Original Article

Association of Polymorphisms of *STAT6* and SO_2 with Chinese Childhood Asthma: a Case-control Study*

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Abstract

Objective To investigate the association of polymorphisms of *STAT6* gene and air pollutants of PM_{10}, NO_2, and SO_2, with asthma in Chinese children.

Methods 418 subjects aged 14 years and under were recruited in a case-control study. The association between *STAT6* polymorphisms and childhood asthma were tested by allele frequency, genotype analysis, and MDR analysis. Exposure to outdoor air pollutants was estimated by a 5-day moving average level. Statistical analyses were performed with SAS 9.1 software.

Results Only 3 alleles of GT repeats at exon 1 of *STAT6* were found in Chinese children. C258T and T710C were 2 new SNPs of *STAT6* at 3'-UTR. Children who carried T allele of C258T were more common in asthma children than in control subjects (P<0.05). The MDR analysis showed that GT repeats, C258T and T710C of *STAT6* polymorphisms interacted together in leading to susceptibility to childhood asthma among Chinese people. After confounding factors were controlled, such as SNP C258T, family history of asthma, frequency of influenza within a year, the 5-day average of SO_2 was tested to be a key risk factor of asthma in Chinese children (P<0.05).

Conclusion Chinese children differed in polymorphisms of *STAT6* and in its relation with childhood asthma.

Key words: Polymorphism; *STAT6*; Air pollution; Asthma; Childhood; SO_2

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INTRODUCTION

STAT6 (signal transducer and activator of transcription 6) is located at chromosome 12q13.3. Genome wide association study and genetic susceptibility studies showed that polymorphisms of genes at 12q13-q24 were closely associated with bronchial hyper-responsiveness, increased serum IgE level, and cell counts of eosinophils[1-2]. Many candidate genes located in the above region were believed to be associated with asthma and allergic diseases, which included *STAT6*, *ITGB7* (integrin-β7), *LTA4H* (leukotriene A4 hydrolase), *IGF1* (insulin-like growth factor 1), *NFYB* (β unit of nuclear factor-Y), and *NOS1* (neuronal nitric oxide synthase), etc[3-9]. *STAT6* was recognized as one of the most promising candidate genes of asthma. Dinucleotide repeat polymorphism (GT repeat) in exon1 of *STAT6* gene had been tested to be significantly associated with susceptibilities to

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asthma among the British, Indians, and the Japanese[10-13]. In addition, it was associated with increased total serum IgE level among the British and Germans[9-11]. Other polymorphisms of *STAT6* gene, such as G2964A polymorphism of *STAT6* at 3'-UTR and C1570T at intron 18 were also reported to be related with asthma susceptibilities[6,14-19]. It is widely accepted that different population might have different susceptibilities. Apart from that different races might have different susceptibilities, adults and children might be also different in susceptibilities. Except for association of G2964A with adult asthma of the Chinese[14-18], no research has been reported to study *STAT6* polymorphisms and asthma of Chinese children. Due to the importance of *STAT6* gene in regulating and activating Th2 cells which were believed to be closely related with asthma physiopathology, it's necessary to repeat testing the association of *STAT6* gene polymorphisms and childhood asthma in Chinese children. In order to investigate distinct genetic susceptibilities to asthma of Chinese children, we tested in the present study the association of childhood asthma with polymorphisms of *STAT6* genes in Chinese children. Association between environmental risk factors, genetic variations of *STAT6*, and Chinese childhood asthma were also tested.

MATERIALS AND METHODS

Study Population

A non-matched case control study was designed to study asthma risks of Chinese Han children. Between September 2007 and August 2008, 223 unrelated Chinese Han children aged 14 years and under with physician-diagnosed asthma were enrolled from 2 pediatric medical centers (Beijing Children's Hospital and Capital Institute of Pediatrics). The participation rates of the 2 medical centers were 85% and 87% respectively. The diagnosis of asthma was made according to the criteria for childhood asthma established by Chinese Society of Respiratory Diseases[20]. 197 non-asthmatic controls without respiratory and neuronal diseases were recruited from children visiting the same hospital for surgery. IgE levels of subjects were tested by the hospital where the child was enrolled in this study. Asthma children who were not diagnosed for the first time were excluded. In addition, subjects who were patients with immune deficiency or with severe cardiovascular diseases were also excluded from this study. Parents of subjects were investigated through written questionnaires that included core questions of ISSAC (The International Study of Asthma and Allergies in Childhood) questionnaire and some indoor risks of asthma. We obtained informed consents from all parents of qualified study subjects. This study was approved by the Committee of Ethics at the Institute of Environmental Health and Related Product Safety, Chinese Center for Disease Control and Prevention.

Assessment of Serum IgE Level

Serum IgE levels of subjects were tested by the hospital where the child was enrolled in this study. IgE and concentrations of outdoor air pollutants (PM_{10}, SO_2, and NO_2) were logarithm transformed to base 10 before analysis. A child was considered atopic by 1 or more positive antigen-specific IgE to common aeroallergens (Dermatophagoides pteronyssinus, Dermatophagoides farinae, cat or dog danders, Alternaria tenuis, cockroach, mixed tree pollen, and mixed grass pollen).

SNP Screening and Genotyping

DNA was extracted from peripheral blood leukocytes by using Biomed Whole Blood Genomic DNA Extraction Kit (Biomed, Beijing, China). Exon 1 and 3'-UTR were chosen as target DNAs. The genomic region of interests was amplified by multiplex polymerase chain reactions (PCRs). Primers of PCR and sequencing were designed by Primer Premier 5.0 (Premier, Canada). 5'-GAGGGACC-TGGGTAGAAGGAGAAGC-3' and 5'-GGCACTCCTTCA-CCTCCTTTCCCT-3' were sequences of sense primer and antisense primer of exon1; 5'-AGACCTGCTC-TGGACACTTGCTC-3' and 5'-CTCCCAGGCAGCATTG-GAGGTGT-3' were primers for 3'-UTR of *STAT6*. 563 bp and 932 bp were PCR products of the first exon and 3'-UTR respectively. These PCRs were performed in a total volume of 50 μL containing 100 ng DNA, 2.5 mmol/L dNTPs, 10X Pyrobest™ buffer with 2.5 mmol/L $MgCl_2$, 10 pmol of each primer and 2.0 unit Pyrobest™ DNA Polymerase (Takara Bio Inc, JP). DNA specimen were denatured at 95 °C for 4 min followed by 30 cycles of being denatured at 95 °C for 30 s, annealed for 30 s, an extension at 72 °C for 120 s, and a final extension at 72 °C for 10 min. Target DNA sequences were tested by Beijing Biomed Co., Ltd with ABI-3730*xl* DNA Analyzers.

To insure repeatability of sequences, 60 DNA specimen (30 cases and 30 controls) were amplified by another DNA Polymerase (Pfu DNA Polymerase,

Tiangen Biotech Co Ltd, Beijing) and then sent to Sangon Biotech Co Ltd (Shanghai) for sequencing. SNPs were identified by sequence assembly with Genetool Lite 1.0 software. SNP associations with childhood asthma were tested by allele frequency, genotype analysis, and MDR (multifactor dimensionality reduction) analysis. Genotype distributions were tested by Hardy-Weinberg equilibrium (HWE) and genotype simulations.

Assessment of Outdoor Air Pollutant Exposure

API (air pollution index) of every interested district was downloaded daily from the webpage of the Ministry of Environmental Protection of the People's Republic of China. Air pollutant exposure levels of subjects were calculated by API of every related section. Concentrations of air pollutants were multiplied by 1000 and then logarithm transformed to base 10. Exposure levels were estimated by 5-day moving average levels of air pollutants which were calculated by the average level of a specific day and that of 4-consecutive day ahead.

Statistical Analyses

Results were expressed as proportions or means and standard deviations (SD). Characteristics of subjects were compared by using the *t*-test or ANOVA for numerical data, and chi-square for categorical variables. The associations between SNPs and dichotomous phenotypes were analyzed by using multivariate logistic regression. Gene-gene interaction was estimated by the MDR analysis. The best model was selected for its accuracy, cross-validation (CV) consistency, and statistical significance. The model was statistically significant when the permutation test *P*-value was less than 0.05. The high-risk and low-risk genotypes in the model were determined by the MDR analysis also. When the ratio of asthma children to control children was equal to or greater than 1.0, the genotypes were defined as high-risk. However, if the threshold was lower than 1.0, the genotypes were defined as low-risk. The MDR analysis was performed by mdr 2.0_beta_8.3. Age, sex, family history of asthma, individual influenza frequency within a year, and ETS (environmental tobacco smoking) exposure were adjusted as covariates. A stepwise analysis was used to test the association of asthma with genetic risks and environmental risks including air pollutants of PM_{10}, SO_2, and NO_2. Effects greater than 0.10 were retained in the stepwise models; effects less than 0.10 were removed. All comparisons were two-tailed, and *P* values less than 0.05 were considered significant.

RESULTS

Study Population

The mean (SD) age of patients and controls was 5.7 (2.6) years and 6.8 (2.6) years, respectively. Atopy was more common among cases (65.0%) than controls (27.1%; $P<0.001$). No significant differences was found in age, gender, birth weight, weeks of pregnancy, residential area, apartment size, floor, cooking fuels and heating methods ($P>0.05$)[21]. Table 1 summarized demographic and allergic characteristics of subjects.

Table 1. Demographic Characteristics of Asthma and Control Subjects

Characteristics	Asthmatics (%)	Controls (%)	*P* Value
Age			0.084
⩽3	18.6	10.6	
>3−7	53.4	57.3	
>7−⩽14	28.0	32.1	
Sex			0.210
Male	71.3	65.3	
Female	28.7	34.7	
Birth Weight			0.244
<2.5kg	1.7	2.8	
2.5-4.2kg	96.1	92.2	
>4.2kg	2.2	5.0	
Weeks of Pregnancy			0.422
<37w	5.1	7.1	
37-42w	94.9	92.9	
>42w	0.0	0.0	
Allergy Test (Positive)			
Dust Mite	32.5	11.1	<0.001
Mixed Tree Pollen	11.7	6.1	0.242
Mixed Grass Pollen	13.6	8.6	0.260
Alternaria Tenuis	40.3	11.6	<0.001
Cockroach	11.7	7.6	0.326
Cat/Dog	14.1	9.6	0.325

Note. Data was analyzed by Chi-square.

SNP Detection

About 96.1% DNA specimen were qualified in sequencing. Sequence of the same DNA specimen with different DNA polymerase was the same, so was with the different company. All SNPs found followed HWE ($P=0.324$, 0.561, and 0.139

respectively). Table 2 summarized allele frequencies of rs71802646, C258T, and T710C. rs71802646 (GT repeats tandem) was found to be the only SNP at exon1. 3 alleles of rs71802646 (GT repeat polymorphism) were found, and they were 13 GT repeats (A1, 38.0%), 14 GT repeats (A2, 2.7%), and 15 GT repeats (A3, 61.7%). A little more A1 carriers (37.5%) were found in controls than in asthma children (36.7%); however, the allele frequencies were not statistically different (P>0.05), neither were allele frequencies of A2 and A3 (P>0.05). Allele frequencies of GT repeats were not associated with atopy either (P>0.05).

No allele frequency of registered SNPs had been found greater than 0.05 in the interested region of 3'-UTR of *STAT6*. However, C258T and T710C were found to be 2 new SNPs in 3'UTR of *STAT6* by BLAST (Basic Local Alignment Search Tool). C258T and T710C had been submitted to NCBI dbSNP. C258T was registered as ss185319345 and T710C as ss185319352. Carriers of T allele of C258T were more common in asthma (42.5%) than in control subjects (27.9%) (P<0.05). Allele frequencies of T710C were not statistically different in asthma and control subjects (P>0.05).

Genotype Analysis

Table 3 summarized genotype distributions of C258T and T710C. Genotypes of C258T and T710C were simulated in dominant, co-dominant, over-dominant, recessive, and log-additive model. Adjusted for age and sex as covariates, asthma diagnosis was not closely associated with genotypes of C258T, neither was T710C (P>0.05).

Table 2. Allele Frequency of Stat6 Polymorphisms

SNPs	Variation	Position in the Gene	Alleles	Asthmatics	Controls	*P* Value
rs71802646	-202_-199 del4	Exon 1	A1	82	72	0.724
			A2	7	4	
			A3	124	116	
C258T	258 C>T	3'-UTR	T	94	55	0.002
			C	127	142	
T710C	710 T>C	3'-UTR	C	67	46	0.109
			T	154	151	

Note. Data was analyzed by Mantel-Haenszel chi square; SNP of rs71802646 is the polymorphism of GT repeats; del, deletion which is a type of polymorphism.

Table 3. Association of SNP Genotypes in *STAT6* at 3'-UTR and Childhood Asthma

	Genotype	Asthmatics *N* (%)	Controls *N* (%)	OR* (95% CI)	*P* Value
C258T	Co-dominant model				
	CC	118 (53.4)	109 (55.3)	1.00	Ref
	CT	81 (36.6)	74 (37.6)	0.98 (0.64,1.48)	0.911
	TT	22 (10.0)	14 (7.1)	0.73 (0.35,1.51)	0.394
	Recessive model				
	TT	22 (10.0)	14 (7.1)	1.00	Ref
	CC/CT	199 (90.0)	183 (92.9)	0.73 (0.36,1.50)	0.396
T710C	Co-dominant model				
	CC	58 (26.3)	47 (23.9)	1.00	Ref
	TC	111 (50.2)	110 (55.8)	0.87 (0.54,1.41)	0.568
	TT	52 (23.5)	40 (20.3)	1.05 (0.62,1.96)	0.731
	Over-dominant model				
	TC	111 (50.2)	110 (55.8)	1.00	Ref
	CC/TT	110 (49.8)	87 (44.2)	1.21 (0.81,1.79)	0.353

Note. *Data was analyzed by logistic regression, controlled for age and gender as covariates. OR, odd ratio; CI, confidence interval; *N*, sample size.

Association of Genotypes of STAT6 with Serum Total IgE Level

Associations of *STAT6* SNP genotypes with the serum total IgE level were summarized in Table 4. As to the association of GT repeats polymorphisms with the serum total IgE level, no significant difference was found among different allele carriers (P>0.05). No close association of genotypes of C258T and T710C with serum total IgE level was found either (P>0.05).

Table 4. Association of Serum Total IgE and Polymorphisms of C258T, T710C, and GT Repeats

Polymorphisms		*N*	Log_{10}IgE (IU/mL)	*P* Value
C258T	CC	229	2.20±0.48	0.971
	CT	158	2.20±0.43	
	TT	31	2.16±0.50	
T710C	CC	103	2.26±0.42	0.383
	TC	223	2.20±0.46	
	TT	92	2.16±0.50	
GT Repeats	A1	131	2.25±0.44	0.278
	A2	10	2.03±0.51	
	A3	223	2.21±0.49	

Note. Data was analyzed by GLM procedure and Student-Newman-Keuls Test.

Gene-gene Interaction and Asthma

We tested association of childhood asthma with epistasis (gene-gene interaction) of polymorphisms GT repeats (rs71802646), C258T and T710C by MDR analysis. With 10-fold cross-validation, 3 models were selected by higher CV consistency and testing accuracy. Table 5 summarized MDR analysis results of gene-gene interaction of *STAT6* polymorphisms. 3-locus model which included GT repeats, C258T, and T710C was tested to be the best model for estimating asthma susceptibilities and epistasis of *STAT6* polymorphisms. Figure 1 shows a weak main effect of GT repeat polymorphisms and clear interaction effects of C258T and T710C. Carriers of $TT_{C258}A1CC_{T710C}$, $TC_{C258T}A3CC_{T710C}$, $TT_{C258T}A3CC_{T710C}$, $CC_{C258T}A3TC_{T710C}$, $CC_{C258T}A1TT_{T710C}$, $CC_{C258T}A2TT_{T710C}$, $CC_{C258T}A3TT_{T710C}$, $TT_{C258T}A2TT_{T710C,}$ and $TT_{C258T}A1TT_{T710C}$ were in higher risks of childhood asthma with the odd ratio being 1.95 (95% CI: 1.31-2.91). GT repeat polymorphisms, C258T, and T710C in *STAT6* were tested to be interacted in the association with childhood asthma (P<0.05). T710C synergistically effected on asthma with GT repeat polymorphisms and C258T, whereas GT repeat polymorphisms might be counteracted by the effects of C258T.

Table 5. *STAT6* Polymorphisms Interactive Effects on Childhood Asthma

Model	SNP Interaction	CV Consistency	Accuracy	OR (95% CI)	*P* Value
1 locus	GT Repeats	8/10	0.55	1.48 (0.99, 2.23)	0.057
2 locus	GT Rrpeats C258T	4/10	0.56	1.62 (1.07, 2.44)	0.022
3 locus	GT Rrpeats C258T T710C	10/10	0.58	1.95 (1.31, 2.91)	0.001

Note. Data was analyzed by the MDR analysis; CV, cross validation; OR, odd ratio; CI, confidence interval.

Outdoor Air Pollutants, Polymorphisms of STAT6, and Asthma

To test effects of genetic variations of *STAT6* and environmental risk factors on Chinese childhood asthma, a stepwise analysis was used to establish a multivariate model. GT repeat polymorphisms, T710C and C258T were chosen as genetic risk factors. In addition, age, gender, family history of asthma, environmental tobacco smoking exposure in the family, and the individual frequency of influenza within a year were chosen as covariates in the model. PM_{10}, NO_2, and SO_2 were chosen as representatives of outdoor air pollutants, and the exposure levels were estimated by 5-day moving average levels of air pollutants. Table 6 summarized the association between *STAT6* polymorphisms, environmental risk factors and childhood asthma. Logistic regression showed that C258T genotypes, family history of asthma, individuals with >10 times flu a year, and short-term wave of 5-day average concentration of SO_2 were important risks for childhood asthma in Chinese children.

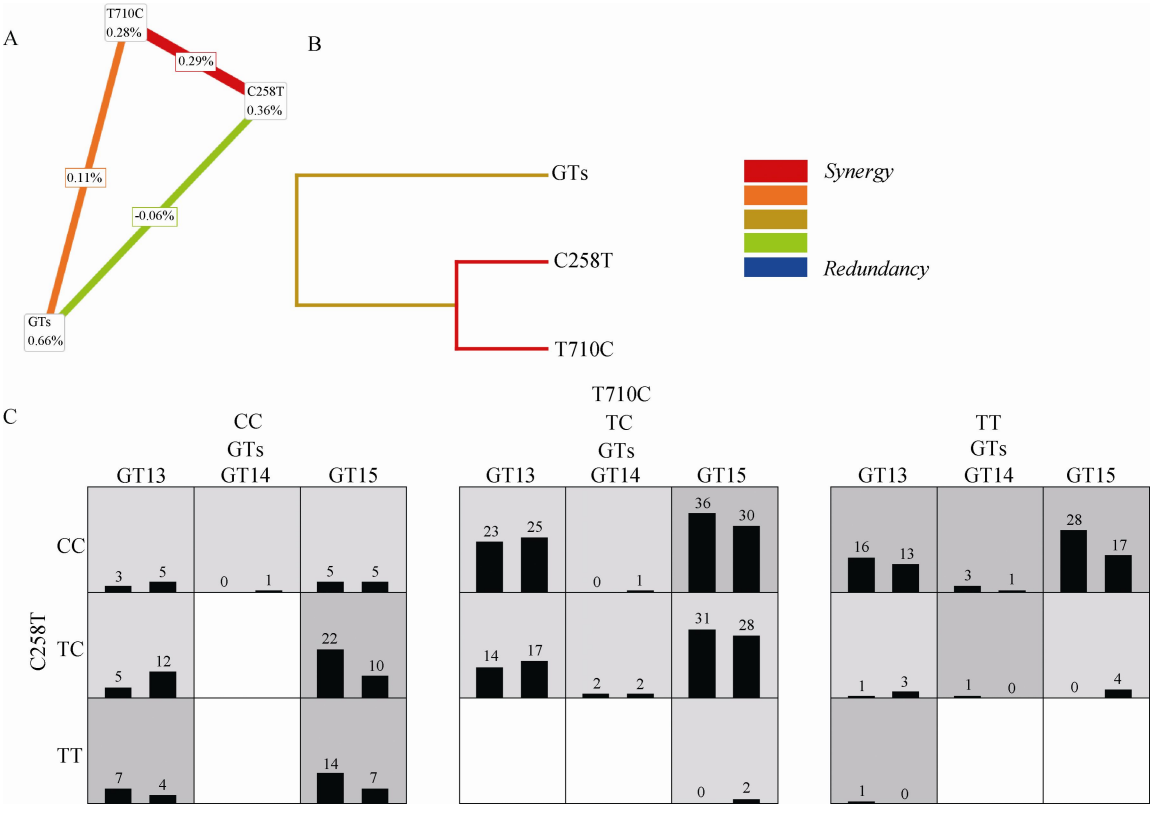

Figure 1. Entropy and graphical model of gene-gene interaction. This figure illustrated gene-gene interaction of 3 polymorphisms in STAT6 gene in relation with childhood asthma. (A) Percentage less than 0.0 indicated redundancy, whereas percentage more than 0.0 indicated synergy. (B) Red or orange lines suggested synergistic effects, and green or blue lines suggested counteractive effects. (C) Asthmatics (left bars) and controls (right bars) were tested for each multilocus genotype combination. Dark-grey cells and light-grey cells indicate "high-risk" and "low-risk" for asthma respectively. Cells in white color were cells without subjects of those genotype combinations. GT13-GT15 were alleles of A1, A2, and A3 respectively.

Table 6. Association of Outdoor Air Pollutant and Polymorphisms of *STAT6* with Asthma

Risk Factors	Estimate	Standard Error	Chi-Square	OR(95% CI)	*P* Value
C258T (CC carriers *vs*. TT&TC carriers)	-0.421	0.236	3.18	0.66 (0.41,1.04)	0.070
Family history of asthma (yes *vs*. no)	0.858	0.270	10.06	2.36 (1.39,4.01)	0.002
Freq. of flu (>10 times *vs*. <6 times)	1.75	0.385	20.53	5.74 (2.69,12.11)	<.0001
SO_2 (every 10 μg/m^3)	0.784	0.301	6.78	2.19 (1.21,3.95)	0.009

Note. Data was analyzed by logistic regression, a stepwise analysis, α=0.10. C258T, a polymorphism of stat6 gene; CC/TT, homozygote carriers, TC, heterzygote carriers; OR, odd ratio; CI, confidence interval; Freq. of flu, the individual counts of influenza within a year; SO_2, 5-day average level of SO_2.

DISCUSSION

GT repeats of exon1 in *STAT6* were reported to be associated with asthma, allergic disease, and serum total IgE level in some populations including our neighbors like the Japanese and Indians[6,10-12]. It's reasonable to confirm the association of *STAT6* polymorphism with asthma in Chinese children and

it'll be helpful for exploration of distinguishing asthma susceptibilities of the Chinese. By sequencing and sequence assembly, we found that GT repeat polymorphisms were also found in the first exon of *STAT6* in Chinese children. However, the types of alleles were quite distinct, and the association with asthma was different from that of populations in other countries. It's even different from that of our neighbor countries. We only found 3 types of GT repeats, and they were A1 (13 GT repeats), A2 (14 GT repeats), and A3 (15 GT repeats). The Japanese had four types of GT repeats (A1, A2, A3, and A4:16 GT repeats) and Indian people have 5 types of GT repeats as reported (A1, A2, A3, A4, and A5:17 GT repeats)[10-11]. A1 carriers were more in asthmatics and allergic disease patients than in controls among the Japanese. A3 carriers were significantly more in Indian asthmatics than in controls (OR=1.76:1.18-2.60), and A4 carriers were significantly more in Indian controls than in asthmatics (OR=0.33:0.19-0.57)[12]. Unlike our neighbors, the percentage of A1 carriers in asthma children was not found significantly different from that in control subjects in Chinese people. No close association of GT repeat polymorphisms with childhood asthma had been found in Chinese children. Also, no association was found with total IgE level or atopy either.

STAT6 is believed to be a promising candidate gene. Apart from GT repeat polymorphisms of exon1, SNP G2964A in 3'-UTR was also reported to be associated with asthma. Five independent studies had explored the association of SNP G2964A with asthma in Chinese Han and Li nationalities. Except for one study, the other four studies reported that SNP G2964A was not associated with susceptibility to asthma in Chinese population[14-18]. Due to the important regulation in transcription and different genetic susceptibilities of different population, it's necessary to test the association of polymorphisms of *STAT6* at 3'-UTR with asthma in Chinese children. Although there might be some difference in genetic susceptibilities between adults and children, we assumed that SNP G2964A might not be crucial in childhood asthma diagnosis in Chinese people. We therefore selected the target DNA of 3'-UTR without 2964 (G>A) position.

We did not find registered SNPs in NCBI dbSNP Build 130 with allele frequency greater than 0.05. However, we found 2 new SNPs in 3'-UTR of *STAT6* gene which were not recorded in dbSNP Build 130. We've submitted the SNPs and got the new registered numbers of ss185319345 (C258T) and ss185319352 (T710C) in dbSNP Build 131. Although genotypes of C258T and T710C were not tested to be independently associated with childhood asthma, T allele carriers of C258T were more common in asthma children than in control subjects (P<0.05). As asthma is a complicated disease, asthma susceptibility might not be predicted only by one SNP distribution. The MDR analysis was developed to test gene-gene interactions, which were not suitable for the logistic analysis. CV consistency and accuracy were used to be criteria for selecting multifactor models. Although GT repeat polymorphism and T710C were not associated with asthma independently, the MDR analysis showed that GT repeats, C258T, and T710C interacted together in susceptibilities to asthma in Chinese children. The result reminds us that we need to further screen the whole gene sequence to determine genetic susceptibilities of *STAT6* to asthma.

Asthma is believed to be closely associated with genetic risks. However, asthma was reported to have various environmental triggers. We've found interactive effects of *STAT6* polymorphisms on asthma and would like to further explore the association of *STAT6* polymorphisms and environmental risks with asthma in Chinese children. Except for common confounding factors of age, gender, family history of asthma, ETS exposure in the family, and the individual frequency of influenza within a year, we input 5-day averages of NO_2, PM_{10}, and SO_2 as environmental risks. Trigging effects of outdoor air pollutants on asthma might be lagged for several days when the levels were below exposure limits. In reference to the association of lung functions, we selected 5-day moving averages as estimates of air pollutants[22]. To our surprise, apart from family history and influenza, genotypes of C258T and 5-day average concentration of SO_2 were tested to be important risk factors for childhood asthma, although SNP C258T was not statistically significant in the model (P=0.070). Compared with other risk factors in the model, SNP C258T of *STAT6* did not play a key role in effects on asthma. We might infer that some genes other than *STAT6* might be more promising candidate genes of asthma in Chinese children, although we need further research on relationship of susceptibilities to asthma with genetic variations of *STAT6*.

As to asthma triggers of air pollutants, SO_2 had been reported to be asthma triggers by some reports, although it was controversial[22]. Our results verified that SO_2 might be an important asthma risk when

there were short-term waves of SO_2 in the air. Except for SO_2, PM_{10}/$PM_{2.5}$, and NO_2 were also reported as asthma risks[23]. However, PM_{10} and NO_2 were not included in our model, since our investigation was between Sept. 2007 and Aug. 2008, which included the span of Beijing Olympic Games. PM_{10} and NO_2 levels were decreased significantly in the period according to API statistics by restricting vehicles and some other pollution-control measures. In addition, we could not tell the $PM_{2.5}$ percent in the total amount of particles. Therefore, whether air pollutants (PM_{10}, etc) might be key asthma triggers in Chinese children or not needs to be further explored.

In summary, key asthma risk factors of Chinese children might be quite distinct from those of other populations. Due to increasing prevalence of asthma in recent decades, findings from our study show the urgent need for systematic research on specific genetic susceptibilities to asthma and key environmental asthma triggers for Chinese people, which will be critical in control and treatment of asthma in Chinese children.

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