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Single nucleotide polymorphisms predisposing to asthma in children of Mauritian Indian and Chinese Han ethnicity

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Abstract

Our objective was to investigate the distributions of six single nucleotide polymorphisms (SNPs) *MS4A2 E237G*, *MS4A2 C-109T*, *ADRB2 R16G*, *IL4RA I75V*, *IL4 C-590T*, and *IL13 C1923T* in Mauritian Indian and Chinese Han children with asthma. This case-control association study enrolled 382 unrelated Mauritian Indian children, 193 with asthma and 189 healthy controls, and 384 unrelated Chinese Han children, 192 with asthma and 192 healthy controls. The SNP loci were genotyped using polymerase chain reaction (PCR)-restriction fragment length polymorphism for the Chinese Han samples and TaqMan real-time quantitative PCR for the Mauritian Indian samples. In the Mauritian Indian children, there was a significant difference in the distribution of *IL13 C1923T* between the asthma and control groups (P = 0.033). The frequency of *IL13 C1923T T/T* in the Mauritian Indian asthma group was significantly higher than in the control group [odds ratio (OR) = 2.119, 95% confidence interval = 1.048-4.285]. The Chinese Han children with asthma had significantly higher frequencies of *MS4A2 C-109T T/T* (OR = 1.961, P = 0.001) and *ADRB2 R16G A/A* (OR = 2.575, P = 0.000) than the control group. The *IL13 C1923T* locus predisposed to asthma in Mauritian Indian children, which represents an ethnic difference from the Chinese Han population. The *MS4A2 C-109T T/T* and *ADRB2 R16G A/A* genotypes were associated with asthma in the Chinese Han children.

Key words: Asthma; Single nucleotide polymorphisms; Mauritian Indian; Chinese Han

Introduction

Asthma, one of the most common chronic respiratory diseases of childhood, is characterized by reversible airflow obstruction due to chronic inflammation of the airways (1). It is thought to be caused by a combination of genetic and environmental factors (2). In the last decade, analysis of single nucleotide polymorphisms (SNPs) has become the newest approach for detection and localization of the genetic determinants of asthma (3,4).

Elevated levels of total immunoglobulin (Ig)E and allergy-specific IgE are hallmarks of allergic inflammation (5). Many genetic studies have shown that the *C-109T* polymorphism of *MS4A2* encoding the β chain of the high-affinity IgE receptor is associated with increased plasma IgE levels (6) and the release of proinflammatory factors in asthmatic airways (7,8). Interleukin (IL)-13 may promote the differentiation and survival of eosinophils and mast cells and induce the isotype switching of IgE. The T allele of the *IL13 C1923T* locus is significantly associated with increased risk of asthma (9), and *rs1295686* is associated with the dysregulation of total IgE (10). *ADRB2 R16G*, a polymorphism of the β2-adrenergic receptor gene, may be strongly associated with airway hyperresponsiveness following activation by β2-adrenoceptor agonists (11). Association of the IL-4 and IL-4 receptor alpha chain (*IL4RA*) gene with asthma has also been reported in some studies (12,13).

In our research, we genotyped six SNP loci from the five candidate genes (*IL13*, *IL4*, *IL4RA*, *MS4A2*, *ADRB2*) in Mauritian Indian and Chinese Han populations in order to determine their association with asthma.

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Material and Methods

Subjects

This case-control association study included 382 unrelated Mauritian Indian children, 193 with asthma and 189 healthy controls, and 384 unrelated Chinese Han children, 192 with asthma and 192 healthy controls. The children with asthma were recruited at the Sir Seewoosagur Ramgoolam National Hospital of Mauritius and the Asthma Clinic of Shanghai Xin Hua Hospital in China. They were 3 to 12 years of age, evenly divided between males and females and fulfilled the guidelines of the American Thoracic Society for the diagnosis of asthma (14). The control group consisted of unrelated healthy volunteers, 18 to 22 years of age, evenly divided between males and females, had no symptoms or history of allergy or other pulmonary diseases, and had no first-degree relatives with a history of asthma or atopy. All subjects provided written informed consent.

The study design was approved by the Ethics Committees of the Ministry of Life and Quality of Life of the Republic of Mauritius and Shanghai Xin Hua Hospital in China, and was conducted according to the Declaration of Helsinki and all subsequent revisions.

Genotyping

Genomic DNA was collected and isolated from oral mucosa swabs using a DNA extraction kit (Tiangen, China). The six SNP loci from the Chinese Han population were detected using polymerase chain reaction (PCR)-restriction fragment length polymorphism (15,16). PCR amplification of the corresponding genomic region surrounding each SNP locus was performed in a TaKaRa PCR thermal cycler (TaKaRa TP600, China). The reaction was performed in a final volume of 10 μL including 2.05 μL commercial PCR master mix (TaKaRa Ex Taq), 5 pmol of each primer, and 10 ng genomic DNA. Cycling conditions included 1 cycle at 95°C for 5 min, 40 cycles at 95°C for 30 s, melting temperature for 45 s, 72°C for 1 min, and a final extension at 72°C for 10 min. To avoid contamination, negative controls were included in each PCR. PCR-amplified products were detected by 2% agarose gel electrophoresis. Six different restriction enzymes were used to digest the PCR products of the six loci. A 10-μL reaction mixture containing 0.5 U restriction enzyme (NEB, China) and 5 μL PCR products was incubated for 16 h. Digested products were analyzed by 4% agarose gel electrophoresis and photographed using the Automatic Digital Gel Imaging system (Tanon-3500, China).

The six SNP loci from the Mauritian Indian population were genotyped by TaqMan real-time quantitative PCR (ABI 9700, Applied Biosystems,USA). The reaction was performed on a 384-well plate. Each well contained 6-FAM (6-carboxyfluorescein)- and VIC (4,7,2′-trichloro-7′-phenyl-6-carboxyfluorescein)-labeled probes. The total volume of 10 μL included 10 ng genomic DNA, 5 μL TaqMan Universal Master Mix (Applied Biosystems), 0.2 μL TaqMan SNP Genotyping Assay Mix, and 2.5 μL RNase. Cycling conditions included 1 cycle of 95°C for 10 min, 40 cycles at 95°C for 15 s, and 60°C for 60 s. The Assay IDs of rs569108, rs1441586, rs1042713, rs1805010, rs2243250, rs1295686 were C___900116_10, C___1842226_10, C___2084764_20, C___2769554_10, C___16176216_10, and C___8932053_10, respectively. Negative controls were included in each PCR to avoid contamination. After PCR, each sample was automatically analyzed by measuring allele-specific final fluorescence in the ABI Prism 9700HT detection system, using the SDS 2.2 software for allele discrimination (Applied Biosystems).

Statistical analysis

Hardy-Weinberg equilibrium and genotype distributions between asthma children and healthy controls were analyzed by the chi-square test. All statistical analyses were done with the SPSS 18.0 software (IBM Corporation, USA). Two-tailed P values of 0.05 or less were considered to be statistically significant.

Results

Genotype distributions with asthma in Mauritian Indian population

As shown in Table 1, there was a significant difference in the distribution of *IL13 C1923T* in asthma and control groups ($P = 0.033$), and the frequency of *IL13 C1923T T/T* in the asthma group was significantly higher than in the control group [odds ratio (OR) = 2.119, 95% confidence interval (95%CI) = 1.048-4.285]. No statistically significant differences were found in genotype distributions of the other five loci (*MS4A2 E237G*, *MS4A2 C-109T*, *ADRB2 R16G*, *IL4RA I75V*, and *IL4 C-590T*) between the two groups ($P > 0.05$).

Genotype distributions with asthma in Chinese Han population

Significant differences were found in the distribution of *MS4A2 C-109T* ($P = 0.001$) and *ADRB2 R16G* ($P = 0.000$) between the asthma and control groups. The asthma group had significantly higher frequencies of *MS4A2 C-109T T/T* (OR = 1.961, 95%CI = 1.31-2.94), and *ADRB2 R16G A/A* (OR = 2.575, 95%CI = 1.66-3.99) than the control group. There were no statistically significant differences in genotype distributions of the other four loci (*MS4A2 E237G*, *IL4RA I75V*, *IL4 C-590T*, and *IL13 C1923T*) between the 2 groups ($P > 0.05$; Table 2).

Discussion

In the first part of this study, we genotyped six SNP loci from five candidate genes in Mauritian Indian children

Table 1. Distribution of SNPs in the Mauritian Indian population.

SNP/Group	n	Genotype			P	Odds ratio (95%CI)
C1923T		CC	CT	TT	0.033	2.119 (1.048-4.285)
Control	186	0.414	0.516	0.07		
Asthma	182	0.429	0.434	0.137		
E237G		AA	AG	GG	0.992	0.984 (0.678-1.643)
Control	188	0.868	0.127	0.005		
Asthma	192	0.885	0.109	0.005		
I75V		AA	AG	GG	0.151	1.436 (0.875-2.436)
Control	188	0.340	0.473	0.186		
Asthma	186	0.328	0.425	0.247		
C-590T		CC	CT	TT	0.288	1.471 (0.72-3.006)
Control	189	0.667	0.259	0.074		
Asthma	190	0.542	0.353	0.105		
R16G		AA	AG	GG	0.811	1.056 (0.678-1.643)
Control	188	0.229	0.484	0.287		
Asthma	192	0.885	0.503	0.298		
C-109T		CC	CT	TT	0.98	0.994 (0.591-1.67)
Control	188	0.351	0.463	0.186		
Asthma	189	0.291	0.524	0.185		

CI: confidence interval; SNP: single nucleotide polymorphism.

suffering from asthma. A significant difference in the distribution of *IL13 C1923T* was found between the asthma and control groups. The frequency of *IL13 C1923T T/T* homozygote in the asthma group was significantly higher than in the control group. The results indicate that *IL13 C1923T* may be associated with asthma in Mauritian Indian children and the homozygous *IL13 C1923T T/T* alleles may be responsible for the development of asthma.

We also genotyped these six SNPs in the Chinese Han population and found significant differences in the distribution of *MS4A2 C-109T* and *ADRB2 R16G* between

Table 2. Distribution of SNPs in the Chinese Han population.

SNP/Group	n	Genotype			P	Odds ratio (95%CI)
C1923T		CC	CT	TT	0.16	1.650 (0.880-3.097)
Control	192	0.266	0.589	0.146		
Asthma	192	0.260	0.646	0.094		
E237G		AA	AG	GG	0.042	2.379 (1.009-5.613)
Control	192	0.708	0.198	0.094		
Asthma	192	0.724	0.234	0.042		
I75V		AA	AG	GG	0.524	1.177 (0.713-1.941)
Control	192	0.286	0.500	0.214		
Asthma	192	0.333	0.479	0.188		
C-590T		CC	CT	TT	0.745	0.932 (0.609-1.426)
Control	192	0.026	0.313	0.661		
Asthma	192	0.036	0.286	0.677		
R16G		AA	AG	GG	0.00	2.575 (1.664-3.985)
Control	192	0.156	0.396	0.448		
Asthma	192	0.240	0.521	0.240		
C-109T		CC	CT	TT	0.001	1.961 (1.307-2.942)
Control	192	0.125	0.302	0.573		
Asthma	192	0.125	0.469	0.406		

CI: confidence interval; SNP: single nucleotide polymorphism.

the asthma and control groups, suggesting that these two SNPs may be related to asthma in Chinese Han children. Compared with the control group, the asthma group had significantly higher frequencies of *MS4A2 C-109T T/T* and *ADRB2 R16G A/A*, indicating that *C-109T T/T* and *R16G A/A* homozygotes might be responsible for the development of asthma.

In our study, no significant association of *MS4A2 E237G*, *IL4RA I75V* and *IL4 C-590T* with asthma was shown in the two ethnic populations, which contrasts with findings in other ethnic groups. One study performed in central China reported that the T allele of the *IL13 C1923T* locus was significantly associated with increased risk of pediatric asthma (17). However, in our study the *IL13 C1923T* polymorphism was associated with asthma only in the Mauritian Indian and not in the Chinese Han population. We did not find a common locus predisposing to asthma in the two ethnically different populations.

In conclusion, our study indicates *IL13 C1923T* as a predisposing locus to asthma in Mauritian Indian children and *MS4A2 C-109T T/T* and *ADRB2 R16G A/A* as associated with asthma in Chinese Han children.

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