.6 (20.3–59.6)	4.4 (0–8.8)	<0.0001
3% ODI events·h^{-1}	27.9 (16.2–61.3)	3.5 (0–7.6)	<0.0001
RH-PAT index	1.75 (1.46–3.91)	1.82 (1.41–2.83)	0.61
Blood			
Creatinine mg·dL^{-1}	0.8 (0.6–1.0)	0.8 (0.6–1.0)	0.41
TC mg·dL^{-1}	189 (130–299)	184 (132–293)	0.78
HDL-C mg·dL^{-1}	53 (41–66)	52 (38–68)	0.13
TG mg·dL^{-1}	95 (44–215)	98 (58–304)	0.30
CRP mg·dL^{-1}	0.1 (0.0–0.5)	0.0 (0.0–0.4)	0.07
Glucose mg·dL^{-1}	96 (75–133)	96 (83–140)	0.23
Adrenaline pg·mL^{-1}	16 (5–45)	16 (5–36)	0.38
Noradrenaline pg·mL^{-1}	257 (82–521)	216 (113–529)	0.31
L-PGDS ng·mL^{-1}	480.4 (323.2–567.4)	466.0 (323.9–599.2)	0.92
Urine			
Morning L-PGDS ng·mg^{-1} of creatinine	591.2 (227–3274.1)	317.8 (130.3–1587.6)	0.007

Data are presented as median (range), unless otherwise stated. BMI: body mass index; BP: blood pressure; AHI: apnoea/hypopnoea index; ODI: oxygen desaturation index; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides; CRP: C-reactive protein.

difficult to obtain urine in which L-PGDS and catecholamine levels could be measured at the same time. Therefore, even though we knew that plasma catecholamine levels were unstable and easily variable, the usefulness of which was difficult to understand, we measured plasma catecholamine levels. In the present study, urinary L-PGDS concentrations were also correlated with plasma adrenaline and noradrenaline. Therefore, sympathetic overactivity in OSA [40, 41] might also induce increases in urinary L-PGDS. The relationship between urinary catecholamine and L-PGDS should be studied in the future. We also found that HDL-C was a significant determinant of morning urinary L-PGDS. MIWA *et al.* [42] reported that

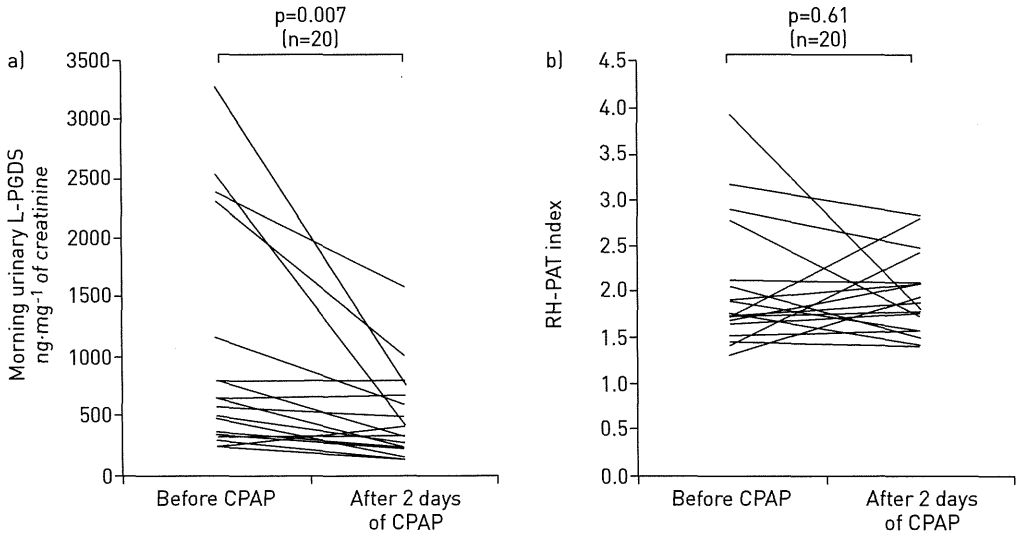

FIGURE 4 Change in a) morning urinary lipocalin-type prostaglandin D synthase (L-PGDS) concentrations and b) reactive hyperaemia peripheral arterial tone (RH-PAT) index before and after 2 days of continuous positive airway pressure (CPAP). Individual data are presented.