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

K. Ramphul*, J. Lv*, L. Hua, Q.H. Liu, D.Z. Fang, R.X. Ji and Y.X. Bao
Department of Pediatrics, Xin Hua Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

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.

Correspondence: Y.X. Bao, Department of Pediatrics, Xin Hua Hospital, Shanghai Jiaotong University School of Medicine, 1665 Kongjiang Road, Shanghai 200092, China. Fax: +86-21-6579-5173. E-mail: smilebao@sjtu.edu.cn

*These authors contributed equally to this study.

Received December 6, 2013. Accepted February 11, 2014. First published online April 4, 2014.
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 Ramaoolam 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 TP800, 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 of 95°C for 5 min, 40 cycles at 95°C for 10 s, 60°C for 15 s, and 72°C for 60 s. 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...
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 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.

### 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.

Acknowledgments

Research supported in part by grants from the National Natural Science Foundation of China (#30972750 and #30872805), the Key Technology R&D Program from the Science and Technology Commission of Shanghai (#10DZ1951000) and a grant from Xin Hua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine in 2012 (#12QYJ03).

References

1. Accordini S, Corsico A, Cerveri I, Gislaton D, Gulsvik A, Janson C, et al. The socio-economic burden of asthma is substantial in Europe. Allergy 2008; 63: 116-124, doi: 10.1111/j.1398-9995.2007.01523.x.
2. Sengler C, Lau S, Wahn U, Nickel R. Interactions between genes and environmental factors in asthma and atopy: new developments. Respir Res 2002; 3: 7, doi: 10.1186/rr179.
3. Denham S, Koppelman GH, Blakey J, Wijkstra M, Ferreira MA, Hall IP, et al. Meta-analysis of genome-wide linkage studies of asthma and related traits. Respir Res 2008; 9: 38, doi: 10.1186/1465-9921-9-38.
4. Zhang Y, Zhang J, Huang J, Li X, He C, Tian C, et al. Polymorphisms in the transforming growth factor-beta1 gene and the risk of asthma: A meta-analysis. Respir Med 2010, 15: 643-650, doi: 10.1111/j.1440-1843.2010.01748.x.
5. Oettgen HC, Geha RS. IgE regulation and roles in asthma pathogenesis. J Allergy Clin Immunol 2001; 107: 429-440, doi: 10.1067/mii.2001.113759.
6. Kim SH, Bae JS, Holloway JW, Lee JT, Suh CH, Nahm DH, et al. A polymorphism of MS4A2 (-109T>C) encoding the beta-chain of the high-affinity immunoglobulin E receptor (FcepsilonR1beta) is associated with a susceptibility to aspirin-intolerant asthma. Clin Exp Allergy 2006; 36: 877-883, doi: 10.1111/j.1365-2223.2006.02443.x.
7. Kim YK, Park HW, Yang JS, Oh SY, Chang YS, Shin ES, et al. Association and functional relevance of E237G, a polymorphism of the high-affinity immunoglobulin E receptor beta chain gene, to airway hyper-responsiveness. Clin Exp Allergy 2007; 37: 592-598, doi: 10.1111/j.1365-2223.2007.02680.x.
8. Cui T, Wang L, Wu J, Xie J. The association analysis of FcepsilonRIbeta with allergic asthma in a Chinese population. Chin Med J 2003; 116: 1875-1878.
9. Lachheb J, Chelbi H, Ammar J, Hamzaoui K, Hamzaoui A. Promoter polymorphism of the IL-18 gene is associated with atopic asthma in Tunisian children. Int J Immunogenet 2008; 35: 63-68, doi: 10.1111/j.1744-313X.2007.00738.x.
10. Granada M, Wilk JB, Tuzova M, Strachan DP, Weidinger S, Albrecht E, et al. A genome-wide association study of plasma total IgE concentrations in the Framingham Heart Study. J Allergy Clin Immunol 2012; 129: 840-845, doi: 10.1016/j.jaci.2011.09.029.
11. Tiotiu A, Todea D, Tiotiu D, Sandor V. The distribution and polymorphism of beta-2-adrenoreceptors in the bronchi. Pneumologia 2005; 54: 177-180.
12. Li Y, Guo B, Zhang L, Han J, Wu B, Xiong H. Association between C-589T polymorphisms of interleukin-4 gene promoter and asthma: a meta-analysis. Respir Med 2008; 102: 984-992, doi: 10.1016/j.rmed.2008.02.008.
13. Risma KA, Wang N, Andrews RP, Cunningham CM, Ericksen MB, Bernstein JA, et al. V75R576 IL-4 receptor alpha is associated with allergic asthma and enhanced IL-4 receptor function. J Immunol 2002; 169: 1604-1610.
14. American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, November 1986. Am Rev Respir Dis 1987; 136: 225-244, doi: 10.1164/ajrccm/136.1.225.
15. Kumar R, Barbadic M. Oncogene detection at the single cell level. Oncogene 1988; 3: 647-651.
16. Aron Y, Swierzczewski E, Lockhart A. A simple and rapid micromethod for genomic DNA extraction from jugal epithelial cells. Application to human lymphocyte antigen typing in one large family of atopic/asthmatic probands. Allergy 1994; 49: 788-790, doi: 10.1111/j.1398-9995.1994.tb02105.x.
17. Wu X, Li Y, Chen Q, Chen F, Cai P, Wang L, et al. Association and gene-gene interactions of eight common single-nucleotide polymorphisms with pediatric asthma in middle China. J Asthma 2010; 47: 238-244, doi: 10.3109/02770900903509099.

www.bjournal.com.br
Braz J Med Biol Res 47(5) 2014