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Association between candidate genes and lung function growth in Chinese asthmatic children

T. F. Leung*, I. H. S. Chan†, G. W. K. Wong*, C. Y. Li*, N. L. S. Tang†, E. Yung* and C. W. K. Lam†

Departments of *Paediatrics and †Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong

Clinical and Experimental Allergy

Summary

Background Asthma is caused by a complex interaction between multiple candidate genes and environmental factors. The Childhood Asthma Management Program reported lung function decline in a significant proportion of Caucasian asthmatic children, but such a relation has not been studied in other populations. Our group recently reported that interleukin-13 (*IL13*), interleukin-4 receptor- α and thymus and the activation-regulated chemokine interacted to influence asthma and raised plasma total IgE. However, there has not been any study that has addressed the genetic influences for longitudinal lung function growth.

Objective We studied the longitudinal changes in spirometric variables in Chinese asthmatic children, and investigated the influence and interactions between eight different loci in six candidate genes as well as environmental factors affecting lung function growth in these children.

Methods Spirometry was performed at baseline and study completion. Genotyping was performed by restriction fragment length polymorphism. Multi-factor dimensionality reduction (MDR) was used to detect any gene–gene or gene–environment interaction.

Results We prospectively followed 131 Chinese children, aged 9.9 (3.0) years, for 4.5 (0.8) years. Their mean (standard deviation) baseline forced expiratory volume in 1 s (FEV₁) was 98.6 (20.6)% of predicted, and FEV₁ to forced vital capacity (FVC) ratio was 77.8 (11.3)%. FEV₁ and FVC increased by 210 (115) and 248 (148) mL/year during this study, and these changes were significantly larger among males ($P < 0.0001$). Univariate analysis revealed a significant association between annual FEV₁ change and C1570T of signal transducer and activator of transcription 6 gene (*STAT6*; $P = 0.009$). Linear regression confirmed this finding ($P = 0.041$). Using MDR, we detected a significant 3-locus interaction between *IL13* R130Q, *ADRB2* R16G and *STAT6* C1570T for determining change in FVC ($P = 0.045$).

Conclusion Our data suggest that *STAT6* may influence lung function growth in asthmatic children. We also found significant interactions among several atopy-related genetic polymorphisms for influencing FVC change.

Keywords asthma, candidate gene, children, gene interaction, growth, spirometry, *STAT6*

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Correspondence:

Ting-Fan Leung, Department of Paediatrics, The Chinese University of Hong Kong, 6/F, Clinical Sciences Building, Prince of Wales Hospital, Shatin, Hong Kong.
E-mail: ttleung@cuhk.edu.hk

Introduction

Asthma is one of the most common chronic respiratory diseases. It is a complex disease resulting from interactions between multiple genes and environmental factors [1, 2]. The Childhood Asthma Management Program (CAMP) reported its experience on long-term follow-up for these patients, and found that a proportion of asthmatic children had worsening lung function with time. A number of demographic and clinical factors (e.g. age,

baseline lung function) might be useful in predicting significant lung function decline in these Caucasian children [3]. On the other hand, the genetic determinants for this secular change in lung function of asthmatic children have not been explored. Although many genes have been reported to be associated with asthma [4–6], not a single polymorphic marker or gene locus was found to have a strong and independent effect on asthma susceptibility [7, 8]. Therefore, searching for candidate genes for asthma phenotypes requires a thorough understanding of

gene–gene and gene–environment interactions. Traditionally, gene–gene interactions in complex diseases have been examined by logistic regression, multi-locus linkage disequilibrium and Hardy–Weinberg equilibrium testing. These statistical methods require a large sample size, which greatly limits their general application [9]. Hahn *et al.* [10] developed a software called multi-factor dimensionally reduction (MDR) to detect high-order gene–gene interactions in case–control studies with relatively small samples. Our group was the first to apply MDR for asthma phenotypes, and found R130Q of interleukin-13 gene (*IL13*) together with I50V of the interleukin-4 receptor- α gene (*IL4RA*) and C-431T of thymus and the activation-regulated chemokine gene (*TARC*), respectively, to confer increased risks for asthma and increased plasma total IgE concentration in Chinese children [11].

Apart from these three genes, we also genotyped in this study the single nucleotide polymorphisms (SNPs) T1309C and C1570T of the signal transducer and activator of transcription 6 (*STAT6*) and G-401A of regulated on activation, normal T cell expressed and secreted (*RANTES*). *STAT-6* is an important signalling molecule in the type 2 T-helper lymphocyte (Th2)-related pathway, and polymorphic markers of *STAT6* conferred risk for asthma and atopy in various ethnic groups. Different combinations of alleles in genes (including *STAT6*) along the Th2 pathway also increased the risk of developing asthma and elevated serum IgE levels [12–14]. On the other hand, *RANTES* attracts atopy-related leucocytes to sites of allergic inflammation. SNPs at positions –28 and –401 of *RANTES* promoter were associated with near-fatal asthma in Chinese children and asthma diagnosis in the Caucasian population [15–17]. Our previous study found –28G to be rare in Chinese, but asthmatic children who were homozygous for –401A had poorer lung function [18].

We also studied two functional SNPs, R16G and E27Q, in the β_2 -adrenergic receptor gene (*ADRB2*) [19] that

modulates airway smooth muscle tone. The β -adrenergic response by the genotype clinical trial indicated that R16G affected the long-term response to β -agonist treatment [20]. In the multi-centre Human Genome Epidemiology review, these *ADRB2* SNPs were shown to interact with each other and were also associated with altered risks for asthma in children [21]. In this study, the relation between longitudinal 'growth' in spirometric variables and these eight SNPs (Table 1) in our Chinese asthmatic children were analysed by linear regression, and the possible interactions between these loci and environmental factors were examined using the MDR method.

Patients and methods

Study population

This study recruited a cohort of unrelated, southern Chinese children aged 5–15 years with asthma, diagnosed according to the American Thoracic Society guideline [22], from paediatric clinics of a university teaching hospital in Hong Kong. The same investigator (T.F.L.) prospectively followed these patients for 4 years. All subjects were seen at least every 6 months for assessment of their asthma control, exposure to environmental factors and medication use. They were free from any self-reported respiratory tract infection for 4 weeks, and any asthmatic exacerbation for 3 months, before spirometric measurements. Their anti-asthma treatments also remained unchanged for at least 3 months before spirometry. Information on the presence of potential environmental risk factors for asthma and allergy [e.g. environmental tobacco smoke (ETS) exposure, early feeding practice and pet-keeping] was collected using questionnaire items adapted from the International Study of Asthma and Allergy of Childhood [23]. Subjects' parents gave written consent, and the Clinical Research Ethics Committee of our university approved this study.

Table 1. Candidate gene polymorphisms included in this study

Chromosomal location	Candidate gene	Polymorphism	Minor allele	Reported associations	MAF in our patients
5q31–33	<i>IL13</i>	R130Q	130Q	IgE, asthma	0.407
16p12	<i>IL4RA</i>	I50V	50V	Atopy, IgE, atopic asthma	0.458
12q13–14	<i>STAT6</i>	T1309C	C	IgE	0.296
		C1570T	T		0.338
16q13	<i>TARC</i>	C-431T	T	Atopy, asthma	0.338
17p12–q11	<i>RANTES</i>	G-401A	A	Asthma, atopy, lung function	0.345
5q32–34	<i>ADRB2</i>	R16G	16G	Asthma, IgE, BHR, bronchodilator response	0.439
		E27Q	27E		0.118

BHR, bronchial hyper-responsiveness; MAF, minor allele frequency; *IL13*, interleukin-13; *IL4RA*, interleukin-4 receptor- α ; *STAT6*, signal transducer and activator of transcription 6; *TARC*, thymus and the activation-regulated chemokine; *RANTES*, regulated on activation, normal T cell expressed and secreted; *ADRB2*, β_2 -adrenergic receptor gene.

Measurement of allergen-specific immunoglobulin E

Specific IgE antibodies to *Dermatophagoides pteronyssinus*, cat and mixed cockroaches in plasma were measured by AutoCAP (Phadia, Uppsala, Sweden), and a concentration ≥ 0.35 kIU/L was considered to be positive. Children with at least one positive allergen-specific IgE were classified as atopic [24].

Lung function assessment

All subjects underwent lung function assessments at baseline and then at least yearly until the completion of a 4-year follow-up period using COMPACT II spirometer (Vitalograph, Buckingham, UK) and according to the American Thoracic Society guideline [25]. Their measured forced expiratory volumes in 1 s (FEV₁) and forced vital capacity (FVC) were compared with published references for local Chinese children [26]. Short-acting and long-acting β -agonists were stopped for at least 6 and 12 h, respectively, before spirometry. Inhaled corticosteroids (ICS) were allowed, but dosage had to be unchanged within 3 months before spirometric measurements.

Single nucleotide polymorphism genotyping

Polymerase chain reaction and restriction fragment length polymorphism were used to genotype the eight SNPs in this study (Table 1). The genotyping protocols were described in detail in our published studies [11, 18, 27, 28]. The results obtained by these molecular assays were confirmed by direct sequencing for each SNP in 15 randomly selected samples using Big Dye Terminator Cycle sequencing kits with an ABI-310 gene sequencer (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

The allele frequencies for eight SNPs were estimated by the gene-counting method, and χ^2 was used to examine the Hardy–Weinberg equilibrium. The associations of annual growths of FEV₁ and FVC with individual genotypes for each SNP or environmental factors were analysed by univariate analysis of variance (ANOVA). The loci showing significant associations with these two phenotypes (with $P < 0.1$) were then tested with multi-variate linear regression for any independent association, adjusting for age, gender, change in body height during the study period, ICS use *ever* (at baseline or during the study period) and respective baseline spirometric parameters as covariates.

MDR (v0.3.1, Computational Genetics Laboratory, Dartmouth Medical School, Hanover, NH, USA or <http://www.epistasis.org>) was used to examine any gene–gene or gene–environment interaction for annual changes in

FEV₁ and FVC. This statistical method determines one model, either single or multi-locus, that most successfully predicts a phenotype from several loci and/or environmental factors that can be divided into high-risk and low-risk combinations [10, 11, 29]. Briefly, cross-validation (CV) consistency and prediction error were calculated for each combination of a pool of genetic polymorphisms and/or environmental factors. When CV consistency is maximal for one model and prediction error is minimal for another, statistical parsimony is used to choose the best model. We determined statistical significance by comparing the average prediction error from the observed data with the distribution of average prediction errors under the null hypothesis of no association derived empirically from 5000 permutations. The null hypothesis was rejected when the P -value derived from the permutation test was 0.05 or lower. In the present analysis, absolute changes in FEV₁ and FVC were categorized into upper, middle and lower tertiles. MDR was then applied to analyse any association between individual SNPs or environmental factors and FEV₁ or FVC changes in the upper tertile (i.e. low risk) and lower tertile (i.e. high risk).

Results

Subjects

One hundred and thirty-one asthmatic children were recruited and Table 2 describes their demographic and clinical characteristics. The mean [standard deviation (SD)] age of these patients at study baseline was 9.9 (3.0) years, and two-thirds of them were males. They were followed-up for 4.5 (0.8) years before the end-of-study spirometric measurements. Seventy-five (57.3%) patients had *ever* received ICS treatment during this study, and 11 (14.7%) of them had a concurrent long-acting β -agonist. None of these patients received a leukotriene modifier throughout the study period. Their median (inter-quartile range) change in FEV₁ and FVC during this study was 14.8 (8.7–20.5)% and 13.0 (7.8–19.8)%, respectively. Only two (1.5%) patients had $\geq 1\%$ decline in FEV₁ per year of follow-up.

Influence of environmental and genetic factors on lung function growth

The longitudinal changes in FEV₁ and FVC were significantly larger among males than females ($P < 0.0001$ for both). The respective mean (SD) values were 0.345 (0.112) vs. 0.137 (0.083) mL/year for FEV₁, and 0.292 (0.142) vs. 0.158 (0.115) mL/year for FVC. These parameters, on the other hand, were not associated with ETS exposure, pet keeping, breastfeeding ever or ICS use ever (results not shown). Table 1 reports the minor allele frequencies of eight SNPs in our patients. All of them followed

Table 2. Demographic and clinical features of 131 participants

Characteristic	Value
Age at recruitment, years	9.9 ± 3.0
Male gender (%)	88 (67.2)
ETS exposure* (%)	43 (32.8)
Pet-keeping* (%)	5 (3.8)
Breastfeeding ever (%)	38 (29.0)
ICS treatment ever* (%)	75 (57.3)
Positive allergen-specific IgE [†]	
<i>Dermatophagoides pteronyssinus</i> (%)	116 (88.5)
Cat (%)	19 (14.5)
Mixed cockroaches (%)	42 (32.1)
Atopy (with ≥1 allergen-specific IgE) [†] (%)	118 (90.1)
Baseline spirometry [‡]	
FEV ₁ (%)	98.6 ± 20.6
FVC (%)	119.9 ± 28.1
FEV ₁ to FVC ratio	0.78 ± 0.11
End-of-study spirometry [‡]	
FEV ₁ (%)	91.5 ± 16.6
FVC (%)	103.6 ± 14.4
FEV ₁ to FVC ratio	0.81 ± 0.14
Annual changes in spirometric variables	
FEV ₁ (mL/year)	210 ± 115
FVC (mL/year)	248 ± 148

Results expressed as either number (percentage) or mean ± SD.

*Refer to either baseline or throughout the study period.

[†]Blood samples available in 130 patients.

[‡]Results compared with published sex- and height-matched reference values [26].

ETS, environmental tobacco smoke; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; ICS, inhaled corticosteroid.

Hardy–Weinberg equilibrium. The overall success rate of our genotyping was 99.3%, with failure in defining results only for seven SNPs. Table 3 summarizes the relation between the changes in both FEV₁ and FVC and individual SNPs. *STAT6* C1570T was significantly associated with the annual 'growth' of FEV₁ ($P=0.009$). Multivariate linear regression was then performed to analyse genetic loci that were possibly linked to the two spirometric variables. The association between change in FEV₁ and *STAT6* C1570T remained significant ($\beta=0.164$; $P=0.041$) after adjusting for age, gender, change in body height, baseline FEV₁ and ICS use ever as covariates. None of the polymorphisms was significantly associated with FVC change.

Multi-factor dimensionality reduction analysis

Table 4 summarizes the interactions among studied SNPs for spirometric indices. There was no significant gene–gene interaction for FEV₁ change as analysed using MDR. In contrast, *IL13* R130Q, *ADRB2* R16G and *STAT6* C1570T interacted and were associated with FVC change

in our patients (CV consistency 9.6, prediction error 32.8%; $P=0.045$). MDR was repeated after including the important environmental factors of tobacco smoke exposure, pet keeping and breastfeeding ever. There was no significant gene-by-environment interaction with these factors for either FEV₁ or FVC changes (data not shown).

Discussion

In this study, 131 Chinese asthmatic children were followed prospectively for 4 years. Their allergic sensitization and exposures to environmental factors such as ETS and pets were recorded, and eight SNPs in six asthma candidate genes were characterized. We reported for the first time the data on annual lung function 'growth' in these children. Both FEV₁ and FVC increased on follow-up in most cases, whereas a significant decline in FEV₁ was found only in two patients. Environmental factors did not influence longitudinal changes in spirometric variables over this study period. On the other hand, C1570T of *STAT6* may be a useful genetic predictor of longitudinal FEV₁ change in our Chinese children. This study also found significant interactions among a number of SNPs to modulate FVC change.

Pathological findings of airway remodelling can be found in bronchial biopsies from both adults and children with asthma [30, 31]. Clinically, adult asthmatics had an accelerated loss of lung function as compared with those without asthma [32, 33]. Such information is lacking for children with asthma. Data from CAMP suggested that about one-quarter of the children with mild–moderate asthma showed a progressive decline in pre-bronchodilator FEV₁ of ≥1% per year of asthma duration [3, 34]. Younger age, male sex, clinical centre and higher post-bronchodilator FEV₁ predicted this significant reduction of FEV₁ during follow-up. Lung function changes were similar among patients who received ICS, nedocromil or placebo. Similar findings were reported in a cohort of steroid-naïve children with asthma who were followed for up to 7 years [35]. On the other hand, only two of our Chinese patients showed this significant degree of lung function decline. Further research should delineate the mechanisms leading to this slow lung growth velocity in children with asthma. Besides, the reasons for this inter-ethnic difference in lung function decline remain unclear as all studies were conducted in asthmatic children for at least 4 years. The main difference between our study and CAMP was that our patients were older than the latter one (mean age 9.9 vs. 8.9 years), and CAMP results found this factor to be significantly related to lung function decline [3]. Overall, there is a lack of reproducible, longitudinal data that describe normal lung growth in children. This limitation renders it difficult to evaluate the natural history of asthma in children.

Table 3. Association of gene polymorphisms with indices of annual growth of spirometric variables ($n = 131$)

SNP	N	Spirometric values (mL/year) in relation to genotypes*			P [†]
<i>IL13</i> R130Q	129	RR ($n = 45$)	RQ ($n = 63$)	QQ ($n = 21$)	
FEV ₁		0.199 (0.116)	0.222 (0.116)	0.211 (0.109)	0.603
FVC		0.237 (0.151)	0.256 (0.157)	0.253 (0.116)	0.799
<i>IL4RA</i> I50V	131	II ($n = 35$)	IV ($n = 72$)	VV ($n = 24$)	
FEV ₁		0.179 (0.092)	0.226 (0.117)	0.207 (0.131)	0.129
FVC		0.210 (0.120)	0.273 (0.153)	0.227 (0.158)	0.072
<i>STAT6</i> T1309C	130	T/T ($n = 63$)	T/C ($n = 57$)	C/C ($n = 10$)	
FEV ₁		0.217 (0.115)	0.208 (0.122)	0.166 (0.053)	0.430
FVC		0.236 (0.133)	0.268 (0.163)	0.189 (0.127)	0.208
<i>STAT6</i> C1570T	130	C/C ($n = 63$)	C/T ($n = 46$)	T/T ($n = 21$)	
FEV ₁		0.238 (0.108)	0.171 (0.117)	0.205 (0.107)	0.009
FVC		0.271 (0.160)	0.214 (0.130)	0.244 (0.134)	0.115
<i>TARC</i> C-431T	130	C/C ($n = 56$)	C/T ($n = 60$)	T/T ($n = 14$)	
FEV ₁		0.188 (0.121)	0.223 (0.112)	0.226 (0.079)	0.203
FVC		0.224 (0.147)	0.261 (0.149)	0.263 (0.117)	0.352
<i>RANTES</i> G-401A	129	G/G ($n = 55$)	G/A ($n = 59$)	A/A ($n = 15$)	
FEV ₁		0.192 (0.107)	0.232 (0.116)	0.178 (0.103)	0.086
FVC		0.219 (0.135)	0.269 (0.157)	0.244 (0.114)	0.163
<i>ADRB2</i> R16G	131	RR ($n = 39$)	RG ($n = 69$)	GG ($n = 23$)	
FEV ₁		0.213 (0.094)	0.211 (0.122)	0.202 (0.128)	0.932
FVC		0.232 (0.128)	0.258 (0.159)	0.247 (0.147)	0.702
<i>ADRB2</i> E27Q	131	QQ ($n = 104$)	QE ($n = 23$)	EE ($n = 4$)	
FEV ₁		0.211 (0.106)	0.212 (0.141)	0.167 (0.188)	0.747
FVC		0.246 (0.146)	0.259 (0.156)	0.240 (0.181)	0.909

*Values are given as mean (SD).

†Analysed by ANOVA.

FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; SNP, single nucleotide polymorphism; *IL13*, interleukin-13; *IL4RA*, interleukin-4 receptor- α ; *STAT6*, signal transducer and activator of transcription 6; *TRAC*, thymus and the activation-regulated chemokine; *RANTES*, regulated on activation, normal T cell expressed and secreted; *ADRB2*, β_2 -adrenergic receptor gene.

Table 4. Multi-locus interaction models by MDR method for the decrease in FEV₁ and FVC

FEV ₁					FVC			
Model	Loci combination	CV consistency	Prediction error (%)	P*	Loci combination	CV consistency	Prediction error (%)	P*
2 locus	<i>STAT6</i> T1309C, <i>STAT6</i> C1570T	3.3	55.0	0.979	<i>IL4RA</i> I50V, <i>STAT6</i> C1570T	5.6	48.0	0.779
3 locus	<i>IL4RA</i> I50V, <i>ADRB2</i> R16G, <i>RANTES</i> G-401A	4.7	52.6	0.915	<i>IL13</i> R130Q, <i>ADRB2</i> R16G, <i>STAT6</i> C1570T	9.6	32.8	0.045
4 locus	<i>IL13</i> R130Q, <i>IL4RA</i> I50V, <i>RANTES</i> G-401A, <i>STAT6</i> C1570T	5.6	44.4	0.546	<i>IL13</i> R130Q, <i>ADRB2</i> R16G, <i>RANTES</i> G-401A, <i>STAT6</i> C1570T	9.0	34.1	0.069
5 locus	<i>TARC</i> C-431T, <i>IL4RA</i> I50V, <i>ADRB2</i> R16G, <i>RANTES</i> G-401A, <i>STAT6</i> C1570T	4.8	56.1	0.981	<i>IL13</i> R130Q, <i>IL4RA</i> I50V, <i>ADRB2</i> R16G, <i>RANTES</i> G-401A, <i>STAT6</i> C1570T	6.1	39.5	0.283

*Based on 5000 permutations.

CV, cross-validation; *IL13*, interleukin-13; *IL4RA*, interleukin-4 receptor- α ; *STAT6*, signal transducer and activator of transcription 6; *TRAC*, thymus and the activation-regulated chemokine; *RANTES*, regulated on activation, normal T cell expressed and secreted; *ADRB2*, β_2 -adrenergic receptor gene.

Our study shows C1570T in *STAT6* to be an important genetic marker of FEV₁ 'growth' in asthmatic children. The human *STAT6*, which belongs to the STAT family of transcription factors, maps to chromosome 12q13.3-q14.1 and contains 23 exons [36]. STAT-6 is

involved in the signalling of IL-4 and IL-13 – two crucial Th2 cytokines involved in the pathogenesis of asthma and allergy [37, 38]. Increased numbers of activated Th2 cells, mast cells and eosinophils, both in bronchial mucosa and bronchoalveolar lavage fluid, are constant features of

asthma. Besides, *STAT6*^{-/-} mice failed to develop bronchial hyper-responsiveness after allergen provocation, produced no detectable levels of serum IgE and lacked a typical Th2 cytokine response [39]. Although our previous study could not find any association between T1309C and C1570T of *STAT6* and asthma diagnosis or plasma IgE [27], Duetsch *et al.* [12] reported a significant association between these SNPs and total IgE levels in Caucasians using sib-pair analysis. Besides, Kabesch *et al.* [14] studied the genetic associations for a panel of Th2 pathway-related genes (i.e. *IL13*, *IL4*, *IL4RA* and *STAT6*) in German children. By combining polymorphisms in all these genes, these investigators found that the risks for having asthma and high serum IgE levels increased 16.8- and 10.8-fold, respectively, as compared with the maximum effect of any single SNP. The present study provides further evidence that *STAT6* plays a crucial role in modulating asthma phenotypes.

The pathogenesis of asthma and allergy involves a delicate network of distinct but inter-related inflammatory cells and immunological pathways, the most widely cited of which being the Th2-specific immunity [40]. Our finding of a gene-gene interaction between R130Q in *IL13*, R16G in *ADRB2* and C1570T in *STAT6* for FVC change is thus not surprising, and that suggests epistasis among these candidate genes. Unlike breast cancer in which a particular gene locus exerted a main effect [29], asthma does not have a typical pattern of Mendelian inheritance that can be explained by a linear model [41]. MDR has been successfully applied to delineate gene-gene interactions for studies of complex traits that contained relatively small samples [29, 42, 43]. Our recent studies found by MDR and logistic regression that *IL13* and *IL4RA* interacted to confer asthma risk, and *IL13* and *TARC* for raised plasma total IgE, in Chinese children [11] although the effects of individual SNPs for the respective phenotypes were insignificant [24, 28, 44]. These findings also support the sensitivity of MDR in detecting polygenic interactions among multiple loci.

A major limitation of this study relates to its relatively small sample size. As for this study, a significant gene-gene interaction was detected under the three-locus model with *IL13* R130Q, *ADRB2* R16G and *STAT6* C1570T to influence FVC change (Table 4). Owing to the relatively small sample size, we did not assign patients into different combinations of the three SNPs. Otherwise, each SNP combination would contain very few subjects for any accurate comparison. We tried to minimize this problem by applying MDR to ascertain any gene-gene or gene-environment interaction. This statistical method is useful in detecting high-order gene-gene interactions for studies of complex traits that included relatively small samples between 177 and 686 [29, 43, 44]. Multiple factors are pooled under MDR into high-risk and low-risk groups, effectively reducing the genetic or environmental predic-

tors from *n* dimensions to one dimension. This new, one-dimensional multi-factor variable is evaluated for its ability to predict phenotypes through CV and permutation testing [10, 29, 45]. The MDR approach is non-parametric (i.e. no hypothesis about the value of a statistical parameter is made) and model-free (i.e. no assumption for any inheritance model). Using simulated data, Ritchie *et al.* [29, 45] showed that MDR has high power to identify gene-gene interactions in the presence of 5% genotyping error, 5% missing data or a combination of both. Another limitation is the lack of replication of our study findings in other independent populations, which would otherwise add strength to these results. Lastly, this study has chosen only one to two SNPs per candidate gene. As these numbers of SNPs were not sufficient to capture most genetic information of the candidate genes, this study cannot exclude that genes other than *STAT6* (and also *IL13* and *ADRB2* as suggested by MDR) are associated with lung function growth in children. Future studies should elaborate on our findings by including more 'haplotype tagging' SNPs in the studied candidate genes.

In summary, this study describes for the first time the annual 'growth' of spirometric variables in Chinese children with asthma. These parameters are affected by subjects' gender, but are not related to any environmental factor. In contrast, the C1570T polymorphism of *STAT6* is associated with annual FEV₁ change in these children. By implementing the MDR method, this study demonstrates significant gene-gene interactions between SNPs in *IL13*, *ADRB2* and *STAT6* for FVC change in these patients.

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