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ORIGINAL RESEARCH

Lower FEV$_1$ in non-COPD, nonasthmatic subjects: association with smoking, annual decline in FEV$_1$, total IgE levels, and *TSLP* genotypes

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Abstract: Few studies have investigated the significance of decreased FEV$_1$ in non-COPD, nonasthmatic healthy subjects. We hypothesized that a lower FEV$_1$ in these subjects is a potential marker of an increased susceptibility to obstructive lung disease such as asthma and COPD. This was a cross-sectional analysis of 1505 Japanese adults. We divided the population of healthy adults with no respiratory diseases whose FEV$_1$/FVC ratio was ≥70% (n = 1369) into 2 groups according to their prebronchodilator FEV$_1$ (% predicted) measurements: <80% (n = 217) and ≥80% (n = 1152). We compared clinical data – including gender, age, smoking habits, total IgE levels, and annual decline of FEV$_1$ – between these 2 groups. In addition, as our group recently found that *TSLP* variants are associated with asthma and reduced lung function, we assessed whether *TSLP* single nucleotide polymorphisms (SNPs) were associated with baseline lung function in non-COPD, nonasthmatic healthy subjects (n = 1368). Although about half of the subjects with lower FEV$_1$ had never smoked, smoking was the main risk factor for the decreased FEV$_1$ in non-COPD, nonasthmatic subjects. However, the subjects with lower FEV$_1$ had a significantly higher annual decline in FEV$_1$ independent of smoking status. Airflow obstruction was associated with increased levels of total serum IgE ($P = 0.029$) and with 2 functional *TSLP* SNPs (corrected $P = 0.027–0.058$ for FEV$_1$% predicted, corrected $P = 0.015–0.033$ for FEV$_1$/FVC). This study highlights the importance of early recognition of a decreased FEV$_1$ in healthy subjects without evident pulmonary diseases because it predicts a rapid decline in FEV$_1$ irrespective of smoking status. Our series of studies identified *TSLP* variants as a potential susceptibility locus to asthma and to lower lung function in non-COPD, nonasthmatic healthy subjects, which may support the contention that genetic determinants of lung function influence susceptibility to asthma.

Keywords: airflow obstruction, asthma, chronic obstructive pulmonary disease, pulmonary function test, thymic stromal lymphopoietin

Introduction

Pulmonary function is among the most important health indicators, being a strong predictor of long-term morbidity and mortality.[1] Impaired pulmonary function is a strong risk factor for the development of asthma and COPD and a marker of disease severity. The diagnosis and assessment of COPD recommended by Global Initiative for Obstructive Lung Disease (GOLD) guidelines are based on the degree of airflow obstruction as assessed by spirometry.[2] Any arbitrary classification of a continuous variable, such as FEV$_1$ and the FEV$_1$/FVC ratio, results in a group of patients with borderline phenotypes,[3] and classification based on the FEV$_1$ and FEV$_1$/FVC criteria established by the GOLD guidelines may underemphasize the potential significance of the "unclassified" subgroup of individuals who have a decreased FEV$_1$ and a normal

FEV_1/FVC ratio. Early-life origin of COPD has recently been emphasized,[4,5] and there is evidence that pathological abnormalities of COPD may be present even in the absence of airflow obstruction.[6] However, the clinical characteristics of individuals with a decreased FEV_1 and a normal FEV_1/FVC ratio are not well established, and the validity of classifying these individuals as "normal" is uncertain.

Chronic stimulation of the innate immune system by microbes, inhaled allergens, or components of tobacco smoke is involved in the inflammation and remodeling of airways that underlies asthma and COPD,[7,8] and certain genetic variants may play a role in the common pathogenesis of both asthma and COPD.[9,10] TSLP is an innate cytokine released by primary human epithelial cells in response to certain microbial products, physical injury, cigarette smoke, or inflammatory cytokines,[11,12] and TSLP has an important role in the initiation of allergic and adaptive airway inflammation through innate pathways that initially activate airway epithelium.[13] Patients with asthma have higher concentrations of TSLP in their lungs.[14,15] Moreover, TSLP is overexpressed in the bronchial mucosa in patients with COPD, which suggests that TSLP not only plays a key role in allergic inflammation, but may also play a role in nonallergic airway inflammation.[15] Our group has recently identified the *TSLP* gene as an asthma susceptibility gene.[16] Since it has been suggested that genetic determinants of lung function influence susceptibility to asthma as well as COPD,[17] our previous finding may indicate a role of TSLP in the development of airflow obstruction that originates in earlier life.

The two aims of the current study are to describe the characteristics of healthy subjects with lower FEV_1 using a large dataset from healthy, non-COPD, nonasthmatic subjects which includes valid lung function measurements, chest roentgenograms, and responses about respiratory symptoms, and to investigate the role of functional polymorphisms at the *TSLP* gene on baseline lung function defined by FEV_1% predicted and FEV_1/FVC in these healthy subjects.

Methods
Subjects
This was a cross-sectional analysis of 1505 Japanese men and women aged 22 to 81 years who visited the Tsukuba Medical Center for annual medical checkup from June 2008 to May 2009. Detailed information on respiratory health, lifestyle, and exposure conditions to environmental irritants such as tobacco smoking, allergens, and air pollutions was collected. Lung function data, chest radiographic findings, and the results of a standard clinical examination were also available

for all participants. Based on a detailed questionnaire on pulmonary symptoms, a physical examination including pulmonary auscultation and chest roentgenogram, we carefully excluded the presence of pulmonary diseases such as asthma, COPD, chronic bronchitis, old tuberculosis, and pulmonary fibrosis.

Our diagnosis of asthma basically relied on a positive response to the following questions: "Have you ever been told by a doctor that you have asthma" and "Do you still have asthma?" in addition to the presence of asthmatic symptoms such as wheezing, cough, dyspnea, and chest tightness or the current use of anti-asthmatic medications. Chronic bronchitis was defined as self-report of cough or phlegm during the day or at night on most days for as much as 3 months each year for 2 or more years. Clinical diagnosis of COPD was defined as reporting of any respiratory symptom (exertional breathlessness, chronic cough, regular sputum, frequent winter bronchitis, or wheeze) and evidence of airway obstruction on spirometry (both $FEV_1/FVC < 0.7$ and $FEV_1 < 80\%$ predicted). Subjects who reported previously doctor-diagnosed chronic bronchitis or emphysema were also excluded. Pulmonary tuberculosis may cause similar respiratory symptoms and chronic airflow limitation to those seen with COPD. Thus, we carefully excluded subjects with scarring on chest radiograph and/or previous history of pulmonary tuberculosis treatment.

Classification of the subjects
Of the 1505 subjects, 113 subjects with a history of asthma, COPD, chronic bronchitis, interstitial pneumonia, or pulmonary tuberculosis were excluded; 1392 (92.5%) subjects who had no pulmonary diseases were eligible to participate in the study. None of these 1392 subjects were taking bronchodilators such as β-agonists, anticholinergics, antileukotrienes, methylxanthines, or inhaled corticosteroids. Of the 1392 subjects without respiratory diseases, 23 subjects had $FEV_1/FVC < 70\%$. To characterize the group of individuals with decreased FEV_1 (predicted $FEV_1 < 80\%$) and normal ratio of FEV_1/FVC, we divided the 1369 healthy subjects into 2 subgroups according to their prebronchodilator FEV_1 (% predicted): $<80\%$ (lower FEV_1, n = 217) and $\geq80\%$ (higher FEV_1, n = 1152).

Of these subjects, 365 healthy subjects with normal lung function were used as the control group in our previous genetic study of *TSLP* for adult asthma.[16] The flowchart of the current study is shown in Figure 1.

The Medical Ethics Committees of the University of Tsukuba (Tsukuba, Japan) and the Tsukuba Medical Center
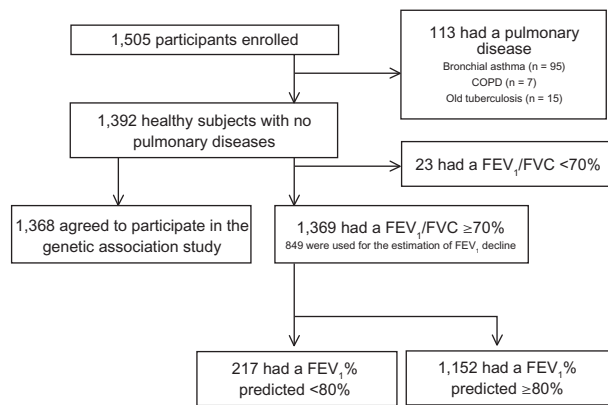
Figure 1 Recruitment and enrollment of study participants. Of the 1,505 participants, 113 subjects with a history of asthma, COPD, chronic bronchitis, or pulmonary tuberculosis were excluded, and 4 of those excluded were identified as having 2 or 3 pulmonary diseases. Of the 1,392 subjects without respiratory diseases, 23 subjects had FEV$_1$/FVC < 70%. To characterize the group of individuals with decreased FEV$_1$ (predicted FEV$_1$ < 80%) and normal ratio of FEV$_1$/FVC, we divided 1,369 healthy subjects whose FEV$_1$/FVC was ≥70% into 2 subgroups according to their prebronchodilator FEV$_1$ (% predicted): <80% (lower FEV$_1$, n = 217) and ≥80% (higher FEV$_1$, n = 1,152). We also examined the relationship between *TSLP* single nucleotide polymorphisms and baseline lung function using 1,368 healthy subjects, who agreed to provide a DNA sample for genotyping. Of the 1,392 healthy subjects, the median number of measurements was 9 (range 4–18 measurements) and the median number of years in retrospective follow-up was 11 (range 4–23 years) for the 866 subjects who had an annual checkup for 4 or more years of the retrospective follow-up and had at least 4 separate pulmonary function test measurements. Valid estimates of the annual decline of FEV$_1$ were obtained for 849 healthy subjects with FEV$_1$/FVC > 70% or for 827 healthy subjects with FEV$_1$/FVC > lower limit of normal.

(Tsukuba, Japan) both approved the study, and each subject provided written informed consent.

Pulmonary function test (PFT)

Spirometry performance was required to meet the criteria by the Japanese Respiratory Society (JRS).[18] Data for FEV$_1$, FVC, and FEV$_1$/FVC ratio were available for all participants. Lung function was measured in the standing position using an electronic spirometer (Autospiro series; Minato Medical Science Co., Ltd, Osaka, Japan). No bronchodilation was applied. Well-trained spirometry technicians continuously monitored the procedure and reviewed flow-volume curves to ensure adherence to standards. Participants performed up to 3 forced expiratory maneuvers to obtain acceptable maneuvers; FEV$_1$ and FVC values taken to characterize each participant were the maximum results obtained from acceptable maneuvers. FEV$_1$ and FVC were expressed as percentage of predicted values according to the prediction equations of the JRS. FEV$_1$/FVC ratio was calculated using actual values. Calibration was checked weekly with a 2-L syringe. Calibration checks were also undertaken as specified by the manufacturer. All available longitudinal data of FEV$_1$ were collected retrospectively to evaluate the annual decline of FEV$_1$ for every participant.

Genotyping

A total of 23 polymorphisms in the *TSLP* gene have been identified in the Japanese population.[19] Three tag SNPs in *TSLP*, rs3806933, rs2289276, and rs2289278, were genotyped using the TaqMan allele-specific amplification method (Applied Biosystems, Foster City, CA) as described previously.[16]

Statistics

Descriptive statistics were expressed as mean ± standard deviation (SD). We compared clinical data – gender, age, body mass index (BMI), smoking habits (never smoker, ex-smoker, and current smoker), smoking index, total serum IgE levels, atopy (defined as at least 1 positive response to 14 inhaled antigens), and annual decline of FEV$_1$ – between 2 groups (the lower FEV$_1$ group and the higher FEV$_1$ group) using a 2-tailed Student's *t*-test or Pearson's chi-square test as appropriate. Smoking index was calculated by multiplying smoking dose (cigarettes per day) and duration (years smoked), and was categorized into 3 groups: 0, 0 to 200, and >200 cigarette-years.

The use of a fixed threshold to define airway obstruction is associated with some extent of misclassification.[21] Therefore, in addition to the fixed values of FEV$_1$/FVC (70%) and FEV$_1$ (80% of predicted), we also used the age- and sex-specific lower limit of normal (LLN) as an alternative threshold for the FEV$_1$/FVC ratio and FEV$_1$ to categorize 1392 healthy subjects without pulmonary diseases. We used spirometry reference equations for FEV$_1$/FVC and FEV$_1$ for men and women from a large Japanese population-based study (1863 never-smoking adults from ages 18 to 95 years).[22] The LLNs for FEV$_1$/FVC and FEV$_1$ were calculated by subtracting 1.645 × residual standard deviation (RSD) from the predicted means assuming a Gaussian distribution of the residuals.

Values of the annual decline of FEV$_1$ were computed for each individual across the repeated measurements using a linear mixed-effect model. We included only those subjects who took the annual checkup for at least 4 years of retrospective follow-up and who had at least 4 separate PFT measurements to obtain an accurate estimate; among 1392 healthy subjects, 866 subjects had valid estimates of the annual decline of FEV$_1$. The median number of measurements was 9 (range 4–18 measurements) and the median number of years in retrospective follow-up was 11 (range 4–23 years) for the 866 subjects. We used a random intercept to take into account the heterogeneity across subjects and the correlation induced by having repeated observations on the same subjects. We chose 25 years of

age as the starting point for the analyses because maximum lung function would be achieved before that age and lung function is thought to be in decline by that age.[20] Estimation of an annual decline of FEV_1 was performed using SYSTAT software, version 13 (Systat Software, Inc., Chicago, IL). Valid estimates of the annual decline of FEV_1 were obtained for 849 subjects with $FEV_1/FVC > 70\%$ or for 827 subjects with $FEV_1/FVC > LLN$.

General linear models were used to assess associations between *TSLP* polymorphisms and pulmonary function measurements after adjusting for gender, age, BMI, FVC% predicted, smoking status, smoking index, and total serum IgE levels (log-transformed). We used the Hardy-Weinberg equilibrium (HWE) program[23] to compare observed numbers of genotypes with the numbers of genotypes expected under HWE. In the association study, we applied a Bonferroni correction on the *P*-values. As we tested 3 polymorphisms, we used $P = 0.05/3 = 0.0167$ as a threshold for significance.

For haplotype analyses, we used the Haplo.score program, which adjusted for covariates and calculated simulation

P values for each haplotype.[24] P values < 0.05 were considered to indicate statistical significance.

Results

The characteristics of the population studied are shown in Table 1. The individual values of FEV_1 and FEV_1/FVC for all healthy subjects (n = 1392) are shown in Figure 2. Based on GOLD guidelines, 217 subjects who had a reduced FEV_1 ($<80\%$ of predicted) could not be classified as having COPD because they had a FEV_1/FVC ratio $\geq70\%$. In cross-sectional analyses, the percentage of males in the lower FEV_1 group was significantly higher than the percentage of males in the higher FEV_1 group. No significant differences in the following factors were observed between the 2 groups – lower FEV_1 and higher FEV_1: age (range), 51.1 (25–78) vs 50.1 (22–78); BMI, 23.1 vs 23.0; and atopy, 57.6% vs 58.0% for the lower FEV_1 group and the higher FEV_1 group, respectively. Among subjects who had a normal FEV_1/FVC ratio and a decreased $FEV_1\%$ (the lower FEV_1 group), 48.8% had never smoked. Nevertheless, smokers were more common in the lower FEV_1 group than in the higher FEV_1 group ($P < 0.0001$).

Table 1 Characteristics of the participants at baseline[a]

Variables	$FEV_1\%$ predicted $<80\%$ (N = 217)	$FEV_1\%$ predicted $\geq80\%$ (N = 1152)	*P* value
Age range (years)	51.1 ± 10.5 (25–78)	50.1 ± 9.3 (22–78)	0.20
Male sex (%)	125 (57.6)	503 (43.7)	0.0002
BMI	23.1 ± 3.2	23.0 ± 3.1	0.72
FVC (L)	2.73 ± 0.66	3.25 ± 0.77	<0.0001
FVC% predicted (%)	84.0 ± 9.2	104.0 ± 11.5	<0.0001
FEV_1 (L)	2.18 ± 0.53	2.72 ± 0.64	<0.0001
FEV_1/FVC (%)	80.1 ± 5.5	83.8 ± 5.0	<0.0001
Smoking habits[b] (N, %)			<0.0001
Never smokers	106 (48.8)	759 (65.9)	
Ex-smokers	59 (27.2)	245 (21.3)	
Current smokers	52 (24.0)	148 (12.8)	
Smoking index[c] (N, %)			<0.0001
0	106 (48.8)	759 (65.9)	
0–200	32 (14.8)	139 (12.1)	
>200	79 (36.4)	254 (22.0)	
Serum IgE (log IU/mL)	1.86 ± 0.60	1.76 ± 0.58	0.029
Atopy (%)	125 (57.6)	668 (58.0)	0.94
FEV_1 decline (mL/year)[d]	32.3 ± 27.0	20.8 ± 25.0	<0.0001
Never smokers (N = 538)	32.4 ± 25.2 (N = 65)[e]	18.8 ± 23.1 (N = 473)[f]	<0.0001
Ex- and current smokers (N = 311)	32.3 ± 28.6 (N = 73)	24.8 ± 28.2 (N = 238)	0.048

Notes: [a]Data are presented as mean ± SD unless otherwise indicated; [b]Subjects were classified as never smokers, ex-smokers, or current smokers based on questionnaire responses; [c]Smoking index was calculated for current and past smokers by multiplying smoking dose (cigarettes per day) and duration (years smoked); [d]To obtain an accurate estimate of the annual decline of FEV_1, we selected only subjects who took the annual checkup for at least 4 years of retrospective follow-up and who had at least 4 separate pulmonary function test measurements (n = 849); [e]When FEV_1 decline was compared within the lower FEV_1 group (n = 138) between never smokers and ex- or current smokers, no difference was found (32.4 [SD 25.2] mL/year for 65 never smokers and 32.3 [28.6] mL/year for 73 current or ex-smokers, $P > 0.5$); [f]In the higher FEV_1 group (n = 711), a significant difference in the annual decline of FEV_1 was found between never smokers and ex- or current smokers (18.8 [SD 23.1] mL/year for 473 never smokers and 24.8 [28.2] mL/year for 238 current or ex-smokers, $P = 0.0026$).
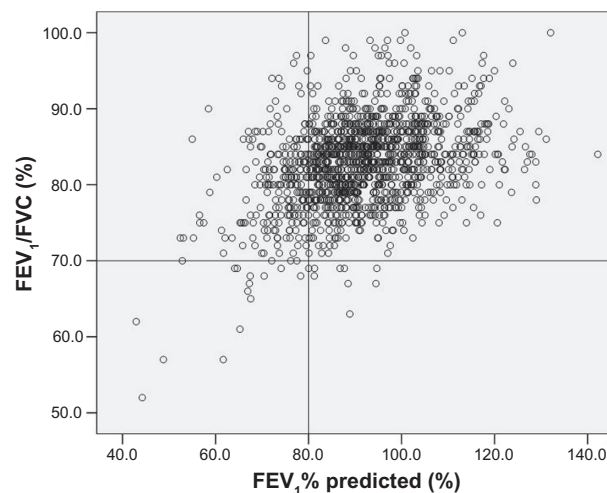
Figure 2 Plot of individual values of FEV$_1$% predicted and FEV$_1$/FVC. Data from 1392 healthy adults with no respiratory diseases.

The smoking index was also significantly higher in the lower FEV$_1$ group than in the higher FEV$_1$ group ($P < 0.0001$). Levels of serum total IgE were higher in the lower FEV$_1$ group ($P = 0.029$). Annual decline of FEV$_1$ calculated by a linear mixed regression averaged 32.3 mL/year for the lower FEV$_1$ group and 20.8 mL/year for the higher FEV$_1$ group ($P < 0.0001$). When FEV$_1$ decline was compared within the lower FEV$_1$ group (n = 138) between never smokers and ex- or current smokers, no difference was found according to smoking status (32.4 [SD 25.2] mL/year for 65 never smokers and 32.3 [28.6] mL/year for 73 current or ex-smokers, $P > 0.5$). In contrast, in the higher FEV$_1$ group (n = 711), a significant difference in the annual decline of FEV$_1$ was found between never smokers and ex- or current smokers (18.8 [SD 23.1] mL/year for 473 never smokers and 24.8 [28.2] mL/year for 238 current or ex-smokers, $P = 0.0026$).

Among 1392 healthy subjects, 866 subjects had a valid estimate of the annual decline in FEV$_1$. When these 866 subjects were studied, the annual decline of FEV$_1$ between lower FEV$_1$ group and higher FEV$_1$ group was also significantly different irrespective of how we define the lower FEV$_1$ groups (FEV$_1$% < 80% predicted or FEV$_1 <$ LLN). In addition,

when 61 healthy subjects with FEV$_1$/FVC $<$ LLN were excluded from the analysis, the annual decline in FEV$_1$ consistently differed between higher and lower groups, as the lower FEV$_1$ group was defined using the cut-off of FEV$_1 <$ LLN (Table 2).

DNA for a genetic association study was available for 1368 of the 1392 healthy subjects with no pulmonary diseases. All SNPs investigated were within HWE ($P > 0.05$). The overall success rate for genotyping was 99.3%. The genotypic frequency of *TSLP* gene polymorphisms among the healthy Japanese participants (n = 1368) are shown in Table 3. SNP rs3806933 was associated with a lower FEV$_1$% predicted (corrected $P = 0.036$) and with a lower FEV$_1$/FVC ratio (corrected $P = 0.033$). SNP rs2289276 was also associated with a lower FEV$_1$/FVC (corrected $P = 0.029$). Both associations were driven by carriers of 2 minor alleles (recessive models); significant associations were noted with a lower FEV$_1$% predicted and with a lower FEV$_1$/FVC at rs3806933 (corrected $P = 0.027$ and corrected $P = 0.015$, respectively) and at rs2289276 (corrected $P = 0.042$ and corrected $P = 0.015$, respectively). In contrast, there was no association between the genotype at rs2289278 and the FEV$_1$% predicted or the FEV$_1$/FVC phenotype. We did not observe any significant association between any one of these SNPs and the annual decline in FEV$_1$.

As recent studies have shown sex-specific associations between variants of the *TSLP* gene and asthma,[25] levels of total serum IgE and specific IgE antibody toward cockroach,[26] we further conducted a sex-stratified analysis; however, we could not determine the sex-specific effect on these associations (data not shown). We also did not find convincing evidence that the effect of *TSLP* genetic variants in FEV$_1$% predicted or FEV$_1$/FVC was modified by environmental exposure to tobacco smoke in the study population (data not shown).

The haplotype composed of these 3 variants was significantly associated with FEV$_1$/FVC, with a P value of 0.018 from 1000 simulations of a global score test, as implemented

Table 2 Association between FEV$_1$ and a decline of FEV$_1$ in healthy subjects

Criteria for defining normal FEV$_1$/FVC ratio	Criteria for defining the higher FEV$_1$ group	Annual FEV$_1$ decline[d] (lower FEV$_1$ group)	Annual FEV$_1$ decline[d] (higher FEV$_1$ group)	P
FEV$_1$/FVC $\geq$ 70% (n = 1369)[a]	FEV$_1$% $\geq$ 80%	32.3 $\pm$ 27.0 (n = 138)	20.8 $\pm$ 25.0 (n = 711)	$<$0.0001
FEV$_1$/FVC $\geq$ LLN (n = 1331)[b]	FEV$_1$ $\geq$ LLN	31.1 $\pm$ 29.8 (n = 84)	21.1 $\pm$ 24.3 (n = 743)	0.0005
None (n = 1392)[c]	FEV$_1$% $\geq$ 80%	34.2 $\pm$ 29.3 (n = 147)	21.0 $\pm$ 25.1 (n = 719)	$<$0.0001
None (n = 1392)[c]	FEV$_1$ $\geq$ LLN	36.1 $\pm$ 34.3 (n = 106)	21.5 $\pm$ 24.5 (n = 760)	$<$0.0001

Notes: Valid estimates of the annual decline of FEV$_1$ were obtained for 849,[a] 827,[b] and 866[c] healthy subjects, respectively; [d]mL/year.
Abbreviation: LLN, lower limit of normal.

Table 3 Associations between *TSLP* genotype at 3 single nucleotide polymorphisms (SNP) and lung function

SNP		N	FEV$_1$% predicted (%) (95%CI)	Nominal P value	FEV$_1$/FVC (%) (95%CI)	Nominal P value
rs3806933	CC	721	92.04 (91.57–92.51)	0.012[a]	83.29 (82.89–83.68)	0.011[a]
	CT	526	91.15 (90.60–91.71)		82.46 (81.99–82.92)	
	TT	112	90.95 (89.75–92.15)		82.49 (81.48–83.49)	
rs3806933	CC	721	92.04 (91.57–92.51)	0.0089[a]	83.29 (82.89–83.68)	0.0050[a]
	CT + TT	638	91.12 (90.62–91.62)		82.46 (82.04–82.88)	
rs2289276	CC	777	91.97 (91.52–92.43)		83.26 (82.88–83.65)	
	CT	488	91.13 (90.55–91.70)	0.019	82.42 (81.94–82.90)	0.0096[a]
	TT	91	90.95 (89.61–92.29)		82.44 (81.32–83.56)	
rs2289276	CC	777	91.97 (91.52–92.43)		83.26 (82.88–83.65)	
	CT + TT	579	91.10 (90.57–91.63)	0.014[a]	82.43 (81.98–82.87)	0.0049[a]
rs2289278	CC	844	91.38 (90.94–91.82)		82.73 (82.36–83.10)	
	CG	466	91.97 (91.38–92.56)	NS[b]	83.21 (82.72–83.71)	NS
	GG	52	92.22 (90.44–93.99)		82.80 (81.32–84.28)	
rs2289278	CC	844	91.38 (90.94–91,82)	NS	82.73 (82.36–83.10)	NS
	CG + GG	518	91.99 (91.43–92.56)		83.17 (82.70–83.64)	

Notes: [a]$P < 0.05$ after Bonferroni correction; [b]NS, not significant; some subjects could not be genotyped for technical reasons.

in Haplo.score.[24] The haplotype most strongly associated with lower FEV$_1$/FVC was rs3806933C/rs2289276C/rs2289278C (haplotype-specific scores = –2.79, $P = 0.0018$) on the basis of 10,000 simulations; this haplotype showed significantly increased promoter activity in a previous study.[16] For FEV$_1$% predicted, haplotype analysis did not further improve the significance compared with the single SNP analysis.

Discussion

We studied prebronchodilator lung function results from a large number of Japanese adults without pulmonary disease. This study showed that the group of individuals who have normal FEV$_1$/FVC ratio and decreased FEV$_1$ mainly consists of smokers with higher cumulative tobacco consumption. In addition, this group was characterized by higher levels of total serum IgE and an increased annual decline in FEV$_1$. Furthermore, this study provided preliminary evidence that functional SNPs in the *TSLP* gene influence FEV$_1$ and FEV$_1$/FVC in healthy subjects independently of the impact of tobacco smoke, emphasizing a distinct role for TSLP in airway obstruction that is a potentially important shared risk factor for asthma and COPD.

Although smoking is a major, modifiable risk factor, nonsmokers are similarly at risk for lung function decline; there was no difference in the annual FEV$_1$ decline between smokers (32.4 mL/year) and nonsmokers (32.3 mL/year) in the lower FEV$_1$ group. In a study with 20-year follow-up, average decline in FEV$_1$ of 2496 healthy adults without airflow obstruction was 18 mL/year,[27] which is compatible with that of 1152 healthy adults with normal FEV$_1$ in the

current study. An analysis of the Framingham Offspring Cohort also showed that the mean FEV$_1$ decline was 19.6 mL for 666 males and 17.6 mL for 912 females in healthy never smokers.[28] In contrast, in a study of 3-year follow-up, the mean yearly absolute decline of FEV$_1$ in adult patients with mild asthma without severe asthma-related events was from 27 mL to 34 mL,[29] which was similar to that found in 217 healthy adults with lower FEV$_1$ in the current study. Never smokers comprise a substantial proportion of individuals with COPD, which has been associated with a prior diagnosis of asthma, a family history of asthma, and respiratory infections in childhood.[4,5,30] Therefore, our findings indicate that the group of healthy subjects with lower FEV$_1$ is a mixture of individuals who are susceptible to 1 or more irritants including microbial infections, allergens, or tobacco smoking. The lower FEV$_1$ in otherwise healthy adults may be a marker for an increased FEV$_1$ decline and an increased risk for chronic inflammatory lung diseases such as asthma and COPD, indicating the importance of recognizing decreased FEV$_1$ in targeting intervention efforts among smokers and nonsmokers alike.

The mechanism that mediates the association of the *TSLP* gene with the lower levels of FEV$_1$ in healthy subjects is not defined. The level of FEV$_1$ at a given time point in an individual depends on 2 potentially independent processes: the maximum lung function obtained during development, and the rate of decline of lung function with age. Because we failed to see genetic effects of *TSLP* on the annual decline of FEV$_1$, the association between basal lung function and *TSLP* genotype may be the result of abnormalities in

lung development, rather than accelerated decline of lung function. TSLP is released predominantly by bronchial epithelial and smooth muscle cells in response to certain microbial products, allergen-associated molecules, tobacco smoke, physical injury, or inflammatory cytokines.[11,31] TSLP integrates environmental stresses with an initial activation of resident innate immune cells, followed by activation of the adaptive immune system. Having a predisposition toward exaggerated TSLP responses against innocuous environmental antigens, individuals with particular *TSLP* genotypes would be expected to have impaired lung development which could lead to increased susceptibility to altered lung functions as adults.

Significant associations of an altered lung function in healthy subjects were obtained with the same *TSLP* SNPs, rs3806933 (–847C/T) and rs2289276 (–82C/T), which were associated with asthma susceptibility in our previous study;[16] the same alleles were associated with both asthma and lower FEV$_1$ in non-COPD nonasthmatic subjects. The rs3806933 site is in the promoter region of the *TSLP* gene, and the minor allele has been shown to create a binding site for the transcription factor activating protein (AP)-1. The variant enhances AP-1 binding to the regulatory element and increases promoter-reporter activity of *TSLP* in response to poly(I:C) stimulation in normal human bronchial epithelium.[19] AP-2α protein, a possible transcriptional repressor, binds to sequences containing the rs2289276 (–82C/T) SNP with a higher affinity to –82C (on the protective allele).[16] Our previous and current findings are consistent with a detrimental role of increased TSLP expression in pulmonary function and the pathogenesis of chronic inflammatory lung diseases. However, there remains a possibility that functional SNPs, other than rs3806933 and rs2289276, which are in linkage disequilibrium with either or both rs3806933 and rs2289276, may be truly responsible for our findings.

Recently, 2 studies have reported genome-wide association studies for lung function, using cross-sectional spirometric measurements in healthy individuals.[32,33] They identified 6 genetic loci newly associated with natural variation in lung function, and it has been suggested that genetic determinants of lung function influence susceptibility to asthma and COPD. However, the list of the top 2000 SNPs associated with FEV$_1$ and FEV$_1$/FVC in these studies did not include any SNPs that are located in and around the *TSLP* gene including 3 SNPs examined in the current study. Failure to observe a SNP-for-SNP replication in ethnically diverse populations is not uncommon, and can result from a variation in allele frequencies, population admixture, heterogeneity of the phenotype, and environmental factors.

Our study has several limitations that require discussion. Importantly, we do not have information about postbronchodilator FEV$_1$ for these subjects. For the clinical identification of early stages of COPD, the GOLD provided an international standard for diagnosis based on FEV$_1$/FVC ratio <0.70 measured by postbronchodilator spirometry,[2] and the use of prebronchodilator measurements prevents the identification of reversible obstruction. Reversible obstructions, however, are mostly undiagnosed asthma, and in this study, we carefully excluded asthma by a detailed questionnaire on pulmonary symptoms (cough, sputum, exertional dyspnea, wheeze), and physical examinations including pulmonary auscultation. In addition, prebronchodilator lung function has been used in many epidemiological studies and has been shown to have value in predicting health outcomes on a population level.[5,21,34]

Another limitation of our study is the retrospective nature of the FEV$_1$ follow-up. The follow-up periods and numbers of spirometry measurements varied among subjects. This variation may have contributed to a biased estimation of the annual decline in FEV$_1$ among subjects; however, we used a linear mixed-effects model to control for correlations among repeated measures within each subject, and we limited the analysis to include only subjects who had at least 4 separate FEV$_1$ measurements during 4 or more years follow-up. Nonetheless, our results will need to be replicated in other, ideally prospective, studies with large cohorts.

Third, it is unclear whether the excess decline in FEV$_1$ found in this study led to significantly more asthma or COPD development. However, it is important to recognize that excess decline of 10 to 15 mL/year in the lower FEV$_1$ group compared with the higher FEV$_1$ group would lead to an excess loss of 200 to 300 mL in 20 years, which would be expected to affect COPD incidence, particularly in subjects whose baseline lung function is lower. Serial measurements of FEV$_1$ can presumably identify COPD at a point in time where the impact of the disease is generally limited. Our study suggests that prebronchodilator FEV$_1$ may be a good marker of disease susceptibility even when the subjects have a normal FEV$_1$/FVC ratio, allowing much earlier detection of COPD. Further prospective studies will determine whether lower FEV$_1$ in healthy subjects without pulmonary diseases is associated with the future development of asthma or COPD.

In conclusion, although the FEV$_1$/FVC ratio is a relatively sensitive index of mild airflow obstruction, it seems that

lower FEV_1 in non-COPD, nonasthmatic subjects may be of some help in identifying subjects at risk for these chronic inflammatory pulmonary diseases. Given the increasing societal burden of airflow obstruction, early detection of an altered lung function in asymptomatic adults is important. The demonstration of increased annual decline of FEV_1, even in healthy adults with lower FEV_1, should alert clinicians to the importance of case detection in subjects at risk, irrespective of their smoking status. This study may suggest the need for a definition of COPD that is not exclusively based on spirometry. In addition, we identified *TSLP* as associated with natural variation in lung function, bringing new insight into our understanding of the genetic basis of lung development and related airway disorders, such as asthma and COPD.

Acknowledgments

Disclosure

The authors report no conflicts of interest.

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