Interleukin-13 and RANTES polymorphisms in relation to asthma in children of Chinese Han nationality

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Summary

Background: **IL-13 (interleukin-13) and RANTES (Regulated upon Activation, Normal T cells Expressed and Secreted) are the important asthma inflammatory mediators.**

Objective: **The present study aimed to investigate the single and combined associations between the polymorphism (SNP) loci in *IL-13 and RANTES* genes with the development of asthma in children of Chinese Han nationality.**

Methods: **The risk associated with genotypes of three *IL-13* SNPs and two *RANTES* SNPs was determined by the χ^2 test in 384 children with asthma and an equal number of healthy controls matched by sex.**

Results: **Between the experimental and control groups, no statistically significant differences (P >0.05) were found in genotype distribution and allele frequency among three loci (IL-13 C-1112T, IL-13 C1923T, and *RANTES A-403G*). However, significant diversity was observed among *IL-13 A2044G* (P =0.0001) and *RANTES G-28C* (P =0.0001). Moreover, the frequency of *IL-13 A2044G A/A* and *RANTES G-28C G/G* in the asthma group was significantly higher than in the control group (odds ratio [OR] =2.59, P =0.0001; OR =3.00, P =0.0001, respectively). Carriers of both *IL-13 A2044G A/A* and *RANTES G-28C G/G* have a more significant risk for developing asthma than those with only a single polymorphism.**

Conclusions: **The three loci (*IL-13 C-1112T, IL-13 C1923T,* and *RANTES A-403G*) make little contribution to the development of asthma in children of Chinese Han nationality. *IL-13 A2044G* and *RANTES G-28C* are significantly associated with childhood asthma. *IL-13 A2044G A/A* and *RANTES G-28C G/G* have a significant and combined effect on the development of asthma. *(Asian Pac J Allergy Immunol 2013;31:247-52)***

Key words: *Asthma, interleukin 13, RANTES, polymorphisms, Chinses Han nationality*

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Submitted date: 19/3/2012

Accepted date: 7/1/2013

Introduction

Asthma is the most common chronic inflammatory lung diseases of childhood worldwide. Because it is a common and frequently encountered respiratory disease in children with increasing incidence and mortality, the impact of asthma on society is substantial. With the development of molecular biology and genetics recently, increasing research indicates that asthma is a complex genetic disorder.[1-2] Several candidate genes for asthma have been described, among which IL-13 (interleukin-13) and RANTES (Regulated upon Activation, Normal T cells Expressed and Secreted) are the important genes associated with inflammation.[3-5] Besides, through reviewing 118 genes associated with asthma or atopy, Ober et al.[6] found that IL-13 and RANTES genes were duplicated in 6 or more studies and thus are believed to be the genes most likely to be associated with asthma and atopy.

The *IL-13* gene is located on chromosome 5q31-33, which contains a number of genes involved in the mechanism of allergic asthma.[7] IL-13 has a number of actions relevant to the asthmatic diathesis, such as its role in promoting the differentiation and survival of eosinophils and mast cells, and in inducing the IgE isotype switch. IL-13 also has the ability to stimulate the production of RANTES and eotaxin in epithelial cells, recruiting eosinophils into the lung and causing airway inflammation.[5,8] The *RANTES* gene lies on chromosome 17q11.2-q12.[9] RANTES is a RANTES chemokine that can induce

recruitment of eosinophils and their up-regulation into the airways of asthmatic children causing inflammation.[10] Thus, IL-13 and RANTES interact to cause the airways inflammation in the development of asthma.

Single-nuleotide polymorphisms (SNPs) are the most common source of genetic variation in populations.[11] Analysis of the genetic variarions that contribute to susceptibility for common diseases will shed light on the development of diagnostics and therapeutics, and disease risk can be modelled as the product of risks of many independent risk variations.[12] Thus, we assessed five SNP loci (*IL-13 C-1112T, C1923T, A2044G*, and *RANTES G-28C, A-403G*) in the *IL-13* and *RANTES* genes for a single and combined association with the development of asthma in Chinese Han children.

Methods

Subjects

The study population comprised 768 unrelated individuals of Chinese Han nationality (384 patients with asthma and an equal number of healthy controls). Informed consent was obtained in all cases. The study design conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institutional Ethical Committee of Xin Hua hospital.

All patients were recruited from the Asthma Clinic of Xin Hua hospital, which is affiliated to Shanghai Jiaotong University School of Medicine. Each patient had at least one or more active asthma symptom. Asthma was defined by the following two criteria: two or more episodes of wheezing and shortness of breath during the last year; reversibility of the wheezing and dyspnea, either spontaneously or by bronchodilator treatment as defined by the guidelines of the American Thoracic Society. The diagnosis of asthma in participants was confirmed clinically by a pediatrician who had treated the children for more than two years. Because wheezing is often associated with viral respiratory infection in young children, we excluded children younger than 3 years of age.[13] The 384 patients ranged from 3 to 12 years of age, with 192 boys and 192 girls.

Individuals enrolled as control subjects were healthy students from Shanghai Huadong University and Shanghai Normal University. The inclusion criteria of the control group were as follows: absence of symptoms or history asthma; no symptoms or history of other pulmonary diseases; no symptoms or history of allergy; and absence of first-degree relatives with a history of asthma and atopy.The ratio of males to females was 1:1.and their ages ranged from 18 to 22 years. Control individuals were older, which allowed a longer period for exclusion of asthma.

Genotype Detection

Oral mucosa swab samples were collected for genomic DNA extraction using TIANamp Swab DNA Kit (Tiangen,Benjing,China).The five SNPs (*IL-13 C-1112T, A2044G, C1923T*;*RANTES G-28C, A-403G*) were genotyped using the Taqman real-time quantative polymerase chain reaction (*ABI 9700*, Applied Biosystems, Foster City, CA). The reaction was performed in a 384-well plate format. Each well contained FAM-labeled and VIC-labeled probes. The total volume of 10 μL included 10 ng of genomic DNA, 5 μL of Taqman Universal Master Mix (Applied Biosystems, Foster City, CA), 0.2 μL of TaqMan® SNP Genotyping Assay Mix, and 2.5μL of RNase. Cycling conditions included 1 cycle at 95ºC for 10 minutes, 40 cycles at 95ºC for 15 seconds, and at 60ºC for 60 seconds. To avoid contamination, negative controls were included in each PCR reaction. After PCR, the results from each sample were automatically determined by measuring allele-specific final fluorescence in an ABI Prism 9700HT detection system, using the SDS 2.2 software for allele discrimination (Applied Biosystem).

Statistical Analysis

The Hardy-Weinberg equilibrium was estimated using the χ^2 test. The differences between the experimental and control groups in genotype distribution were analyzed using the χ^2 test. A P value of 0.05 or less was considered statistically significant. All statistical analyses were performed using SPSS version 16.0 software (SPSS Inc, Chicago, Illinois, USA).

Results

Single effect of the five SNP loci on the development of asthma in children of Chinese Han nationality

No significant differences (p >0.05) were found between the experimental and control groups in genotype distribution among the three loci (*IL-13 C-1112T, IL-13 C1923T*, and *RANTES A-403G*) (Table 1). However, a significant diversity was observed among *IL-13 A2044G* (χ^2 =13.112, P =0.0001 and *RANTES G-28C* (χ^2 =13.190, P =0.0001. The frequencies of *IL-13 A2044G A/A* and *RANTES G-28C G/G* in the asthma group were significantly

Table 1. Analysis of the associations between IL-13 C1923T, C-1112T and RANTES A-403G and Asthma [n (%)]

SNP	rs number	Group	N	Genotype			χ2	*P* value
				C/C	C/T	T/T		
IL-13 C1923T	1295686	Control	384	179 (46.6)	169 (44.0)	36 (9.4)		
		Patients	384	174 (45.3)	164 (42.7)	46 (12.0)	1.365	0.505
				C/C	C/T	T/T		
IL-13 C-1112T	1800925	Control	384	288 (75)	80 (20.8)	16 (4.2)		
		Patients	384	285 (74.2)	84 (21.9)	15 (3.9)	0.146	0.930
				A/A	A/G	G/G		
RANTES A-403G	2107538	Control	384	52 (13.5)	183 (47.7)	149 (38.8)		
		Patients	384	50 (13.0)	186 (48.4)	148 (38.5)	0.067	0.967

Abbreviations：SNP, single-nucleotide polymorphisms; rs, reference SNP

higher than those in the control group (odds ratio [OR] =2.59, *P* =0.0001; OR=3.00, *P* =0.0001, respectively) (Table 2).

Combined effects of IL-13 A2044G and RANTES G-28C loci on the development of asthma in children of Chinese Han nationality

Different genotype combinations were marked separated according to whether they carried *IL-13 A2044G A/A* or *RANTES G-28C G/G* homozygosity (Table 3). The carriers of both A/A and G/G showed a more significant risk of developing asthma than those with either a single SNP.

Discussion

Asthma is a common clinical syndrome resulting from several factors such as immunity, environment and heredity. Genetic predisposition is probably caused by a characteristic pattern of polymorphism in multiple genes involved in the regulation of the allergic reaction.[14] Such polymorphism patterns may be identified and be of predictive use for childhood asthma diagnosis.

With the completion of the Human Genome Project, analysis of single-nucleotide polymorphisms (SNPs) has become the newest approach in the detection and localization of the genetic determinants of human disease. Polymorphisms within IL-13 and RANTES genes have shown an association with the development of asthma, based on populations such as Korea, the United Kingdom and Spain.[15-19] However, there is no report on the single and combined associations of *IL-13 A2044G, C-1112T, C1923T* and *RANTES G-28C, A-403G* with asthma in children of Chinese Han Nationality.

We found that there were significant differences between the experimental and control groups in genotype distribution between *IL-13 A2044G* and *RANTES G-28C*, and the frequency of *IL-13 A2044G A/A* and *RANTES G-28C G/G* in the asthma group was significantly higher than in the control group. Therefore, *IL-13 A2044G* and *RANTES G-28C* were the relevant SNPs for asthma in our population set, in which the homozygotic *A2044G A/A* and *G-28C G/G* alleles were responsible for the development of asthma in children of Chinese Han nationality, which was consistent the meta-analysis result pubilshed by Yang H and Zhao D, respectively. They found that IL13 A2044G and RANTES G-28C were associated with an increased risk of athma in Asian populations.[20-21] We also observed that carriers of both *A044G A/A* and *G-28C G/G* had a more significant risk of developing asthma than those with either a single SNP, thus suggesting that synergism existed between the *A2044G A/A* and *G-28C G/G* genotypes.

Numerous studies have revealed that the *IL-13 A2044G* SNP markedly increases the risk of developing asthma,[16,17,22,23] and is associated with elevated level serum IgE and a high eosinophil count.[24-25] RANTES G-28C located in the promoter region may enhance transcriptional activity and result in subsequent RANTES overexpression in lung cells, which affects the development of asthma by recruiting more neutrophils and eosinophils into the airways, causing inflammation.[13,18] Moreover, RANTES G-28C may play an important role in asthma predisposition and in the severity of airway obstruction.[13] However, our study is the first report of the combined effects of *IL-13 A2044G* and *RANTES G-28C* on the development of asthma. RANTES is expressed by airway epithelial cells, and IL-13 regulates RANTES production by airway epithelial cells.[5,26,27] Polymorphisms in IL-13 and RANTES genes, such as IL-13 A2044G and RANTES G-28C, may interact and influence the regulation of RANTES secretion by IL-13. A previous

Table 2. Analysis of the associations between IL-13 A2044G and RANTES G-28C and Asthma [n (%)]

SNP	rs number	Group	N	Genotype			$\chi 2$	*P* value	Odds Ratio (95%CI)
				A/A	A/G	G/G			
IL-13 A2044G	20541	Control	384	21 (5.5)	164 (42.7)	199 (51.8)			2.59[1]
		Patients	384	50 (13.0)	154 (40.1)	180 (46.9)	13.052[1]	0.0001[1]	(1.52~4.40)
				G/G	G/C	C/C			
RANTES G-28C	2280788	Control	384	14 (3.6)	60 (15.6)	310 (80.7)			3.00[2]
		Patients	384	39 (10.2)	63 (16.4)	282 (73.4)	12.667[2]	0.0001[2]	(1.59～5.60)

report has shown that *IL-13 A2044G* can alter the primary structure of protein and produce the mutant IL-13 which is more active than WT IL-13.[28-29] Thus, we conclude that the airway muscle cells and epithelial cells can overexpress RANTES by regulation of the mutant IL-13, and RANTES is greatly over expressed through interaction between the mutant IL-13 and RANTES G-28C. Further studies will need to be conducted on this topic.

In our study, we also found that no significant difference between existed between the experimental and control groups in genotype distribution among the three loci (*IL-13 C-1112T, C1923T* and *RANTES A-403G*), indicating that these SNPs had no relationship with the development of asthma in children of Han nationality. However, previous reports have shown an association between the pathogenesis of asthma and *IL-13 C-1112T*[22] and *RANTES A-403G.*[19] The reason for this discrepancy may be the diversity of genetic backgrounds among populations of different nationalities or the sample sizes of our study. Further studies are necessary to determine whether this is the case. We found no reports of an association betweenSNP *IL-13 C1923T* and childhood asthma.

Genetic determinants are an attractive approach to elucidating the inheritable causes of asthma and atopy. Much greater emphasis is focused on the single effect of an SNP locus on asthma than the assessment of combined loci that may lead to the development of asthma or atopy. As a multi-genetic disorder, the pathogenesis of asthma is likely to be associated with the interaction of many SNP loci.[1,25] Future studies will seek to enlarge sample sizes and increase SNP loci for interaction analyses in Chinese Han children and multifactor dimensionality reduction (MDR) will be utilized to set up a predisposing gene model[30-31] for screening children with a high risk of developing asthma.

Acknowledgement

1.Quanhua Liu and Li Hua contributed to the article equally. 2.Grant sponsor: The National Natural Science Foundation of China, General Program (contract grant number: 30972750); Science and Technology Commission of Shanghai Municipality, Key Technologies R&D Program (contract grant number: 10DZ1951000); Selection and cultivation of young teachers in colleges and universities of Shanghai, special fund for scientific research (contract grant number: jdy10116).

Conflict of interest

The authors declare that they have no conflicts of interest.

Table 3. Analysis of the combined effects of IL-13 A2044G A/A and RANTES G-28C G/G on asthma [*n* (%)]

Group	No.	A/A+G/G	nonA/A+G/G	A/A+nonG/G	nonA/A+nonG/G
Patients	384	32 (8.3)	7 (1.8)	18 (4.7)	327 (85.2)
Control	384	7 (1.8)	7 (1.8)	14 (3.6)	356 (92.7)
χ^2		17.235[1]	0.025[2]	0.858[3]	
P		0.0001[1]	0.875[2]	0.354[3]	
OR (95% *CI*)		4.62 (2.07～10.34)[1]	1.09 (0.39～3.07)[2]	1.38 (0.70～2.73)[3]	

[1]A/A+G/G group compared with non/A+nonG/G group;

[2] nonA/A+G/G group compared with non/A+nonG/G group;

[3] A/A+nonG/G group compared with nonA/A+nonG/G group.

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