Received: 8 June 2018 | Revised: 6 August 2018 | Accepted: 18 August 2018

DOI: 10.1002/jcla.22675

RESEARCH ARTICLE

WILEY

Interleukin-7 gene polymorphism rs766736182 associates with the risk of asthma in children

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Background: Recently studies uncovered associations between polymorphisms of interleukin genes and the risk of asthma. However, the relationship between polymorphisms of interleukin-7 gene and the risk of children asthma has not been discovered yet. This study aims to investigate the relationship between single nucleotide polymorphisms (SNPs) on interleukin-7 gene and the risk of children asthma.

Methods: We genotyped eight SNPs of interleukin-7 gene in blood samples from 437 asthma patients and 489 healthy controls to analyze potential associations of these SNPs with the risk of asthma in children.

Results: A missense SNP rs766736182 (odds ratio (OR) = 2.185, 95% confidence interval (CI) = 1.561-2.252, *P*-value = 8.69468E-19) of the interleukin-7 gene is associated with the risk of children asthma.

Conclusions: This study reveals that SNP rs766736182 of interleukin-7 is the risk factor for children asthma and implies potential role of immune system in the pathogenesis of children asthma.

KEYWORDS

children asthma, interleukin-7, polymorphism

1 | INTRODUCTION

Asthma is a common long-term respiratory disease, which is characterized by variable and recurring symptoms, reversible airflow obstruction, and bronchospasm.[1] Asthma typically begins in childhood and occurs throughout patients' life. The incidence rates of asthma in children vary between regions and countries, the average of which is 11.7% and 14.1% in 6-7 year and 13-14 year, respectively.[2] Asthma is caused by a combination of genetic and environmental factors. Exposing to air pollution and allergens are most common environmental factors. Cytokines drive the inflammatory processes and are the most important genetic factors that underlying pathophysiological mechanisms of asthma.[3]

Interleukin genes encode cytokines important for inflammatory processes.[4,5] The single nucleotide polymorphisms (SNPs) of several interleukin genes, such as interleukin-1,[6] interleukin-2,[7] and interleukin-6,[8] associate with the risk of asthma in childhood. Interleukin-7 encodes a cytokine important for B- and T-cell development, which functions as a pre-pro-B cell growth-stimulating factor and a cofactor for V(D)J rearrangement of the T-cell receptor beta (TCRB) during early T-cell development. However, it is still unclear whether SNPs in interleukin-7 are associated with asthma.

To study whether polymorphism of interleukin-7 gene associates with the risk of asthma, we genotyped eight SNPs (rs766736182, rs762037062, rs1545228, rs2583759, rs1441850, rs1119642, rs11777564, and rs145230246) of interleukin-7 gene in blood samples from 437 asthma patients and 489 age-matched healthy children. The statistical analysis revealed only SNP rs766736182 of interleukin-7 but no other seven SNPs is significantly associated with the risk of asthma, which suggested a potential role of interleukin-7 gene polymorphism in the pathogenesis of asthma.

2 | MATERIALS AND METHODS

2.1 | Study design and patients

The case-control study consisted of 437 asthma patients [216 boys and 221 girls with a mean age ± SD of 4.1 ± 3.16 years] and 489 healthy controls [227 boys and 224 girls with a mean age ± SD of 4.62 ± 3.69 years] from Xuzhou Children's Hospital. Patients with asthma were diagnosed by clinical medical specialists according to diagnosis guidelines of the American Thoracic Society for asthma.[9] The age-matched healthy boys and girls in control group had no symptoms or history of allergy or asthma or other pulmonary diseases. After a full rationalization of the procedure, written consents were obtained from legal guardians of all participants. The ethics council of Xuzhou Children's Hospital approved this study according to the declaration of national capital.

2.2 | Genotyping

Blood samples were drawn from patients and healthy controls to extract DNA using a QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, CA). Selected eight SNPs in the interleukin-7 gene were captured using a PCR reaction system and were confirmed by ABI-PRISM 3730 genetic analyzer (Sequenom, Inc.) via sequencing. These eight SNPs were selected from the dbSNP database (www.ncbi.nlm.nih.gov/SNP), in which rs766736182 and rs762037062 are missense SNPs, rs1545228 and

TABLE 1 Information of asthma patients and age-matched controls

	Patients (n = 437)	Control (n = 451)
Age (y)	4.1 ± 3.16	4.62 ± 3.69
Male	216	227
Female	221	224
Mild asthma	239	NA
Moderate asthma	157	NA
Severe asthma	41	NA
Population		
Han	428	436
Others	9	15
Family history		
Yes	5	0
No	432	451

NA, Not available.

TABLE 2 Allele and genotype frequencies of SNPs in patients and age-matched controls

Group	Allele frequency		*P*	Genotype frequency			*P*	H- W
rs766736182	A	G		AA	AG	GG		
Patients	629	245	8.6947E-19	215	199	23	2.5015E-18	0.026926
Control	751	151		312	127	12		0.976958
rs1545228	A	G		AA	AG	GG		
Patients	540	334	0.35107282	155	230	52	0.47801731	0.056868
Control	557	345		165	227	59		0.379922
rs2583759	A	G		AA	AG	GG		
Patients	581	293	0.14481981	187	207	43	0.34335729	0.422815
Control	583	319		179	225	47		0.152863
rs1441850	A	G		AA	AG	GG		
Patients	543	331	0.153396	167	209	61	0.35699542	0.943474
Control	544	358		159	226	66		0.611194
rs1119642	C	T		CC	CT	TT		
Patients	489	385	0.20223551	139	211	87	0.34278251	0.912631
Control	518	384		146	226	79		0.870155
rs11777564	A	G		AA	AG	GG		
Patients	717	157	0.3511808	299	119	19	0.79955693	0.282424
Control	740	162		309	122	20		0.219136
rs762037062	C	T		CC	TC	TT		
Patients	705	169	0.067751	285	135	17	0.15504635	0.979676
Control	746	156		307	132	12		0.886651
rs145230246	G	T		GG	TG	TT		
Patients	489	385	0.14238464	135	219	83	0.33764696	0.940992
Control	522	380		153	216	82		0.931151

SNP, single nucleotide polymorphism.

TABLE 3 Subgroup comparisons of allele and genotype frequencies of rs766736182

Group	*P*-value	
	Allele frequency	Genotype frequency
Mild asthma vs Moderate asthma	0.128723	0.237615
Mild asthma vs Severe asthma	0.688137	0.380183
Moderate asthma vs Severe asthma	0.761681	0.397457

rs2583759 locate at 3'UTR, rs1441850, rs1119642, and rs11777564 locate at intron region, and rs145230246 locates at 5'UTR.

2.3 | PCR system

The 50 μL PCR reaction system is as follows: 2 μL DNA, 2 μL of primers, 0.25 μL Taq enzyme, 4 μL dNTP, 5 μL PCR buffer, and add water to a final volume of 50 μL to build the reaction mixture. Thereafter, initial denaturation for 5 minutes at 94°C. Thirty-nine amplification cycles were performed using the following conditions: 94°C for 30 s, 52.7°C for 50 s, 72°C for 45 s. Finally, it is the 5 minutes extension at 72°C.

2.4 | Statistical analysis

The chi-squared test was performed to decide genotype frequencies and allele differences between asthma patients and healthy controls, and the odds ratio (OR) with a 95% confidence interval (CI) was used to decide relative risk. Hardy-Weinberg equilibrium (HWE) tests were for data quality control using R. linkage disequilibrium (LD) was measured by R^2. All *P*-values are two-tailed value and are statistical significant when <0.05.

3 | RESULTS

3.1 | rs766736182 associates with the risk of asthma

The case-control study involved 437 asthma patients (216 boys and 221 girls with a mean age ± SD of 4.1 ± 3.16 years) and 489 age-matched healthy controls (227 boys and 224 girls with a mean age ± SD of 4.62 ± 3.69 years (Table 1). Distributions of the genotypes of the eight SNPs in all age-matched healthy controls and in most of the patients were in Hardy-Weinberg equilibrium (HWE), except for rs766736182 in asthma patients (Table 2). The rs766736182 showed significant differences between asthma patients and healthy controls in both allele frequencies and genotype frequencies (*P*-value < 0.05). The other seven of tested SNPs showed no significant differences in either allele frequencies or genotype frequencies between asthma patients (n = 437) and controls (n = 489) for seven of tested SNPs (Table 2). The G allele frequency of rs766736182 was higher in asthma patients than that in controls (*P*-value = 8.69468E-19, OR = 2.185, 95% confidence interval CI = 1.561-2.252). The genotype frequencies of rs766736182 (*P*-value = 8.69468E-19) was significantly different between asthma patients and controls, respectively. The other seven of the tested SNPs showed no significant differences in allele frequencies or genotype frequencies between asthma patients (n = 437) and controls (n = 489) for seven of the tested SNPs (Table 2). Moreover, no significant differences in allele frequencies or genotype frequencies of rs766736182 were observed between asthma patients in different stages (Table 3).

3.2 | Linkage disequilibrium analysis

The linkage disequilibrium (LD) analysis revealed that only rs1441850 and rs1545228 showed strong LD with each other (R^2 > 0.8) (Table 4). Other SNP pairs only showed modest or weak LD, as evidenced by low R^2 value, especially for SNP pairs involving rs145230246, rs766736182, and rs762037062 (Table 4). It suggests that most of analyzed SNPs perform their function independently in the studied population, which make building haplotypes unnecessary in this study.

4 | DISCUSSION

Asthma is a long-term respiratory disease characterized by variable and recurring symptoms, reversible airflow obstruction, and

TABLE 4 The linkage disequilibrium(R^2) among the SNPs

R^2	rs1545228	rs2583759	rs145230246	rs1441850	rs1119642	rs11777564	rs766736182	rs762037062
rs1545228	1	0.649	0	0.995	0.014	0.061	0	0
rs2583759	0.649	1	0	0.647	0	0	0	0.013
rs145230246	0	0	1	0	0	0	0	0
rs1441850	0.995	0.647	0	1	0.014	0.062	0.215	0
rs1119642	0.014	0	0	0.014	1	0.001	0	0
rs11777564	0.061	0	0	0.062	0.001	1	0	0
rs766736182	0	0	0	0.215	0		1	0
rs762037062	0	0.013	0	0	0	0	0	1

SNP, single nucleotide polymorphism.

bronchospasm, and begins in childhood.[1] Recent studies suggested that genetic factors, such as single nucleotide polymorphisms (SNPs) of genes, might contribute to the risk of many diseases,[10-12] as well as children asthma.[13] In this study, we found significant association between missense SNP rs766736182(*P*-value = 8.69468E-19, OR = 2.185, 95% confidence interval CI = 1.561-2.252) of interleukin-7 gene and the risk of children asthma.

Interleukin-7 is a cytokine important for B- and T-cell development. It stimulates the differentiation of multipotent (pluripotent) hematopoietic stem cells into lymphoid progenitor cells and plays important roles in many types of diseases.[14-18] Interleukin-7 binds to the interleukin-7 receptor, a heterodimer consisting of interleukin-7 receptor alpha and common gamma chain receptor, which results in a cascade of signals important for T-cell development within the thymus and survival within the periphery.[19] The airway response to allergen is associated with the generation of interleukin-7, which may contribute to airway inflammation in asthma by promoting enhanced eosinophil activation and survival.[20] However, it is still unclear whether SNPs of interleukin-7 gene are associated with asthma. This study aims to investigate whether SNPs of interleukin-7 are associated with risk of asthma in 437 asthma patients and 489 healthy controls. The results show that SNPs rs766736182 of interleukin-7 but no other SNPs are associated with the risk of asthma in Chinese Han population.

The SNP rs766736182 on interleukin-7 gene is a missense variation, which causes a substitution of Lys to Glu on the 175th position of the amino acid sequence of interleukin-7 protein. This study implies that this Lys175Glu substitution may alter the function of interleukin-7 protein, thereafter alters the progress of airway inflammation and affect the incidence of asthma. Moreover, it further suggests that polymorphisms of interleukin-7 are involved in immune-mediated mechanisms underlying pathological process of children asthma, which would promotes novel potential therapeutics of children asthma.[21-23]

In conclusion, this study reveals the association of interleukin-7 SNP rs766736182 with the risk of children asthma, which improves our knowledge on the pathogenesis of children asthma and promotes future diagnosis and therapeutics for asthma in children.

AUTHORS CONTRIBUTION

Junhua Cao, Zhenguang Li, Chonglin Zhang, Qiang Ji, Chuanling Zhang, and Tong Qian performed the experiments. Junhua Cao, Zhenguang Li, and Chonglin Zhang analyzed the data. Junhua Cao and Lijun Tian wrote and edited the article.

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How to cite this article: Cao J, Tian L, Li Z, et al. Interleukin-7 gene polymorphism rs766736182 associates with the risk of asthma in children. *J Clin Lab Anal*. 2019;33:e22675. https://doi.org/10.1002/jcla.22675