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Original Article

CHI3L1 is correlated with childhood asthma

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Abstract: Objective: The aim of this study was to assess the association between *CHI3L1* gene two single nucleotide polymorphisms (SNPs) (rs12141494 and rs4950928) and susceptibility to pediatric asthma in a Chinese Han population. Methods: The case-control study was carried out on 115 children with asthma and 108 healthy controls. Genotypes of rs12141494 and rs4950928 within *CHI3L1* gene were determined with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach. Chi-square test was employed to analyze the differences of genotype and allele frequencies of the two polymorphisms between case and control groups. Odds ratios (ORs) and 95% confidence intervals (CIs) were applied to explain relative susceptibility of pediatric asthma. Results: The frequencies of genotypes and alleles of rs4950928 in *CHI3L1* gene showed a significant difference between asthmatic children and healthy controls ($P<0.05$). However, no significant differences were found in *CHI3L1* SNP rs12141494 between two groups ($P>0.05$). Conclusion: Our results suggested that rs4950928 in *CHI3L1* gene was associated with pediatric asthma risk in a Chinese Han population, while rs12141494 was not.

Keywords: *CHI3L1*, polymorphism, pediatric asthma, PCR-RFLP

Introduction

Asthma is one of the most common chronic disease of the airways caused by respiratory inflammation, which is characterized by recurrent attacks of breathlessness and wheezing. It has been investigated to affect as many as 300 million people worldwide [1]. Asthma is a leading case of morbidity of children and has affected up to 10% of all children less than 18 years old in the USA [2]. In China, a current survey has estimated that the total asthma incidence of pediatric asthma aged 0-14 years old in cities has increased significantly and reached 3.02% [3]. Allergens and other environmental exposures such as tobacco smoke and air pollution are considered as the common factors of asthma in children. In addition, asthma also has a closely relationship with genetic polymorphisms [4]. The asthma prevalence is estimated to be significantly different among various regions, cities, ages, and genders. However, the pathologic and genetic mechanism have not been completely understood.

Chitinase3-like1 (CHI3L1), often referred to as YKL-40 or human cartilage glycoprotein-39 (HCgp-39), is a glycoprotein which is known to be a pro-inflammatory cytokine of chitinase [5, 6]. YKL-40 is a secreted protein with a molecular weight of 40 KD, which is produced by vatious cells including cancer cells, neutrophils and activated macrophages and plays a role in inflammation, cell proliferation, differentiation, protection against apoptosis, stimulation of angiogenesis and regulation of extracellular tissue remodeling [7]. *CHI3L1*, the encoding gene of YKL-40, is located on human chromosome 1q32.1 [7]. Plasma levels of YKL-40 increases not only in the patients with primary or advanced cancer, but also in patients with many other disease characterized by inflammation. Ridker et al. have investigated that the single-nucleotide polymorphisms (SNPs) in *CHI3L1* gene are determinants of YKL-40 levels [8].

The occurrence of asthma involves in multiple genetic disorders [9, 10]. *CHI3L1* is a novel gene related to asthma occurrence. Chupp et al. have reported that the chitinase-like protein YKL-40 is found in elevated quantities in the serum and lungs in the patients with asthma [11]. Genetic studies have also suggested that

Table 1. Primer sequences of *CHI3L1* gene rs12141494 and rs4950928 polymorphisms

SNP	Primer sequences		Size (bp)
rs12141494	Upstream	5'-TCCAGCTATTGCAGAGTATT-3'	256
	Downstream	5'-CCTTACTCCTGGGTTTCC-3'	
rs4950928	Upstream	5'-CTTGCCAGCCTCGTGATT-3'	190
	Downstream	5'-AGCAGAGCAGGGCAGGGTG-3	

variations in *CHI3L1* gene contribute to the pathogenesis of asthma. However, the researches about association between *CHI3L1* SNPs and asthma are major concentrated in foreign groups, few studies have been reported in Chinese population.

Various of genetic variants have been identified in *CHI3L1* gene in which a number of them are associated with plasma YKL-40 levels [12]. In the present study, we selected two SNPs including rs12141494 and rs4950928, and explored the distributions of *CHI3L1* gene polymorphisms in a case-control groups. Furthermore, the correlation of *CHI3L1* gene polymorphisms with pediatric asthma susceptibility had also been evaluated.

Methods and materials

Objects of study

This case-control study included 223 unrelated individuals including 115 children with asthma and 108 healthy controls. This research was consented and approved by Ethics committee of Department of Internal Medicine, Xi'an Children's Hospital. Sample collection is conformed to ethics criteria of national human genome research. Informed consent was obtained from all parents or their guardians. All participants were Chinese Han population.

The 115 children with asthma were aged between 3 and 12 years old, including 54 females and 61 males. All patients were clinically stable and had at least one active asthma symptom. Asthma patients were those admitted to Department of Internal Medicine, Xi'an Children's Hospital from June 2013 to May 2014 and were diagnosed according to the Global Initiative for Asthma guidelines. 108 age and gender-marched healthy children were recruited as controls during the same time. They were also aged between 3 and 12 years old, including 49 females and 59 males. All healthy children had no respiratory symptoms such as cough, sputum and no allergic diseases such as asthma, allergic rhinitis, atopic dermatitis and so on. Besides, their parents had no history of allergies.

Sample collection and DNA extraction

2 ml peripheral venous blood were collected from every child, anticoagulated by 0.5% EDTA (pH 8.0). Genomic DNA was extracted by DNA extraction kit (Tiangen, Beijing, China), and stored at -20°C for standby application.

PCR amplifications and genetic typing assay

The genotypes of *CHI3L1* gene rs12141494 and rs4950928 were examined by Mass ARRAY-IPLEX. Primer sequences for rs12141494 and rs4950928 were designed by Primer Premier 5.0, and synthesized by Sangon Biotech (Shanghai, China) (**Table 1**). PCR amplification was performed in a total volume of 25 μl containing 5 μl 10 × Buffer, 2 μl template DNA, 1 μl upstream primer, 1 μl downstream primer, 0.5 μl Taq DNA polymerase, 2 μl dNTP, and 13.5 μl deionized sterile water. PCR procedures were as the following: 94°C initial denaturation for 5 min; followed by 35 cycles of denaturing at 95°C for 30 s, annealing at 55°C for 30 s and extension for 30 s at 72°C; finally extension at 72°C for 10 min. PCR products were examined by agarose gel electrophoresis. Then the PCR products were recovered purified and resin desalination. Finally put the chip filled with treated PCR products into mass spectrometers to observe the genotypes.

Statistical analysis

All statistical analysis were performed using PASW statistics 18.0 statistical software. The representativeness of cases and controls were tested by Hardy-Weinberg equilibrium (HWE) test. Genotype and allele frequencies of rs1129055 and rs2715267 polymorphisms were calculated by direct counting. Comparisons of the asthmatic and control groups were conducted via Chi-square test. Correlation of *CD86* gene polymorphisms with sepsis susceptibility was assessed by odds ratios (ORs) and 95%

Table 2. Genotype and allele distributions of *CHI3L1* gene rs12141494 and rs4950928 polymorphisms in case and control groups

Genotype/Allele	Case *n*=115 (%)	Control *n*=108 (%)	X^2	*P*	OR (95% CI)
rs12141494					
GG	39 (33.91)	50 (46.30)	-	-	1
GA	52 (45.22)	41 (37.96)	2.661	0.138	1.626 (0.905-2.920)
AA	24 (20.87)	17 (15.74)	2.434	0.134	1.810 (0.856-3.829)
G	130 (56.52)	141 (65.28)	-	-	1
A	100 (43.48)	75 (34.72)	3.582	0.065	1.446 (0.986-2.120)
rs4950928					
CC	63 (54.78)	75 (69.44)	-	-	1
CG	42 (36.52)	27 (25.00)	4.262	0.041	1.852 (1.028-3.335)
GG	10 (8.70)	6 (5.56)	1.632	0.290	1.984 (0.683-5.762)
C	168 (73.04)	177 (81.94)	-	-	1
G	62 (26.96)	39 (18.06)	5.038	0.031	1.675 (1.065-2.634)

confidence intervals (CIs). Two sided *P*-values less than 0.05 were considered as statistically significance.

Results

HWE test

Genotype and allele distributions of *CHI3L1* gene rs12141494 and rs4950928 were shown in **Table 2**. Distributions of the polymorphisms in two groups were all according to HWE test, indicating the representativeness of participants.

Distributions of CD86 gene polymorphisms

As shown in **Table 2**, three genotypes of GG, GA and AA were verified in rs12141494, the frequencies were 33.91%, 45.22%, 20.87% in case group and 46.30%, 37.96%, 15.74% in control group respectively, in which GA and AA genotype frequencies increased in cases compared with controls. Furthermore, G and A allele frequencies were 56.52%, 43.48% in cases and 65.28%, 34.72% in controls respectively, while A allele carriers were more in cases than in controls. Even so, the differences had no statistical significance (*P*>0.05), which suggested that *CHI3L1* gene rs12141494 might had no obvious association with pediatric asthma occurrence.

The same as shown in **Table 2**, three genotypes of CC, CG and GG were observed in rs4950928. The frequencies were 54.78%, 36.52%, 8.70% in cases and 69.44%, 25.00%, 5.56% in controls respectively, in which CG and GG genotype frequencies increased significantly in case group compared with control group (*P*<0.05). Meanwhile, C and G allele frequencies were 73.04%, 26.96% in cases and 81.94%, 18.06% in healthy controls. Obviously, in patients group G allele carriers were more than that in control group, and the differences were statistically significant (P<0.05). All results demonstrated that the mutation of C to G of *CHI3L1* gene rs4950928 might have a positive correlation with pediatric asthma susceptibility and G allele might be a risk factor for pediatric asthma occurrence (OR=1.675, 95% CI= 1.065-2.634).

Discussion

Asthma is a chronic disease characterized by recurrent attacks of breathlessness and wheezing, which severely affects children's growth and mental health. It has been reported to be affected by multiple and diverse interacting factors, such as environmental and psychosocial exposures, complex traits, and genetic variations. A number of studies have been performed to explore the association of genetic variations and asthma susceptibility, such as *ADAM33*, *ADAM12*, *PTPN22*, interleukin-23 (*IL-23*), *IL-10* and so on [13-16]. Up to now, although childhood asthma has been controllable, its prevalence is still rising [17]. Therefore, to explore the pathogenesis of asthma is the basic way for disease prevention and control of asthma.

CHI3L1 gene widely expressed in human body. Its coding protein is a chitinase amyloid (YKL-40), which plays active role in anti-infective defense and repair responses. YKL-40 is also called HcGP-39 or BRP-39 in mice and Ines Mack et al. have suggested that YKL-40 might involve in pediatric lung diseases such as pediatric asthma [18]. A major study has suggested

that variations in *CHI3L1* gene might influence the YKL-40 levels [8]. Lin YS er al. have reported that *CHI3L1* gene SNP were correlated with the development of cervical pre-cancerous lesions and invasive cancer in Taiwanese women [19]. However, Zheng JL et al. have found that *CHI3L1* gene polymorphisms are correlated with serum YKL-40 levels, but not with the prevalence or severity of coronary artery disease (CAD) in a Chinese population [20].

Genetic studies have also suggested that variations in *CHI3L1* gene might contribute to the pathogenesis of asthma. Kjaergaard et al. have identified 59 genetic variations in *CHI3L1* gene and fifteen of them are reported to be associated with plasma YKL-40 levels [12]. Rs4950928 is located on the promoter region of *CHI3L1* gene. This variation is correlated with serious disease, and it might be a risk factor for asthma risk [21, 22]. Various studies have performed, but the risk allele reported in the previous studies has been differed. Ober et al. have identified the C allele in rs4950928 as the risk allele for asthma, whereas G allele have been reported to be associated with atopic asthma in a European population [23, 24]. Rs12141494 is a novel intronic SNP which is located on intron 6 of *CHI3L1* gene. Gomez et al. have first explored its association with asthma severity and found rs12141494 is linked to severe asthma traits [25]. In china, Tsai et al. have explored the association of the *CHI3L1* tagSNPs including rs903358, rs7542294, rs946259, rs880633, rs12128727, rs1538372, rs10399805, rs10399931, rs6691378, and rs946261 with asthma risk in a Taiwan population [26]. But few studies have been reported in the Chinese Han population.

In the present study, we explored the correlation of two *CHI3L1* gene SNPs (rs12141494 and rs495092) with pediatric asthma susceptibility in a Chinese Han population. In our Chinese Han cohort, no statistically significant differences were detected in the genotype and allele distributions of CHI3L1 gene rs12141494 between asthma children and healthy controls. Rs12141494 is a novel intronic SNP and has only been investigated by Gomez et al. in American [25], thus future studies in other more ethnic groups are warranted to determine its effect on asthma. However, significant differences have been found in rs4950928 genotype and allele distributions between case and control group. As shown in **Table 2**, CG genotype and G allele frequencies increased significantly in cases compared with controls. It demonstrated that we speculated that *CHI3L1* gene rs4950928 was associated with pediatric asthma susceptibility and G allele might be the risk factor for the disease occurrence in our cohort of 223 Chinese Han population. The risk factor was consistent with the results performed in the Freiburg and Chicago populations [24], while inconsistent with the research done by Ober et al. [23].

In the summary, our study confirmed the novel SNP in *CHI3L1* gene, although it seemed not be associated with pediatric asthma susceptibility. Besides, *CHI3L1* gene rs4950928 was supposed to be correlated with pediatric asthma risk. The variable site G allele might increase the risk for pediatric asthma occurrence. And it is the first time to explore the association between *CHI3L1* gene rs12141494 and rs4950928 and pediatric asthma risk in the Chinese population. These observations suggested that genetic variation in *CHI3L1* gene played an important role in the occurrence and development of pediatric asthma, but further studies in a larger or different populations should be involved to confirm our result.

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Disclosure of conflict of interest

None.

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