

Original Article

Genetic correlation of SOCS3 polymorphisms with infantile asthma: an evidence based on a case-control study

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Abstract: Objective: In order to explore the relevance of SOCS3 gene polymorphisms with infantile asthma and provide evidence for the etiology of infantile asthma, we conducted this case-control study. Methods: A total of 273 children were enrolled for study in this article, including 119 children with asthma and 154 healthy controls frequency-matched with the former in sex and age. The genotyping of SOCS3 rs4969170, rs4969168 polymorphisms in all subjects were performed using TaqMan probe method. Odds ratio (OR) with 95% confidence interval (CI) was used to represent the association strength between SOCS3 polymorphisms and infantile asthma and calculated by χ^2 test which was conducted to check the Hardy-Weinberg equilibrium (HWE) in the control group. Results: The genotypes distributions of SOCS3 polymorphisms in controls conformed to HWE. Compared with GG/GA genotype in SOCS3 rs4969170, AA genotype obviously increased the susceptibility to asthma in children (OR=2.556, 95% CI=1.377-4.744) and A allele also made the same conclusion (OR=2.287, 95% CI=1.311-3.991). Differently in rs4969168, AG and AG/GG genotypes distributions had significant differences in two groups ($P=0.036$, 0.043). This two polymorphisms existed the linkage disequilibrium and the haplotype analysis showed that A-G and A-A haplotypes in rs4969170-rs4969168 increased 1.855 and 0.863 times risk of asthma development in children, respectively. Conclusions: A significant relevance involved in SOCS3 gene polymorphisms and infantile asthma development based on a Chinese Han population.

Keywords: SOCS3, polymorphism, infantile asthma

Introduction

Asthma is one of the most common chronic respiratory disease attacking children [1]. It brings about not only heavy economic burden but also emotional distress on families and children. Moreover, recurrent attacks seriously influence the entire lives of children. Asthma has been demonstrated to be a multifactorial disease, resulting from the hyperactivity and obstruction in response to pollutants, allergens and irritants [2]. Less outdoor exercise and unhealthy diets may be related with the increased morbidity of asthma [3]. Some studies suggested there was tight linkage between asthma morbidity and family-related stress [4]. Also, diet lipid was presented as a risk factor for asthma [5]. The breast milk for asthmatic children contains less polyunsaturated fatty

acids (PUFA) and contrastly, more linoleic acid [6]. Further studies reported that asthma onset was correlated with genetic factors [7-9]. In recent years, lots of attention has been focused on the relationship of genes and asthma aimed to identify high-risk population for this disease, which will contribute to early diagnosis and improved treatment.

Asthma results from immune imbalance regulated by IgE with Th2 in dominance. Cytokines of IL-4, IL-5 and IL-13 produced by Th2 play important roles in inflammatory activity of asthma through activating JAK-STAT signal pathway. Suppressor of cytokine signaling (SOCS) were negatively feedback regulatory proteins of I and II cytokine signal transduction pathway [10]. A experiment in vitro suggested that the SOCS3 level in Th2 was 22 times more than that of

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Table 1. The genetic association analysis between two polymorphisms of SOCS3 gene and infantile asthma susceptibility

Genotype/allele	Case, n (%)	Control, n (%)	OR (95% CI)	P value	
rs4969170	GG/GA	17 (14.29)	46 (29.87)	1.000 (Ref.)	-
	AA	102 (85.71)	108 (70.13)	2.556 (1.377-4.744)	0.002
	G	19 (7.98)	51 (16.56)	1.000 (Ref.)	-
	A	219 (92.02)	257 (83.44)	2.287 (1.311-3.991)	0.003
rs4969168	AA	15 (12.61)	34 (22.08)	1.000 (Ref.)	-
	AG	72 (60.50)	79 (51.30)	2.066 (1.040-4.104)	0.036
	GG	32 (26.89)	41 (26.62)	1.216 (0.547-2.702)	0.631
	AG/GG	104 (87.39)	120 (77.92)	1.964 (1.013-3.808)	0.043
	A	102 (42.86)	147 (47.73)	1.000 (Ref.)	-
	G	136 (57.14)	161 (52.27)	1.217 (0.866-1.711)	0.257

Table 2. The haplotype analysis of SOCS3 gene polymorphisms in infantile asthma

Haplotype site1-site2	Case, 2n=238 (%)	Control, 2n=308 (%)	χ^2 (P)	OR (95% CI)
G-G	19 (7.98)	51 (16.56)	-	1.000 (Ref.)
A-G	117 (49.16)	110 (35.71)	12.831 (0)	2.855 (1.586-5.138)
A-A	102 (42.86)	147 (47.73)	4.433 (0.035)	1.863 (1.038-3.341)

Note: site1: rs4969170; site2: rs4969168.

Th1, which indicates that SOCS3 may be involved in the immune disorder diseases of asthma [11]. Fortunately, Zafra et al. find that SOCS3 silencing attenuates functions of eosinophil, key inflammatory cells in asthma [12]. The above results point out the important role of SOCS3 role in asthma. Increasing studies demonstrated that SOCS3 polymorphisms with the occurrence of idiopathic scoliosis [13], chronic hepatitis C [14] and obesity [15], however, there were few studies about the SOCS3 polymorphisms with asthma susceptibility.

Our study adopted case-control design to analyze the relationship of SOCS3 polymorphisms (rs4969170, rs4969168) with risk of asthma in Chinese Han children. The study will help to improve the diagnosis and treatments of asthmatic patients.

Materials and methods

Study groups

A total of 273 children participated in this case-control study. Among them, 119 with asthma were diagnosed by pathobiology in Xi'an Children's Hospital as the case group. The cases contained 53 boys and 66 girls with the age range of 3-13 years old and the average

age of 6.54±2.37. The other 154 healthy children were as the control group from the physical examination center of Xi'an Children's Hospital in the same period with the cases, including 71 boys and 83 girls with the mean age of 7.12±2.03. All children had no the other immune system diseases beyond the bound of blood. Our study was supported by the Ethics Committee of Xi'an Children's Hospital and written informed consents were obtained from all subjects before collecting blood samples.

DNA extraction

2 ml peripheral venous blood was required from all enrolled children and put in the anticoagulant tube with EDTA. The conventional chloroform-isoamyl alcohol method was used to extract blood genome DNA and finally the DNA samples were stored at -20°C refrigerator for standby application.

The genotyping of SOCS3 rs4969170, rs4969168 polymorphisms in two groups

SOCS3 rs4969170, rs4969168 polymorphisms were conducted the genotyping using the TaqMan probe method. The PCR amplification primers and TaqMan probe sequences were designed, and PCR reaction program was conducted according to the report of Persico et al. in 2008 [16]. The PCR products were sequenced by the Dye Terminator Cycle Sequencing FS Ready Reaction Kit (Perkin-Elmer Applied Biosystems) and a 373A DNA sequencer (Applied Biosystems, Branchberg, NJ).

Statistical analysis of data

All data were represented using $\bar{x} \pm s$ and %, Hardy-Weinberg equilibrium (HWE) of polymorphisms genotypes distributions in the control group were checked by the χ^2 test which was used to calculate the odds ratio (OR) and 95%

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confidence interval (CI). The later usually represented the association intensity between gene polymorphism and disease risk. The statistical analysis was conducted in SPSS 18.0 software. In the meanwhile, the linkage disequilibrium and haplotype analyses of SOCS3 rs4969170, rs4969168 polymorphisms were also performed in this article.

Results

HWE test

Through the χ^2 test, the genotypes distributions of SOCS3 rs4969170, rs4969168 polymorphisms were both consistent with the rule of HWE, the results showed our study population possessed the representativeness.

The comparison of genotype frequencies in SOCS3 polymorphisms between the case and control groups and the association analysis with infantile asthma

As was shown in **Table 1**, AA genotype frequency of rs4969170 was significantly higher in cases than controls (85.71%&70.13%) and the difference reached the significant level ($P=0.002$), similarly, A genotype was also significant difference in cases and controls ($P=0.003$). Therefore, the common genotype AA and allele A in rs4969170 obviously increased the susceptibility to asthma in children, compared with GA/GG genotype and G allele (AA vs. GA/GG: OR=2.556, 95% CI=1.377-4.744; A vs. G: OR=2.287, 95% CI=1.311-3.991).

Differently, heterozygous mutant genotype AG in rs4969168 had an significant higher frequency in the case than control groups (60.50%&50.30%). AG/GG genotype increased 0.964 times risk of infantile asthma development, compared with AA genotype (OR=1.964, 95% CI=1.013-3.808). The SOCS3 rs4969170, rs4969168 might be two independent risk factors in the development of infantile asthma.

The linkage disequilibrium and haplotype analyses of SOCS3 rs4969170 and rs4969168 polymorphisms

In order to further ensure the roles of these two polymorphisms in infantile asthma, the linkage disequilibrium and haplotype were analyzed. In

rs4969170, rs4969168 polymorphisms, a total of three haplotypes were identified, namely G-G, A-G, A-A haplotypes. Among of them, A-G and A-A haplotypes were both associated with the increased risk of asthma development in children, compared with haplotype G-G (OR=2.855, 95% CI=1.586-5.138 and OR=1.863, 95% CI=1.038-3.341, **Table 2**).

Discussion

Asthma is a clinical common disease with great damage for human body and social public health [17, 18]. It can occur in any age, but more than half patients are children less than 12 years old, so infantile asthma is paid attention wildly by the domestic and foreign scholars, especially the roles of genetic factors due to the family heredity of asthma.

Due to *CD14* participating in primary immune and inflammatory responses, Zhang et al. select -159C/T polymorphism in *CD14* to explore the association with asthma in Chinese Han children, the results show that TT genotype carriers have the significantly higher serum IgE level than CC genotype and it may be a genetic risk marker for infantile asthma [19]. Wang et al. find that interleukin-2 and 4 (IL2, IL4) genes polymorphisms are both associated with infantile asthma susceptibility and may be important biomarkers to identify the susceptible population of asthma in children [20]. In addition, others interleukin genes polymorphisms are also studied whether they have the association with infantile asthma, for example, *IL13*, *IL13* and *IL4* receptors genes are proved to be involved in asthma development and the interaction also exists [21]. Foreign scholars of Sharma et al. find that the genetic variants of *TBX21* gene are associated with asthma in Indian children and may be modify the susceptibility to asthma and severity [22].

In this article, we studied the association between the genetic variants of SOCS3 gene (rs4969170, rs4969168) and the susceptibility to asthma in children. The common genotype AA and allele A of SOCS3 rs4969170 polymorphism were found to increase the risk of asthma occurrence. However, the heterozygous mutant genotype AG in rs4969168 showed the significant relevance with asthma development in children, meanwhile, AG/GG genotype was

also proved to be associated with the increased risk for asthma, compared with the genotype AA. The synergistic effect of rs4969170, rs4969168 polymorphisms in *SOCS3* gene was reported for the first time. A-G and A-A haplotypes in rs4969170-rs4969168 were both showed to involve in the risk of asthma occurrence. From the above we can see that A allele in rs4969170 has a more influence on asthma in children than the other elements, but the conclusion needs to be further verified.

In previous studies, the role of *SOCS3* gene in asthma has rarely been reported. Kubo et al. indicate that *SOCS3* is remarkably expressed in Th2 cells and inhibits the differentiation process of Th1 cells simultaneously, which may play a role in atopic asthma [23]. Seki et al. report that the expression level of *SOCS3* mRNA in moderate and severe asthma is obviously higher in cases than controls (qPCR), which suggests that the expression of *SOCS3* is associated with the severity of asthma [24]. However, the genetic variants of *SOCS3* are not referred in asthma. In our study, we selected two common polymorphisms of *SOCS3* in other diseases, however, due to the complication of asthma development, these researches are not enough to ensure its ethology.

In addition, except for genetic factors, environment and biological factors also have an important influence on infantile asthma. Koinis-Mitchell et al. conduct a study and indicate that optimize sleep health may play a protective role in urban children with asthma [25]. Neck circumference is considered to be associated with asthma severity in children according to the study of Hacıhamdioglu et al. [26]. As we all know, obesity increases the risk of asthma in children and Vo et al. point out that vitamin D may related to asthma in children through affecting risk of acute respiratory infection (ARI) and corticosteroid responsiveness which are as the moderators in obesity-asthma pathway [27]. What's more, air pollution, blood lead level, stress, and nutritional status are also reported to be involved in the asthma development of children [28-30].

Therefore, more influence factors are considered to explore the pathology and ethology of asthma in children combined with genetic factors with well-design and enough large sample size in the future.

Disclosure of conflict of interest

None.

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