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Genetic effects of multiple asthma loci identified by genome-wide association studies on asthma and spirometric indices

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Abstract

Background: Genome-wide association study (GWAS) published by GABRIEL consortium identified 10 asthma-associated loci. However, their relationship with lung functions is unclear. This study investigated the association between asthma traits and single-nucleotide polymorphisms (SNPs) of these GWAS loci.

Methods: Rs3894194 and rs9273349 were not genotyped due to unavailable TaqMan assays. Genetic associations of remaining eight SNPs were investigated in 903 school-age asthmatics and 1205 non-allergic controls. Four significant SNPs were then replicated in 479 adult asthmatics and 746 adult controls, and 1341 Chinese preschool children. Meta-analyses were performed by combining data from school-age children and adults. Generalized multifactor dimensionality reduction (GMDR) was used to analyze their interactions for asthma traits.

Results: Childhood asthma was associated with *GSDMB*_rs2305480 (odds ratio [OR] 0.69, 95% confidence interval [CI] 0.57-0.83). *IL13*_rs1295686 was associated with all asthma (OR 1.64, 95% CI 1.16-2.32) and early-onset asthma (OR 1.92, 95% CI 1.20-3.06) in adults, whereas *GSDMB*_rs2305480 was only associated with early-onset asthma (OR 0.69, 95% CI 0.49-0.96). According to meta-analyses, the minor allele of rs2305480 was inversely associated with FEV₁, FVC and FEV₁/FVC ($p < 0.01$). GMDR analyses revealed 2-locus models of *SLC22A5* with *SMAD3* to modulate FEV₁/FVC in both preschool children and adults, with *IL13* to determine FVC in both school-age children and adults, and with *IL2RB* to modulate FEV₁/FVC in school-age children.

Conclusions: *IL13* and *GSDMB* are replicated as asthma genes. Rs2305480 of *GSDMB* is also associated with low FEV₁, FVC and FEV₁/FVC among asthmatics. Moreover, *SLC22A5*, *IL13*, *SMAD3* and *GSDMB* interact to modulate spirometric indices.

Keywords: asthma; epistasis; gene-gene interaction; genome-wide association study; lung function

Asthma is characterized by airway hyperresponsiveness accompanied by intermittent airway obstruction and respiratory symptoms, which results from complex interactions between many genetic and environmental factors (1). The genetic basis of asthma has been extensively studied; various candidate genes have been reported for association with asthma. Not all of these replications, however, have been successfully replicated in different population. The GABRIEL consortium published the largest meta-analysis genome-wide association study (GWAS) in Europeans with 10365 cases of doctor-diagnosed asthma and 16110 controls (2). This GWAS observed associations at genome-wide significance between asthma and single-nucleotide polymorphisms (SNPs) at seven loci, implicating *HLA-DQ*, *IL18R1*, *IL33*, *SMAD3*, *GSDMA*, *GSDMB* and *IL2RB*. Based on a less stringent threshold for significance, this study also identified associations with asthma at three additional loci *SLC22A5*, *IL13*, and *RORA*. Genetic associations with asthma, however, can differ significantly among different ethnic populations (3,4). It is currently unknown whether these associations play an important role in asthma risk in different ethnic populations or in European populations only. On top of that, thus far, not a single factor or gene was found to have a strong and independent effect on asthma (5,6). It is likely that a number of candidate genes interact to determine the susceptibility to asthma traits. Therefore, searching for the interactions between susceptibility genes and environmental factors can provide useful insights to dissect this complex disease. For instance, we demonstrated significant interactions between *IL13* and *IL4RA* for plasma total IgE concentration and between *TARC* and *FCER1B* for peripheral blood (PB) eosinophil count in Chinese asthmatic children (7). In this study, we investigated the relationship between asthma-associated SNPs that were reported in the above

GABRIEL GWAS and asthma diagnosis and intermediate phenotypes in Chinese school-age children and confirmed any genetic association in two independent cohorts. This study also examined multi-locus interactions between SNPs for asthma traits.

Patients and Methods

Study populations

The *school-age children cohort* consisted of unrelated Chinese children aged 5-18 years. Our cases consisted of children with physician-diagnosed asthma from pediatric clinics of our university-affiliated teaching hospital or local community studies for allergies and obesity (8-11). The control group consisted of non-allergic children who attended our outpatient clinics for minor non-respiratory and non-inflammatory illnesses or from the above local community studies. These controls did not have any history of asthma, eczema or allergic rhinitis according to the validated Chinese International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (12). In our *adult cohort*, unrelated Chinese adults aged ≥ 18 years old with physician-diagnosed asthma were recruited from our adult chest clinic (13). Non-allergic controls without any asthma, eczema and allergic rhinitis were recruited from healthy healthcare workers and medical students in our hospital who participated in our study to establish exhaled nitric oxide reference (14). All these subjects were either non-smokers or had a smoking history of less than 10 pack years who quit smoking for at least 1 year. In our *preschool children cohort*, unrelated Chinese children aged 2-7 years were recruited from local nurseries and kindergartens to establish spirometric references (15,16). All subjects were free from any infectious symptom for four weeks before the study, and their Chinese ethnicity was ascertained by reporting from self (adults) or parents (preschool and school-age children). The Clinical Research Ethics Committee of our University approved these studies, and subjects and/or their parents gave informed written consent.

Atopy and spirometric assessments

Plasma total and allergen-specific IgE concentrations were measured by microparticle immunoassay (IMx analyzer, Abbott Laboratories, Abbott Park, IL) and fluorescent enzyme immunoassay (AutoCAP; Phadia AB, Uppsala, Sweden) respectively. Skin prick tests (SPTs) were also performed with locally prevalent allergens. Subjects were considered atopic if they had at least one positive SPT or allergen-specific IgE result. Lung function of preschoolers, school-age children and adults were measured by spirometry according to international guidelines. Details of these assessments are provided in online supplement. Results of spirometric indices were expressed in terms of percent predicted values as compared with appropriate local references (S6-S8).

Genotyping

Genomic DNA was extracted from PB leucocytes in the school-age children and adult cohorts, and from buccal cells in the preschool children cohort. Table S1 summarizes the locations and gene functions of our 10 target SNPs. In the school-age children cohort, all SNPs except rs3894194 and rs9273349 were genotyped by TaqMan genotyping assays (Applied Biosystems, Foster City, CA) using ABI-7900HT thermocycler (Applied Biosystems). Rs3894194 and rs9273349 were not genotyped due to unavailability of TaqMan assays. In both adult and preschool children cohorts, SNP on the most widely replicated asthma locus (*GSDMB*_rs2305480) and the three most consistent SNPs from our school-age children cohort (*SLC22A5*_rs2073643, *IL13*_rs1295686 and *SMAD3*_rs744910) were genotyped by TaqMan assays.

Statistical analysis

Results were expressed as proportions or mean and standard deviation (SD). Hardy-Weinberg equilibrium of SNPs was examined by exact test. The associations between SNPs and categorical and quantitative phenotypes were analyzed by logistic and linear regression respectively. In the meta-

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analysis, the heterogeneity across the school-age and adult cohorts was assessed by Cochran's Q test, with $p \leq 0.10$ being significant that implied apparent heterogeneity (17,18). Data was first evaluated using fixed-effects model (the inverse variance-weighted method), and then random-effects model (19) when Cochran's Q test was significant. All comparisons were made two-tailed using SPSS version 18 (SPSS Inc., Chicago, IL). The nominal significance threshold was set at $p < 0.05$, which was reduced to 0.00625 (0.05/8) for the school-age children and 0.0125 (0.05/4) for adult and preschool children cohorts after Bonferroni corrections for the numbers of SNPs being genotyped. Generalized multifactor dimensionality reduction (GMDR) (20) was used to evaluate epistatic interactions among SNPs for asthma traits. Generalized linear model was then performed to confirm GMDR results. Standard analysis of variance and Student *t* test were then used to compare the asthma phenotypes among different groups assigned by GMDR.

Results

Study populations

The school-age children cohort consisted of 903 asthmatics and 1205 non-allergic controls. The adult cohort consisted of 1225 adults (479 asthmatics and 746 controls) while the preschool children cohort consisted of 1341 subjects. Tables S2-S4 summarize demographic and clinical characteristics of these cohorts.

Asthma traits and individual SNPs

The genotyping efficiency was $\geq 98\%$ for the eight SNPs being successfully assayed in the school-age children cohort and for the four SNPs tested in adult and preschool children cohorts, and all of them followed HWE (see footprint in Table 1). Table 1 illustrates minor allele frequencies (MAFs) of these SNPs. Childhood asthma was associated with rs2305480 of *GSDMB* (odds ratio [OR] 0.69, 95% confidence interval [CI] 0.57-0.83; Table 2), which was consistent with our finding of this SNP only

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for early-onset asthma (OR 0.69, 95% CI 0.49-0.96; Tables S5 and S6) in adults. Rs1295686 of *IL13* was associated with all asthma (OR 1.64, 95% CI 1.16-2.32; Table S7) and early-onset asthma (OR 1.92, 95% CI 1.20-3.06; Table S5).

Table S8 describes the results of meta-analyses. Similar as single cohort analyses, rs1295686 of *IL13* was associated with both early-onset asthma (OR 1.26, 95% CI 1.07-1.49) and all asthma (OR 1.17, 95% CI 1.05-1.30). The corresponding phenotypic associations for rs2305480 of *GSDMB* were also significant (OR 0.69, 95% CI 0.58-0.81 and OR 0.73, 95% CI 0.63-0.85). Meta-analyses also revealed new findings between rs2073643 of *SLC22A5* and early-onset asthma (OR 0.84, 95% CI 0.72-0.99) and all asthma (OR 0.83, 95% CI 0.72-0.96).

Regarding spirometric indices, FEV₁ among asthmatics was lower in those homozygous for minor allele of rs2305480 in *GSDMB* ($p < 0.05$; Table 3) in the school-age children cohort. Such finding was replicated in the adult cohort ($p = 0.001$; Table S9). In this cohort, the same allele was also associated with lower FVC among asthmatics ($p < 0.001$; Table S10). For FEV₁/FVC, weakly significant result was found for *GSDMB* in the school-age children cohort (Table S11) but which could not be replicated in the adult cohort (Table S12). Meta-analyses of school-age children and adult cohorts showed that minor allele of rs2305480 was associated with lower FEV₁, FVC and FEV₁/FVC among asthmatics (Table S13), which were consistent with those of single cohort analyses. Moreover, meta-analyses revealed that higher FVC was weakly associated with minor allele of rs2073643 in *SLC22A5* while higher FEV₁/FVC was weakly associated with minor allele of rs1295686 in *IL13*. On the other hand, among controls in the adult cohort, rs2305480 in *GSDMB* was not associated with any spirometric index (Table S14-S16). In the preschool children cohort, none of the four target SNPs was associated with FEV_{0.5}, FEV_{0.75}, FEV₁, FVC and FEV_n/FVC after adjusting for age, gender, domestic passive smoking exposure and wheeze ever as covariates.

On the other hand, none of the SNPs was associated with atopy, plasma total IgE concentration and FEV₁ reversibility in all three cohorts following Bonferroni correction.

Gene-gene interactions for asthma traits

This study could not identify any significant interactive models for asthma diagnosis (results not shown). In the school-age children cohort, GMDR analyses revealed one three-locus model formed by rs2073643 in *SLC22A5*, rs1295686 in *IL13* and rs744910 in *SMAD3* and one five-locus model for FEV₁ with TA >55% and $p < 0.05$ (Table 4a). The three-locus model was confirmed by generalized linear model ($p \leq 0.001$) adjusting for age, sex and ICS treatment as covariates. One two-locus, one three-locus model and one five-locus model were found for FVC (TA >55% and $p < 0.05$; Table S17). These results were also confirmed by generalized linear model ($p \leq 0.001$). For the two-locus model formed by rs2073643 in *SLC22A5* and rs1295686 in *IL13*, FVC was significantly lower among high-risk than low-risk patients (mean: 1.99 vs 2.02; $p < 0.0001$; Fig S1). With respect to patients' FEV₁/FVC, we found one significant two-locus, one three-locus model and one five-locus model (TA >55% and $p < 0.05$; Table 4b) which were again confirmed by generalized linear model ($p \leq 0.001$). In the two-locus model involving rs2073643 of *SLC22A5* and rs2284033 of *IL2RB*, (FEV₁/FVC)² was lower among high-risk than low-risk patients (mean: 0.668 vs 0.715; $p = 0.003$; Fig S2).

GMDR replicated the same three-locus model for FEV₁ (TA = 56.93% and $p = 0.043$) and two-locus model for FVC (TA = 59.47% and $p = 0.010$; Fig S3) found from school-age children in adults (Table S18). FVC was lower in high-risk than low-risk patients (mean: 88.8% vs 91.8%; $p = 0.043$). Significant two-locus model from rs2073643 in *SLC22A5* and rs744910 in *SMAD3* was found for FEV₁/FVC (TA = 57.36% and $p = 0.022$) (Fig S4).

Among preschoolers, the four tested SNPs did not interact to modulate FEV_{0.5}, FEV_{0.75}, FEV₁ and FVC. However, the same two-locus models formed by rs2073643 in *SLC22A5* and rs744910 in *SMAD3* as found in the adult cohort were found for FEV_{0.75}/FVC (TA = 55.18% and p = 0.017; Fig 1a) and FEV_{0.5}/FVC (TA = 54.40% and p = 0.039) (Table S19). These results were confirmed by generalized linear model (p < 0.001; Fig 1b).

Discussion

This genetic study confirmed the associations between *GSDMB* and both childhood asthma and early-onset asthma in adults. *IL13* was also associated with asthma in adults (adult cohort). Meta-analyses identified associations between asthma and these two loci as well as *SLC22A5*. By GMDR and generalized linear model analyses, we detected novel interactions among SNPs of *SLC22A5*, *IL13*, *GSDMB* and *SMAD3* for spirometric indices including FEV₁ and FVC in asthmatic patients and FEV_{0.75}/FVC and FEV_{0.5}/FVC in preschool children. Nonetheless, none of the SNPs was associated with atopy or plasma total IgE.

SLC22A5 encodes plasma membrane carnitine transporters (21), and functional defect of this gene causes primary systemic carnitine deficiency. As *SLC22A5* is expressed at the apical side of airway epithelial cells in trachea, it might contribute to the transport or module the pharmacological activity of inhaled cationic drugs such as ipratropium bromide (22). *IL13* played crucial roles in asthmatic airway remodelling (23,24). Plasma IL-13 concentration and *IL13* polymorphisms have also been associated with reduced FEV₁ (25,26). *SMAD3*, a transcriptional factor activated by transforming growth factor- β (27), was involved in intracellular mediation of inflammation. *SMAD3*-deficient mice had elevated levels of proinflammatory cytokines in their lungs (28). Several studies reported associations between *GSDMB* and bronchodilator responsiveness (29-31), but the relative contribution of *GSDMB* to lung function remained poorly understood. Besides epistasis between *GSDMB* and other genes for spirometric indices, our patients with the A allele of rs2305480 in *GSDMB* had significantly

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lower FEV₁, FVC and FEV₁/FVC. Some of these results were consistent with those of our asthma pharmacogenetic study (32), which extended previous studies of *GSDMB* to asthma traits such as lung function.

The A allele of rs2305480 in *GSDMB* was found to be protective allele for asthma while it was associated with lower lung function among asthmatics. On the other hand, this allele was not associated with any lung function parameter among adult controls (Tables S14-S16). We hypothesize that environmental factors such as tobacco smoke exposure may explain this seemingly discrepant findings of the A allele on asthma and lung function. In the French Epidemiological Study on the Genetics and Environment of Asthma, the harmful effect of G allele (our major allele) of *GSDMB* on asthma was only evident in adults who were exposed to smoking (33). In our study, we were unable to evaluate the confounding effects of passive smoke exposure on asthma and spirometric indices in the school-age children cohort as such data was missing in most community subjects who contributed to this cohort. All our subjects in the adult cohort were either non-smokers or ex-smokers for at least one year, which did not allow us to evaluate possible gene-environment interaction between the G allele of *GSDMB*_rs2305480 and current tobacco smoke exposure on subjects' lung function. The same genetic association results between this SNP and spirometric indices was obtained among asthmatics and controls when we adjusted for ex-smoking (5.0% cases and 7.1% controls; Table S3) in the adult cohort. Besides, the smoking behaviour of subjects would change with time as they might quit or reduce smoking upon asthma diagnosis or when their disease control worsened. It is possible that we observed a reverse causation between A allele of rs2305480 and poorer lung function in our adult asthmatics (Table 3). Thus, *GSDMB*_rs2305480 may interact with environmental factors such as drug usage and exposure to smoking among asthmatics to result in lower lung function, which should be further examined in future studies.

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Apart from lung function results, this study also replicated the associations between asthma diagnosis and *IL13* and *GSDMB*. Our result was consistent with those found on 17q21 locus from both the initial GWAS for childhood asthma (2) and a number of replication studies in different ethnic groups (34-36). Among adults in our second replication cohort, *GSDMB* was associated with early-onset but not late-onset asthma (Tables S5 and S6) which was consistent with the results reported by the French Epidemiological Study on the Genetics and Environment of Asthma (33). Similar to *GSDMB*, rs1295686 of *IL13* was mainly associated with early-onset asthma. Although all target SNPs were reported in the large-scale GWAS (2), this study failed to detect genetic associations between asthma and SNPs on genes other than *GSDMB* and *IL13* in multiple Chinese cohorts. In addition, we failed to detect any epistatic interaction among the target genes for asthma susceptibility. These observations highlight the complex nature of asthma pathogenesis involving multiple genes and environmental exposures (33,37). Thus, our lack of significant replicative findings for some genes may be due to their interactive effects with environmental exposures or inadequate study power that results from interethnic differences in MAFs or linkage disequilibrium (LD) patterns.

This study supported substantial interethnic variations in MAFs for SNPs on the eight asthma genes (Table 1). For instance, the minor alleles of rs2073643 in *SLC22A5* and rs744910 in *SMAD3* in our Chinese were major alleles of these SNPs among Caucasians in the GABRIEL study (2). Our group also observed this finding for several other SNPs (32,38,39). Moreover, the MAF of *GSDMB* rs2305480 in our subjects differed by ≥ 0.14 and ≥ 0.21 respectively when compared with HapMap data for Han Chinese and the GABRIEL Consortium. In fact, MAFs of the genotyped SNPs in our subjects were much closer to those of Southern Han Chinese under 1000 Genomes (<http://www.1000genomes.org>).

The genotyping of one SNP for each candidate gene was the major limitation of this study. Hence, our SNPs were insufficient in capturing the genetic information of target genes. Future studies should include tagging SNPs that would be selected by an optimal tagging subset based on LD and haplotype analyses. Owing to interethnic differences in MAFs of chosen SNPs, our combined sample size was also too small to detect SNPs with small effect size and delineate multiple-locus interactive effects for asthma phenotypes (e.g. MAF of 0.003 for *IL33* rs1342326 among controls). We tried to overcome the latter problem by detecting gene-gene interactions using GMDR, which was designed for genetic studies of relatively small sample size (20,40). Apart from that, this study did not examine the confounding effects of environmental factors. Two studies independently found early-life and current tobacco smoke exposures to be important in modulating asthma susceptibility in both children and adults (33,37). Differential environmental exposures between different populations may mask or enhance the effects of different asthma genes, which account for the discrepancy in results of asthma replicative studies.

In conclusions, this study replicated the genetic associations between asthma and *IL13* and *GSDMB*. Additionally, we detected the association between *GSDMB* and lung functions among our asthmatic patients. These results extended the significance of asthma loci *SLC22A5*, *IL13*, *GSDMB* and *SMAD3* to patients' spirometric indices.

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SNP	Alleles (B/b)	School-age children cohort		Adult cohort		Preschool children cohort	MAF from HapMap				MAF from 1000 Genomes					Published data
		Asthma	Controls	Asthma	Controls	All subjects	Han Chinese	Japanese	European	African	Southern					GABRIEL
											Han Chinese	Asian	European	American	African	
<i>SLC22A5</i> / rs2073643	T/C	0.313	0.341	0.307	0.321	0.339	0.209	0.355	0.571	0.372	0.305	0.28	0.469	0.428	0.335	0.55
<i>IL13</i> / rs1295686	C/T	0.369	0.348	0.378	0.338	0.338	0.302	0.297	0.217	0.708	0.36	0.329	0.227	0.398	0.697	0.2
<i>GSDMB</i> / rs2305480	G/A	0.194	0.240	0.228	0.245	0.240	0.384	0.314	0.469	0.066	0.24	0.315	0.476	0.348	0.108	0.45
<i>IL2RB</i> / rs2284033	A/G	0.463	0.453	ND	ND	ND	0.4	0.444	0.61	0.575	0.4	0.386	0.581	0.572	0.616	0.56
<i>IL33</i> / rs1342326	A/C	0.007	0.003	ND	ND	ND	0	0	0.168	0.332	0	0	0.151	0.144	0.36	0.16
<i>RORA</i> / rs11071559	C/T	0.168	0.175	ND	ND	ND	0.081	0.244	0.155	0.558	0.175	0.18	0.133	0.18	0.504	0.14
<i>SMAD3</i> / rs744910	G/A	0.380	0.388	0.329	0.366	0.383	0.4	0.411	0.551	0.3	0.39	0.423	0.521	0.486	0.356	0.51
<i>IL18R1</i> / rs3771166	G/A	0.095	0.087	ND	ND	ND	0.14	0.151	0.407	0.735	0.14	0.15	0.409	0.29	0.675	0.38

Table 1 Interethnic differences in minor allele frequencies of our eight genotyped SNPs

MAF, minor allele frequency; ND, not done

All genotyped SNPs of all subjects in three cohorts were in Hardy-Weinberg equilibrium with $p > 0.05$

Table 2 Association between asthma diagnosis and eight SNPs genotyped in the school-age children cohort

Genotype	Asthma (n=903)	Controls (n=1205)	p-value by Pearson χ^2	OR, 95% CI (<i>P</i> *)		
	n (%)	n (%)		Additive model	Dominant model	Recessive model
<i>SLC22A5</i> /						
rs2073643						
TT	423 (47.2)	512 (42.7)	0.124	0.88, 0.76-	0.84, 0.70-	0.88, 0.65-
TC	385 (43.0)	556 (46.4)		1.01 (0.070)	1.01 (0.057)	1.20 (0.422)
CC	88 (9.8)	130 (10.9)				
<i>IL13</i> /						
rs1295686						
CC	345 (38.6)	512 (43.0)	0.092	1.14, 0.99-	1.24, 1.06-	1.00, 0.75-
CT	439 (49.1)	530 (44.5)		1.30 (0.068)	1.54 (0.012)	1.31 (0.971)
TT	110 (12.3)	150 (12.6)				
<i>GSDMB</i> /						
rs2305480						
GG	584 (64.8)	682 (56.8)	0.001	0.75, 0.63-	0.69, 0.57-	0.85, 0.54-
GA	284 (31.5)	461 (38.4)		0.88	0.83	1.35 (0.496)
AA	33 (3.7)	58 (4.8)		(< 0.001)	(< 0.001)	
<i>IL2RB</i> /						
rs2284033						
AA	251 (27.9)	360 (30.0)	0.521	1.04, 0.91-	1.10, 0.90-	1.00, 0.80-
AG	464 (51.6)	592 (49.3)		1.19 (0.548)	1.34 (0.370)	1.26 (0.973)
GG	184 (20.5)	248 (20.7)				
<i>IL33</i> /						
rs1342326**						
AA	886 (98.7)	1193 (99.4)	0.117	2.07, 0.76-		
AC	12 (1.3)	7 (0.6)		5.65 (0.157)		
CC	0	0				
<i>RORA</i> /						
rs11071559						
CC	622 (68.9)	829 (68.9)		0.96, 0.81-	1.01, 0.83-	0.66, 0.39-

CT	259 (28.7)	327 (27.2)	0.148	1.14 (0.652)	1.23 (0.957)	1.13 (0.132)
TT	22 (2.4)	47 (3.9)				
<i>SMAD3</i> /						
rs744910						
GG	341 (38.0)	448 (37.3)		0.95, 0.83-	0.97, 0.80-	0.88, 0.68-
GA	431 (48.0)	575 (47.8)	0.847	1.09	1.17 (0.729)	1.14 (0.340)
AA	126 (14.0)	179 (14.9)		(0.461)		
<i>IL18R1</i> /						
rs3771166						
GG	721 (81.8)	986 (83.2)		1.06, 0.84-	1.08, 0.85-	0.81, 0.26-
GA	153 (17.4)	192 (16.2)	0.659	1.34 (0.610)	1.38 (0.535)	2.42 (0.713)
AA	7 (0.8)	7 (0.6)				

OR, odds ratio; CI, confidence interval.

* Analyzed by binary logistic regression adjusting for age and gender as covariates.

** Heterozygous vs homozygous

Minor alleles were designated as risk alleles

Table 3 Association between FEV₁ and genotyped SNPs among 451 asthmatics in the school-age children cohort

Genotype	All cases	FEV ₁ (%)	p-value by	P-value*		
	n (%)	Mean ± SD	one-way ANOVA	Additive model	Dominant model	Recessive model
<i>SLC22A5</i> /						
rs2073643						
TT	214 (47.7)	91.1 ± 15.5	0.180	0.173	0.093	0.866
TC	193 (43.0)	94.3 ± 19.6				
CC	42 (9.4)	93.1 ± 16.0				
<i>IL13</i> /						
rs1295686						
CC	163 (36.3)	92.1 ± 19.1	0.847	0.565	0.494	0.876
CT	227 (50.6)	93.0 ± 17.2				
TT	59 (13.1)	93.2 ± 13.2				
<i>GSDMB</i> /						
rs2305480						
GG	298 (66.1)	95.4 ± 19.2	0.076	0.031	0.035	0.034
GA	137 (30.4)	92.4 ± 25.7				
AA	16 (3.5)	91.3 ± 16.0				
<i>IL2RB</i> /						
rs2284033						
AA	123 (27.3)	91.2 ± 15.1	0.366	0.172	0.287	0.244
AG	226 (50.1)	92.5 ± 19.3				
GG	102 (22.6)	94.5 ± 15.9				
<i>IL33</i> /						
rs1342326**						
AA	444 (98.7)	92.7 ± 17.3	0.403	NA	0.353	NA
AC	6 (1.3)	86.7 ± 26.4				
CC	0	NA				
<i>RORA</i> /						
rs11071559						
CC	304 (67.4)	92.3 ± 18.3	0.820	0.493	0.570	0.542
CT	137 (30.4)	93.0 ± 15.7				
TT	10 (2.2)	95.2 ± 15.7				

<i>SMAD3</i> /						
rs744910						
GG	170 (37.9)	94.3 ± 20.0				
GA	217 (48.3)	91.1 ± 16.6	0.210	0.279	0.107	0.894
AA	62 (13.8)	92.8 ± 12.0				
<i>IL18R1</i> /						
rs3771166						
GG	369 (84.2)	92.4 ± 17.2				
GA	66 (15.1)	92.9 ± 19.3	0.343	0.689	0.892	0.200
AA	3 (0.7)	107.2 ± 13.7				

NA, not applicable.

* By linear regression adjusting for age, gender and current ICS treatment as covariates.

** Heterozygous versus homozygous

Minor alleles were designated as risk alleles

Table 4 Multi-locus interaction models for (a) FEV₁ and (b) (FEV₁/FVC)² in school-age children with asthma

(a) FEV₁

Number of locus	SNP combination	CVC	TA (%)	p*
1	<i>SMAD3</i> _rs744910	5	54.83	0.128
2	<i>IL13</i> _rs1295686, <i>SMAD3</i> _rs744910	4	54.50	0.158
3	<i>SLC22A5</i> _rs2073643, <i>IL13</i> _rs1295686, <i>SMAD3</i> _rs744910	9	62.88	0.001

4	<i>SLC22A5</i> _rs2073643, <i>IL13</i> _rs1295686, <i>GSDMB</i> _rs2305480, <i>SMAD3</i> _rs744910	5	60.24	0.008
5	<i>SLC22A5</i>_rs2073643, <i>IL13</i>_rs1295686, <i>GSDMB</i>_rs2305480, <i>IL2RB</i>_rs2284033, <i>SMAD3</i>_rs744910	10	62.18	0.003
6	<i>SLC22A5</i> _rs2073643, <i>IL13</i> _rs1295686, <i>GSDMB</i> _rs2305480, <i>IL2RB</i> _rs2284033, <i>RORA</i> _rs11071559, <i>SMAD3</i> _rs744910	10	56.83	0.077
7	<i>SLC22A5</i> _rs2073643, <i>IL13</i> _rs1295686, <i>GSDMB</i> _rs2305480, <i>IL2RB</i> _rs2284033, <i>RORA</i> _rs11071559, <i>SMAD3</i> _rs744910, <i>IL18R1</i> _rs3771166	10	58.30	0.057
8	<i>SLC22A5</i> _rs2073643, <i>IL13</i> _rs1295686, <i>GSDMB</i> _rs2305480, <i>IL2RB</i> _rs2284033, <i>IL33</i> _rs1342326, <i>RORA</i> _rs11071559, <i>SMAD3</i> _rs744910, <i>IL18R1</i> _rs3771166	10	56.83	0.090

CVC, cross-validation consistency; SNP, single-nucleotide polymorphism; TA, testing accuracy.

* Based on 5000 permutations.

(b) $(FEV_1/FVC)^2$

Number of locus	SNP combination	CVC	TA (%)	p*
1	<i>IL13</i> _rs1295686	8	55.15	0.083
2	<i>SLC22A5</i>_rs2073643, <i>IL2RB</i>_rs2284033	7	56.67	0.043
3	<i>SLC22A5</i> _rs2073643, <i>IL13</i> _rs1295686, <i>IL2RB</i> _rs2284033	8	52.19	0.313
4	<i>SLC22A5</i>_rs2073643, <i>IL13</i>_rs1295686, <i>IL2RB</i>_rs2284033, <i>SMAD3</i>_rs744910	9	57.75	0.027

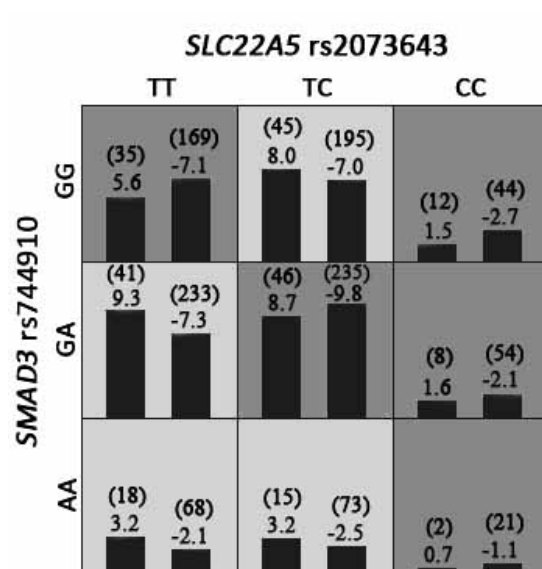
5	<i>SLC22A5</i> _rs2073643, <i>IL13</i> _rs1295686, <i>GSDMB</i> _rs2305480, <i>IL2RB</i> _rs2284033, <i>SMAD3</i> _rs744910	10	57.60	0.038
6	<i>SLC22A5</i> _rs2073643, <i>IL13</i> _rs1295686, <i>GSDMB</i> _rs2305480, <i>IL2RB</i> _rs2284033, <i>RORA</i> _rs11071559, <i>SMAD3</i> _rs744910	10	55.02	0.129
7	<i>SLC22A5</i> _rs2073643, <i>IL13</i> _rs1295686, <i>GSDMB</i> _rs2305480, <i>IL2RB</i> _rs2284033, <i>RORA</i> _rs11071559, <i>SMAD3</i> _rs744910, <i>IL18R1</i> _rs3771166	10	53.99	0.199
8	<i>SLC22A5</i> _rs2073643, <i>IL13</i> _rs1295686, <i>GSDMB</i> _rs2305480, <i>IL2RB</i> _rs2284033, <i>IL33</i> _rs1342326, <i>RORA</i> _rs11071559, <i>SMAD3</i> _rs744910, <i>IL18R1</i> _rs3771166	10	53.41	0.251

CVC, cross-validation consistency; SNP, single-nucleotide polymorphism; TA, testing accuracy.

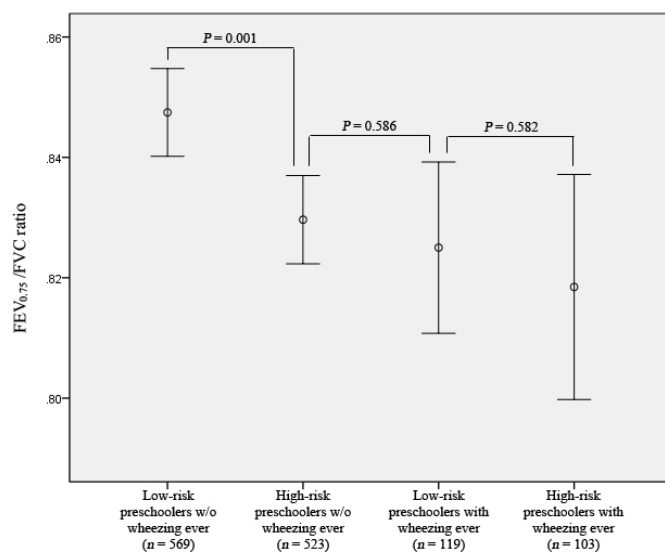
* Based on 5000 permutations.

Fig. 1 (a) Best two-locus model for FEV_{0.75}/FVC of preschoolers in the preschool children cohort. High-risk genotypes are in dark grey and low-risk genotypes are in light grey. Left and right bars represent children with and without wheeze ever respectively. The figures above the bars in each cell are score whereas those in brackets represent the number of subjects in each subgroup. The length of bars represents the absolute values of score. (b) Distribution of FEV_{0.75}/FVC in pre-schoolers with high-risk and low-risk genotypes defined by 2-locus model of *SLC22A5*_rs2073643 and *SMAD3*_rs744910 ($p = 0.0003$ by ANOVA).

(a)



(b)



ONLINE SUPPLEMENT

Genetic effects of multiple asthma loci identified by genome-wide association studies on asthma and spirometric indices

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METHODS

Assessment of atopy

Plasma total IgE concentrations were measured by microparticle immunoassay (IMx analyzer, Abbott Laboratories, Abbott Park, IL) and compared with our local references. Concentrations of specific IgE to *Dermatophagoides pteronyssinus*, cat, dog, cockroach and mixed moulds were measured by fluorescent enzyme immunoassay (AutoCAP; Phadia AB, Uppsala, Sweden), with ≥ 0.35 kIU/l being positive. Skin prick tests (SPTs) were performed with extracts of locally prevalent allergens including house dust mites, mixed cockroaches, cat dander, dog dander and mixed molds (ALK Abelló, Round Rock, TX) (S1-S3). Subjects were considered atopic if they had at least one positive SPT or allergen-specific IgE result.

Spirometric measurements

Asthmatics in the school-age children cohort underwent spirometry (COMPACT II; Vitalograph, Buckingham, UK) to measure forced expiratory volume in 1-second (FEV₁) and forced vital capacity (FVC) before and 30 minutes following inhalation of 200 µg salbutamol (S4). All subjects in adult cohort performed spirometry (S-model; Vitalograph, Buckingham, England) to measure FEV₁ and FVC before and 30 minutes following inhalation of 400 µg salbutamol. Children in preschool children cohort performed incentive spirometry (Master Screen, Jaeger GmbH, Würzburg, Germany) according to international guideline (S5) to

measure forced expiratory volume in 0.5-second ($FEV_{0.5}$), $FEV_{0.75}$, FEV_1 and FVC.

Spirometric results were compared with respective age-specific local references (S6-S8).

Statistical analysis

Generalized multifactor dimensionality reduction (GMDR, version 0.9; <http://www.healthsystem.virginia.edu/internet/addiction-genomics/software/>) was used to determine high and low risk (S9). GMDR is an advancement of MDR (S10) that allows adjustment for discrete and quantitative covariates and is applicable to both continuous and dichotomous phenotypes. In brief, GMDR computes the maximum-likelihood estimates and the score values of all individuals under the null hypothesis. The cumulative score value is calculated within each cell multifactor cell, which is labelled either as high risk if the average score meets or exceeds a pre-assigned threshold of 0 or as low risk if the threshold is not exceeded. In the evaluation of the interactive models, outcome parameters including testing accuracy (TA), cross-validation consistency (CVC) and statistical significance of the model were considered. TA measuring the ability to classify individuals with respect to their score values is used to choose the best model. A TA score of 0.5 indicates that the model predicts no better than chance, whereas a score of 1.0 indicates a perfect prediction. In general, a TA score of at least 0.55 for a model is considered satisfactory. CVC evaluates the consistency with which the selected interaction is classified as the best model among all possible combinations.

Permutation tests are considered the gold standard in multiple testing adjusting as they simultaneously provide unbiased type I error control and high power (S11). This method is used in GMDR to identify the best interactive models. The statistical significance of a model is determined by comparing the average prediction error from the observed data with the distribution of average prediction errors under the null hypothesis of absence of association derived empirically from 5000 permutations. The null hypothesis is rejected when the P value derived from permutation is 0.05 or lower.

In this study, an exhaustive search was performed to test for possible one-locus to eight-locus (discovery cohort) or one-locus to four-locus (first and second replication cohorts) interactions using 10-fold cross-validation. Age, gender and other possible confounders (e.g. ICS usage and disease status) were included as covariates. Standard analysis of variance was used to compare asthma phenotypes among different groups according to GMDR classification while Student t test was used for subgroup analyses. Generalized linear model was then performed to confirm GMDR results. $P < 0.05$ was considered statistically significant. Standard analysis of variance and Student t test were then used to compare the asthma phenotypes among different groups according to the GMDR classification.

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Table S1 Details of ten target SNPs considered in this study

SNP	Name of Gene	Gene Symbol	Position*	Chromosome	Cytogenetic band	Nature of SNP	Major allele	Minor allele
rs2073643	Solute carrier family 22 (organic cation/carnitine transporter), member 5	<i>SLC22A5</i>	131751187	5	5q31.1	Intron	T	C
rs1295686	Interleukin 13	<i>IL13</i>	132023742	5	5q31.1b	Intron	C	T
rs2305480	Gasdermin B	<i>GSDMB</i>	35315722	17	17q12c	Mis-sense mutation	G	A
rs2284033	Interleukin 2 receptor, beta	<i>IL2RB</i>	35863980	22	22q12.3d	Intron	A	G
rs1342326	Interleukin 33 (intergenic)	<i>IL33</i> (intergenic)	6180076	9	9p24.1b	Intergenic/Unknown	A	C
rs11071559	RAR-related orphan receptor A	<i>RORA</i>	58857280	15	15q22.2a	Intron	C	T
rs744910	SMAD family member 3	<i>SMAD3</i>	65233839	15	15q22.33c	Intron	G	A
rs3771166	Interleukin 18 receptor 1	<i>IL18R1</i>	102352654	2	2q12.1a	Intron	G	A
rs3894194	Gasdermin A	<i>GSDMA</i>	35375519	17	17q21.1	Coding exon (18 R/Q)	G	A
rs9273349	Major histocompatibility complex, class II, DQ	<i>HLA-DQ</i>	32733847	6	6p21.32	Downstream	C	T

* Position is based on the number of base pairs from the start of the chromosome.

Table S2 Clinical characteristics of 903 patients and 1205 controls in the school-age cohort

Characteristic	Asthma	Controls
Age, years	11.0 ± 4.1*	13.6 ± 4.5
Male	558 (61.8)*	533 (44.2)
Log ₁₀ -transformed plasma total IgE level	2.61 ± 0.69*	1.86 ± 0.69
Increased plasma total IgE level**	625 (78.5)*	271 (40.2)
Atopy***	580 (75.3)*	254 (37.9)
Spirometric variables		
FEV ₁ , % predicted	92.6 ± 17.5	ND
FVC, % predicted	103.5 ± 20.3	ND
FEV ₁ /FVC	0.823 ± 0.105	ND
FEV ₁ reversibility, %	7.3 ± 11.1	ND
Subjects with reversible FEV ₁	83 (23.4)	ND

FEV₁, forced expiratory volume in 1-second; FVC, forced vital capacity; ND, not done.

Results expressed in mean ± standard deviation or number (percentage).

* p < 0.001.

** Compared with in-house upper limits of normal: 120 kIU/l for 5-6 years, 160 kIU/l for 6-7 years and 180 kIU/l for ≥8 years.

*** Defined as having ≥ 1 positive SPT or RAST to aeroallergens.

Table S3 Demographic and clinical characteristics of 479 asthmatics and 746 controls in the adult cohort

Characteristic	Asthma	Controls
Age, years	44.9 ± 13.8*	48.4 ± 16.2
Male	193 (40.3)*	394 (52.8)
Log ₁₀ -transformed plasma total IgE level	2.15 ± 0.65*	1.60 ± 0.64
Atopy**	341 (79.7)*	379 (51.1)
Ex-smoker	24 (5.0)	53 (7.1)
Spirometric variables		
FEV ₁ , % predicted	88.1 ± 19.8*	104.5 ± 13.7
FVC, % predicted	90.4 ± 17.0*	95.0 ± 13.3
FEV ₁ /FVC	0.801 ± 0.108*	0.890 ± 0.061
FEV ₁ reversibility, %	8.2 ± 8.6*	2.5 ± 6.1
Subjects with reversible FEV ₁	104 (23.5)*	24 (3.2)

FEV₁, forced expiratory volume in 1-second; FVC, forced vital capacity; ND, not done.

Results expressed in mean ± standard deviation or number (percentage).

* p < 0.001.

** Defined as having ≥ 1 positive SPT to aeroallergens.

Table S4 Demographic, clinical and environmental factors of 1341 children in the preschool children cohort

Characteristic	Result
Age, years	4.7 ± 1.0
Male	740 (55.2)
Presence of allergic diseases	
Asthma	71 (5.3)
Wheezing ever	227 (16.9)
Current wheeze	142 (10.6)
Allergic rhinitis	384 (28.7)
Eczema	461 (34.4)
Food allergy (doctor-diagnosed)	53 (4.0)
Passive tobacco smoke exposure	
Current household members	553 (41.2)
Mother smoked during pregnancy	54 (4.0)
Current cat and/or dog keeping	89 (6.7)
Lung function by incentive spirometry	
FEV ₁ , % predicted	97.3 ± 13.4
FEV _{0.75} , % predicted	100.0 ± 15.1
FEV _{0.5} , % predicted	91.7 ± 14.3
FVC, % predicted	102.2 ± 14.4
FEV ₁ /FVC	0.894 ± 0.069
FEV _{0.75} /FVC	0.837 ± 0.087
FEV _{0.5} /FVC	0.716 ± 0.091

FEV_n, forced expiratory volume in *n*-second; FVC, forced vital capacity; ND, not done.

Results expressed in mean ± standard deviation or number (percentage).

Table S5 Association between early-onset asthma (16 years or younger) and four target SNPs in the adult cohort

Genotype	Early-onset asthma (n=207)	All controls (n=746)	p-value by Pearson χ^2	OR, 95% CI (<i>P</i> *)		
	n (%)	n (%)		Additive model	Dominant model	Recessive model
<i>SLC22A5</i> / rs2073643						
TT	101 (49.0)	340 (45.8)	0.257	0.98,	0.85,	1.36,
TC	79 (38.3)	328 (44.2)		0.77-1.25	0.62-1.18	0.82-2.23
CC	26 (12.6)	74 (10.0)		(.857)	(.326)	(.232)
<i>IL13</i> / rs1295686						
CC	82 (39.8)	318 (42.9)	0.082	1.32,	1.23,	1.92,
CT	91 (44.2)	347 (46.8)		1.04-1.68	0.89-1.71	1.20-3.06
TT	33 (16.0)	77 (10.4)		(.025)	(.215)	(.006)
<i>GSDMB</i> / rs2305480						
GG	131 (63.9)	414 (55.5)	0.073	0.75,	0.69,	0.86,
GA	64 (31.2)	298 (39.9)		0.57-1.00	0.49-0.96	0.40-1.83
AA	10 (4.9)	34 (4.6)		(.048)	(.028)	(.689)
<i>SMAD3</i> / rs744910						
GG	87 (42.0)	299 (40.1)	0.560	0.88,	0.92,	0.70,
GA	98 (47.3)	346 (46.4)		0.70-1.12	0.67-1.28	0.42-1.17
AA	22 (10.6)	100 (13.4)		(.306)	(.631)	(.175)

OR, odds ratio; CI, confidence interval.

* Analyzed by binary logistic regression adjusting for age and gender as covariates.

Minor alleles were designated as risk alleles

Table S6 Association between late-onset asthma (after 16 years) and four target SNPs in the adult cohort

Genotype	Late-onset asthma (n=249)	All controls (n=746)	p-value by Pearson χ^2	OR, 95% CI (p*)		
	n (%)	n (%)		Additive model	Dominant model	Recessive model
<i>SLC22A5</i> / rs2073643						
TT	124 (50.4)	340 (45.8)	0.405	0.84,	0.80,	0.83,
TC	102 (41.5)	328 (44.2)		0.67-1.06	0.59-1.07	0.49-1.41
CC	20 (8.1)	74 (10.0)		(.141)	(.129)	(.496)
<i>IL13</i> / rs1295686						
CC	95 (38.5)	318 (42.9)	0.119	1.23,	1.22,	1.50,
CT	115 (46.6)	347 (46.8)		0.99-1.53	0.90-1.64	0.98-2.31
TT	37 (15.0)	77 (10.4)		(.066)	(.202)	(.065)
<i>GSDMB</i> / rs2305480						
GG	142 (57.5)	414 (55.5)	0.330	0.99,	0.91,	1.38,
GA	89 (36.0)	298 (39.9)		0.77-1.26	0.68-1.23	0.74-2.58
AA	16 (6.5)	34 (4.6)		(.905)	(.550)	(.309)
<i>SMAD3</i> / rs744910						
GG	117 (47.4)	299 (40.1)	0.116	0.80,	0.76,	0.75,
GA	104 (42.1)	346 (46.4)		0.64-1.00	0.56-1.01	0.47-1.19
AA	26 (10.5)	100 (13.4)		(.050)	(.061)	(.220)

OR, odds ratio; CI, confidence interval.

* Analyzed by binary logistic regression adjusting for age and gender as covariates.

Minor alleles were designated as risk alleles

Table S7 Association between asthma diagnosis and four target SNPs in the adult cohort

Genotype	Asthma (n=479)	All controls (n=746)	p-value by Pearson χ^2	OR, 95% CI (p*)		
	n (%)	n (%)		Additive model	Dominant model	Recessive model
<i>SLC22A5</i> / rs2073643						
TT	233 (49.1)	340 (45.8)	0.428	0.91,	0.83,	1.06,
TC	192 (40.4)	328 (44.2)		0.76-1.09	0.66-1.05	0.72-1.56
CC	50 (10.5)	74 (10.0)		(.303)	(.121)	(.757)
<i>IL13</i> / rs1295686						
CC	190 (39.9)	318 (42.9)	0.028	1.23,	1.18,	1.64,
CT	212 (44.5)	347 (46.8)		1.04-1.46	0.93-1.49	1.16-2.32
TT	74 (15.5)	77 (10.4)		(.019)	(.180)	(.005)
<i>GSDMB</i> / rs2305480						
GG	287 (60.4)	414 (55.5)	0.054	0.90,	0.81,	1.26,
GA	159 (33.5)	298 (39.9)		0.74-1.09	0.64-1.03	0.75-2.10
AA	29 (6.1)	34 (4.6)		(.273)	(.088)	(.390)
<i>SMAD3</i> / rs744910						
GG	215 (45.1)	299 (40.1)	0.170	0.85,	0.83,	0.76,
GA	210 (44.0)	346 (46.4)		0.71-1.01	0.66-1.05	0.53-1.09
AA	52 (10.9)	100 (13.4)		(.063)	(.122)	(.135)

OR, odds ratio; CI, confidence interval.

* Analyzed by binary logistic regression adjusting for age and gender as covariates.

Minor alleles were designated as risk alleles

Table S8 Meta-analysis of the association between asthma diagnosis and four target SNPs for two cohorts of school-age children and adults

SNP	Major allele	Minor allele*	Early-onset asthma			Total sample (all asthma)		
			OR (95% CI)	p**	p by Cochran's Q test**	OR (95% CI)	p**	p by Cochran's Q test**
<i>SLC22A5</i> / rs2073643	T	C	Additive model					
			0.90 (0.80 – 1.02)	.097	.458	0.89 (0.80 – 0.99)	.040	.759
			Dominant model					
			0.84 (0.72 – 0.99)	.032	.930	0.83 (0.72 – 0.96)	.014	.964
			Recessive model					
			0.99 (0.77 – 1.28)	.949	.150	0.95 (0.75 – 1.20)	.661	.460
<i>IL13</i> / rs1295686	C	T	Additive model					
			1.18 (1.05 – 1.33)	.007	.295	1.17 (1.05 – 1.30)	.004	.475
			Dominant model					
			1.26 (1.07 – 1.49)	.005	.865	1.24 (1.07 – 1.43)	.005	.612
			Recessive model					
			1.32 (0.79 – 2.21)	.284	.018	1.25 (0.86 – 1.82)	.237	.028
<i>GSDMB</i> / rs2305480	G	A	Additive model					
			0.75 (0.65 – 0.86)	<.001	.963	0.80 (0.71 – 0.91)	.001	.161
			Dominant model					
			0.69 (0.58 – 0.81)	<.001	1.000	0.73 (0.63 – 0.85)	<.001	.281
			Recessive model					
			0.85 (0.57 – 1.27)	.430	.988	1.01 (0.72 – 1.43)	.947	.274
<i>SMAD3</i> /	G	A	Additive model					

rs744910	0.93 (0.83 – 1.05)	.253	.596	0.91 (0.82 – 1.01)	.086	.308
	Dominant model					
	0.96 (0.81 – 1.13)	.589	.809	0.91 (0.79 – 1.06)	.216	.322
	Recessive model					
	0.84 (0.67 – 1.06)	.142	.442	0.84 (0.68 – 1.03)	.099	.515

OR, odds ratio; CI, confidence interval.

* Minor alleles were designated as risk alleles.

** p-values are shown for tests of association under a fixed-effects model, with tests of heterogeneity across the cohorts analyzed using Cochran's Q test; tests of association analyzed using a random-effects model when p-value of Cochran's Q test ≤ 0.10 which demonstrated apparent heterogeneity.

Table S9 Association between FEV₁ and four target SNPs for 445 asthmatics in the adult cohort

Genotype	All cases	FEV ₁ (%)	p-value by one-way ANOVA	P-value*		
	n (%)	Mean ± SD		Additive model	Dominant model	Recessive model
<i>SLC22A5</i> / rs2073643						
TT	216 (49.0)	87.1 ± 20.5	0.117	0.212	0.470	0.123
TC	179 (40.6)	87.7 ± 18.3				
CC	46 (10.4)	93.7 ± 21.6				
<i>IL13</i> / rs1295686						
CC	177 (40.0)	88.8 ± 18.4	0.154	0.855	0.531	0.228
CT	195 (44.0)	86.2 ± 19.9				
TT	71 (16.0)	91.1 ± 22.4				
<i>GSDMB</i> / rs2305480						
GG	268 (60.6)	90.7 ± 17.7	0.001	0.001	0.003	0.030
GA	147 (33.3)	84.4 ± 22.2				
AA	27 (6.1)	80.5 ± 21.0				
<i>SMAD3</i> / rs744910						
GG	197 (44.5)	88.5 ± 20.3	0.784	0.779	0.983	0.579
GA	197 (44.5)	87.3 ± 19.3				
AA	49 (11.1)	88.9 ± 19.5				

* Analyzed by linear regression adjusting for age, gender and current ICS treatment as covariates.

Minor alleles were designated as risk alleles

Table S10 Association between FVC and four target SNPs for 445 asthmatics in the adult cohort

Genotype	All cases	FVC (%)	p-value by one-way ANOVA	p-value*		
	n (%)	Mean $\pm$ SD		Additive model	Dominant model	Recessive model
<i>SLC22A5</i> / rs2073643						
TT	216 (49.0)	89.2 $\pm$ 17.5	0.340	.277	.255	.618
TC	179 (40.6)	90.9 $\pm$ 15.9				
CC	46 (10.4)	92.8 $\pm$ 18.8				
<i>IL13</i> / rs1295686						
CC	177 (40.0)	91.5 $\pm$ 16.3	0.130	.746	.343	.512
CT	195 (44.0)	88.4 $\pm$ 16.9				
TT	71 (16.0)	92.1 $\pm$ 18.6				
<i>GSDMB</i> / rs2305480						
GG	268 (60.6)	93.0 $\pm$ 15.5	<0.001	<.001	<.001	.074
GA	147 (33.3)	86.3 $\pm$ 18.3				
AA	27 (6.1)	84.6 $\pm$ 18.9				
<i>SMAD3</i> / rs744910						
GG	197 (44.5)	91.1 $\pm$ 17.3	0.273	.915	.577	.278
GA	197 (44.5)	88.8 $\pm$ 16.6				
AA	49 (11.1)	92.3 $\pm$ 17.0				

* Analyzed by linear regression adjusting for age, gender and current ICS treatment as covariates.

Minor alleles were designated as risk alleles

Table S11 Association between (FEV₁/FVC)² and genotyped SNPs among 451 asthmatics in the school-aged children cohort

Genotype	All cases	(FEV ₁ /FVC) ²	p-value by one-way ANOVA	p-value**		
	n (%)	Mean ± SD		Additive model	Dominant model	Recessive model
<i>SLC22A5</i> / rs2073643						
TT	214 (47.7)	0.690 ± 0.160	0.857	.654	.770	.617
TC	193 (43.0)	0.688 ± 0.169				
CC	42 (9.4)	0.674 ± 0.185				
<i>IL13</i> / rs1295686						
CC	163 (36.3)	0.698 ± 0.171	0.297	.727	.274	.384
CT	227 (50.6)	0.676 ± 0.165				
TT	59 (13.1)	0.706 ± 0.158				
<i>GSDMB</i> / rs2305480						
GG	298 (66.1)	0.683 ± 0.165	0.027	.987	.404	.035
GA	137 (30.4)	0.710 ± 0.168				
AA	16 (3.5)	0.598 ± 0.159				
<i>IL2RB</i> / rs2284033						
AA	123 (27.3)	0.665 ± 0.164	0.169	.085	.066	.346
AG	226 (50.1)	0.695 ± 0.164				
GG	102 (22.6)	0.702 ± 0.175				
<i>IL33</i> / rs1342326*						
AA	444 (98.7)	0.689 ± 0.165	0.082	NA	.098	NA
AC	6 (1.3)	0.570 ± 0.236				
CC	0	NA				
<i>RORA</i> / rs11071559						
CC	304 (67.4)	0.681 ± 0.166	0.308	.085	.123	.244
CT	137 (30.4)	0.699 ± 0.169				
TT	10 (2.2)	0.747 ± 0.128				
<i>SMAD3</i> / rs744910						

GG	170 (37.9)	0.700 ± 0.164				
GA	217 (48.3)	0.685 ± 0.172	0.292	.090	.150	.192
AA	62 (13.8)	0.662 ± 0.153				
<i>IL18R1</i> /						
rs3771166						
GG	369 (84.2)	0.687 ± 0.171				
GA	66 (15.1)	0.692 ± 0.144	0.751	.655	.783	.375
AA	3 (0.7)	0.758 ± 0.070				

NA, not applicable.

* Heterozygous versus homozygous

** By linear regression adjusting for age, gender and current ICS treatment as covariates.

Minor alleles were designated as risk alleles

Table S12 Association between FEV₁/FVC and four target SNPs for 445 asthmatics in the adult cohort

Genotype	All cases	FEV ₁ /FVC	p-value by	p-value*		
	n (%)	Mean ± SD	one-way ANOVA	Additive model	Dominant model	Recessive model
<i>SLC22A5</i> / rs2073643						
TT	216 (49.0)	0.802 ± 0.115	0.124	.623	.779	.121
TC	179 (40.6)	0.792 ± 0.107				
CC	46 (10.4)	0.829 ± 0.082				
<i>IL13</i> / rs1295686						
CC	177 (40.0)	0.798 ± 0.106	0.206	.328	.858	.095
CT	195 (44.0)	0.796 ± 0.105				
TT	71 (16.0)	0.822 ± 0.125				
<i>GSDMB</i> / rs2305480						
GG	268 (60.6)	0.806 ± 0.099	0.354	.212	.443	.106
GA	147 (33.3)	0.795 ± 0.123				
AA	27 (6.1)	0.780 ± 0.115				
<i>SMAD3</i> / rs744910						
GG	197 (44.5)	0.800 ± 0.112	0.881	.609	.459	.939
GA	197 (44.5)	0.803 ± 0.108				
AA	49 (11.1)	0.794 ± 0.100				

* Analyzed by linear regression adjusting for age, gender and current ICS treatment as covariates.

Minor alleles were designated as risk alleles

Table S13 Meta-analysis of the association between lung function phenotypes and four target SNPs for asthmatics in two cohorts of school-age children and adults

SNP	Major allele	Minor allele*	FEV ₁			log ₁₀ FVC			(FEV ₁ /FVC) ²		
			β (SE)	p**	p by Cochran's Q test**	β (SE)	p**	p by Cochran's Q test**	β (SE)	p**	p by Cochran's Q test**
<i>SLC22A5</i> / rs2073643	T	C	Additive model								
			0.062 (0.034)	.065	.961	0.069 (0.033)	.037	.533	-0.004 (0.034)	.917	.592
			Dominant model								
			0.058 (0.034)	.087	.516	0.077 (0.033)	.021	.557	-0.017 (0.034)	.607	.915
			Recessive model								
			0.040 (0.034)	.232	.320	0.024 (0.033)	.472	.710	0.021 (0.034)	.529	.173
<i>IL13</i> / rs1295686	C	T	Additive model								
			0.018 (0.034)	.589	.786	0.016 (0.033)	.634	.235	0.019 (0.034)	.570	.283
			Dominant model								
			0.002 (0.034)	.957	.354	0.017 (0.044)	.706	.051	-0.020 (0.034)	.551	.340
			Recessive model								
			0.032 (0.034)	.339	.450	0.005 (0.033)	.872	.691	0.066 (0.034)	.049	.450
<i>GSDMB</i> / rs2305480	G	A	Additive model								
			-0.128 (0.033)	<.001	.404	-0.048 (0.125)	.702	<.001	-0.023 (0.034)	.500	.475
			Dominant model								
			-0.121 (0.033)	<.001	.492	-0.055 (0.119)	.642	<.001	0.010 (0.034)	.772	.368
			Recessive model								
			-0.102 (0.033)	.002	.932	-0.009 (0.066)	.888	.015	-0.088 (0.034)	.009	.739

SMAD3/ rs744910	G	A	Additive model								
			-0.020 (0.034)	.560	.404	0.017 (0.033)	.615	.627	-0.031 (0.034)	.359	.136
			Dominant model								
			-0.039 (0.034)	.253	.255	-0.020 (0.033)	.555	.759	-0.019 (0.034)	.579	.135
			Recessive model								
			0.016 (0.034)	.630	.760	0.063 (0.033)	.060	.647	-0.035 (0.034)	.297	.423

β , effect-size estimates due to the tested SNP after adjustment for age, gender and current ICS treatment as covariates; SE, standard error.

* Minor alleles were designated as risk alleles.

** p-values are shown for tests of association under a fixed-effects model, with tests of heterogeneity across the cohorts analyzed using Cochran's Q test; tests of association analyzed using a random-effects model when p-value of Cochran's Q test ≤ 0.10 which demonstrated apparent heterogeneity.

Table S14 Association between FEV₁ and four target SNPs for 744 controls in the adult cohort

	All controls	FEV ₁ (%)	p-value	p-value*		
Genotype	n (%)	Mean ± SD	by one-way ANOVA	Additive model	Dominant model	Recessive model
<i>SLC22A5</i> / rs2073643						
TT	338 (45.7)	103.4 ± 13.2	0.127	0.084	0.153	0.161
TC	327 (44.2)	105.1 ± 13.4				
CC	74 (10.0)	106.4 ± 16.7				
<i>IL13</i> / rs1295686						
CC	315 (42.6)	104.4 ± 13.8	0.968	0.636	0.679	0.732
CT	347 (47.0)	104.6 ± 13.7				
TT	77 (10.4)	104.8 ± 13.6				
<i>GSDMB</i> / rs2305480						
GG	412 (55.5)	103.9 ± 13.4	0.239	0.574	0.263	0.273
GA	297 (40.0)	105.5 ± 14.4				
AA	34 (4.6)	102.9 ± 10.7				
<i>SMAD3</i> / rs744910						
GG	299 (40.3)	104.0 ± 13.4	0.024	0.054	0.386	0.009
GA	346 (46.6)	104.0 ± 14.0				
AA	97 (13.1)	108.1 ± 13.2				

* Analyzed by linear regression adjusting for age and gender as covariates.

Minor alleles were designated as risk alleles

Table S15 Association between FVC and four target SNPs for 744 controls in the adult cohort

Genotype	All controls	FVC (%)	p-value by one-way ANOVA	p-value*		
	n (%)	Mean $\pm$ SD		Additive model	Dominant model	Recessive model
<i>SLC22A5</i> / rs2073643						
TT	338 (45.7)	94.0 $\pm$ 12.4	0.194	0.225	0.217	0.545
TC	327 (44.2)	95.8 $\pm$ 13.9				
CC	74 (10.0)	95.6 $\pm$ 14.3				
<i>IL13</i> / rs1295686						
CC	315 (42.6)	94.9 $\pm$ 13.3	0.694	0.284	0.593	0.154
CT	347 (47.0)	94.8 $\pm$ 13.0				
TT	77 (10.4)	96.2 $\pm$ 14.6				
<i>GSDMB</i> / rs2305480						
GG	412 (55.5)	94.3 $\pm$ 13.1	0.168	0.526	0.206	0.215
GA	297 (40.0)	96.1 $\pm$ 13.6				
AA	34 (4.6)	93.5 $\pm$ 11.1				
<i>SMAD3</i> / rs744910						
GG	299 (40.3)	94.8 $\pm$ 17.3	0.020	0.165	0.840	0.012
GA	346 (46.6)	94.2 $\pm$ 13.6				
AA	97 (13.1)	98.4 $\pm$ 12.6				

* Analyzed by linear regression adjusting for age and gender as covariates.

Minor alleles were designated as risk alleles

Table S16 Association between FEV₁/FVC and four target SNPs for 744 controls in the adult cohort

Genotype	All controls	FEV1/FVC	P-value by one-way ANOVA	P-value*		
	n (%)	Mean $\pm$ SD		Additive model	Dominant model	Recessive model
<i>SLC22A5</i>						
/						
rs2073643	338	0.886 $\pm$ 0.064	0.253	0.315	0.357	0.501
TT	(45.7)	0.893 $\pm$ 0.059				
TC	327	0.893 $\pm$ 0.053				
CC	(44.2)					
	74 (10.0)					
<i>IL13</i> /						
rs1295686						
CC	315	0.892 $\pm$ 0.060	0.074	0.333	0.792	0.099
CT	(42.6)	0.891 $\pm$ 0.061				
TT	347	0.875 $\pm$ 0.063				
	(47.0)					
	77 (10.4)					
<i>GSDMB</i> /						
rs2305480						
GG	412	0.890 $\pm$ 0.061	0.641	0.673	0.552	0.814
GA	(55.5)	0.888 $\pm$ 0.061				
AA	297	0.899 $\pm$ 0.057				
	(40.0)					
	34 (4.6)					
<i>SMAD3</i> /						
rs744910						
GG	299	0.888 $\pm$ 0.061	0.771	0.680	0.591	0.962
GA	(40.3)	0.891 $\pm$ 0.060				
AA	346	0.893 $\pm$ 0.064				
	(46.6)					
	97 (13.1)					

* Analyzed by linear regression adjusting for age and gender as covariates.

Minor alleles were designated as risk alleles

Table S17 Multi-locus interaction models for $\log_{10}$ FVC in school-age children with asthma

Number of locus	SNP combination	CVC	TA (%)	p*
1	<i>SLC22A5</i> _rs2073643	6	56.92	0.038
2	<i>SLC22A5</i>_rs2073643, <i>IL13</i>_rs1295686	9	58.60	0.018
3	<i>SLC22A5</i> _rs2073643, <i>IL13</i> _rs1295686, <i>SMAD3</i> _rs744910	6	56.84	0.067
4	<i>SLC22A5</i>_rs2073643, <i>IL13</i>_rs1295686, <i>IL2RB</i>_rs2284033, <i>SMAD3</i>_rs744910	10	57.86	0.039
5	<i>SLC22A5</i>_rs2073643, <i>IL13</i>_rs1295686, <i>GSDMB</i>_rs2305480, <i>IL2RB</i>_rs2284033, <i>SMAD3</i>_rs744910	10	59.89	0.014
6	<i>SLC22A5</i> _rs2073643, <i>IL13</i> _rs1295686, <i>GSDMB</i> _rs2305480, <i>IL2RB</i> _rs2284033, <i>RORA</i> _rs11071559, <i>SMAD3</i> _rs744910	10	50.48	0.458
7	<i>SLC22A5</i> _rs2073643, <i>IL13</i> _rs1295686, <i>GSDMB</i> _rs2305480, <i>IL2RB</i> _rs2284033, <i>RORA</i> _rs11071559, <i>SMAD3</i> _rs744910, <i>IL18R1</i> _rs3771166	10	57.81	0.069
8	<i>SLC22A5</i> _rs2073643, <i>IL13</i> _rs1295686, <i>GSDMB</i> _rs2305480, <i>IL2RB</i> _rs2284033, <i>IL33</i> _rs1342326, <i>RORA</i> _rs11071559, <i>SMAD3</i> _rs744910, <i>IL18R1</i> _rs3771166	10	57.08	0.083

CVC, cross-validation consistency; SNP, single-nucleotide polymorphism; TA, testing accuracy.

* Based on 5000 permutations.

Table S18 Multi-locus interaction models for FEV₁, FVC and FEV₁/FVC for subjects in the adult cohort

Number of locus	SNP combination	CVC	TA (%)	p*
<i>FEV₁</i>				
1	<i>GSDMB_rs2305480</i>	10	59.63	0.003
2	<i>IL13_rs1295686, GSDMB_rs2305480</i>	10	61.36	0.002
3	<i>SLC22A5_rs2073643, IL13_rs1295686, SMAD3_rs744910</i>	9	56.93	0.043
4	<i>SLC22A5_rs2073643, IL13_rs1295686, GSDMB_rs2305480, SMAD3_rs744910</i>	10	59.37	0.019
<i>FVC</i>				
1	<i>GSDMB_rs2305480</i>	10	60.58	0.001
2	<i>SLC22A5_rs2073643, IL13_rs1295686</i>	10	59.47	0.010
3	<i>SLC22A5_rs2073643, IL13_rs1295686, GSDMB_rs2305480</i>	7	53.87	0.197
4	<i>SLC22A5_rs2073643, IL13_rs1295686, GSDMB_rs2305480, SMAD3_rs744910</i>	10	46.25	0.794
<i>FEV₁/FVC</i>				
1	<i>SLC22A5_rs2073643</i>	4	48.23	0.680
2	<i>SLC22A5_rs2073643, SMAD3_rs744910</i>	9	57.36	0.022
3	<i>IL13_rs1295686, GSDMB_rs2305480, SMAD3_rs744910</i>	8	60.56	0.009
4	<i>SLC22A5_rs2073643, IL13_rs1295686, GSDMB_rs2305480, SMAD3_rs744910</i>	10	57.95	0.004

CVC, cross-validation consistency; SNP, single-nucleotide polymorphism; TA, testing accuracy.

* Based on 5000 permutations.

Table S19 Multi-locus interaction models for $FEV_{0.75}/FVC$ and $FEV_{0.5}/FVC$ for children in the preschool children cohort

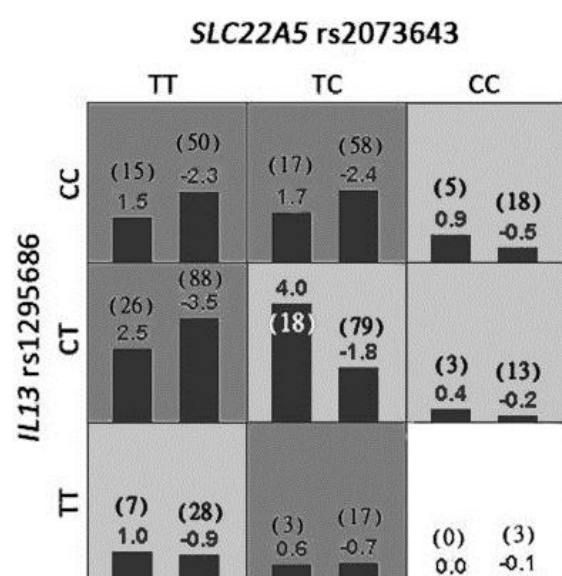
Number of locus	SNP combination	CVC	TA (%)	p*
<i>FEV_{0.75}/FVC</i>				
1	<i>SMAD3</i> _rs744910	4	51.34	0.298
2	<i>SLC22A5</i>_rs2073643, <i>SMAD3</i>_rs744910	10	55.18	0.017
3	<i>SLC22A5</i> _rs2073643, <i>GSDMB</i> _rs2305480, <i>SMAD3</i> _rs744910	8	51.33	0.323
4	<i>SLC22A5</i> _rs2073643, <i>IL13</i> _rs1295686, <i>GSDMB</i> _rs2305480, <i>SMAD3</i> _rs744910	10	47.58	0.819
<i>FEV_{0.5}/FVC</i>				
1	<i>SLC22A5</i> _rs2073643	7	50.03	0.517
2	<i>SLC22A5</i>_rs2073643, <i>SMAD3</i>_rs744910	10	54.40	0.039
3	<i>SLC22A5</i> _rs2073643, <i>GSDMB</i> _rs2305480, <i>SMAD3</i> _rs744910	8	50.60	0.419
4	<i>SLC22A5</i> _rs2073643, <i>IL13</i> _rs1295686, <i>GSDMB</i> _rs2305480, <i>SMAD3</i> _rs744910	10	46.69	0.897

CVC, cross-validation consistency; SNP, single-nucleotide polymorphism; TA, testing accuracy.

* Based on 5000 permutations.

Fig. S1 (a) Best two-locus model for patients' $\log_{10}$ FVC derived from GMDR analysis in the discovery cohort. High-risk genotypes are in dark grey and low-risk genotypes are in light grey, and white cells indicate unclassified genotypes due to small numbers of subjects. Left bars represent asthmatics who have low FEV_1 ($< 80\%$) while right bars are those with normal FEV_1 ($\geq 80\%$). The figures above the bars in each cell are score, whereas those in brackets represent the number of subjects in each subgroup. The length of bars represents the absolute values of score. (b) Distribution of $\log_{10}$ FVC in patients in the discovery cohort with high-risk and low-risk genotypes defined by 2-locus model of *SLC22A5*_rs2073643 and *IL13*_rs1295686 ($p < 0.0001$ by Student *t* test).

(a)



(b)

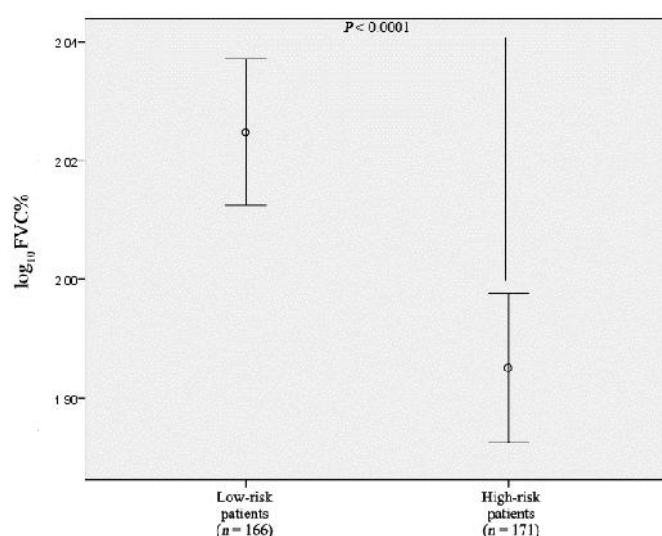
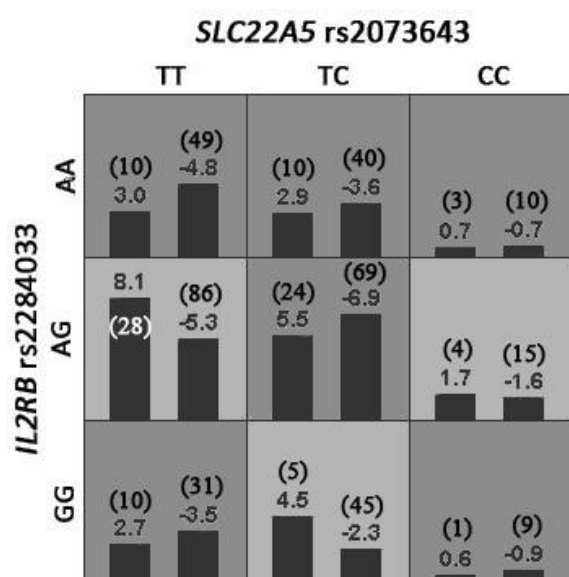


Fig. S2 (a) Best two-locus model for $(FEV_1/FVC)^2$ derived from GMDR analysis of asthmatics in the discovery cohort. High-risk genotypes are in dark grey and low-risk genotypes are in light grey. Left bars represent asthmatics who have low FEV_1 ($< 80\%$) while right bars are those with normal FEV_1 ($\geq 80\%$). The figures above the bars in each cell are score, whereas those in brackets represent the number of subjects in each subgroup. The length of bars represents the absolute values of score. (b) Distribution of $(FEV_1/FVC)^2$ in the discovery cohort with high-risk and low-risk genotypes defined by 2-locus model of *SLC22A5*_rs2073643 and *IL2RB*_rs2284033 ($p = 0.003$ by Student t test).

(a)



(b)

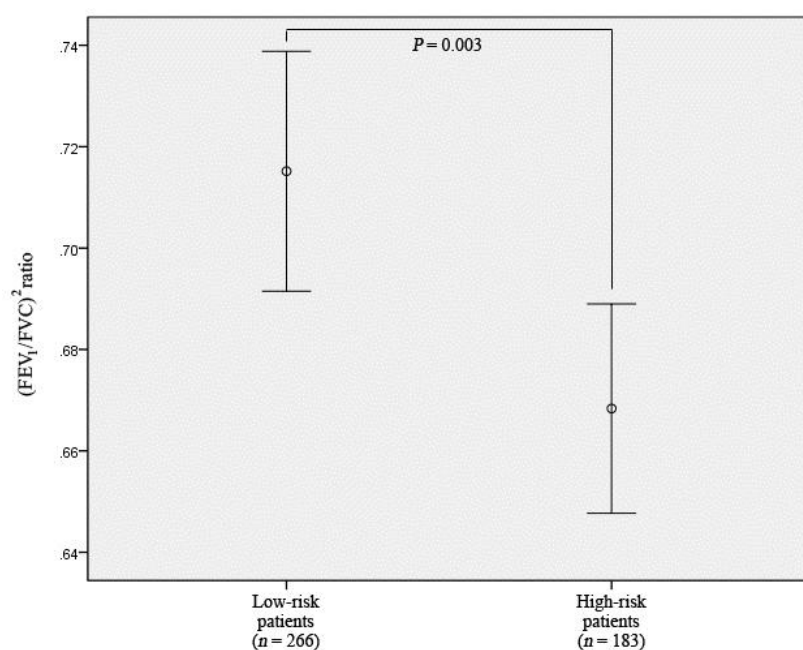
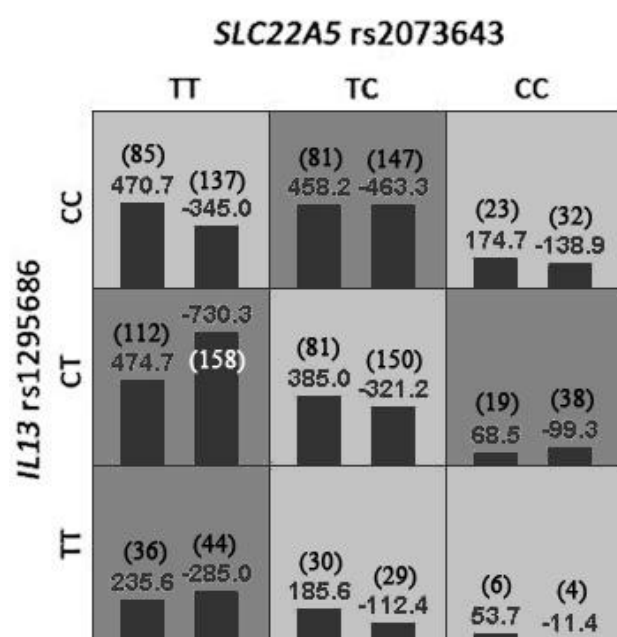


Fig. S3 (a) Best two-locus model for patients' FVC derived from GMDR analysis in the second replication cohort. High-risk genotypes are in dark grey and low-risk genotypes are in light grey. Left bars represent asthmatics while right bars are controls. The figures above the bars in each cell are score, whereas those in brackets represent the number of subjects in each subgroup. The length of bars represents the absolute values of score. (b) Distribution of FVC in subjects in the second replication cohort with high-risk and low-risk genotypes defined by 2-locus model of *SLC22A5*_rs2073643 and *IL13*_rs1295686 ($p < 0.0001$ by ANOVA).

(a)



(b)

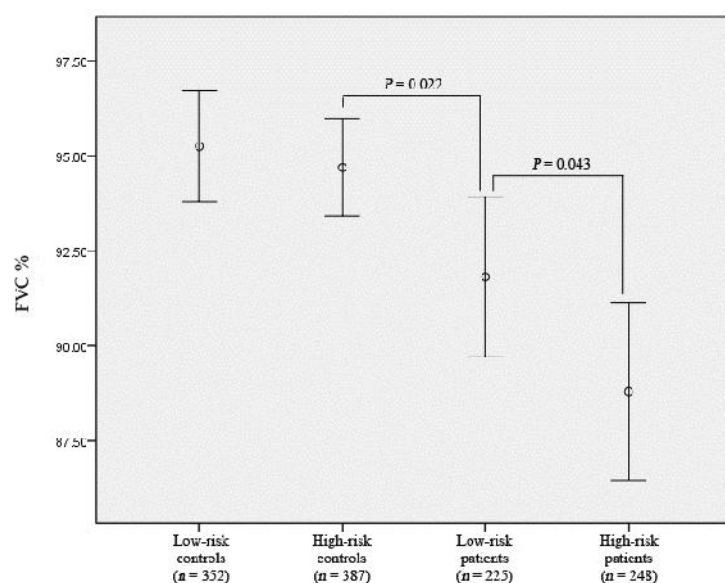
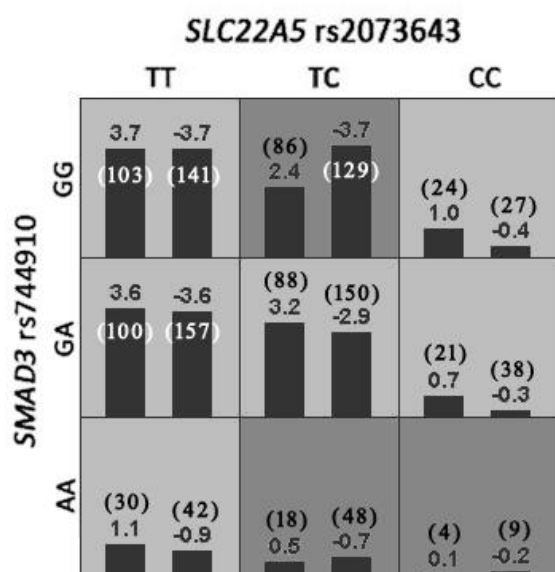


Fig. S4 (a) Best two-locus model for subjects' FEV₁/FVC derived from GMDR analysis in the second replication cohort. High-risk genotypes are in dark grey and low-risk genotypes are in light grey. Left bars represent asthmatics while right bars are controls. The figures above the bars in each cell are score, whereas those in brackets represent the number of subjects in each subgroup. The length of bars represents the absolute values of score. (b) Distribution of FEV₁/FVC in subjects under the second replication cohort with high-risk and low-risk genotypes defined by 2-locus model of *SLC22A5*_rs2073643 and *SMAD3*_rs744910 ($p < 0.0001$ by ANOVA).

(a)



(b)

