Correlation between IL-4 and IL-13 gene polymorphisms and asthma in Uygur children in Xinjiang

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Received June 7, 2018; Accepted November 13, 2018

DOI: 10.3892/etm.2018.7096

Abstract. Correlation between interleukin-4 (IL-4) and interleukin-13 (IL-13) gene polymorphisms and bronchial asthma in Uygur children in Xinjiang, China, and the effects on serum total immunoglobulin E (IgE) were investigated. Thirty-seven child patients with asthma and 29 healthy children were screened. The gene polymorphisms of Arg130Gln in IL-13 and -590C/T in IL-4 gene promoter region were analyzed using the polymerase chain reaction (PCR) and direct gene sequencing; the asthma-related indexes were detected using the enzyme-linked immunosorbent assay, and the relevant indexes were analyzed; moreover, whether there was a synergistic effect between Arg130Gln in IL-13 and -590C/T in IL-4 gene promoter region in the pathogenesis of asthma in children was analyzed. The T/T homozygote genotype frequency and T allele frequency of C-590T gene in IL-4 in the asthma group were significantly higher than those in the control group (45.9 vs. 10.3%, OR=8.91; 63.5 vs. 36.2%, OR=3.07, P<0.05). The A/A genotype frequency and A allele frequency of Arg130Gln locus in IL-13 in the asthma group were obviously higher than those in the control group (54.1 vs. 17.2%, OR=6.29; 67.6 vs. 39.7%, OR=3.17, P<0.05). In the two gene loci, the level of serum IgE in the same genotype in the asthma group was higher than that in the control group (Z=-2.128, -2.050, -2.700 vs. -3.766, -3.799, -3.397; P<0.05). The risk of asthma in carriers of both IL-4 -590C/T TT and Arg130Gln locus AA genotypes was significantly increased compared with that in carriers of either IL-4 -590C/T TT genotype or Arg130Gln locus AA genotype (OR=6.00, P=0.046; OR=4.50, P=0.033; OR=22, P=0.005). The IL-4 -590C/T and Arg130Gln locus gene polymorphisms are associated with the asthma susceptibility and increased serum total IgE in Uygur children in Xinjiang. There is a synergistic effect between the T allele of IL-4 -590C/T locus and the A allele of IL-13 Arg130Gln locus.

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Key words: asthma, IL-4, IL-13, IgE, gene polymorphism, children

Introduction

With the economic development and influence of environmental factors, the incidence rate of asthma has increased dramatically, and this increase is more obvious especially in children. From 1988 to 1990, the cluster sampling investigation showed that the average prevalence rate of asthma in children aged 0-14 years was 0.11-2.03% (1). The second national asthma survey in the twentieth century showed that the average prevalence rate in children was 1.54% around the country, while that in urban children was 0.12-3.34%. The third national epidemiological survey of urban children showed that as of 2013, the proportion of urban children with asthma in total had increased from 1.97% in 2003 to 3.02%, which was significantly higher than before (2). The incidence of asthma in children in Xinjiang is also high. In October 1990, an epidemiological survey of asthma in Urumqi, Altay and Turpan of Xinjiang was conducted by the Xinjiang Children's Asthma Survey Coordination Group found that the total prevalence of asthma in children was 0.52%. It can be seen that the incidence of asthma in Xinjiang is also high, while the prevalence rate of Han nationality is significantly higher than that of Uygur (3). Kere and Laitinen found that the incidence of asthma in children in Xinjiang is also on the rise (4). The pathogenesis of asthma is complex with a strong genetic predisposition. The hereditary mode is not clear, but most scholars believe that asthma is a kind of multi-gene genetic disease, which is the result of interaction among multiple genes and environmental factors. Environmental factors can affect the immune response and cause the asthma symptoms. Immunoglobulin E (IgE) is widely considered as a key trigger factor for airway allergic inflammation. With the rapid development of genetics and molecular biology, it is an important aspect of asthma genetics to determine the susceptibility gene of asthma from the relationship between single nucleotide polymorphism and asthma. Over the years, researchers worldwide have used a large

number of methods to determine that asthma is an extremely complex genetic disease involving a variety of genes, and many chromosomal regions have been found to be associated with the occurrence of asthma, among which 5q31-33, 6q21-23, 11ql3, 12q14-24 and 16p11-12 were important asthma susceptibility regions (4-7). It has been shown that IgE, interleukin-4 (IL-4), IL-4R, IL-13 and TCR are located in these regions. IL-4 and IL-13 genes have been studied intensively in China as well as other countries. These are located and closely linked on the same chromosome (5q3l-q33 and 5q23-31, respectively), and the encoded cytokines are partially the same in the structure and function. At present, a large number of known polymorphic loci have been reported, and it is widely believed that these polymorphic loci are associated with the susceptibility and severity of asthma (6).

Prior studies showed a relationship between IL-4 and IL-13 gene polymorphisms and asthma in children, but the results are not conclusive. At present, relevant studies in China mostly focus on the asthma in Han children, and on single locus and asthma susceptibility and severity, but seldom on the two gene loci at the same time. Considering the interaction between the two genes and their roles, as well as the differences among regions and races, the IL-4 gene -590C/T and IL-13 Arg130Gln gene polymorphisms in 37 child patients with asthma were analyzed via the polymerase chain reaction (PCR). Moreover, the relationship of mutations in IL-4 and IL-13 gene loci with the asthma and serum total IgE level and whether there was a synergistic effect between the two genes in the pathogenesis of asthma was investigated. The results of this study can provide a basis for the prevention, diagnosis and treatment of the disease, and lay a scientific foundation for the prevention and reduction of asthma and related diseases.

Patients and methods

Objects of study

Selection of patients. A total of 37 Uygur child patients with asthma treated or admitted into Outpatient and Emergency Department of Paediatrics, the First Affiliated Hospital of Xinjiang Medical University (Xinjiang, China) from February 2014 to March 2015 were randomly selected as the asthma group.

This study was approved by the Ethics Committee of The Affiliated Yangpu Hospital of Shanghai Tongji University (Shanghai, China). All children enrolled and their guardians were informed of the study and their guardians signed the informed consent.

Diagnostic criteria. Diagnostic criteria of bronchial asthma in children: Guidelines for the Diagnosis and Prevention of Bronchial Asthma in Children (Revised) established by Group of Pneumatology, Society of Pediatrics, Chinese Medical Association in October 2008 (7): i) Wheezing, cough, shortness of breath and chest tightness occur repeatedly, mostly related to the allergen contact, cold air, physical and chemical stimulation, respiratory infections and sports, and they often attack or exacerbate at night and (or) in the early morning; ii) scattered or diffuse expiratory phase-based wheezing rale could be heard in the episode, and the expiratory phase extended; iii) the above symptoms and signs were effectively treated or spontaneously relieved after the anti-asthma treatment; iv) Wheezing, coughs, shortness of breath and chest tightness caused by other diseases were excluded; and v) those with atypical clinical manifestations (such as no obvious wheezing or wheezing rale) should meet at least one of the following criteria: a) positive in bronchial provocation test or exercise challenge test; b) the reversible airway limitation was confirmed: 1) positive in bronchial diastolic test: The forced expiratory volume at 1 sec (FEV1) at 15 min after the inhalation of quick-acting β_2 receptor agonist (such as Salbutamol) increased by ≥12%; 2) effective anti-asthma treatment: FEV1 increased by ≥12% at 1-2 weeks after the treatment with bronchodilator agents and oral (or inhaled) glucocorticoid; c) daily aberration rate of peak expiratory flow (PEF) (continuous monitoring for 1-2 weeks) ≥20%.

Those who met the criteria (i-iv) or criteria (iv) and (v) were diagnosed as asthma.

Screening criteria

Inclusion criteria

Asthma group. i) The patients that met the diagnostic criteria of asthma in children; ii) the patients with asthma symptoms caused by other diseases, such as acute laryngitis, diphtheria, laryngo-tracheal bronchitis, congenital airway malformations, bronchiolitis, bronchial stenosis or softening, airway foreign body and cardiac asthma; iii) patients who did not use antibiotics, hormones or asthmatic agents within 2 weeks prior to their admission; iv) patients who did not suffer from other immune diseases; and v) patients with normal heart and lung functions.

Control group. Children without individual and family anaphylactic diseases and other allergic diseases, without a history of infectious diseases recently and not using antibiotics, any immunomodulator or adrenal glucocorticoid.

Exclusion criteria

Asthma group. i) Patients who did not meet the diagnostic criteria of asthma in children; ii) patients with asthma caused by other diseases, such as acute laryngitis, diphtheria, laryngo-tracheal bronchitis, congenital airway malformations, bronchiolitis, bronchial stenosis or softening, airway foreign body and heart disease; iii) patients using glucocorticoid or immunomodulator for a long period or have started using it recently; iv) patients with primary immune dysfunction; v) patients with cough variant asthma or atypical asthma; vi) patients with cardiopulmonary failure; and vii) patients who did not cooperate and those with non-cooperative parents.

Control group. i) Children who could not cooperate in all relevant examinations; ii) children with an allergic history of eczema, rhinitis and asthma, in the family; and iii) children who used immunomodulator or adrenal glucocorticoid recently.

Research contents and methods

Sample collection and processing. Fasting venous blood (4 ml) was collected in two tubes during the morning hours, of which 2 ml was transferred into anticoagulant tube containing ethylene diamine tetraacetic acid (EDTA) to be used for extraction of genomic DNA. The remaining 2 ml blood

Table I. PCR reaction system (total reaction system: 25 μl).

Reagents	Usage amount (μl)
10X buffer solution	2.5
dNTP	2.0
$MgCl_2$	2.5
10 μmol/l primer	2
Sample DNA	3.0
Taq polymerase	0.25
Deionized water	12.75

was placed into a drying tube, and the serum was extracted and transferred into a 1.5 ml EP tube after centrifugation at 2,000 x g for 8 min at 20˚C. All tubes were stored at -20˚C.

Reagents and equipment

Reagents. Premix Primer STAR HS was purchased from Takara Bio Inc. (Otsu, Japan), agarose powder was purchased from Biowest (Miami, FL, USA), loading buffer was purchased from Takara Bio, Inc., GoldenView nucleic acid dyes were purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd. (Beijing, China) and EZNA Blood DNA kit was purchased from Omega Bio-Tek Inc. (Norcross, GA, USA).

Equipment. NanoDrop 2000 spectrophotometer, Eppendorf Research Plus pipettor (20-200, 10-100 and 0.5-10 μl) and Eppendorf Mastercycler pro gradient polymerase chain reaction (PCR) instrument were purchased from Eppendorf (Hamburg, Germany). DYCP-31DN horizontal electrophoresis tank was purchased from Beijing Liuyi Instrument Factory (Beijing, China). Tanon 1600 digital gel imaging system, Eppendorf 5430R refrigerated centrifuge and ABI 3500xL gene analyzer were purchased from Eppendorf.

DNA extraction and PCR amplification. DNA was extracted from the blood sample using the kit (cat. no. D5032; EZNA Blood DNA kit; Omega Bio-Tek, Inc.).

PCR primers of the two gene loci were synthesized by Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA. PCR primers of -590 locus in IL-4 gene promoter region: forward: 5'-GGATGTGTTTAGGTTCCATTCA-3', reverse: 5'-CCTCCTGGGGAAAGATAGAGTAA-3'. Primer sequences of IL-13 Arg130Gln gene locus: forward: AAGGAATTTTACCCCTCCCTAAC, reverse: GAATGAG ACAGTCCCTGGAAAG.

PCR amplification was performed using Takara LA Taq™ (code no. DRR042A). The systems of IL-4 and IL-13 genes were the same (Table I).

PCR amplification conditions of IL-4 primers: pre-denaturation at 95˚C for 10 min, 94˚C for 30 sec, 72˚C for 1 min, a total of 35 cycles, extension at 72˚C for 5 min. Amplification conditions of IL-13 primers: pre-denaturation at 95˚C for 10 min, 94˚C for 30 sec, 56˚C for 30 sec, 72˚C for 1 min, a total of 35 cycles, extension at 72˚C for 5 min.

Detection of PCR products via agarose gel electrophoresis. Agarose gel (2%) was prepared. PCR product (5 μl) and 6X loading buffer (1 μl) were completely mixed and added into the sample loading hole, followed by electrophoresis for 1 h under the voltage of 150 V. The gel was placed into the digital imaging display system. In case each pair of primer could be amplified into the target band with uniform brightness, good specificity and correct size and location, PCR amplification was deemed as success. If not or significantly non-specific bands appeared, PCR amplification was deemed as failure, and PCR amplification was repeated.

Frozen blood samples were used in this experiment, the concentration of the extracted DNA was low, and bands were not clear in some PCR products, however the sequencing was completed successfully, indicating that the concentration of PCR amplification products was low, but concentration needed for sequencing was reached.

PCR product sequencing and result judgment. PCR products received the base sequencing after detection on the ABI 3500xL gene analyzer and the sequencing results were compared and analyzed using the Mutation Surveyor software to obtain the relevant genotypes.

Detection of serum IL-4, IL-13 and IgE levels via ELISA. Frozen plasma was warmed up, and IL-4, IL-13 and IgE levels were measured using the ELISA kit.

Statistical analysis. The single-sample Kolmogorov-Smirnov normality test was used for the measurement data. Data in normal distribution were presented as the mean ± standard deviation, and independent sample t-test was used for the comparison between the two groups. Data that did not comply with the normal distribution were presented as median (minimum and maximum), and Mann-Whitney test was used for the comparison between the two groups, while Kruskal-Wallis test was used for the comparison among groups. Enumeration data were presented as the frequency [percentage (%)]. The t-test was used for the comparisons of age, height and weight of children between the asthma and control groups, while Chi-square test was used for the comparison of sex. Chi-square test was used for the comparison between the two groups, and the OR value and 95% CI were calculated via logistic regression analysis. Statistical Product and Service Solutions (SPSS) 17.0 (SPSS, Inc., Chicago, IL, USA) was used for the statistical analysis at the inspection level of α=0.05.

Quality control

Data source control. All children enrolled were treated in Department of Pediatrics and Child Healthcare Department in the First Affiliated Hospital of Xinjiang Medical University from February 2014 to March 2015 in strict accordance with the inclusion and exclusion criteria, so as to ensure the reliability of data source.

Professional technical control. Blood samples were obtained from children in a fasting state in the early mooring for inspection. To avoid the influence of the test tube and other factors, the samples were obtained and treated with drying tube and EDTA anticoagulant tube. All the blood samples were sent to Beijing Hyster Technology Co., Ltd. (Beijing, China) for inspection by relevant professional staff.

Table II. Comparison of baseline data of children between the asthma and control groups.

Items	Asthma group (n=37)	Control group (n=29)	t/χ^2 value	P-value
Age (years)	9.00±2.78	8.21±2.72	1.162	0.250
Height (meters)	1.34±0.16	1.32±0.17	0.667	0.507
Weight (kg)	30.70±10.88	26.84±7.91	1.605	0.113
Male	25 (67.6)	18 (62.1)	0.216	0.642

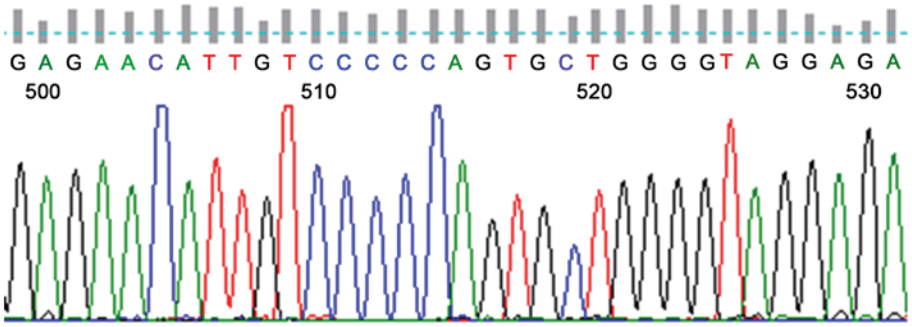

Figure 1. Normal wild-type gene sequencing.

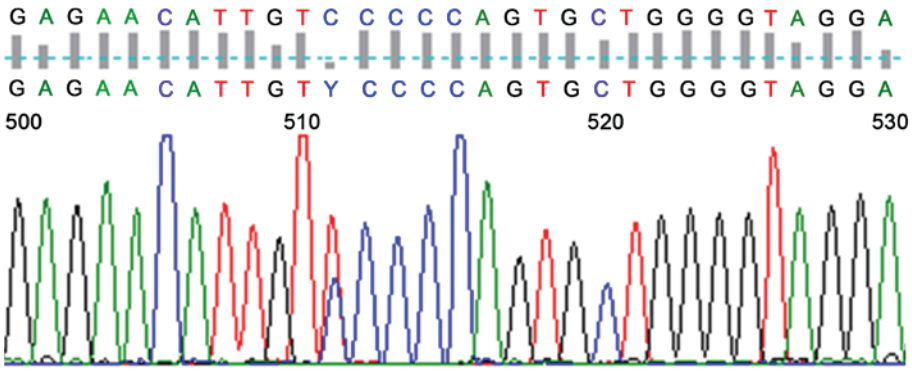

Figure 2. Heterozygous mutant gene sequencing.

Data analysis control. Data were entered by two people at the same time, the results were compared, and the original data were double checked if there was any question to ensure that the input data were true and reliable. After the data input, Excel raw data files were created for data analysis.

Results

Basic data of the patients. The asthma group included 25 males and 12 females aged 4-14 years old with an average of 9.00±2.78 years. The control group included 16 males and 13 females aged 4-14 years with an average of 8.21±2.72 years. There were no significant differences in the age, height, weight or sex (P>0.05) (Table II).

PCR-RFLP detection of IL-4 gene

Detection of PCR amplification product of IL-4 gene -590C/T locus. After PCR amplification, IL-4 gene -590C/T locus of children in the two groups could be amplified into positive fragments with the length of 195 bp.

Polymorphism detection of IL-4 gene-590C/T locus. After PCR amplification of IL-4 gene -590C/T locus gene, sequencing detection was performed, and three genotypes were found: wild homozygous CC, heterozygous mutant CT and homozygous mutant TT (Figs. 1-3).

Genotype frequency and allele frequency distribution of IL-4 gene -590C/T locus in the asthma and control groups. The genotype and each allele frequency of IL-4 gene -590C/T locus had statistically significant differences between the two groups (P=0.007, P=0.002, <0.05). The probability of asthma in children with T/T homozygous genotype was higher than that in children with CC genotype (OR=8.91, 95% CI=1.89, 41.98), and the probability of asthma in children with T allele was significantly higher and was 3.07 times more than that in children with C allele (OR=3.07, 95% CI=1.50, 6.27) (Tables III and IV).

Comparison of serum IgE level between different groups of different genotypes and the same genotype of IL-4

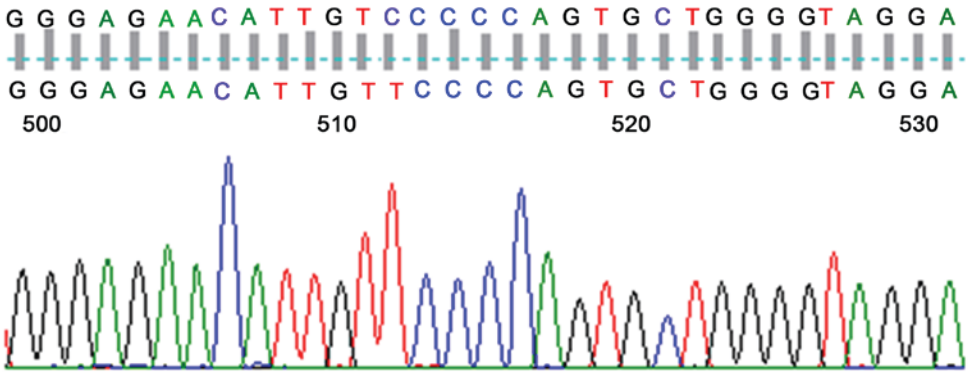

Figure 3. Homozygous mutant gene sequencing.

Table III. -590 Locus genotype distribution in IL-4 gene promoter region in the two groups.

		Genotype				
Groups	n	CC	CT	TT	χ^2 value	P-value
Control group	29	11 (37.9%)	15 (51.7%)	3 (10.3%)	10.009	0.007
Asthma group	37	7 (18.9%)	13 (35.1%)	17 (45.9%)		
OR (95% CI)		1	1.36 (0.41, 4.54)	8.91 (1.89, 41.98)		

Table IV. -590C/T allele frequencies in IL-4 gene promoter region in the asthma and control groups.

		Allele			
Groups	n	C	T	χ^2 value	P-value
Control group	29	37 (63.8%)	21 (36.2%)	9.707	0.002
Asthma group	37	27 (36.5%)	47 (63.5%)		
OR (95% CI)		1	3.07 (1.50, 6.27)		

Table V. Comparison of serum total IgE levels (kIU/l) in different genotypes of IL-4 -590C/T locus in the two groups.

		Genotype		
Groups	n	CC	CT	TT
Control group	29	45.01 (1.91, 192.00)[a]	28.67 (1.65, 1679.00)[a]	309.20 (86.74, 473.40)[a]
Asthma group	37	112.40 (23.57, 2500.00)[a]	852.13 (6.16, 2165.00)[a]	1187.71 (601.20, 2500.00)[b]
Z value	-	-2.128	-2.050	-2.700
P-value	-	0.033	0.040	0.007

The letters [a] and [b] indicate the results of pairwise comparisons in the same group. [a]P>0.05, compared with CC genotypes. [b]P<0.05 compared with CC and CT genotypes.

-590C/T locus. The serum levels of IgE in both groups were analyzed, and results showed that they did not obey the normal distribution. Therefore, the serum IgE level was presented as median, and Mann-Whitney test was used for the comparison between the two groups. The comparison of the same genotype in the two groups suggested that the serum total IgE level in the asthma group was significantly higher than that in the control group (P=0.033, 0.04 and 0.007, <0.05). There was no statistically significant difference in the IgE level among CC, CT and TT genotypes in normal children. In the asthma group, the serum IgE level in TT genotype was significantly higher than that in CC and CT

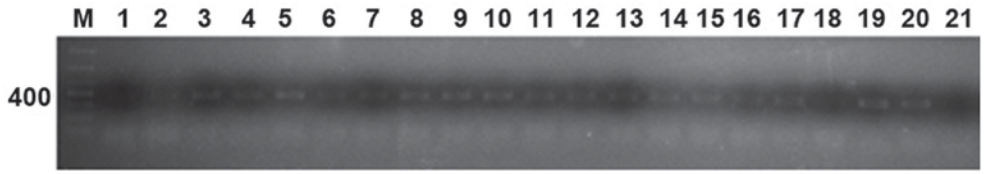

Figure 4. Electrophoretogram of IL-13 Arg130Gln PCR product. M: marker; 1-21 indicate part of the objects of study.

Table VI. IL-13 Arg130Gln genotype frequencies in the asthma and control groups.

		Genotype				
Groups	n	GG	GA	AA	χ^2 value	P-value
Control group	29	11 (37.9%)	13 (44.8%)	5 (17.2%)	9.449	0.009
Asthma group	37	7 (18.9%)	10 (27.0%)	20 (54.1%)		
OR (95% CI)		1	1.21 (0.34, 4.25)	6.29 (1.61, 24.57)		

Table VII. IL-13 Arg130Gln locus allele frequencies in the asthma and control groups.

		Allele			
Groups	n	G	A	χ^2 value	P-value
Control group	29	35 (60.3%)	23 (39.7%)	10.248	0.001
Asthma group	37	24 (32.4%)	50 (67.6%)		
OR (95%CI)		1	3.17 (1.55, 6.49)		

genotypes (Table V). We believe that the elevated level of IgE was associated with the T allele.

PCR-RFLP detection of IL-13 gene

Detection of PCR amplification product of IL-13 Arg130Gln locus. IL-13 gene was amplified via PCR and the amplification products were electrophoresed. The IL-13 Arg130Gln loci in both groups was amplified into positive bands (Fig. 4).

Detection of IL-13 Arg130Gln locus polymorphism. After amplification, IL-13 Arg130Gln locus received the base detection in the genetic analyzer, and three genotypes were detected (GG, GA and AA genotypes).

Genotype frequency and allele frequency distribution in the asthma and control groups. The GG, GA and AA genotype frequencies of IL-13 Arg130Gln locus in the asthma group were 18.9, 27.0 and 54.1%, respectively, and the differences were statistically significant between the two groups (χ^2=9.449, P=0.009; χ^2=10.248, P=0.001). The probability of asthma in patients with AA genotype was 6.29 times higher than that in patients with GG genotype, and the probability of asthma in patients with A allele was 2.17 times higher than that in patients with G allele (OR=6.29, 95%CI=1.61, 24.57; OR=3.17, 95% CI=1.55, 6.49) (Tables VI and VII).

Comparison of serum IgE level between different genotypes of IL-13 Arg130Gln locus. First, the total IgE in the two groups were analyzed, suggesting that they did not obey the normal distribution. Therefore, the serum IgE level was presented as median, and Mann-Whitney test was used for the comparison between the two groups. The comparison of the same genotype in the two groups suggested that the serum total IgE level in the asthma group was significantly higher than that in normal children (P<0.05). In normal children, three different genotypes had no effect on the IgE level; but in the asthma group, the level of serum IgE in patients with AA genotype was obviously higher than that in patients with GG and GA genotypes (Table VIII).

Discussion

At present, it is universally accepted that asthma is a polygene genetic disease with obvious familial aggregation and strong genetic heterogeneity, and its heritability is as high as 60-80% (8). Asthma does not follow the classical Mendel's law of inheritance, which is often caused by the interaction among a variety of genetic and environmental factors and other susceptible factors. There are gene-gene and gene-environment interactions. Studies have shown that cytokines are the messengers of important information among inflammatory cells during the onset of asthma, and determine the type and duration of inflammatory response. Various inflammatory mediators have different effects on the airway, the interaction among them causes and aggravate BHR and airway inflammation, leading to an increase in the level of

Table VIII. Comparison of serum total IgE levels (kIU/l) in different genotypes of IL-13 Arg130Gln locus.

		Genotype		
Groups	n	GG	GA	AA
Control group	29	45.01 (1.65, 1679.00)[a]	28.67 (1.91, 192.00)[a]	82.00 (19.42, 473.40)[a]
Asthma group	37	1106.80 (14.79, 1434.44)[a]	110.35 (6.16, 2500.00)[a]	1338.75 (601.20, 2500.00)[b]
Z value	-	-3.766	-3.799	-3.397
P-value	-	0.001	0.001	0.001

The letters [a] and [b] indicate the results of pairwise comparisons in the same group. [a]P>0.05, compared with GG genotypes. [b]P<0.05 compared with GA and AA genotypes.

IgE, and producing the pathophysiological characteristics of asthma (9). IL-4 and IL-13 play important roles in the regulation of cytokines and signal transduction during the IgE synthesis, and they also play important roles in the regulation of IgE level in the serum and the pathogenesis of asthma.

IL-4 was discovered by Howard *et al* in 1982 and was officially named by the international community in 1986 (10). IL-4 gene is located on human chromosome 5q31-q33 and consists of four exons and three introns, approximately 10 kb in size, composing of 129 amino acid residues (11,12). IL-4 is produced by Th2 cells, which can promote Th cells differentiate into Th2 cells, up-regulate the expression of resting B cells, promote the activated B cells to secrete IgE and IgG1, promote the transformation of IgG to IgE and enhance the IgE-mediated immune response. Besides, IL-4 can also promote the vascular endothelial cell proliferation, increase the expression of vascular cell adhesion molecules 21 in endothelial cells and participate in the pathogenesis of asthma. IL-4 directly or indirectly induces MC and EOS degranulation to produce the bronchial hyperresponsiveness and inflammation, and plays a vital role in the immediate and delayed response of asthma. Also, it has an immunomodulatory effect on B cells, T cells, mastocytes and eosinophils. In 1995, Rosenwasser *et al* (13) reported that the mutant C→T on -590 locus in IL-4 gene promoter region was related with the occurrence of asthma, and T allele can increase the IgE level. In this study, the correlation analysis of C→T on -590 locus in IL-4 gene with asthma in Uygur children in Xinjiang showed that the genotype frequency and allele frequency between the two groups of children had statistically significant differences (χ^2=10.009, P=0.007; χ^2=9.707, P=0.002). In the asthma group, the risk of asthma in children with TT genotype was increased by 7.91 times compared with that in children with CC genotype [(OR=8.91, 95% CI (1.89, 41.98)]. The comparison of the two alleles showed that the risk of asthma in children with T allele was 3.07 times higher than that in children with C allele [(OR=3.07, 95% CI (1.50, 6.27)]. The Mann-Whitney test of serum total IgE levels in both groups revealed that, compared with the control group, the IgE level in children with the same genotype increased dramatically (P=0.033, 0.04 and 0.007, respectively). There was no statistically significant difference in the IgE level between different genotypes in normal children and between CC and CT genotypes in asthma children. These data suggested that the T allele may increase the IgE level and the probability of asthma in children. Vercelli (12) studied the different bases in IL-4 gene promoter region in patients with varying degrees of asthma in Britain and found that T allele has a close relationship with the severity of asthma. Neelofar *et al* (14) found through the study on IL-4 gene polymorphisms in 250 asthma children and 200 normal children that the allele is related to asthma, and it can increase the serum TIgE concentration. The above indicates that the allele of T is closely related to asthma, which is consistent with the results of this study. Liang *et al* (15) showed that IL-4 gene polymorphism was not related to the susceptibility to asthma in adults of Shandong, China. It is inconsistent with the findings of this study, which may be related to changes in the immune system of adults after adulthood.

IL-13 is a cytokine named at the Keystone Cytokine Conference in 1993 and its gene is located on human chromosome 5q23-q31, it consists of 4 exons and 3 introns encoding the protein containing 132 amino acids (16). IL-13 gene is adjacent to IL-4, IL-3, IL-5, IL-9 and GM-CSF genes, forming the cluster of cytokine genes. IL-13 is mainly secreted by the activated $CD4^+$ T cells (Th2), whose biological function is to inhibit monocyte from releasing the inflammatory cytokines and chemical factors, induce B cell proliferation and differentiation, and promote IgE synthesis and expression of some adhesion molecules in endothelial cells. Wu *et al* (17) reported that IL-13 gene promoter locus polymorphism is an important candidate for asthma in children in Guangdong, and its mutant T allele is associated with asthma and can increase the level of IgE in serum. IL-13 gene is polymorphic, and it has been shown that more than ten IL-13 gene loci are closely related to asthma and IgE level in serum. It was (18) found that elevated serum levels of IL-13 were associated with asthma in Uygur children in Xinjiang. It was found in this study that there were 3 genotypes in IL-13 Arg130Gln locus in children with asthma, and the genotype frequencies of GG, GA and AA were 18.9, 27.0 and 54.1%, respectively, while these frequencies in the control group were 37.9, 44.8 and 17.2%. All the differences were statistically significant. Compared with that in children with G allele, the probability of asthma in children with A allele was 2.17 times (OR=3.17) higher, suggesting that the A allele is more important in the susceptibility to asthma. The Mann-Whitney test was performed for the serum total IgE levels in both groups, and the comparison of the IgE level in children with the same genotype showed that the level of

IgE in children with asthma was higher than that in normal children (P<0.001). There were no statistically significant differences in IgE levels among AA, GA and GG genotypes in normal children, but the differences were statistically significant among AA and GG and GA genotypes in the asthma group, indicating that IL-13 Arg130Gln locus base mutation is associated with the serum total IgE level. In the study on IL-13 -1112C/T locus polymorphisms in local asthma and normal children, it was found that it was related to the asthma, but the mutant T gene had no significant correlation with the increased serum TIgE level. Tursun *et al* (18) found that IL-13 Arg130Gln mutation was not a susceptibility gene of asthma in children in Changchun, China, but this mutation could be related to the elevated level of IL-13 in the serum, and the mutant homozygotes might be associated with the serum IgE level. Howard *et al* (19) and Kim *et al* (20) studied the locus in IL-13 gene promoter region and showed a significant relationship with the occurrence of asthma in local children. These results suggest that the Arg130Gln locus of IL-13 gene plays an important role in the pathogenesis of asthma, which is consistent with the conclusion of this study. However, the BHR level among asthma children of different genotype was not compared. We will try to solve this problem in future studies.

In conclusion, asthma is a polygene genetic disease, and the interaction between environmental and genetic factors is closely related to the occurrence of this disease, which is also a common chronic respiratory disease in childhood (21). The single pathogenic gene may have a little impact on the occurrence of asthma (22), so in the future study on asthma-related genes, it is needed to study the effects of different loci in different genes on asthma deeply and study whether there is a synergistic effect among various genes in the occurrence of asthma, providing more accurate treatment. In this study, the polymorphisms of IL-4 (C-590T) and IL-13 Arg130Gln were studied to understand the susceptibility genes of Uygur children with asthma in Xinjiang and their effects on the serum total IgE level and understand whether there was a synergistic effect between the two gene loci in the occurrence of asthma. However, this study had some limitations. The number of effective samples was limited, and there was a possibility of false negative. Moreover, the relevance of illness degree in the asthma group after stratification was not further studied and it is necessary to increase the sample size and further analyze the severity of illness and family history. The pathogenesis of asthma is complex, and the exact mechanisms of IL-4 and IL-13 gene polymorphisms and asthma still need to be further studied. Only by studying the gene polymorphism and gene expression on the whole we can better study the pathogenesis of asthma from the genetic and immunological perspectives, and provide a theoretical basis for the prevention and treatment of the disease.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

JHZ drafted the manuscript. JHZ and MZ were mainly devoted to collecting and interpreting the data. JHZ, MZ and YNW revised it critically for important intellectual content. YNW and XYZ were responsible for the conception and design of the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of The Affiliated Yangpu Hospital of Shanghai Tongji University (Shanghai, China). Signed informed consents were obtained from the guardians of the child patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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