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Identification of IL13 C1923T as a Single Nucleotide Polymorphism for Asthma in Children from Mauritius

Kamleshun Ramphul, MD, Li Hua, PhD, Yi Xiao Bao, PhD, Jing Yang Li, MD, Quan Hua Liu, PhD, Ruo Xu Ji, PhD, and Ding Zhu Fang, PhD

Background: Research increasingly suggests that asthma is a familial and hereditary disorder and that genetic and environmental factors play a key role in its pathogenesis.
Objective: The aim of this study was to investigate the associations between 10 single nucleotide polymorphism (SNP) loci in the development of asthma in children from the Mauritian population.
Methods: The study population consisted of 193 children with asthma and 189 healthy controls from the Mauritian population. Asthma was diagnosed in accordance with the American Thoracic Society criteria. TaqMan real-time quantitative polymerase chain reaction was used to detect the genotypes of the SNP loci.
Results: No statistically significant differences ($p > 0.05$) were found between the experimental and control group in genotype distribution among nine of the loci (MS4A2 E237G, MS4A2 C-109T, ADRB2 R16G, IL4RA Q551R, IL4RA I75V, IL4 C-590T, IL13 A2044G, IL13 C-1112T, and CHI3L1 C-131G). However, the frequency of IL13 C1923T TT in the asthma group was significantly higher than in the control group (odds ratio = 2.119, $p = 0.033$) suggesting that carriers of IL13 C1923T TT in the Mauritian population may have a more significant risk of developing asthma.
Conclusion: The nine loci have little contribution to the development of childhood asthma in the Mauritian population. IL13 C1923T TT has been detected to be the susceptible genotype and may have a significant effect on the pathogenesis of childhood asthma in the Mauritian population.

Introduction

Asthma is one of the most common chronic inflammatory lung diseases, and it is believed to affect 300 million people worldwide,[1] including 10 million children.[2] Its incidence and mortality among the pediatric groups is on the rise, and its impact on society is substantial. Recent developments in molecular biology and genetics suggest that asthma is an hereditary disorder based on several factors, including genes.[3,4]

A significant amount of research has been done over the last decade to find gene loci predisposing to asthma and other atopic diseases. The elevated levels of total immunoglobulin (Ig) E and allergy-specific IgE are hallmarks of allergic inflammation.[5] Ober *et al.* recently reviewed 118 genes associated with asthma or atopy, and 25 of these have been replicated in at least six studies and thus are believed to be the susceptible genes most likely to be associated with asthma and atopy.[6] The current study examined the roles of 10 single nucleotide polymorphisms (SNPs; MS4A2 E237G, MS4A2 C-109T, ADRB2 R16G, IL4RA Q551R, IL4RA I75V, IL4 C-590T, IL13 A2044G, IL13 C1923T, IL13 C-1112T, and CHI3L1 C-131G) with the development of asthma in children from the Mauritian population. The C-109T polymorphism of MS4A2 encoding the β chain of the high-affinity IgE receptor has been associated with increased plasma IgE levels[7] and the release of proinflammatory factors in asthmatic airways.[8,9] The IL-13 gene is found on chromosome 5q31–33 and has been significantly associated with allergic asthma.[10] The T allele of the IL13 C1923T and IL13 C1112T have both shown to be significantly associated with increased risk of asthma,[11,12] and rs1295686 is involved in the dysregulation of total IgE.[13] ADRB2 R16G, a polymorphism of the β2-adrenergic receptor (β2AR) gene, may be strongly associated with hyper-responsiveness of the airway following activation by β2-adrenoceptor agonists.[14] Associations of IL-4 and the IL-4 receptor alpha chain (IL4RA) gene with asthma have also been reported in some studies,[15,16] and CH131L has been shown to be a susceptibility gene for asthma and bronchial hyper-responsiveness.[17] Recent meta-analysis conducted by Nie *et al.* has shown

Department of Pediatrics, Xin Hua Hospital, Shanghai, China.

that IL4RA Q551R polymorphism predisposes children but not adults to asthma, and its exact role in the pathogenesis is still not conclusive.[18]

Methods

Subjects

This study included 382 unrelated individuals from Mauritius (193 children with asthma and 189 adult healthy controls). Informed consent was obtained from the parents or guardians.

All patients were recruited from the Sir Seewoosagur Ramgoolam National Hospital. The 193 children with asthma were aged between 3 and 12 years old, and there was an even distribution of males and females. All asthma patients were clinically stable and had at least one active asthma symptom. Asthma was defined according to the guidelines of the American Thoracic Society.[19] The patient group consisted of children who had been diagnosed with asthma for at least 1 year. They had also had more than two asthma episodes that included symptoms such as wheezing during the past year, and were still following treatment for their asthma. The criteria for a diagnosis of asthma were: (1) frequent wheezing attributable to certain allergens or irritants; (2) bilateral wheezing on auscultation during the asthma attack, prolonged expiration; (3) reversible airway obstruction responding to bronchodilators; (4) ruling out other diseases that can present with wheezing, chest tightness, and cough.

The control participants were healthy volunteers aged between 18 and 22 years old, with an even distribution between males and females. The criteria for the control group were: no symptom or history of other pulmonary diseases, no symptoms or history of allergy, and no first-degree relatives with a history of asthma or atopy. The age group of 18–22 years old allowed enough time to rule out the possibility that the control subjects could develop asthma or any signs of allergies. The control participants and the asthma patients were not related to each other.

Approval of this study was obtained from the Ethical Committee by the Ministry of Life and Quality of Life of the Republic of Mauritius.

Genotype detection

Genomic DNA was collected and isolated from an oral mucosa swab using a DNA extraction kit (Tiangen, Beijing, China). The 10 SNP loci were genotyped by TaqMan real-time quantitative polymerase chain reaction (RT-PCR; 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), 0.2 μL of TaqMan SNP genotyping assay mix, and 2.5 μL of RNase. Cycling conditions included one cycle at 95°C for 10 min, 40 cycles at 95°C for 15 sec, and 40 cycles at 60°C for 60 sec. Negative controls were included in each PCR reaction to avoid any contamination. After RT-PCR, each sample was automatically determined by measuring allele-specific final fluorescence in the ABI Prism 9700HT detection system, using the SDS 2.2 software for allele discrimination (Applied Biosystems). The assay ID for each SNP is listed in Table 1.

Table 1. Assay ID for Each SNP

Primer	*Assay ID*
rs569108	C____900116_10
rs1441586	C___1842226_10
rs1042713	C___2084764_20
rs1801275	C___2351160_20
rs1805010	C___2769554_10
rs2243250	C__16176216_10
rs20541	C___2259921_20
rs1295686	C___8932053_10
rs1800925	C___8932056_10
rs4950928	C__27832042_10

SNP, single nucleotide polymorphism.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows v19 (IBM Corp., Armonk, NY). The Hardy–Weinberg equilibrium was estimated using the chi-square test. Differences in genotype distribution between the experimental and control group were analyzed using the chi-square test. A p-value of <0.05 was considered statistically significant.

Results

No statistically significant differences ($p>0.05$) were found between the experimental and control group in genotype distribution among nine of the loci (MS4A2 E237G, MS4A2 C-109T, ADRB2 R16G, IL4RA Q551R, IL4RA I75V, IL4 C-590T, IL13 A2044G, IL13 C-1112T, and CHI3L1 C-131G). However, the frequency of IL13 C1923T TT in the asthma group was significantly higher than in the control group (odds ratio = 2.119, $p=0.033$) suggesting that carriers of IL13 C1923T TT in the Mauritian population may have a more significant risk of developing asthma (Table 2).

Discussion

Asthma is considered to be the most common chronic childhood disease, and its pathogenesis is extremely complex and not fully understood, although the correlation of a variety of genetic loci and multiple environmental factors have been suggested as significant determinants of this disease.[20,21] The current study was based on children aged between 3 and 12 years and who had been diagnosed with asthma for at least 1 year. They were also still following treatment for their recent episodes of wheezing.

Following the completion of the Human Genome Project, analysis of SNPs has become the newest approach in the detection and localization of the genetic determinants of human disease. During the past decades, numerous studies have been done to assess the role of specific genes and SNPs in the development of the asthmatic phenotype. Polymorphisms within IL-13 genes have been associated with the development of asthma in populations from Korea,[22] the UK,[23] and Spain.[24] This study did not find a significant difference in IL13 C1923T TT between the experimental and control group. Wu *et al*. suggested that the T allele of the IL-13 C1923T was significantly associated with an increased risk of asthma in pediatric asthma patients in Middle

Table 2. Distribution of SNPs in Children of Mauritian Population

SNP	*rs Number*	*Group*	n	*Genotype*			p-*Value*	*OR (95% CI)*
				AA	AG	GG		
IL13 A2044	20541	Control	189	0.063	0.492	0.444		1.199
		Patients	189	0.115	0.396	0.490	0.78	(0.801–1.794)
				AA	AG	GG		
ADRB2 R16G	1042713	Control	188	0.229	0.4840.287		1.056	
		Patients	191	0.199	0.503	0.298	0.811	(0.678–1.643)
				AA	AG	GG		
FcER1 E237G	569108	Control	189	0.868	0.127	0.005		0.984
		Patients	192	0.885	0.109	0.005	0.991	(0.678–1.643)
				CC	CT	TT		
IL13 C1923T	1295686	Control	186	0.414	0.516	0.07		2.119
		Patients	182	0.429	0.434	0.137	0.033	(0.678–1.643)
				CC	CT	TT		
FcER1 C-109T	1441586	Control	188	0.351	0.463	0.186		0.994
		Patients	189	0.291	0.524	0.185	0.78	(0.591–1.67)
				CC	CT	TT		
IL13 C-1112T	1800925	Control	187	0.594	0.369	0.037		1.13
		Patients	190	0.632	0.326	0.042	0.816	(0.401–3.182)
				AA	AG	GG		
IL4RA Q551R	1801275	Control	189	0.593	0.365	0.042	0.865	
		Patients	190	0.521	0.442	0.037	0.784	(0.307–2.436)
				AA	AG	GG		
IL4RA I75V	1805010	Control	188	0.340	0.473	0.186		1.056
		Patients	191	0.328	0.425	0.247	0.151	(0.678–1.643)
				CC	CT	TT		
IL4 C-590T	2243250	Control	189	0.667	0.259	0.074		1.471
		Patients	190	0.542	0.353	0.105	0.288	(0.72–3.006)
				CC	CT	TT		
CHI3L1 C-131G 4950928	Control	189	0.64	0.333	0.026		1.187	
		Patients	192	0.635	0.333	0.031	0.78	(0.356–3.958)

rs, reference SNP; OR, odds ratio; CI, confidence interval.

China.[25] However, no research has previously been done to investigate the genes and SNPs predisposing to asthma in the Mauritian population. The rs1295686 has been associated with the dysregulation of total IgE,[26] which is a major factor in the development of asthma,[27] but its role in the pathogenesis of asthma for the pediatric population has not been fully understood.

No statistically significant differences ($p > 0.05$) were found between the experimental and control group in genotype distribution among nine of the loci (MS4A2 E237G, MS4A2 C-109T, ADRB2 R16G, IL4RA Q551R, IL4RA I75V, IL4 C-590T, IL13 A2044G, IL13 C-1112T, and CHI3L1 C-131G). This contradicts previous reports showing an association between the pathogenesis of asthma and IL4 C-590T,[15] IL13 C-1112T,[28] IL4RA I75V,[29] MS4A2 E237G,[30] ADRB2 R16G, and MS4A2 C-109T.[31] This may be due to the diverse genetic backgrounds in populations of different nationalities or to the small size of the present sample. Further investigations could be considered with different nationalities.

Asthma is a multi-genetic disorder, and its pathogenesis is usually associated with the interaction of several SNP loci.[32,33] Further studies on Mauritian children with a larger sample size and a wider SNP loci choice should be done to set up a possible molecular model for the prediction and diagnosis of asthma in the Mauritian population.

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Address correspondence to:
Yi Xiao Bao, PhD
Department of Pediatrics
Xin Hua Hospital
1665 Kong Jiang Road
Shanghai 200092
China

E-mail: drsmilebao@163.com

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