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Trajectory of Spirometric and Exhaled Nitric Oxide Measurements in Chinese Schoolchildren with Asthma

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Running head: Lung function growth in childhood asthma

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Abstract

Background: Lung function growth occurs in most asthmatic children. A subgroup has subnormal lung function trajectory, but such data is limited in children. This prospective study characterized longitudinal changes of spirometric indices and fractional exhaled nitric oxide level (FeNO) among asthmatic children and identified their genetic and environmental determinants.

Methods: Chinese asthmatic children recruited from paediatric clinics underwent 5-year follow-up for pre-bronchodilator spirometric indices and FeNO. Fourteen asthma-associated single-nucleotide polymorphisms (SNPs) were genotyped. Generalized estimating equation was used to analyze longitudinal changes of spirometric indices and FeNO.

Results: 193 asthmatic children, aged 9.7 (1.9) years, had significant annual decline of 1.3% for FVC and annual increase of 1.2% and 3.6% for FEV₁/FVC and FEF₂₅₋₇₅ respectively. Patients who received inhaled corticosteroid (ICS) had 2.4% lower baseline FEV₁/FVC but 0.81% higher annual increase in FEV₁. Body mass index (BMI) was associated inversely with FEV₁/FVC but positively with FEV₁% and FVC% changes. Asthma exacerbation was associated with lower FEV₁% and FVC% but not their longitudinal changes. When classified by FEV₁ curve, one-quarter of patients had reduced lung function growth which was associated with female gender and lower spirometric and higher FeNO values at baseline. *IL33*_rs1342326 was associated with spirometric indices and FeNO, whereas *GSDMB*_rs2305480 was significantly associated with FEV₁/FVC change.

Conclusion: Asthmatic children have annual decline in FVC and increase in FEV₁/FVC and FEF₂₅₋₇₅. Their lung function trajectory is influenced by gender, ICS treatment, BMI and asthma exacerbations. *IL33* and *GSDMB* may be candidate genes for their lung function growth.

Keywords: asthma; determinant; exhaled nitric oxide; genetics; lung function trajectory

Lung function of normal children, measured by the spirometric index FEV₁, is characterized by rapid increase in adolescence, a leveling-off or plateauing in early adulthood, and a gradual decline into middle and old age (1). Decreased FEV₁ is a clinically important predictor for later chronic airway obstruction and mortality (2). Although asthmatic children usually have normal lung function growth, some of them had impaired lung function in terms of reduced growth, early decline, rapid decline, or a combination of these patterns during adolescent and early adulthood. However, disease duration and severity as well as asthma exacerbations were reported to be risk factors for impaired lung function in these children (3,4), which predispose them to have faster lung function decline in adulthood. The patterns of serial lung function growth and decline in asthmatic children were closely related to the risks for the abnormal lung-function growth in adulthood that might lead to other long-term respiratory morbidities (5). However, there is limited data on the lung function trajectory of asthmatic children. Both environmental influences and host genetics are important determinants for asthma traits including childhood lung function. This study aimed to characterize lung function trajectory of Chinese children with asthma and identify the environmental and genetic determinants for longitudinal changes of their spirometric indices and FeNO values.

Methods

Study population

This prospective study recruited Chinese asthmatic children aged 6-12 years from pediatric allergy clinic of our university-affiliated teaching hospital. Their asthma was diagnosed according to American Thoracic Society criteria (6), and treated according to Global Initiative

for Asthma guideline (<http://ginasthma.org/>). All patients were followed regularly and their spirometric indices and exhaled nitric oxide concentrations (FeNO) were prospectively measured annually for five years. Details of clinical, lung function and FeNO assessments were described in the Online Supplement. The Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee approved this study, and subjects and their parents gave informed consent to participate.

Selection and genotyping of SNPs

Ten top asthma-associated SNPs from GWAS reported by the GABRIEL Consortium (7), and four other SNPs on 17q21 locus with significant asthma associations in our studies (8,9) and two *IL33* SNPs (see Online Supplement), were genotyped in our patients. Out of 16 selected SNPs, primers of TaqMan assays could not be designed for *GSDMA*_rs3894194 and *HLA-DQ*_rs9273349. Table S1 provides details of the remaining 14 SNPs, which were genotyped in genomic DNA extracted from patients' peripheral blood leucocytes or buccal swabs by TaqMan genotyping assays (Applied Biosystems, Foster City, CA) using ABI-7900HT thermocycler (Applied Biosystems).

Statistical analysis

Generalized estimating equation (GEE) was used to analyze longitudinal changes in spirometric indices and FeNO as well as the associations between these lung function changes and SNPs and environmental factors, adjusting for sex, age, year of visit, ICS treatment ever (at baseline and during follow-up), total number of asthma exacerbations, body mass index (BMI) and household smoking exposure before visit as covariates. Besides, this study categorized patients into normal growth (NG) and reduced growth (RG) groups based on the patterns of their lung function growth for prebronchodilator FEV₁ when

compared with our population references (1,10). The associations between RG and the above patient and environmental factors were analyzed by χ^2 or Fisher exact test as appropriate. Significant factors identified by univariate analyses were confirmed by multivariable logistic regression after adjustment for the above covariates. The level of significance for any genetic association was lowered to 0.005 to adjust for multiple statistical testing.

Results

Study population

One hundred and ninety three asthmatic children with mean (standard deviation [SD]) age of 9.7 (1.9) years were recruited. Table 1 summarizes their clinical characteristics. Eighty-three percent of patients had well-controlled asthma over the five years. They had a mean of 3.9 episodes of asthma exacerbations. Tables S2 and S3 compare subgroups by gender and ICS treatment. Boys were more atopic (93.1% vs 81.0%; $p=0.017$) and had higher FEV₁% (mean [SD]: 94.0 [12.2] vs 86.8 [13.3]; $p<0.001$) and FVC% (102.2 [13.6] vs 97.1 [12.9]; $p=0.012$) than girls at baseline. Patients who received ICS treatment had more asthma exacerbations (5.1 [4.2] vs 2.5 [2.8]; $p<0.0001$). At baseline, ICS-treated patients had lower FEV₁/FVC than those without ICS (81.6 [9.8] vs 84.4 [7.6]; $p=0.026$).

Longitudinal changes of spirometric indices

Fig. 1 describes longitudinal changes of spirometric indices and FeNO in our asthmatic children respectively. After adjusting for covariates, there was no change in FEV₁% ($p=0.832$) but a significant annual decline of 1.3% for FVC% ($p<0.001$) and significant increase of 1.2% and 3.6% per year for FEV₁/FVC and FEF₂₅₋₇₅% respectively ($p<0.001$ for both). This study also observed a significant annual increase of 7.1 ppb for FeNO ($p=0.001$).

Table 2 summarises the effects of demographic, clinical and environmental factors on

lung function changes. Whereas males had 5.0% higher FEV₁% than females in any single year (p=0.006; Fig. S1), we did not detect significant gender disparity in longitudinal changes of any spirometric index. Compared with those without ICS treatment throughout the 5-year period, patients who received ICS treatment had a greater annual increase of 0.81% for FEV₁% (p=0.047; Fig. S2) and lesser decline of 1.05% per year for FVC% (p=0.011; Fig. S3). ICS-treated patients had 2.4% lower FEV₁/FVC than those without ICS treatment (p=0.043; Fig. S4). There was no difference in longitudinal change of FEF₂₅₋₇₅% change in relation to ICS treatment (Fig. S5). BMI was associated inversely with FEV₁/FVC (p=0.022) but positively with longitudinal changes of FEV₁% and FVC% (p=0.001 for both). Besides, the total number of asthma exacerbations over the 5-year period was associated with lower FEV₁% (p=0.025) and FVC% (p=0.043) but not with longitudinal change in any spirometric index.

Following the published criteria (1,10), 46 (23.8%) of our patients had reduced lung function growth during the 5-year period. Table 3 summarizes the relationship between lung function growth and patients' clinical factors as well as baseline spirometry and FeNO values. Patients with reduced lung function growth had lower FEV₁, FVC, FEF₂₅₋₇₅ and FEV₁/FVC (p<0.001 for all) as well as higher FeNO (p=0.049) at baseline. Female patients and those with lower BMI, ≥ 3 asthma exacerbations and poorer asthma control had reduced lung function growth. Logistic regression revealed reduced lung function growth to be associated only with the female gender (odds ratio 2.07, 95% confidence interval 1.02-4.20; p=0.043) after adjustment for age, BMI, ICS treatment ever, occurrence of asthma exacerbations, asthma control status and household smoking exposure as covariates.

Longitudinal changes in FeNO values

The average mean of FeNO of all patients at baseline was 55.1 ppb (Table 1). Regarding FeNO changes, patients who experienced at least one asthma exacerbation had a greater annual increase of 0.74 ppb (95% CI 0.29-1.18) for FeNO ($p=0.001$). The average mean FeNO of atopic and non-atopic patients at baseline were 58.7 ppb and 35.6 ppb respectively (Table S4). By GEE analyses, FeNO of atopic patients was 37.8 ppb (95% CI 20.5-55.0) higher than that of non-atopic patients in any single year ($p<0.001$), but there was no significant association between atopy and longitudinal change of FeNO ($p=0.558$).

Genetic effects on spirometric and FeNO changes

Genomic DNA was available from 162 patients for SNP genotyping. All SNPs had high genotyping efficiency ($> 98\%$) and followed Hardy-Weinberg equilibrium (Table S5). Tables 4 and S6-S8 summarize the associations between 14 genotyped SNPs and lung function changes. We found that minor allele of *IL33*_rs1342326 was associated with higher increase in FEV₁% ($B=2.948$; $p<0.001$; Table S6), FEV₁/FVC ($B=0.802$; $p<0.001$; Table 4) and FEF₂₅₋₇₅% ($B=2.848$; $p<0.001$; Table S7) and less rapid decline in FVC% ($B=2.107$; $p<0.001$; Table S8). Besides, minor allele of *GSDMB*_rs2305480 was associated with greater increase in FEV₁/FVC ($B=1.088$, $p<0.001$) and lower FEV₁/FVC in any single year ($B=-2.99$, $p=0.010$; Table 4). The minor allele of *SMAD3*_rs744910 was weakly inversely associated with change in FEV₁/FVC ($B=-0.456$, $p=0.027$; Table 4) which became insignificant after Bonferroni correction. Table S9 summarizes the effects of SNPs on FeNO change, which showed significant association between minor allele of *IL33*_rs1342326 and higher increase in FeNO ($B=3.726$; $p=0.001$).

Discussion

To our knowledge, this study represented the first report of long term FeNO trajectory of asthmatic children which also delineated longitudinal changes of their spirometric indices. During the five years of follow-up, these school-age asthmatics had significant increase in FeNO, FEV₁/FVC and FEF₂₅₋₇₅% but decrease in FVC%, and FEV₁% did not change with time. Adopting published criteria, 46 (23.8%) of our patients had reduced lung function growth, which was associated female gender, lower baseline spirometric indices and higher baseline FeNO. Regarding determinants of FeNO changes, patients who experienced asthma exacerbations and those with *IL33_rs1342326* had greater annual increase in FeNO.

The ADEPT study evaluated the longitudinal stability of asthma biomarkers in which Caucasian patients with mild, moderate and severe asthma were assessed at 5 visits over 12 months (11). FEV₁/FVC ratio showed excellent reproducibility while pre-bronchodilator FEV₁ and FVC were only moderately reproducible. FeNO demonstrated good reproducibility. Our study offered much longer follow-up of up to five years that could more accurately delineate the trajectory of spirometric and FeNO measurements of asthmatic patients during childhood (Fig. 1). Our group had reported higher FeNO values in Chinese than Caucasians (12-14), and this study extended this observation by providing novel data on FeNO trajectory in children with substantially higher FeNO values. We detected a significant increase in FeNO among asthmatic children over the 5-year period, which was in line with previous findings of positive correlation between age and FeNO during childhood (14,15). Asthma exacerbations were positively associated with more rapid increase of 0.74 ppb for FeNO over time. The question whether longitudinal change of FeNO might better reflect the degree of asthma control warrants further investigation.

Based on age-specific FEV₁ growth curves, the Childhood Asthma Management Program (CAMP) categorized children recruited at 5-12 years into the following four patterns

of lung function growth: normal growth with a normal plateau or maximum not yet reached (NG), normal growth with early decline (NG/ED), reduced growth with a normal plateau or maximum not yet reached (RG), and reduced growth with early decline (RG/ED) (1,16). The numbers of subjects being assigned into these four groups were: 28.2% for NG, 20.0% for NG/ED, 26.7% for RG and 20.1% for RG/ED. Thus, about half of these CAMP participants had RG. Our study followed the same criteria for classifying NG and RG in our asthmatic children, although it was not possible to assess subjects' lung function plateau because they were only followed for 5 years when most of them have not reached their adulthood. Only one-quarter of our asthmatic children were classified into RG, which was much lower than the CAMP cohort probably because most of their children suffered from persistent asthma whereas 45% of our patients did not require ICS treatment (Table 1). Regarding the identified risk factors, both studies found RG to be associated with low baseline spirometric indices although contrasting gender effect was found (males in CAMP and females in this study).

Among our school-age patients, the total number of asthma exacerbations over 5-year period was inversely associated with $FEV_1\%$ and $FVC\%$ but not FEV_1/FVC and $FEF_{25-75}\%$. These findings supported the association between asthma exacerbations and diminished lung function in childhood asthma (17). On the other hand, the lack of $FEF_{25-75}\%$ change might suggest that asthma exacerbations resulted in airflow obstruction mainly on large but not medium-to-small airways. Despite the known anti-inflammatory actions of ICS, previous studies on its benefit on lung function trajectory were inconclusive (18,19). We found that ICS treatment resulted in more rapid increase in $FEV_1\%$ and slower decline in $FVC\%$ of our patients. On the other hand, we did not detect any association between ICS treatment and $FEF_{25-75}\%$ changes probably because of the suboptimal delivery of ICS to patients' medium-to-small airways.

Several SNPs from published asthma genes might modulate lung function of asthmatic children. Our previous smaller study followed asthmatic children for four years, and identified *STAT6* to be a possible modifier of their lung function growth (20). Nonetheless, this gene has not been consistently replicated to be an important asthma gene. Asthma genes and SNPs genotyped in the present study were selected from a large asthma GWAS (7). Firstly, *IL33*_rs1342326 was associated with longitudinal change in FEV₁%, FVC%, FEV₁/FVC, FEF₂₅₋₇₅% and FeNO (Tables 4 and S5-S8). Nonetheless, two other tagging SNPs of this gene were not associated with lung function trajectory in this study. Given the low MAF (0.003) of *IL33*_rs1342326 (Table S4), it is possible that our observed associations for this SNP were false-positive findings. Nonetheless, there is strong biological plausibility for *IL33* to modulate airway functions (21,22). Prefontaine et al. found increased expression of IL-33 in lung tissue from asthmatics especially those with severe disease (23). A recent GWAS reported *IL33* to be an important candidate gene for severe childhood asthma exacerbations (24). Thus, longitudinal studies with larger sample size are needed to elucidate the importance of *IL33* as an asthma gene for lung function trajectory.

Many studies reported genes on 17q21 locus, such as *ORMDL3* and *GSDMB*, to be the most widely replicated asthma genes in different ethnic groups (7,8,25). The recent GWAS also reported *GSDMB* to be associated with severe asthma exacerbations in Swedish preschool children (24). However, their importance in modulating lung function remains poorly studied. This study showed that *GSDMB*_rs2305480 was associated with patients' FEV₁/FVC at any single year as well as longitudinal changes in this spirometric index, the former of which was consistent with what we found in our previous cross-sectional study (25). In a recent GWAS, McGeachie et al. evaluate the genetic underpinnings of lung function growth in 581 CAMP participants with asthma (16). They reported an intergenic SNP (rs4445257) on chromosome 8 that interacted with the distal gene encoding Cub and Sushi

multiple domain 3 gene (*CSMD3*) to be strongly associated with the normal growth with early decline pattern. It would be useful to replicate the genetic association of this SNP in our subjects and other populations.

In conclusions, we observed significant annual decrease in FVC% and increase in FEV₁/FVC and FEF₂₅₋₇₅% among Chinese school-age children with asthma. ICS-treated patients and those with higher BMI had more rapid increase in FEV₁% and less rapid decline in FVC% over the 5-year period. Besides, patients with more asthma exacerbations had lower FEV₁% and FVC%. Asthma exacerbation was a determinant of FeNO changes in asthmatic children. The possible effects of asthma genes including *IL33* and *GSDMB* on lung function trajectory should be investigated in larger prospective genetic studies.

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Author contributions

TFL obtained funding and designed this study, carried out the analyses and wrote the first version of this manuscript. MFT collected clinical data, performed genotyping experiments and analysed study data. ASYL and GWKW followed patients and collected clinical data. WWST supervised data analyses. HYS helped with spirometric and FeNO measurements and SNP genotyping. All authors approved the submitted version of this manuscript.

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Conflicts of interest

All authors declared no competing interests.

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- Accepted Article
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Table 1 Clinical and laboratory characteristics of our 193 asthmatic patients

Characteristic ^a	Result
Male (<i>n</i> , %)	121 (62.7)
Age at baseline, year	9.7 ± 1.9
Eczema comorbidity (<i>n</i> , %)	94 (50.3)
Family history of allergies (<i>n</i> , %)	144 (74.6)
Atopy ^b (<i>n</i> , %)	146 (75.6)
BMI at baseline, kg/m ²	17.4 ± 3.0
Furry pet keeping (<i>n</i> , %)	10 (5.2)
Presence of household smokers (<i>n</i> , %)	51 (26.4)
ICS treatment ever (<i>n</i> , %)	106 (54.9)
Total number of asthma exacerbations	3.9 ± 3.9
Asthma control during 5-year follow-up ^c	
Well control (<i>n</i> , %)	161 (83.4)
Moderate control (<i>n</i> , %)	19 (9.8)
Poor control (<i>n</i> , %)	13 (6.7)
FeNO at baseline, ppb	55.1 ± 42.8
Spirometric indices at baseline	
FEV ₁ (% predicted)	91.3 ± 13.1
FVC (% predicted)	100.3 ± 13.5
FEV ₁ /FVC, %	82.9 ± 9.0
FEF ₂₅₋₇₅ (% predicted)	68.4 ± 25.6

BMI, body mass index; ICS, inhaled corticosteroid; FeNO, fractional exhaled nitric oxide concentration; FEV₁, forced expiratory volume in 1-second; FVC, forced vital capacity; FEF₂₅₋₇₅, maximal mid-expiratory flow.

^a Expressed in mean ± SD unless stated otherwise.

^b Defined as ≥ one positive SPT or RAST to aeroallergens.

^c Well control defined as uncontrolled asthma in ≤ 1 year by ACT or C-ACT; moderate control as uncontrolled asthma in 2-3 years; poor control as uncontrolled asthma in 4-5 years.

Table 2 Effects of different demographic and clinical factors on spirometric indices in our asthmatic children

Spirometric index	Effect on any single year		Effect on longitudinal change	
	B (95% CI), %	p ^b	B (95% CI), % per year	p ^b
Sex ^a				
FEV ₁ %	5.26 (1.64 - 8.89)	0.004	-0.44 (-1.26 - 0.38)	0.297
FVC%	2.23 (-1.38 - 5.83)	0.226	-0.18 (-1.01 - 0.66)	0.680
FEV ₁ /FVC	1.05 (-1.27 - 3.38)	0.375	-0.28 (-0.75 - 0.18)	0.234
FEF ₂₅₋₇₅ %	5.78 (-0.27 - 11.83)	0.061	0.63 (-0.80 - 2.05)	0.388
ICS treatment ^a				
FEV ₁ %	-2.81 (-6.51 - 0.89)	0.137	0.81 (0.01 - 1.60)	0.047
FVC%	0.10 (-3.63 - 3.84)	0.956	1.05 (0.24 - 1.87)	0.011
FEV ₁ /FVC	-2.41 (-4.74 - -0.077)	0.043	-0.24 (-0.72 - 0.23)	0.314
FEF ₂₅₋₇₅ %	-3.24 (-9.77 - 3.29)	0.331	-0.96 (-2.39 - 0.48)	0.192
BMI ^a				
FEV ₁ %	-0.30 (-0.87 - 0.28)	0.312	0.25 (0.10 - 0.39)	0.001
FVC%	0.16 (-0.39 - 0.72)	0.562	0.24 (0.10 - 0.37)	0.001
FEV ₁ /FVC	-0.39 (-0.73 - -0.06)	0.022	0.02 (-0.06 - 0.09)	0.615

FEF ₂₅₋₇₅ %	0.12 (-0.81 - 1.05)	0.804	0.06 (-0.16 - 0.27)	0.603
Asthma exacerbations				
FEV ₁ %	-0.61 (-1.15 - -0.08)	0.025	0.10 (-0.03 - 0.22)	0.128
FVC%	-0.40 (-0.83 - -0.01)	0.043	0.09 (-0.02 - 0.20)	0.093
FEV ₁ /FVC	-0.27 (-0.63 - 0.08)	0.135	0.01 (-0.07 - 0.10)	0.752
FEF ₂₅₋₇₅ %	-0.58 (-1.49 - 0.32)	0.208	0.01 (-0.21 - 0.23)	0.901

BMI, body mass index, ICS, inhaled corticosteroid.

^a Females and patients without ICS treatment were designated as reference groups for sex and ICS treatment respectively. Results for BMI and asthma exacerbations were calculated based on effect size per additional one kg/m² and one episode of asthma exacerbation respectively.

^b Analyzed by GEE after adjustment for covariates.

Table 3 Relationship between clinical factors and patients' lung function growth

Characteristic ^a	NG (n = 147)	RG (n = 46)	p ^b
Male (n, %)	99 (67.3)	22 (47.8)	0.017
Age at baseline, year	9.6 ± 2.0	9.8 ± 1.8	0.689
Eczema comorbidity (n, %)	72 (49.0)	22 (47.8)	0.891
Family history of allergies (n, %)	107 (72.8)	37 (80.4)	0.298
Atopy (n, %)	114 (77.6)	32 (69.6)	0.271
BMI at baseline, kg/m ²	17.6 ± 3.1	16.7 ± 2.3	0.035
Furry pet keeping (n, %)	8 (5.4)	2 (4.3)	0.770
Presence of household smokers (n, %)	42 (28.6)	9 (19.6)	0.227
ICS treatment ever (n, %)	78 (53.1)	28 (60.9)	0.353
Total number of asthma exacerbations	3.7 ± 3.8	4.5 ± 3.8	0.231
Any asthma exacerbation within 5 years	120 (81.6)	39 (84.8)	0.625
≥ 3 asthma exacerbations within 5 years	67 (45.6)	30 (65.2)	0.022
Asthma control during 5-year follow-up ^c			
Well control (n, %)	128 (87.1)	33 (71.7)	0.016
Poor or moderate control (n, %)	19 (12.9)	13 (28.3)	
FeNO at baseline, ppb	51.5 ± 38.7	66.2 ± 52.5	0.049
Spirometric indices at baseline			
FEV ₁ (% predicted)	95.3 ± 10.8	78.5 ± 11.3	<0.001
FVC (% predicted)	102.9 ± 12.8	92.0 ± 12.4	<0.001
FEV ₁ /FVC, %	84.3 ± 8.0	78.3 ± 10.3	<0.001
FEF ₂₅₋₇₅ (% predicted)	72.9 ± 25.9	54.1 ± 18.3	<0.001

BMI, body mass index; ICS, inhaled corticosteroid; FeNO, fractional exhaled nitric oxide concentration; FEV₁, forced expiratory volume in 1-second; FVC, forced vital capacity; FEF₂₅₋₇₅, maximal mid-expiratory flow; NG, normal growth; RG, reduced growth.

^a Expressed in number (percentage) or mean ± SD.

^b Analyzed by χ^2 or Fisher exact test as appropriate.

^c Well control defined as uncontrolled asthma in ≤ 1 year by ACT or C-ACT; moderate control as uncontrolled asthma in 2-3 years; poor control as uncontrolled asthma in 4-5 years.

Table 4 Associations between 14 genotyped SNPs and longitudinal changes in FEV₁/FVC

SNP	Major allele	Minor allele ^a	Genotype			Effect on any single year		Effect on longitudinal change	
			AA (n, %)	Aa (n, %)	aa (n, %)	Coefficient ^b (95% CI), %	p ^c	Coefficient ^b (95% CI), % / year	p ^c
<i>SLC22A5</i> _rs2073643	T	C	79 (49.1)	66 (41.0)	16 (9.9)	-0.06 (-1.85-1.73)	0.948	-0.06 (-0.43-0.31)	0.762
<i>IL13</i> _rs1295686	C	T	66 (41.0)	69 (42.9)	26 (16.1)	0.24 (-1.50-1.97)	0.790	0.03 (-0.36-0.41)	0.897
<i>GSDMB</i> _rs2305480	G	A	102 (63.0)	56 (34.6)	4 (2.5)	-2.99 (-5.27--0.71)	0.010	1.09 (0.60-1.58)	<0.001
<i>IL2RB</i> _rs2284033	A	G	42 (25.9)	85 (52.5)	35(21.6)	-0.67 (-2.41-1.07)	0.449	0.08 (-0.26-0.43)	0.638
<i>IL33</i> _rs1342326	A	C	160 (99.4)	1 (0.6)	0 (0)	0.36 (-1.89-2.61)	0.755	0.80 (0.53-1.08)	<0.001
<i>RORA</i> _rs11071559	C	T	105 (65.2)	53 (32.7)	4 (2.5)	-0.58 (-2.63-1.46)	0.576	0.09 (-0.38-0.57)	0.697
<i>SMAD3</i> _rs744910	G	A	58 (35.8)	79 (48.8)	25 (15.4)	-0.34 (-2.08-1.39)	0.697	-0.46 (-0.86--0.05)	0.027
<i>IL18R1</i> _rs3771166	G	A	137 (85.1)	22 (13.7)	2 (1.2)	-1.14 (-4.75-2.48)	0.538	-0.18 (-0.88-0.52)	0.616
<i>ORMDL3</i> _rs7216389	T	C	103 (64.0)	55 (34.1)	3 (1.9)	-0.49 (-2.82-1.84)	0.681	0.08 (-0.46-0.61)	0.771

<i>ORMDL3</i> _rs17608925	T	C	138 (85.7)	23 (14.3)	0 (0)	1.30 (-2.18-4.77)	0.464	-0.48 (-1.28-0.32)	0.242
<i>GSDMA</i> _rs1007654	G	A	89 (56.0)	61 (38.4)	9 (5.7)	-0.32 (-2.43-1.80)	0.771	0.26 (-0.22-0.74)	0.286
<i>TOP2A</i> _rs11650680	C	T	94 (58.4)	55 (34.2)	12 (7.5)	0.52 (-1.19-2.22)	0.552	0.31 (-0.17-0.79)	0.210
<i>IL33</i> _rs10815370	A	C	130 (81.8)	28 (17.6)	1 (0.6)	-0.29 (-3.55-2.99)	0.864	0.02 (-0.54-0.59)	0.937
<i>IL33</i> _rs1929996	G	C	133 (82.6)	27 (16.8)	1 (0.6)	0.22 (-3.06-3.50)	0.895	-0.07 (-0.63-0.48)	0.795

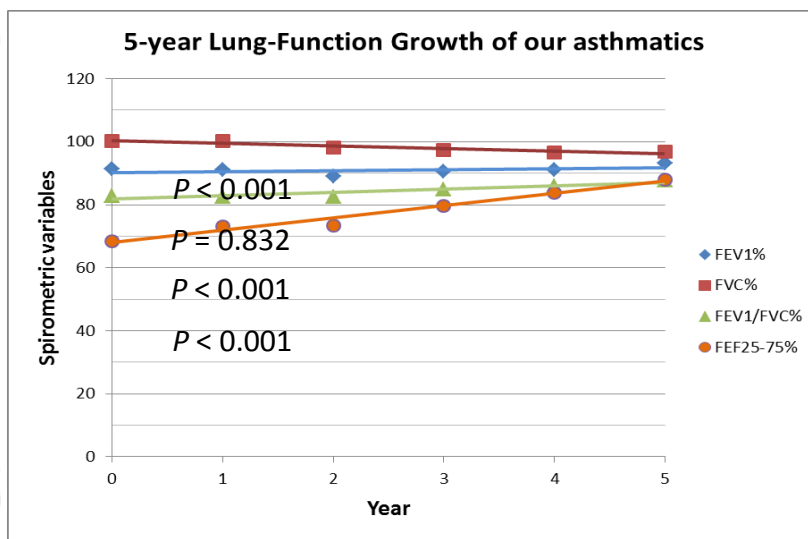
^a Minor alleles were designated as risk alleles.

^b Effect size per additional copy of the risk allele.

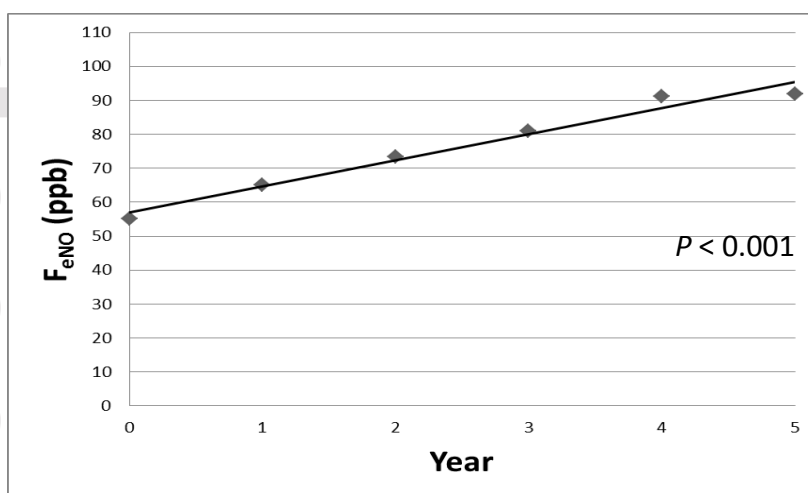
^c Analyzed by GEE after adjusting for sex, year of visit, age, ICS treatment ever, total number of asthma exacerbations, BMI and household smoking exposure as covariates.

Figure 1 Longitudinal changes in (a) spirometric indices and (b) FeNO over five years in our asthmatic children. *P*-values were calculated by GEE after adjustment for sex, age, ICS treatment ever, total number of asthma exacerbations, BMI and presence of household smokers as covariates.

(a)



(b)



ONLINE SUPPLEMENT

Trajectory of Spirometric and Exhaled Nitric Oxide Measurements in Chinese Schoolchildren with Asthma

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Methods

Patient assessments

Environmental exposures (e.g. household smoking, pet keeping), use of asthma medications such as inhaled corticosteroid, occurrence of asthma exacerbations were also recorded. Asthma exacerbations were defined according to published criteria (S1). Atopy status was assessed by skin prick tests (SPTs; ALK Abelló, Round Rock, TX) or measurement of plasma allergen-specific concentrations (Phadia AB, Uppsala, Sweden) to locally important aeroallergens including house dust mites, cockroach, cat, dog and mixed moulds (S2-S4). Patients were considered atopic if they were positive for at least one SPT or allergen-specific IgE tests. At every visit, asthma status of these patients was assessed by Asthma Control Test (ACT) or Childhood ACT (C-ACT) (S5,S6) when scores ≤ 19 were considered as being “uncontrolled”. During the 5-year period, patients’ asthma was classified as being well controlled (uncontrolled asthma in ≤ 1 year), moderately controlled (uncontrolled asthma in 2-3 years, and poorly controlled (uncontrolled asthma in 4-5 years).

Spirometric and exhaled nitric oxide measurements

All patients underwent pre-bronchodilator spirometry (COMPACT II; Vitalograph, Buckingham, UK) to measure forced expiratory volume in 1-second (FEV₁), forced vital capacity (FVC) and forced expiratory flow at 25-75% of FVC (FEF₂₅₋₇₅) according to international recommendations (S7). These spirometric indices were expressed as percentages of predicted values from local references (S8). Fractional exhaled nitric oxide concentration (FeNO) was measured at an expiratory flow of 50 ml/sec using a chemiluminescence analyzer (NOA280i; Sievers Instruments, Boulder, CO) according to published guideline (S9). The mean FeNO value of three reproducible measurements was recorded.

Selection and genotyping of SNPs

In view of low minor allele frequency (MAF) of *IL33* SNP rs1342326, two tagging SNPs of this gene were also selected by HaploView version 4.2 (Daly Lab, Cambridge, MA) based on HapMap database for southern Han Chinese (HapMap Data Rel 27 PhaseII+III, Feb09, on NCBI B36 assembly, dbSNP b126) at pairwise $r^2 \geq 0.9$ for LD for all SNPs located 5-kb upstream and downstream of *IL33*_rs1342326 with MAF ≥ 0.05 .

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Table S1 Details of chromosomal location and target genes for our 14 genotyped SNPs

SNP	Name of Gene	Gene Symbol	Position ^a	Chromosome	Cytogenetic band	Nature of SNP	Major allele	Minor allele
rs2073643	Solute carrier family 22 (organic cation/carnitine transporter), member 5	<i>SLC22A5</i>	132387596	5	5q31.1	Intron	T	C
rs1295686	Interleukin 13	<i>IL13</i>	131995843	5	5q31.1b	Intron	C	T
rs2305480	Gasdermin B	<i>GSDMB</i>	38062196	17	17q12c	Mis-sense mutation	G	A
rs2284033	Interleukin 2 receptor, beta	<i>IL2RB</i>	37534034	22	22q12.3d	Intron	A	G
rs1342326	Interleukin 33 (intergenic)	<i>IL33</i> (intergenic)	6190076	9	9p24.1b	Intergenic/Unknown	A	C
rs11071559	RAR-related orphan receptor A	<i>RORA</i>	61069988	15	15q22.2a	Intron	C	T
rs744910	SMAD family member 3	<i>SMAD3</i>	67446785	15	15q22.33c	Intron	G	A
rs3771166	Interleukin 18 receptor 1	<i>IL18R1</i>	102986222	2	2q12.1a	Intron	G	A
rs7216389	Orosomucoid 1 (ORM1)-like 3 (<i>S. cerevisiae</i>)	<i>ORMDL3</i>	39913696	17	17q21.1	Intron	T	C
rs17608925	Orosomucoid 1 (ORM1)-like 3 (<i>S. cerevisiae</i>)	<i>ORMDL3</i>	39926578	17	17q21.1	Intron, upstream	T	C
rs1007654	Gasdermin A	<i>GSDMA</i>	39955101	17	17q21.1	Promoter	G	A

rs11650680	DNA topoisomerase II, alpha isozyme	<i>TOP2A</i>	40422984	17	17q21.1	Promoter	C	T
rs10815370	Interleukin 33	<i>IL33</i>	6194831	9	9p24.1b	Upstream	A	C
rs1929996	Interleukin 33	<i>IL33</i>	6187636	9	9p24.1b	Intergenic	G	C

^a Chromosomal positions according to NCBI dbSNP b144.

Table S2 Comparison of clinical and laboratory characteristics between male and female asthmatic patients

Characteristic ^a	Males (n = 121)	Females (n = 72)
Age at baseline, year	9.7 ± 1.9	9.6 ± 2.0
Eczema comorbidity (n, %)	62 (52.1)	35 (48.6)
Family history of allergies (n, %)	85 (80.2)	61 (88.4)
Atopy ^b (n, %)	95 (93.1) [†]	51 (81.0)
BMI at baseline, kg/m ²	17.7 ± 2.9 [†]	16.8 ± 2.9
Furry pet keeping (n, %)	5 (4.5)	5 (7.1)
Presence of household smokers (n, %)	32 (29.1)	19 (27.1)
ICS treatment during follow-up (n, %)	67 (55.4)	38 (53.5)
Total number of asthma exacerbations	3.9 ± 4.0	3.9 ± 3.6
Asthma control during follow-up ^c		
Well control (n, %)	103 (85.1)	58 (80.6)
Moderate control (n, %)	12 (9.9)	7 (9.7)
Poor control (n, %)	6 (5.0)	7 (9.7)
FeNO at baseline, ppb	55.3 ± 38.6	54.7 ± 49.3
Spirometric indices at baseline		
FEV ₁ (% predicted)	94.0 ± 12.2 [‡]	86.8 ± 13.3
FVC (% predicted)	102.2 ± 13.6 [†]	97.1 ± 12.9
FEV ₁ /FVC, %	83.0 ± 8.0	82.6 ± 10.5
FEF ₂₅₋₇₅ (% predicted)	70.1 ± 26.9	65.7 ± 23.2

ICS, inhaled corticosteroid; FEV₁, forced expiratory volume in 1-second; FVC; forced vital capacity; FEF₂₅₋₇₅, forced expiratory flow at 25-75% of FVC.

^a Expressed in mean ± SD unless stated otherwise

^b Defined as ≥ one positive SPT or RAST to aeroallergens.

^c Well control defined as uncontrolled asthma in ≤ 1 year by ACT or C-ACT; moderate control as uncontrolled asthma in 2-3 years; poor control as uncontrolled asthma in 4-5 years.

[†] 0.01 < p < 0.05; [‡] p < 0.001

Table S3 Comparison of clinical and laboratory characteristics between patients who received and did not receive ICS treatment ever during the 5-year follow-up

Characteristic ^a	With ICS treatment (n = 106)	Without ICS treatment (n = 87)
Male (n, %)	67 (63.2)	54 (62.1)
Age at baseline, year	9.8 ± 1.9	9.5 ± 2.0
Eczema comorbidity (n, %)	60 (57.1) [†]	34 (39.5)
Family history of allergies (n, %)	80 (81.6)	66 (85.7)
Atopy ^b (n, %)	64 (90.1)	42 (85.7)
BMI at baseline, kg/m ²	17.4 ± 3.3	17.3 ± 2.5
Furry pet keeping (n, %)	5 (5.0)	5 (6.3)
Presence of household smokers (n, %)	25 (25.0)	26 (32.5)
Total number of asthma exacerbations	5.1 ± 4.2 [‡]	2.5 ± 2.8
Asthma control during follow-up ^c		
Good control (n, %)	83 (78.3)	78 (89.7)
Moderate Control (n, %)	14 (13.2)	5 (5.7)
Bad Control (n, %)	9 (8.5)	4 (4.6)
FeNO at baseline, ppb	50.4 ± 41.6	61.8 ± 43.9
Spirometric indices at baseline		
FEV ₁ (% predicted)	89.7 ± 13.8	93.2 ± 11.9
FVC (% predicted)	100.1 ± 14.3	100.5 ± 12.7
FEV ₁ /FVC, %	81.6 ± 9.8 [†]	84.4 ± 7.6
FEF ₂₅₋₇₅ (% predicted)	66.4 ± 25.9	70.8 ± 25.2

ICS, inhaled corticosteroid; FEV₁, forced expiratory volume in 1-second; FVC; forced vital capacity; FEF₂₅₋₇₅, forced expiratory flow at 25-75% of FVC.

^a Expressed in mean ± SD unless stated otherwise

^b Defined as ≥ one positive SPT or RAST to aeroallergens.

^c Well control defined as uncontrolled asthma in ≤ 1 year by ACT or C-ACT; moderate control as uncontrolled asthma in 2-3 years; poor control as uncontrolled asthma in 4-5 years.

[†] 0.01 < p < 0.05; [‡] p < 0.001

Table S4 Comparison of clinical and laboratory characteristics between atopic and non-atopic patients

Characteristic ^a	Atopic (<i>n</i> = 146)	Non-atopic (<i>n</i> = 19)
Male (<i>n</i> , %)	95 (65.1) [†]	7 (36.8)
Age at baseline, year	9.8 ± 1.9	9.0 ± 2.2
Eczema comorbidity (<i>n</i> , %)	86 (58.9) [†]	15 (78.9)
Family history of allergies (<i>n</i> , %)	115 (84.6)	13 (86.7)
BMI at baseline, kg/m ²	17.2 ± 2.9	17.3 ± 3.1
Furry pet keeping (<i>n</i> , %)	10 (6.8)	0 (0)
Presence of household smokers (<i>n</i> , %)	38 (27.3)	4 (23.5)
ICS treatment during follow-up (<i>n</i> , %)	82 (56.2)	9 (47.4)
Total number of asthma exacerbations	4.0 ± 4.0	3.7 ± 4.0
Asthma control during follow-up ^c		
Well control (<i>n</i> , %)	122 (83.6)	17 (89.5)
Moderate control (<i>n</i> , %)	14 (9.6)	1 (5.3)
Poor control (<i>n</i> , %)	10 (6.8)	1 (5.3)
FeNO at baseline, ppb	58.7 ± 42.9	35.6 ± 38.2
Spirometric indices at baseline		
FEV ₁ (% predicted)	91.3 ± 13.2	87.7 ± 11.5
FVC (% predicted)	101.3 ± 14.1 [†]	93.9 ± 9.0
FEV ₁ /FVC, %	82.0 ± 9.0	85.3 ± 8.8
FEF ₂₅₋₇₅ (% predicted)	66.4 ± 25.2	70.9 ± 23.8

ICS, inhaled corticosteroid; FEV₁, forced expiratory volume in 1-second; FVC; forced vital capacity; FEF₂₅₋₇₅, forced expiratory flow at 25-75% of FVC.

^a Expressed in mean ± SD unless stated otherwise

^b Defined as ≥ one positive SPT or RAST to aeroallergens.

^c Well control defined as uncontrolled asthma in ≤ 1 year by ACT or C-ACT; moderate control as uncontrolled asthma in 2-3 years; poor control as uncontrolled asthma in 4-5 years.

[†] *p* < 0.01

Table S5 Results on genotyping efficiencies and minor allele frequencies for 14 SNPs

SNP	Genotyping efficiency (%)	Major Allele	Minor Allele	MAF
<u>SNPs reported by GABRIEL Consortium</u>				
<i>SLC22A5</i> _rs2073643	99.4	T	C	0.299
<i>IL13</i> _rs1295686	99.4	C	T	0.369
<i>GSDMB</i> _rs2305480	100	G	A	0.196
<i>IL2RB</i> _rs2284033	100	A	G	0.484
<i>IL33</i> _rs1342326	99.4	A	C	0.003
<i>RORA</i> _rs11071559	100	C	T	0.187
<i>SMAD3</i> _rs744910	100	G	A	0.399
<i>IL18R1</i> _rs3771166	99.4	G	A	0.073
<u>Additional SNPs on 17q21 locus</u>				
<i>ORMDL3</i> _rs7216389	99.4	T	C	0.188
<i>ORMDL3</i> _rs17608925	99.4	T	C	0.070
<i>GSDM1</i> _rs1007654	98.1	G	A	0.248
<i>TOP2A</i> _rs11650680	99.4	C	T	0.248
<u>Additional <i>IL33</i> SNPs</u>				
<i>IL33</i> _rs10815370	98.1	A	C	0.094
<i>IL33</i> _rs1929996	99.4	G	C	0.090

SNP, single nucleotide polymorphism; MAF, minor allele frequency.

All SNPs were in Hardy-Weinberg equilibrium ($p > 0.05$).

Table S6 Associations between 14 genotyped SNPs and longitudinal changes in FEV₁%

SNP	Major allele	Minor allele ^a	Genotype			Effect on any single year		Effect on longitudinal change	
			AA (n, %)	Aa (n, %)	aa (n, %)	Coefficient ^b (95% CI), %	P ^c	Coefficient ^b (95% CI), % / year	P ^c
<i>SLC22A5</i> _rs2073643	T	C	79 (49.1)	66 (41.0)	16 (9.9)	-0.73 (-3.90-2.44)	0.652	-0.13 (-0.80-0.55)	0.712
<i>IL13</i> _rs1295686	C	T	66 (41.0)	69 (42.9)	26 (16.1)	1.69 (-1.10-4.48)	0.234	-0.20 (-0.82-0.42)	0.520
<i>GSDMB</i> _rs2305480	G	A	102 (63.0)	56 (34.6)	4 (2.5)	1.92 (-1.86-5.70)	0.320	-0.32 (-1.11-0.48)	0.434
<i>IL2RB</i> _rs2284033	A	G	42 (25.9)	85 (52.5)	35(21.6)	0.34 (-2.30-2.98)	0.800	-0.15 (-0.73-0.43)	0.617
<i>IL33</i> _rs1342326	A	C	160 (99.4)	1 (0.6)	0 (0)	-1.19 (-5.74-3.36)	0.608	2.95 (2.52-3.38)	<0.001
<i>RORA</i> _rs11071559	C	T	105 (65.2)	53 (32.7)	4 (2.5)	-0.99 (-4.36-2.37)	0.564	0.43 (-0.34-1.19)	0.276
<i>SMAD3</i> _rs744910	G	A	58 (35.8)	79 (48.8)	25 (15.4)	0.17 (-2.74-3.08)	0.910	-0.14 (-0.74-0.47)	0.657
<i>IL18R1</i> _rs3771166	G	A	137 (85.1)	22 (13.7)	2 (1.2)	-9.37 (-23.34-4.60)	0.189	-0.53 (-3.32-13.81)	0.230

<i>ORMDL3</i> _rs7216389	T	C	103 (64.0)	55 (34.1)	3 (1.9)	2.33 (-1.51-6.16)	0.234	-0.41 (-1.23-0.42)	0.335
<i>ORMDL3</i> _rs17608925	T	C	138 (85.7)	23 (14.3)	0 (0)	4.55 (-0.76-9.86)	0.093	-0.32 (-1.52-0.89)	0.602
<i>GSDMA</i> _rs1007654	G	A	89 (56.0)	61 (38.4)	9 (5.7)	0.20 (-3.41-3.81)	0.915	-0.13 (-0.95-0.69)	0.755
<i>TOP2A</i> _rs11650680	C	T	94 (58.4)	55 (34.2)	12 (7.5)	2.09 (-0.89-5.06)	0.170	0.06 (-0.57-0.70)	0.844
<i>IL33</i> _rs10815370	A	C	130 (81.8)	28 (17.6)	1 (0.6)	-1.48 (-6.98-4.02)	0.598	0.57 (-0.54-1.67)	0.314
<i>IL33</i> _rs1929996	G	C	133 (82.6)	27 (16.8)	1 (0.6)	-2.01 (-7.53-3.52)	0.477	0.55 (-0.58-1.68)	0.344

^a Minor alleles were designated as risk alleles.

^b Effect size per additional copy of the risk allele.

^c Analyzed by generalized estimating equation (GEE) after adjusting for sex, year of visit, age, ICS treatment ever, total number of asthma exacerbations, BMI and household smoking exposure as covariates.

Table S7 Associations between 14 genotyped SNPs and longitudinal changes in FEF₂₅₋₇₅%

SNP	Major allele	Minor allele ^a	Genotype			Effect on any single year		Effect on longitudinal change	
			AA (n, %)	Aa (n, %)	aa (n, %)	Coefficient ^b (95% CI), %	P ^c	Coefficient ^b (95% CI), % / year	P ^c
<i>SLC22A5</i> _rs2073643	T	C	79 (49.1)	66 (41.0)	16 (9.9)	-1.82 (-7.46-3.81)	0.526	-0.16 (-1.29-0.97)	0.782
<i>IL13</i> _rs1295686	C	T	66 (41.0)	69 (42.9)	26 (16.1)	1.75 (-3.39-6.89)	0.504	-0.37 (-1.48-0.74)	0.515
<i>GSDMB</i> _rs2305480	G	A	102 (63.0)	56 (34.6)	4 (2.5)	1.19 (-5.36-7.73)	0.723	0.58 (-0.88-2.03)	0.438
<i>IL2RB</i> _rs2284033	A	G	42 (25.9)	85 (52.5)	35(21.6)	-1.20 (-5.84-3.44)	0.611	0.15 (-0.95-1.25)	0.785
<i>IL33</i> _rs1342326	A	C	160 (99.4)	1 (0.6)	0 (0)	-7.41 (-15.44-0.62)	0.070	2.85 (2.04-3.66)	<0.001
<i>RORA</i> _rs11071559	C	T	105 (65.2)	53 (32.7)	4 (2.5)	-3.26 (-9.12-2.60)	0.275	0.73 (-0.72-2.18)	0.324
<i>SMAD3</i> _rs744910	G	A	58 (35.8)	79 (48.8)	25 (15.4)	-1.30 (-6.42-3.82)	0.619	-0.76 (-1.90-0.39)	0.195
<i>IL18R1</i> _rs3771166	G	A	137 (85.1)	22 (13.7)	2 (1.2)	2.11 (-6.80-11.01)	0.643	-1.58 (-3.30-0.14)	0.071

<i>ORMDL3</i> _rs7216389	T	C	103 (64.0)	55 (34.1)	3 (1.9)	1.97 (-4.98-8.91)	0.579	0.60 (-0.96-2.16)	0.452
<i>ORMDL3</i> _rs17608925	T	C	138 (85.7)	23 (14.3)	0 (0)	4.70 (-4.54-13.95)	0.319	-1.06 (-3.29-1.17)	0.351
<i>GSDMA</i> _rs1007654	G	A	89 (56.0)	61 (38.4)	9 (5.7)	3.00 (-3.64-9.63)	0.377	-0.01 (-1.40-1.37)	0.988
<i>TOP2A</i> _rs11650680	C	T	94 (58.4)	55 (34.2)	12 (7.5)	3.96 (-0.86-8.79)	0.108	0.03 (-1.22-1.28)	0.965
<i>IL33</i> _rs10815370	A	C	130 (81.8)	28 (17.6)	1 (0.6)	2.89 (-6.32-12.11)	0.538	-0.89 (-2.66-0.89)	0.327
<i>IL33</i> _rs1929996	G	C	133 (82.6)	27 (16.8)	1 (0.6)	3.84 (-5.46-13.14)	0.418	-1.23 (-2.95-0.49)	0.162

^a Minor alleles were designated as risk alleles.

^b Effect size per additional copy of the risk allele.

^c Analyzed by generalized estimating equation (GEE) after adjusting for sex, year of visit, age, ICS treatment ever, total number of asthma exacerbations, BMI and household smoking exposure as covariates.

Table S8 Associations between 14 genotyped SNPs and longitudinal changes in FVC%

SNP	Major allele	Minor allele ^a	Genotype			Effect on any single year		Effect on longitudinal change	
			AA (n, %)	Aa (n, %)	aa (n, %)	Coefficient ^b (95% CI), %	P ^c	Coefficient ^b (95% CI), % / year	P ^c
<i>SLC22A5</i> _rs2073643	T	C	79 (49.1)	66 (41.0)	16 (9.9)	-0.78 (-3.73-2.16)	0.603	-0.10 (-0.71-0.51)	0.747
<i>IL13</i> _rs1295686	C	T	66 (41.0)	69 (42.9)	26 (16.1)	1.66 (-1.20-4.53)	0.255	-0.28 (-0.91-0.34)	0.377
<i>GSDMB</i> _rs2305480	G	A	102 (63.0)	56 (34.6)	4 (2.5)	2.69 (-1.10-6.48)	0.164	-0.38 (-1.18-0.42)	0.349
<i>IL2RB</i> _rs2284033	A	G	42 (25.9)	85 (52.5)	35(21.6)	0.88 (-1.71-3.45)	0.506	-0.20 (-0.78-0.38)	0.503
<i>IL33</i> _rs1342326	A	C	160 (99.4)	1 (0.6)	0 (0)	-1.83 (-6.20-2.54)	0.411	2.11 (1.66-2.55)	<0.001
<i>RORA</i> _rs11071559	C	T	105 (65.2)	53 (32.7)	4 (2.5)	-0.37 (-3.64-2.90)	0.826	0.30 (-0.46-1.05)	0.440
<i>SMAD3</i> _rs744910	G	A	58 (35.8)	79 (48.8)	25 (15.4)	0.72 (-2.13-3.58)	0.619	0.34 (-0.28-0.96)	0.284
<i>IL18R1</i> _rs3771166	G	A	137 (85.1)	22 (13.7)	2 (1.2)	1.53 (-3.00-6.06)	0.508	-0.48 (-1.56-0.60)	0.380

<i>ORMDL3</i> _rs7216389	T	C	103 (64.0)	55 (34.1)	3 (1.9)	3.05 (-0.66-6.75)	0.107	-0.49 (-1.33-0.35)	0.253
<i>ORMDL3</i> _rs17608925	T	C	138 (85.7)	23 (14.3)	0 (0)	3.37 (-1.92-8.67)	0.212	0.10 (-1.14-1.34)	0.873
<i>GSDMA</i> _rs1007654	G	A	89 (56.0)	61 (38.4)	9 (5.7)	0.27 (-2.79-3.34)	0.861	-0.35 (-1.06-0.37)	0.340
<i>TOP2A</i> _rs11650680	C	T	94 (58.4)	55 (34.2)	12 (7.5)	1.41 (-1.48-4.30)	0.338	0.26 (-0.83-0.31)	0.371
<i>IL33</i> _rs10815370	A	C	130 (81.8)	28 (17.6)	1 (0.6)	-1.28 (-7.45-4.90)	0.685	0.63 (-0.44-1.71)	0.685
<i>IL33</i> _rs1929996	G	C	133 (82.6)	27 (16.8)	1 (0.6)	-2.58 (-8.46-3.30)	0.389	0.76 (-0.33-1.85)	0.174

^a Minor alleles were designated as risk alleles.

^b Effect size per additional copy of the risk allele.

^c Analyzed by generalized estimating equation (GEE) after adjusting for sex, year of visit, age, ICS treatment ever, total number of asthma exacerbations, BMI and household smoking exposure as covariates.

Table S9 Associations between 14 genotyped SNPs and longitudinal changes of FeNO

SNP	Major allele	Minor allele ^a	Genotype			Effect on any single year		Effect on longitudinal change	
			AA (n, %)	Aa (n, %)	aa (n, %)	Coefficient ^b (95% CI), %	P ^c	Coefficient ^b (95% CI), % / year	P ^c
<i>SLC22A5</i> _rs2073643	T	C	79 (49.1)	66 (41.0)	16 (9.9)	-2.35 (-8.15-3.46)	0.429	-0.11 (-1.33-1.10)	0.852
<i>IL13</i> _rs1295686	C	T	66 (41.0)	69 (42.9)	26 (16.1)	1.85 (-3.52-7.22)	0.499	-0.54 (-1.71-0.63)	0.364
<i>GSDMB</i> _rs2305480	G	A	102 (63.0)	56 (34.6)	4 (2.5)	2.84 (-4.46-10.14)	0.446	0.88 (-0.75-2.52)	0.289
<i>IL2RB</i> _rs2284033	A	G	42 (25.9)	85 (52.5)	35(21.6)	-1.27 (-6.07-3.52)	0.602	0.14 (-1.08-1.36)	0.827
<i>IL33</i> _rs1342326	A	C	160 (99.4)	1 (0.6)	0 (0)	-7.56 (-16.04-0.93)	0.081	2.70 (1.63-3.77)	<0.001
<i>RORA</i> _rs11071559	C	T	105 (65.2)	53 (32.7)	4 (2.5)	-3.18 (-9.29-2.94)	0.308	0.92 (-0.61-2.46)	0.238
<i>SMAD3</i> _rs744910	G	A	58 (35.8)	79 (48.8)	25 (15.4)	-3.31 (-8.64-2.03)	0.225	-0.41 (-1.61-0.80)	0.509
<i>IL18R1</i> _rs3771166	G	A	137 (85.1)	22 (13.7)	2 (1.2)	2.49 (-7.18-12.17)	0.613	-1.40 (-3.19-0.38)	0.124

<i>ORMDL3</i> _rs7216389	T	C	103 (64.0)	55 (34.1)	3 (1.9)	3.37 (-3.97-10.71)	0.368	0.88 (-0.78-2.53)	0.300
<i>ORMDL3</i> _rs17608925	T	C	138 (85.7)	23 (14.3)	0 (0)	5.83 (-4.07-15.72)	0.249	-0.74 (-2.85-1.37)	0.492
<i>GSDMA</i> _rs1007654	G	A	89 (56.0)	61 (38.4)	9 (5.7)	3.59 (-3.68-10.86)	0.334	0.37 (-1.10-1.85)	0.620
<i>TOP2A</i> _rs11650680	C	T	94 (58.4)	55 (34.2)	12 (7.5)	3.55 (-1.48-8.58)	0.166	0.33 (-0.88-1.54)	0.595
<i>IL33</i> _rs10815370	A	C	130 (81.8)	28 (17.6)	1 (0.6)	1.41 (-7.98-10.79)	0.769	-0.99 (-2.84-0.87)	0.297
<i>IL33</i> _rs1929996	G	C	133 (82.6)	27 (16.8)	1 (0.6)	2.43 (-7.05-11.90)	0.616	-1.35 (-3.15-0.45)	0.141

^a Minor alleles were designated as risk alleles.

^b Effect size per additional copy of the risk allele.

^c Analyzed by generalized estimating equation (GEE) after adjusting for sex, year of visit, age, ICS treatment ever, total number of asthma exacerbations, BMI and household smoking exposure as covariates.

Figure S1 Five-year change in FEV₁% between male and female children with asthma. P-values were calculated by GEE adjusting for year of visit, age at baseline, ICS treatment ever, total number of asthma exacerbations, BMI and household smoking exposure as covariates.

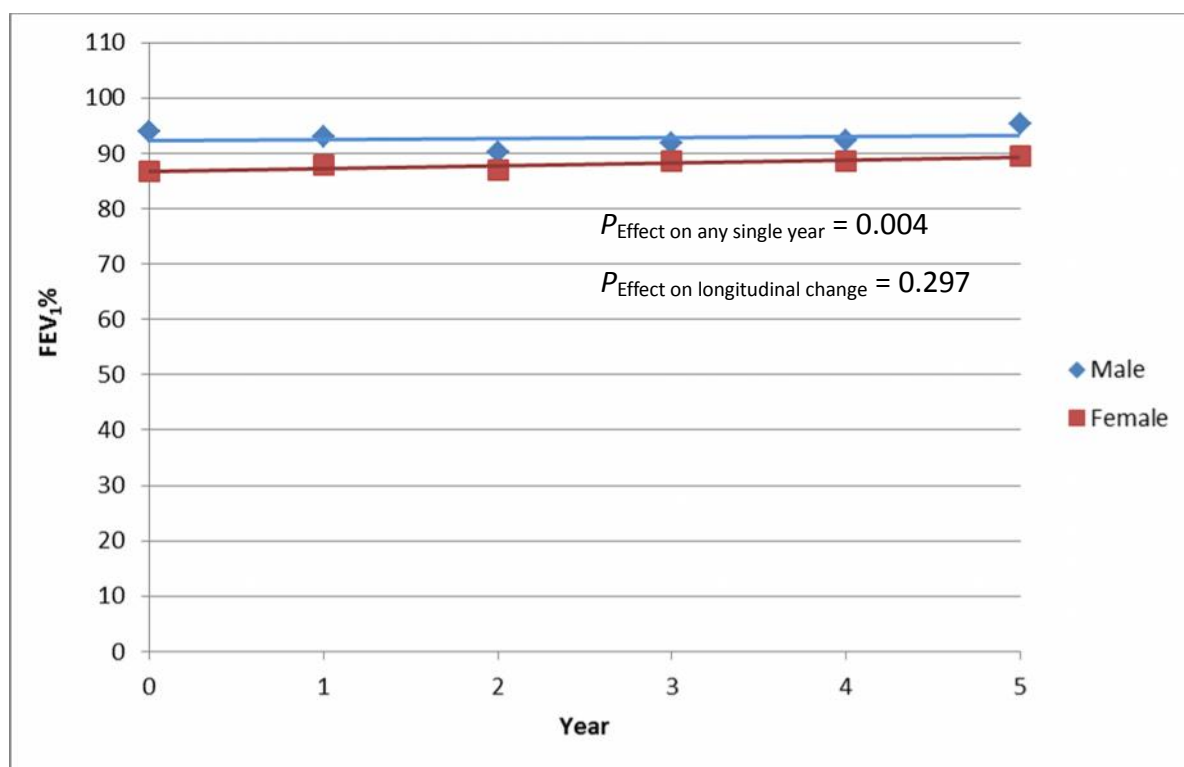


Figure S2 Five-year change in FEV₁% in patients who received and did not receive ICS treatment during follow-up. P-values were calculated by GEE.

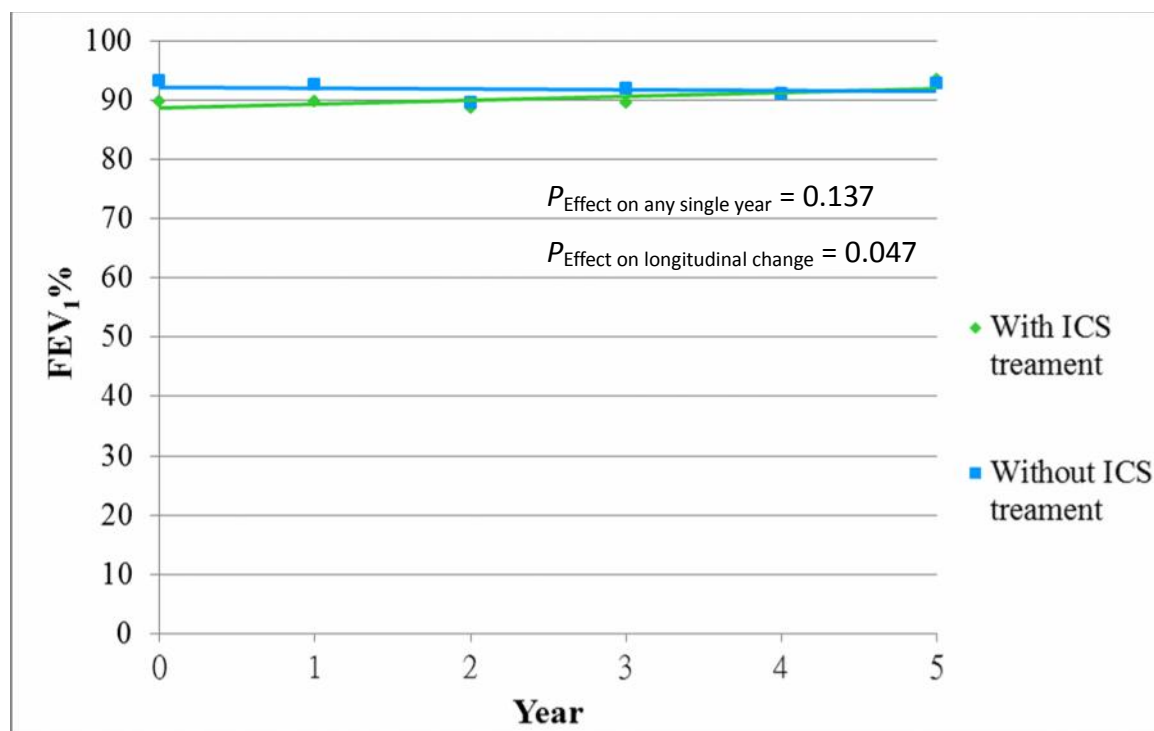


Figure S3 Five-year change in FVC% in patients who received and did not receive ICS treatment. P-values were calculated by GEE.

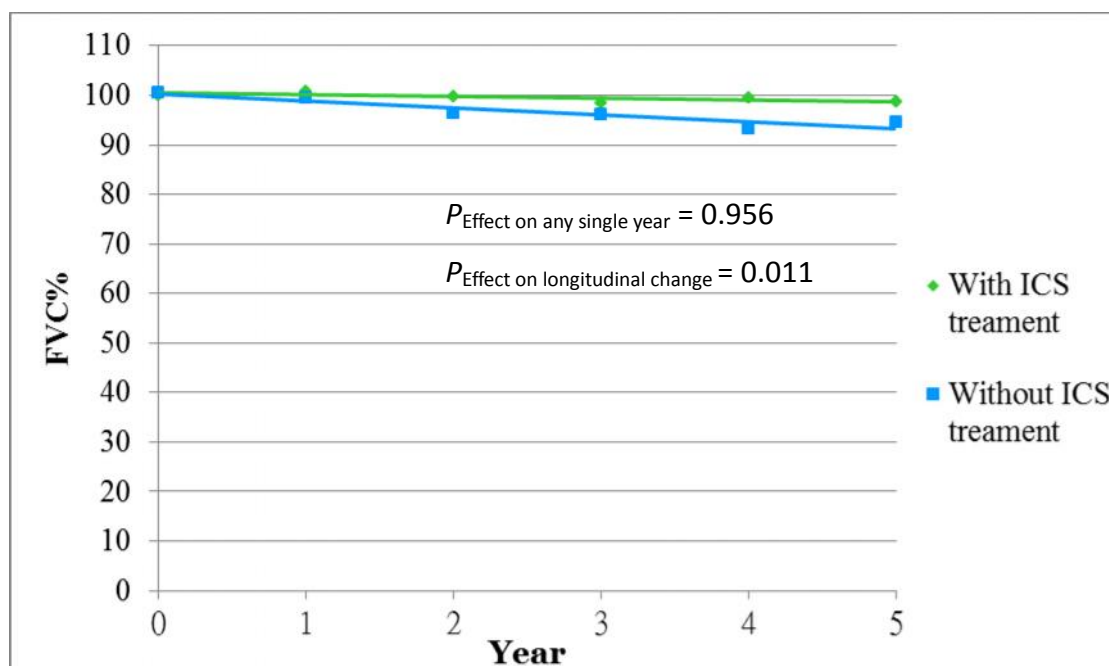


Figure S4 Five-year change in FEV₁/FVC in patients who received and did not receive ICS treatment. P-values were calculated by GEE.

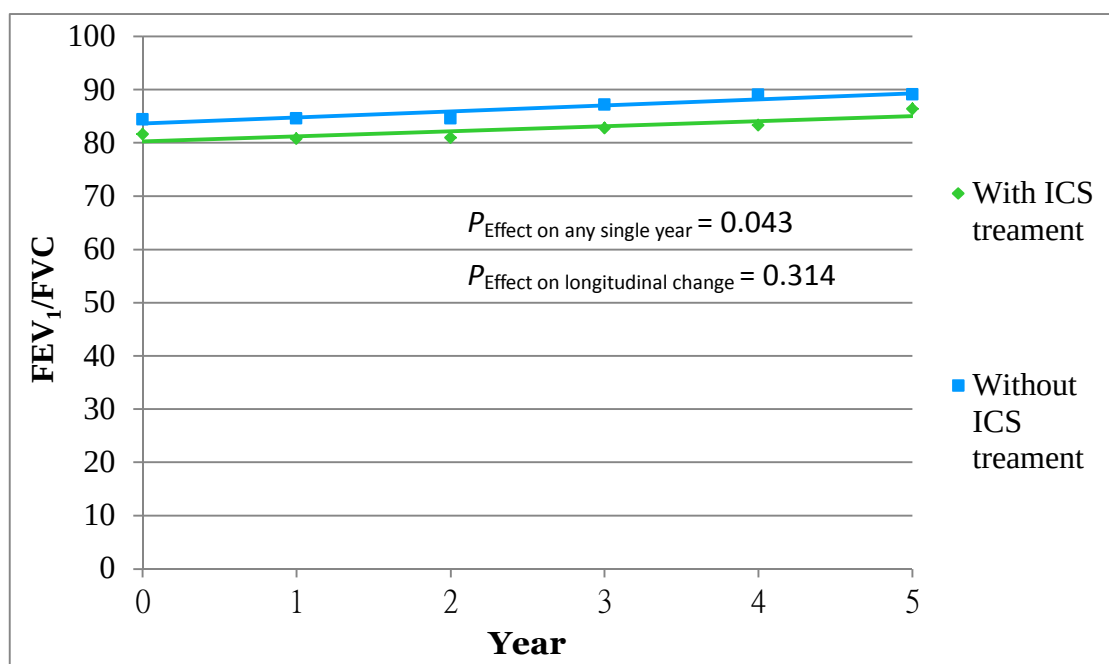


Figure S5 Five-year change in FEF₂₅₋₇₅% in patients who received and did not receive ICS treatment. P-values were calculated by GEE.

