ORIGINAL ARTICLE

Single-Nucleotide Polymorphisms in Genes Predisposing to Asthma in Children of Chinese Han Nationality

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Abstract

Background: Research increasingly suggests that asthma is a familial and hereditary disorder in the pathogenesis of which genetic and environmental factors play an important role.
Objective: To investigate the single and combined associations between 8 single-nucleotide polymorphism (SNP) loci in 5 genes and the development of asthma in children of Chinese Han nationality.
Methods: The study population comprised 192 children with asthma and an equal number of healthy controls. Asthma was diagnosed in accordance with American Thoracic Society criteria. Polymerase chain reaction-restriction fragment length polymorphism was used to detect the genotypes of the SNP loci.
Results: No statistically significant differences (*P*>.05) were found between the experimental and control group in genotype distribution among 6 loci (IL-13 C−1112T, IL-13 C1923T, IL-4 C−590T, IL-4RA I75V, FcεR1ß E237G, and ß2-ADR Q27E). However, significant diversity was observed among FcεR1ß C−109T (*P*=.002) and ß2-ADR R16G (*P*=.000). Furthermore, the frequency of FcεR1ß C−109T T/T and ß2-ADR R16G A/A in the asthma group was significantly higher than in the control group (odds ratio [OR]=1.96, *P*=.001; OR=2.58, *P*=.000, respectively). Carriers of both FcεR1ß C−109T T/T and ß2-ADR R16G A/A had a more significant risk of developing asthma than those with only a single polymorphism.
Conclusion: The 6 loci (IL-13 C–1112T, IL-13 C1923T, IL-4 C–590T, IL-4RA I75V, FcεR1ß E237G and ß2-ADR Q27E) make little contribution to the development of asthma in children of Chinese Han nationality. FcεR1ß C–109T and ß2-ADR R16G are significantly associated with childhood asthma. FcεR1ß C–109T T/T and ß2-ADR R16G A/A have a significant and combined effect on the development of asthma.

Key words: Asthma. Single nucleotide polymorphism. IL-13. IL-4. IL-4 receptor. Fc receptor. Adrenoreceptor.

Resumen

Antecedentes: La investigación sugiere de forma creciente que el asma es un desorden familiar y hereditario en la patogénesis en la cual juegan un importante papel los factores genéticos y ambientales.
Objetivo: Investigar las asociaciones simples y combinadas entre 8 polimorfismos de nucleótido simple (SNP) situados en 5 genes y el desarrollo de asma en niños de nacionalidad china de la etnia Han.
Métodos: La población estudiada comprende 192 niños con asma y un número idéntico de sujetos control. El asma se diagnosticó de acuerdo con los criterios de la Sociedad Torácica Americana. Se empleó la reacción en cadena de la polimerasa aplicada al polimorfismo en la longitud de fragmentos de restricción para detectar los genotipos de los loci SNP.
Resultados: No se hallaron diferencias estadísticamente significativas (*P*>0,05) entre el grupo experimental y el control en la distribución de los genotipos entre los 6 loci (IL-13 C1112T, IL-13 C1923T, IL-4 C590T, IL-4RA I75V, FcεRß E237G, y ß2-ADR Q27E). Sin embargo, se observó una diversidad significativa entre FcεR1ß C–109T (*P*=0,002) y ß2-ADR R16G (p=0,000). Además, la frecuencia de FcεR1ß C–109T T/T y ß2-ADR R16G A/A en el grupo de asma fue significativamente superior al grupo control (odds ratio [OR]=1,96, *P*=,001; OR=2,58, *P*=0,000, respectivamente). Los portadores de FcεR1ß C–109T T/T y ß2-ADR R16G A/A tenían un riesgo significativo mayor de desarrollar asma comparados con aquellos con un solo polimorfismo simple.
Conclusión: Los 6 loci (IL-13 C-1112T, IL-13 C1923T, IL-4 C-590T, IL-4RA I75V, FcεR1ß E237G y ß2-ADR Q27E) aportan una pequeña contribución al desarrollo del asma infantil en niños Chinos de la etnia Han. FcεR1ß C–109T y ß2-ADR R16G están significativamente asociados con el asma infantil. FcεR1ß C–109T T/T y ß2-ADR R16G A/A tienen un efecto combinado significativo en el desarrollo del.

Palabras clave: Asma. Polimorfismos de nucleótido simple. IL-13. IL-4. Receptor de IL-4. Receptor Fc. Adrenorreceptor.

Introduction

Asthma is one of the most common chronic inflammatory lung diseases worldwide. Its incidence and mortality are increasing among children and its impact on society is substantial. Recent developments in molecular biology and genetics suggest that asthma is hereditary disorder based on several factors, including genes [1,2].

For the past 10 years, we have been studying the gene loci predisposing to asthma and other atopic disorders. Ober [3] recently reviewed 118 genes associated with asthma or atopy, and 25 of these have been duplicated in 6 or more studies and thus are believed to be the susceptible genes most likely to be associated with asthma and atopy. 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.

We assessed 8 SNP loci (IL-13 C–1112T, C1923T, IL-4 C–590T, IL-4RA I75V, FcεR1ß C–109T, E237G, ß2-ADR R16G, Q27E) in 5 candidate genes (IL-13, IL-4, IL-4RA, FcεR1ß, ß2-ADR) for single and combined association with the development of asthma in Chinese Han children.

Methods

Study Participants

The study population comprised 384 unrelated individuals

Table 1. Overview of the 8 SNPs Genotyped in Our Study Using PCR-RFLP

SNP Name	rs Number	Primers	Tm, ºC	PCR Product	Restriction Enzyme	Digested Product Length
IL-13 C–1112T	1800925	Forward 5'>GGAATCCAGCATGCCTTGTGAGG<3' Reverse 5'>GTCGCCTTTTCCTGCTCTTCCCGC<3'	65	246	BstUI	TT: 246 bp CC: 223 bp, 23 bp CT: 246 bp, 223 bp, 23 bp
IL-13 C1923T	1295686	Forward 5'>AATGAGACAGTCCCTGGAAAG<3' Reverse 5'>CCGCCTACCCAAGACATTT<3'	54	302	BsaAI	TT: 294 bp CC: 230 bp, 64 bp CT: 294 bp, 230 bp, 64 bp
IL-4 C–590T	2243250	Forward 5'>ACTAGGCCTCACCTGATACG<3' Reverse 5'>GTTGTAATGCAGTCCTCCTG<3'	56	254	BsmFI	TT: 254 bp CC: 210 bp, 44 bp CT: 254 bp, 210 bp, 44 bp
IL-4RA 175V	1805010	Forward 5'>GGCAGGTGTGAGGAGCATCC<3' Reverse 5'>GCCTCCGTTGTTCTCAGGTA<3'	65	273	Rsal	AA: 273 bp GG: 254 bp, 19 bp AG: 273 bp, 254 bp, 19 bp
FcεR1ß C–109T	1441586	Forward 5'>GTGGGGACAATTCCAGAAGA<3' Reverse 5'>CCGAGCTGTCCAGGAATAAA<3'	60	382	Tru9I	CC: 221 bp, 161 bp TT: 182 bp, 161 bp, 39 bp CT: 221 bp, 182 bp, 161 bp, 39 bp
FcεR1ß E237G	569108	Forward 5'>GAGGTAAGTCTCTTGAGCG<3' Reverse 5'>CTTATAAATCAATGGGAGGAAACA<3'	55	173	BseXI	GG: 173 bp AA: 150 bp, 23 bp AG: 173 bp, 150 bp, 23 bp
ß2–ADR R16G	1042713	Forward 5'>GCCTTCTTGCTGGCACCCCAT<3' Reverse 5'>CAGACGCTCGAACTTGGCCATG<3'	66	168	NcoI	AA: 168 bp GG: 150 bp, 23 bp AG: 173 bp, 150 bp, 23b
ß2–ADR Q27E	1042714	Forward 5'>CGCCTTCTTGCTGGCACCCAAT<3' Reverse 5'>CCAGTGAAGTGATGAAGTAGTT<3'	60	203	BseXI	GG: 203 bp CC: 139 bp, 64 bp GC: 203 bp, 139 bp, 64 bp

Abbreviations: IL, interleukin; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; rs, reference SNP; SNP, single nucleotide polymorphism; Tm, melting temperature.

of Chinese Han nationality (192 patients with asthma and an equal number of healthy controls). Informed consent was obtained in all cases.

All patients were recruited from the Asthma Clinic of Xin Hua hospital, which is affiliated to the Shanghai Jiaotong University School of Medicine. The 192 patients were aged 3 to 12 years (50% boys and 50% girls). Each patient had at least 1 active asthma symptom. Asthma was defined by the following criteria: 2 or more episodes of wheezing and shortness of breath during the past year, and reversibility of wheezing and dyspnea, either spontaneously or using bronchodilators, as defined by the guidelines of the American Thoracic Society [4].

The control participants were healthy students from Shanghai Huadong University and Shanghai Normal University. They were aged 18 to 22 years (96 men and 96 women). The inclusion criteria for the control group were as follows: no symptoms or history of asthma, no symptoms or history of other pulmonary diseases, no symptoms or history of allergy, and no first-degree relatives with a history of asthma or atopy.

Genotype Detection

Genomic DNA was isolated from an oral mucosal swab using a DNA extraction kit (Tiangen, Beijing, China). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was applied to detect the genotypes of the 8 SNP loci [5-7]. The reference SNP (rs) number, primers, melting temperature, PCR product length, restriction enzyme, and digested products length of each SNP are described in Table 1. All the SNPs were performed under the same conditions. PCR amplification of the corresponding genomic region surrounding each SNP locus was performed in a TaKaRa PCR thermal cycler (TaKaRa TP600, Dalian, China). The reaction was performed in a final volume of 10 μL including 2.05 μL of a commercial PCR master mix (TaKaRa Ex Taq, Dalian, China), 5 pmol of each primer, and 10 ng of genomic DNA. Cycling conditions included 1 cycle at 95ºC for 5 minutes, 40 cycles at 95ºC for 30 seconds, melting temperature for 45 seconds, 72ºC for 1 minute, and a final extension at 72ºC for 10 minutes. To avoid contamination, negative controls were included in each PCR reaction. PCR-amplified products were detected by 2% agarose gel electrophoresis. Eight different restriction enzymes were used to digest the PCR products of the 8 loci. A 10-μL reaction mixture containing 0.5 units of restriction enzyme (NEB, Beijing, China) and 5 μL of PCR products was incubated for 16 hours. Digested products were analyzed by 4% agarose gel electrophoresis and photographed using the Automatic Digital Gel Imaging system (Tanon-3500, Shanghai, China).

Statistical Analysis

The Hardy-Weinberg equilibrium was estimated using the χ^2 test. Differences in genotype distribution between the experimental and control group were analyzed using the χ^2 test. A P value of .05 or less was considered statistically significant. All statistical analyses were performed using SPSS version 13.0 software (SPSS Inc, Chicago, Illinois, USA).

Results

Single Effect of the 8 SNP Loci on the Development of Asthma in Children of Chinese Han Nationality

No statistically significant differences (P>.05) were found between the experimental group and the control group in

Table 2. Analysis of Association Between FcεR1ß C–109T and ß2-ADR R16G and Asthma

SNP	Group	N	Genotype			χ^2	*P* Value	Odds Ratio (95% CI)
			C/T	C/C	T/T			
C–109T	Control	192	90 (46.9)	24 (12.5)	78 (40.6)	10.671	.001	1.96 (1.31-2.94)
	Experimental	192	58 (30.2)	24 (12.5)	110 (57.3)			
			G/G	A/G	A/A			
R16G	Control	192	46 (24.0)	100 (52.1)	46 (24.0)	18.470	.000	2.58 (1.66-3.99)
	Experimental	192	30 (15.6)	76 (39.6)	86 (44.8)			

Abbreviations: CI, confidence interval; SNP, single-nucleotide polymorphism.
[a] Data are expressed as n (%). Analyses were performed using a 2×2 table for C–109T SNP with homozygotic T/T vs. others in cases and controls, and for R16G SNP with homozygotic A/A vs. others in cases and controls.

Table 3. Analysis of the Combined Effects of FcεR1ß C–109T T/T and ß2-ADR R16G A/A on Asthma

Group	Number	Non-T/T+non-A/A	T/T+non-A/A	Non-T/T+A/A	T/T+A/A
Control	192	82 (42.7)	64 (33.3)	32 (16.7)	14 (7.3)
Experimental	192	60 (31.3)	46 (24.0)	22 (11.5)	64 (33.3)
χ^2		32.421[a]	30.432[b]	23.984[c]	
P Value		0.000[a]	0.000[b]	0.000[c]	
OR (95% CI)		6.25 (3.21-12.18)[a]	6.36 (3.19-12.70)[b]	6.65 (3.01-14.70)[c]	

Abbreviations: CI, confidence interval; OR, odds ratio.
[a] T/T+A/A group compared with non T/T + non A/A group
[b] T/T+A/A group compared with T/T + non A/A group
[c] T/T+A/A group compared with non T/T + A/A group

genotype distribution in 6 loci (IL-13 C–1112T, IL-13 C1923T, IL-4 C–590T, IL-4RA I75V, FcεR1ß E237G, and ß2-ADR Q27E). However, significant diversity was observed between FcεR1ß C–109T (χ^2=12.366, P=.002) and ß2-ADR R16G (χ^2=18.762, *P*=.000). The frequency of FcεR1ß C–109T T/T and ß2-ADR R16G A/A in the asthma group was significantly higher than those in the control group (Table 2).

Combined Effects of FcεR1ß C–109T and ß2-ADR R16G Loci on the Development of Asthma in Children of Chinese Han Nationality

Different genotype combinations were designated according to FcεR1ß C–109T T/T, or ß2-ADR R16G A/A homozygosity (Table 3). The carriers of both T/T and A/A had a more significant risk of developing asthma than those with either a single SNP or no SNP.

Discussion

Asthma is a common clinical syndrome resulting from several factors such as immunity, heredity, and environment. A number of genes have been proposed as causing or contributing to the development of asthma. In all cases, chronic inflammation coupled with the rise of total immunoglobulin (Ig) E and allergy-specific IgE levels leads to airway hyperresponsiveness, which is a hallmark clinical feature of allergic asthma.

During the past 10 years, numerous studies have begun to assess the contribution of specific genes and SNPs to the development of the asthmatic phenotype. Twenty-five genes associated with asthma have been duplicated in 6 or more samples [3]. Ten genes have been duplicated in over 10 independent samples, thus providing the strongest candidate genes for asthma or atopy. In view of the diversity of these genes, we chose 8 SNP loci in 5 candidate genes from the 10 most likely candidates to determine the role of these genes in the development of asthma and atopy in children of Han nationality.

We found that there were significant differences between the experimental and control group in genotype distribution between FcεR1ß C–109T and ß2-ADR R16G, and the frequency of FcεR1ß C–109T T/T and ß2-ADR R16G A/A in the asthma group was significantly higher than in the control group. Therefore, FcεR1ß C–109T and ß2-ADR R16G were the susceptible SNPs for asthma in our population, in which the homozygotic C–109T T/T and R16G A/A alleles were responsible for the development of asthma. We also observed that carriers of both C–109T T/T and R16G A/A had a more significant risk of developing asthma than those with either a single SNP or no SNP, thus suggesting that synergism existed between the C–109T T/T and R16G A/A genotypes.

Kim SH [8] demonstrated that the FcεR1ß–109T allele was associated with higher promoter activity of MS4A2 encoding the ß chain of the high-affinity IgE receptor (FcεR1ß), and that the FcεR1ß C–109T SNP may increase expression of MS4A2 by mast cells, leading to enhanced release of proinflammatory mediators in the asthmatic airway. Moreover, Cui et al [9] have confirmed that homozygosity for the –109T allele is associated with increased total plasma IgE levels in allergic asthma patients. Many studies have shown that ß2-ADR R16G SNP contributes strongly to hyperresponsiveness in the airways, and to the antagonism to bronchodilating agents through receptor downregulation [10]. However, our study is the first report of the combined effects of C–109T and R16G on the development of asthma.

We found no statistically significant differences between the experimental and control group in genotype distribution in 6 loci (IL-13 C–1112T, IL-13 C1923T, IL-4 C–590T, IL-4RA I75V, FcεR1ß E237G and ß2-ADR Q27E), thus 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 [11], IL-4 C–590T [12], IL-4RA I75V [13], FcεR1ß E237G [14], and ß2-ADR Q27E [15] SNPs. Such varied conclusions may be due to the diverse genetic backgrounds in populations of different nationalities or to the small size of our sample. Further studies are necessary to determine whether this is the case. We found no reports of the association between the SNP 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 effect of an SNP at 1 locus on asthma than on the effect of SNPs at combined loci. As asthma is a multigenetic disorder, its pathogenesis

seems to be associated with the interaction of several SNP loci [16,17]. Future studies on Chinese Han children should have large sample sizes and analyze more SNP loci. Multifactor dimensionality reduction should be applied to set up a molecular model for the prediction and diagnosis of asthma [18].

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