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Single nucleotide polymorphisms in the ORM1-like 3 gene associated with childhood asthma in a Chinese population

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ABSTRACT. Single nucleotide polymorphism (SNP)-based genome-wide association studies have revealed that polymorphisms of the ORM1-like 3 (ORMDL3) gene are associated with childhood asthma. We investigated genetic associations of SNPs in and around the ORMDL3 gene with childhood asthma in a Chinese population. Genomic DNA was extracted from peripheral venous blood drawn from 152 subjects with childhood asthma and from 190 control subjects. SNP genotyping was performed with the MassARRAY system (Sequenom) by means of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Among the six SNPs, only the genotype frequencies of rs7216389 were significantly different between asthmatic children and controls. Asthmatic children had a significantly higher frequency of T alleles [odds ratio (OR) = 1.653, 95% confidence interval (95%CI) = 1.170-2.333] in rs7216389, than controls. The TT genotype of rs7216389 was found to be a significant risk factor for childhood asthma by logistic regression analysis (OR = 1.704, 95%CI = 1.105-

2.628). There was no significant association between the TT genotype of rs7216389 and clinical features of childhood asthma. We conclude that the ORMDL3 gene influences childhood asthma and that the TT genotype of the rs7216389 polymorphism is associated with childhood asthma in the Chinese population.

Key words: Childhood asthma; Single nucleotide polymorphisms; ORM1-like 3 (ORMDL3) gene; Genetic association studies; Case-control design; Chinese population

INTRODUCTION

Asthma is one of the most common chronic airway disorders among children throughout the world. As its incidence and mortality are increasing, asthma has created a substantial impact on modern society, especially in China (Chen, 2003). In 2004, statistical studies showed that there were more than 25 million asthmatic patients in China, which included almost 10 million children (Chen, 2004). Asthma is characterized as a multifactorial disease with various unidentifiable causes underlying its development and manifestation. So far, the exact mechanism of asthma is still poorly understood, although both genetic and environmental factors are involved (Ober and Hoffjan, 2006). Environmental conditions, such as poor indoor and outdoor air quality, are risk factors of asthma, but they are much more clearly related to the provocation of asthma attacks and to asthma severity than they are to the prevalence of asthma (Strachan, 2000). However, genetic factors play an important role in the development of asthma, and the heritability of asthma has been estimated to vary between 36 and 79% (Koppelman, 2006; Barton et al., 2009).

In recent years, more than 200 asthma candidate genes have been proposed by different approaches, such as candidate gene-association study and positional cloning (Ober and Hoffjan, 2006; Zhang et al., 2008). A whole-genome association study identified 12 single nucleotide polymorphisms (SNPs) related to childhood asthma, where 7 of them mapped to a locus on chromosome 17q21, which determines the expression of the ORM1-like 3 (ORMDL3) gene; the link between the ORMDL3 gene and the onset of childhood asthma has been observed in two independent Caucasian cohorts (Moffatt et al., 2007). In particular, the strongest sign was detected for the SNP rs7216389 in the ORMDL3 gene, which is a member of a family of genes responsible for encoding transmembrane proteins of the endoplasmic reticulum (Moffatt et al., 2007).

Subsequently, the relevance between SNPs of the ORMDL3 gene and childhood asthma has been confirmed in multiple ethnic groups, such as Mexicans, Puerto Ricans, and African-Americans (Galanter et al., 2008). The SNP rs7216389, which controls the expression of the ORMDL3 gene, has been one of the most extensively studied polymorphisms. It was reported that the SNP rs7216389 is associated with the risks of asthma susceptibility and exacerbations among Scottish children and young adults (Tavendale et al., 2008). Another study of the SNP rs7216389 in a Japanese population supported the importance of ORMDL3 in asthma (Hirota et al., 2008). Moreover, it has been found that asthma diagnosis is associated with rs11650680 and five other SNPs, including rs7216389 in a Hong Kong Chinese population (Leung et al., 2009). The replication studies to define the genetic associations between polymorphisms of the ORMDL3 gene and childhood asthma in the Chinese population are in-

adequate. Studies on the association between SNPs of the ORMDL3 gene and clinical features of childhood asthma in the Chinese population have not yet been reported. The objectives of this study were to identify the relevance of SNPs in and around the ORMDL3 gene to childhood asthma, and then to investigate the associations between SNPs of the ORMDL3 gene and clinical features of childhood asthma in the Chinese population.

MATERIAL AND METHODS

Subjects

All the subjects in our study were recruited from Children's Hospital of Chongqing Medical University, including 152 independent Chinese asthmatic children from the outpatient clinic, which had been diagnosed with asthma according to the guidelines of the Global Initiative for Asthma (Bateman et al., 2008), and 190 inpatient children who suffered from non-asthmatic diseases and with no symptoms or history of asthma or other allergic diseases, such as rhinitis and eczema, no symptoms or history of other pulmonary diseases, and no first-degree relatives with a history of asthma. Patients with neurologic, ophthalmic, endocrine, or immunologic diseases were excluded from our study. A 2-mL venous blood sample was collected from each subject and used for genotyping assays. All cases and controls were free of any infectious symptoms for 4 weeks before the study. The study was approved by the Ethics Committee of Children's Hospital of Chongqing Medical University and all subjects and their parents gave written informed consent.

Target polymorphisms of the ORMDL3 gene

According to the HapMap Date Phase III CHB (Han Chinese in Beijing, China) data, the SNPs whose r^2 value and cut-off ≥ 0.8 were named tag SNPs. Six tag SNPs in and around the ORMDL3 gene were selected by using the TAGGER program available in the Haploview version 4.2 software (Broad Institute of MIT and Harvard, Cambridge, MA, USA), including rs7216389 (C/T), rs8076131 (A/G), rs12603332 (T/C), rs3744246 (T/C), rs11650680 (T/C), rs9894164 (T/C). The sequence and other more detailed information of each SNP with the individual rs accession numbers are available in NCBI databases.

DNA extraction and genotyping

The blood sample was collected in sterile anticoagulant tubes. Genomic DNA was extracted from peripheral venous blood by using a DNA Extraction kit (Qiagen, Germany) according to manufacturer instructions. Primers for PCR and single-base extension were designed by the Assay Designer software package (Sequenom). The MassARRAY system (Sequenom) based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry method (MALDI-TOF) was used to perform SNP genotyping according to manufacturer instructions. The completed genotyping reactions were spotted onto a 352-well spectroCHIP (Sequenom) by using a MassARRAY Nanodispenser (Samsung) and determined by MALDI-TOF. Genotype calling was performed in real-time with the MassARRAY RT version 3.0.0.4 software and analyzed by the MassARRAY Typer version 3.4 software (Sequenom).

Pulmonary function tests

Pulmonary function tests were performed by using MasterScope children spirometry (Jaeger, Germany). The measurement of forced expiratory volume in 1 s, forced vital capacity, and peak expiratory flow were recorded.

Skin prick tests (SPTs)

SPTs were performed for the following 13 common indoor inhalants (Solu prick SQ, ALK-Abello, Denmark): *Dermatophagoides pteronyssinus*, *D. farinae*, *Bromia tropicalis*, *Canis familiaris*, *Felis domesticus*, *Blattella germanica*, *American cockroach*, *mold mix I*, *mold mix IV*, *Artemisia vulgaris*, *Ambrosia artemisifolia*, *Pollens IV*, *Pollens I*. Glycerinated normal saline and histamine dihydrochloride (10 mg/mL) were used as negative and positive control, respectively. The positive SPTs were defined as a wheal diameter of at least 3 mm (Adinoff et al., 1990) greater than the negative control.

Measurement of serum total IgE

Serum total IgE levels were determined with a human IgE enzyme-linked immunosorbent assay (ELISA) kit (BioCheck, USA). All values were then transformed to $\log_{10}$ scale for analysis, in accordance with the recognized logarithmic distribution of total serum IgE in the general population.

Statistical analysis

All polymorphisms were tested for Hardy-Weinberg equilibrium (HWE), and a locus was considered to be in HWE if $P > 0.001$. The genotype and allele frequencies were obtained by direct counting. Differences in genotype and allele distributions between the asthmatic children and controls were analyzed by the chi-square test. The associations between allele frequencies of the ORMDL3 gene and childhood asthma were estimated by computing the odds ratio (OR) and the 95% confidence interval (95%CI). The relevance between genotypes of asthma-related SNPs in the ORMDL3 gene and childhood asthma and the association of asthma-related genotypes in the ORMDL3 gene with clinical features of childhood asthma were tested by logistic regression analysis. All statistical analyses were performed by SPSS version 11.5 and $P \leq 0.05$ was considered to be statistically significant.

RESULTS

Clinical parameters of the subjects

A total of 342 subjects were involved in our study, including 152 asthmatic children (5.9 ± 2.5 years) and 190 controls (6.0 ± 2.5 years). The clinical parameters of both groups are summarized in Table 1. There were no significant differences in the distribution of age and gender between the asthmatic children and controls ($P > 0.05$).

Table 1. Clinical parameters of subjects.

	Asthmatic children N = 152 (%)	Controls N = 190 (%)	P
Age (years, mean ± SD)	5.872 ± 2.543	6.038 ± 2.526	0.910
Gender			
Male	92 (60.5)	110 (57.9)	
Female	60 (39.5)	80 (42.1)	0.623

SD = standard deviation.

Genotype and allele frequencies of ORMDL3

All alleles and genotypes of tag SNPs were in accordance with HWE in each group. There were significant differences in the genotype frequencies of rs7216389 between asthmatic children and controls (P = 0.018), and the asthmatic children showed a higher TT genotype frequency. No significant differences in genotype frequencies of the other 5 SNPs were found between asthmatic children and controls (P > 0.05) (Table 2). Compared to controls, asthmatic children showed significantly higher T allele frequency (P = 0.04; OR = 1.653; 95%CI = 1.170-2.333) in rs7216389. No significant difference was detected in the distribution of the alleles in the other 5 SNPs among asthmatic children when compared to controls (P > 0.05) (Table 3).

Table 2. Genotype frequencies of 6 SNPs in the ORMDL3 gene between asthmatic children and controls.

rs	Genotype	Asthmatic children	Controls	χ^2	Pearson's P
rs7216389	CC	7 (0.046)	21 (0.111)		
	CT	53 (0.349)	79 (0.416)		
	TT	92 (0.605)	90 (0.474)	8.020	0.018
rs8076131	AA	91 (0.599)	102 (0.537)		
	AG	53 (0.349)	77 (0.405)		
	GG	8 (0.053)	11 (0.058)	1.326	0.515
rs12603332	CC	90 (0.592)	101 (0.532)		
	TT	7 (0.046)	10 (0.053)		
	CT	55 (0.362)	79 (0.416)	1.255	0.534
rs3744246	CC	94 (0.618)	103 (0.542)		
	TT	7 (0.046)	9 (0.047)		
	CT	51 (0.336)	78 (0.411)	2.116	0.347
rs11650680	CC	88 (0.579)	115 (0.605)		
	TT	7 (0.046)	9 (0.047)		
	CT	57 (0.375)	66 (0.347)	0.281	0.869
rs9894164	TT	151 (1.000)	190 (1.000)		

A = adenine; C = cytosine; G = guanine; T = thymine.

Table 3. Allele frequencies of 6 SNPs in the ORMDL3 gene between asthmatic children and controls.

rs	Allele	Asthmatic children	Controls	χ^2	Pearson's P	Odds ratio	95%CI
rs7216389	T	237 (0.780)	259 (0.682)				
	C	67 (0.220)	121 (0.318)	8.143	0.040	1.653	1.170-2.333
rs8076131	A	235 (0.773)	281 (0.739)				
	G	69 (0.227)	99 (0.261)	1.026	0.311	1.200	0.843-1.708
rs12603332	T	69 (0.227)	99 (0.261)				
	C	235 (0.773)	281 (0.739)	1.026	0.311	0.833	0.586-1.186
rs3744246	T	65 (0.214)	96 (0.253)				
	C	239 (0.786)	284 (0.747)	1.414	0.234	0.805	0.562-1.152
rs11650680	T	71 (0.234)	113 (0.221)				
	C	233 (0.766)	265 (0.779)	0.151	0.698	1.074	0.749-1.538
rs9894164	T	302 (1.000)	380 (1.000)				

95%CI = 95% confidence interval.

Associations of polymorphism in rs7216389 with clinical features of asthmatic children

The TT genotype of rs7216389 was found to be a risk factor of childhood asthma by logistic regression analysis (P = 0.016; OR = 1.704; 95%CI = 1.105-2.628) (Table 4). A high frequency of the TT genotype of rs7216389 was found in patients with a family history of asthma. However, no significant associations were detected between the TT genotype of rs7216389 and clinical features of asthmatic children (P > 0.05) (Table 5).

Table 4. Associations of genotypes of rs7216389 with childhood asthma.

Genotype	b	P	Odds ratio	95%CI
TT	0.533	0.016	1.704	1.105-2.628
TC	-0.285	0.206	0.752	0.484-1.169
CC	-0.945	0.036	0.389	0.161-0.941

b = regression coefficient; 95%CI = 95% confidence interval.

Table 5. Associations of the TT genotype of rs7216389 with clinical features of asthmatic children.

Clinical features	TT genotype of rs7216389			
	b	P	OR	95%CI
Age	-0.110	0.276	0.896	0.735-1.092
Gender	0.071	0.845	1.074	0.527-2.187
Asthma onset time	0.097	0.363	1.102	0.870-1.359
Family history	0.638	0.084	1.892	0.918-3.901
With rhinitis	0.047	0.899	1.048	0.506-2.173
With eczema	0.106	0.768	1.112	0.549-2.252
With urticaria	0.285	0.534	1.330	0.541-3.270
Total serum IgE level	0.001	0.176	1.001	0.999-1.003
PEF (% predicted)	0.010	0.529	1.010	0.981-1.040
FEV1 (% predicted)	-0.011	0.748	0.989	0.923-1.059
FVC (% predicted)	0.005	0.888	1.005	0.944-1.070
With allergy to *Dermatophagoides pteronyssinus*	0.358	0.689	1.430	0.248-8.266
With allergy to *Dermatophagoides farinae*	0.385	0.721	1.469	0.178-12.133

b = regression coefficient; OR = odds ratio; 95%CI = 95% confidence interval; PEF = peak expiratory flow; FEV1 = forced expiratory volume in 1 s; FVC = forced vital capacity.

DISCUSSION

Previous studies with a genome-wide association analysis identified the ORMDL3 gene as a potential candidate gene for childhood asthma (Moffatt et al., 2007). Subsequently, the associations between the ORMDL3 gene and asthma were established in distinct ethnic groups. Thus, the ORMDL3 gene became a member of genes associated with childhood asthma in multiple populations and replicated at the gene level, such as IL-4, IL-13, CD14, ADRB2, FcER1B, and IL-4RA (Hoffjan and Ober, 2002). However, there is a paucity of literature as to the link between polymorphisms of the ORMDL3 gene and childhood asthma in Chinese.

In our study, the associations of six polymorphisms in and around the ORMDL3 gene with childhood asthma, and the relevance of asthma-related SNP to clinical features of asthmatic children in a Chinese population were investigated to assess possible role of the ORMDL3 gene in the pathogenesis of childhood asthma. Significant differences in genotype and allele frequencies between asthmatic children and controls were detected

only in rs7216389 of all six SNPs. The findings of the different distributions of genotypes and alleles in rs7216389 between asthmatic children and controls are consistent with the studies conducted in multiple populations, such as Japanese (Hirota et al., 2008), Scottish (Tavendale et al., 2008), European (Moffatt et al., 2007; Sleiman et a1., 2008; Galanter et a1., 2009), African American (Galanter et al., 2008), and Chinese in Hong Kong (Leung et a1., 2009). The significantly higher T allele frequencies of rs7216389 in asthmatic children is consistent with the study by Halapi et al. (2010), which reported that the association of rs7216389-T was confined to cases with early onset asthma, particularly in early childhood. Although rs7216389 is located within the first intron of the GSDML gene, the focus has been on the fact that the SNP is strongly associated with the expression of the neighboring gene ORMDL3, not just the GSDML gene itself (Moffatt et al., 2007). The biological mechanism behind these associations remains to be explained.

In this study, the TT genotype of rs7216389 was found as a risk factor of childhood asthma by logistic regression analysis. High frequency of the TT genotype of rs7216389 was found in patients with family history of asthma, but there was no statistical significance. To develop the phenotype-specific early diagnosis of childhood asthma, the relevance between the TT genotype of rs7216389 and clinical features of asthmatic children was analyzed, but no significant associations were found. Total serum IgE levels and parent-reported urticaria or eczema were also not related to the TT genotype of rs7216389, which was similarly reported by Tavendale et al. (2008).

No significant differences in the genotype frequencies of the other five SNPs were found compared to controls. Moreover, no significant differences were detected in the distribution of the alleles in the other five SNPs comparing the asthmatic children to controls. These findings were inconsistent with several previous studies that reported that those five polymorphisms were associated with childhood asthma in different ethnic groups. Many factors can affect the results of such kind of studies, for example, sample sizes, linkage or case-control association studies and ethnic differences, and these factors are most likely to be responsible for the divergent results (Scirica and Celedón, 2007).

Functional analysis of ORMDL family genes was insufficient in the present study. It is known that the majority of these genes are responsible for encoding transmembrane proteins of the endoplasmic reticulum (Hjelmqvist et al., 2002). A recent study demonstrated that ORMDL3 alters endoplasmic reticulum-mediated Ca^{2+} homeostasis and facilitates the unfolded-protein response, and the study provided a first insight into the molecular mechanism explaining the association of ORMDL3 with proinflammatory diseases (Cantero-Recasens et al., 2010). Another study identified the Orm family proteins as critical mediators of sphingolipid homeostasis and raised the possibility that sphingolipid misregulation contributes to the development of childhood asthma (Breslow et al., 2010). However, further study will be needed to investigate the specific role of the ORMDL3 gene in the pathogenesis of childhood asthma.

In conclusion, our results highlight the role of the ORMDL3 as a gene for childhood asthma susceptibility in the Chinese population. It was found that rs7216389 polymorphism was related to childhood asthma in the Chinese population. Additionally, individuals carrying the rs7216389 TT genotype may have an increased susceptibility to childhood asthma. Our research findings may provide a new perspective to investigate the pathogenesis of childhood asthma and may lead to the development of new early diagnosis approaches of childhood asthma.

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