

Original Article

Association between interleukin-13 gene polymorphism and neonatal asthma in Han nationality Chinese population

Aiying Wan, Baozhen Yao, Xiao Zhang, Li Li

Department of Paediatrics, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei, China

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Abstract: Background and objective: Interleukin-13 (*IL-13*) is an important cytokine secreted from type 2 helper T lymphocytes. Previous studies showed that polymorphisms in the *IL-13* gene were associated with allergic asthma. The relationship between this marker and neonatal asthma has not been evaluated. So the aim of this study is to analyze the association of *IL-13* gene polymorphisms (rs20541 and rs1800925) and the risk of neonatal asthma. Methods: This case-control study included 103 neonatal asthma patients and 125 healthy controls that were matched with the patients by age and gender. The genotyping of *IL-13* gene polymorphisms was conducted with the method of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The distribution difference of SNP genotypes and alleles between case and control groups was checked by the chi-square test and genotype frequencies difference in control group was assessed by Hardy-Weinberg equilibrium (HWE). Associations between *IL-13* gene polymorphisms and neonatal asthma risk were evaluated using Chi-square test and presented by odds ratio and 95% confidence intervals (CIs). Results: The genotype distributions of selected controls in *IL-13* polymorphisms conformed to HWE. Both CT genotype and TT genotype of rs1800925 were frequently detected in cases than in controls, and they were associated with the occurrence of neonatal asthma. Rs1800925 T allele may act as a risk factor for neonatal asthma. Beside, both GA genotype and AA genotype of rs20541 were frequently detected in cases than in controls, but they were not associated with the occurrence of neonatal asthma. In contrast, GT and TT genotypes apparently correlated with the risk of neonatal asthma. However, we did not find significant difference between the cases and controls based on allele. At the same time, these two polymorphisms presented the linkage disequilibrium (LD) and the haplotype T-G obviously increased the risk of neonatal asthma. Conclusions: *IL-13* gene rs1800925 polymorphism was associated with neonatal asthma susceptibility. T-G haplotype of rs1800925 and rs20541 might influence the occurrence of neonatal asthma.

Keywords: *IL-13*, polymorphism, neonatal asthma

Introduction

Asthma is one of the most common respiratory disorders encountered in both children and adults. The prevalence of asthma has rapidly increased over the last few decades to epidemic proportions and there are approximately 300 million people worldwide [1]. If urgent action is not taken, mortality of asthma is estimated to increase by almost 20% in the next 10 years. Development of asthma is multifactorial and depends on interactions between multiple susceptibility genes and environmental factors [2-4]. However, the risk of developing asthma tends to run in families, and heritability of asthma has been estimated as 60% [5]. Thus, host

genetic susceptibility may play a crucial role in asthma, especially in neonatal asthma. Therefore, we focused on the effect of genetic factor on neonatal asthma.

IL-13 is a potent pleiotropic cytokine that is produced by activated CD4 T cells [6]. The beneficial effects of *IL-13* include switching B cells to produce immune globulin E (IgE) and promoting the secretion of major histocompatibility complex (MHC) class II molecules. In addition, *IL-13* can inhibit the production of inflammatory cytokines such as *IL-1 α* , *IL-4R*, *IL-8*, and tumor necrosis factor (TNF)- α [7]. The human *IL-13* is encoded by a gene located on chromosome 5q31 region and encompasses 4.6 kb, which contains 4 exons and 3 introns.

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Table 1. Technical data for the analysis of single nucleotide polymorphisms (SNPs) in the human *IL-13* gene

| SNP | Primer sequence | Annealing temperature |
|-----------|------------------------------------|-----------------------|
| Rs1800925 | For. 5'-GGTTTCTGGAGGACTTCTAGGTA-3' | 66 °C |
| | Rev. 5'-GCAGAATGAGTGCTGTGGAG-3' | |
| Rs20541 | For. 5'-TGCTTTTCGAAGTTTCAGTGGGA-3' | 62 °C |
| | Rev. 5'-CATGTCCGAGACACCAAATC-3' | |

IL-13 has been demonstrated to be the central mediator of allergic asthma [8, 9]. Huang et al. [10] found that the expression of *IL-13* was increased in the allergen-challenged bronchoalveolar lavage (BAL) in asthmatic patients. Similarly, Prieto et al. [11] observed a significant increase in the expression of *IL-13* mRNA in BAL cells enriched for alveolar macrophages of the asthmatic patients. Recently, Saha and coworkers suggested that *IL-13* over-expression in sputum and bronchial biopsy specimens was a feature of severe asthma [12]. Collectively, these results indicated that *IL-13* might have an important role in the pathophysiology of asthma. However, the role of *IL-13* gene in the neonatal asthma disease is unknown.

Quite a few single nucleotide polymorphisms (SNPs) have been identified for *IL-13* gene is associated to asthma. For example, *IL-13* SNP rs20541 is a common coding SNP in exon 4, which is located at position 130 and resulted in a change from G to A. *IL-13* SNP rs20541 has been reported to be associated with an increase in the expression of *IL-13* in patients with asthma [13, 14]. Rs1800925 is another common SNP of *IL-13* and associated with asthma, located in the 5' flanking region, which usually causes C to T substitution [15]. However, the roles of *IL-13* +2044G/A and -1112C/T polymorphisms (rs20541 and rs1800925) on risk of neonatal asthma were still unknown.

Therefore, in the present study we aimed to investigate the relationship between rs20541 and rs1800925 polymorphism of *IL-13* gene and the presence of neonatal asthma in a group of Chinese subjects. For this purpose, genetic polymorphisms of *IL-13* in neonatal asthma patients were evaluated and compared. Importantly, we performed genotyping and statistical analysis in two independent populations, which might give us some solid insight.

Materials and methods

Ethics statement

Approval for this study was obtained before it was initiated from the Ethics Committee of Yidu Central Hospital in January 2006. All subjects recruited for this study were Han Chinese in origin. Written informed consent was

obtained from each participant including the guardians on the behalf of the minor participants and next of kin, care takers or guardians on the behalf of participants whose ability to consent was compromised.

The selection of study population

In the present case-control study, 103 neonatal asthma patients (cases) and 125 newborns (controls) without any disease were enrolled between 2006 and 2011. All the newborns are descendants from Northern Han population of China and recruited from the Yidu Central Hospital, located in the north of China. A diagnosis of neonatal asthma was confirmed pathologically by two independent experienced pathologists. They were 49 girls and 54 boys. The control group consisted of 58 girls and 67 boys from the same hospital with the cases in the same period. The controls were no significant difference with the cases in age and sex. They all performed the comprehensive physical examination.

DNA extraction and amplification

3 ml peripheral venous blood sample was collected in the morning from all study subjects after an 8 h fasting period and put in the anticoagulative tube with EDTA 2Na (Meus, Piove di Sacco Italy) to centrifuge for serum. And then genome DNA of all samples was extracted using blood genome DNA extraction kit bought from Beijing TIANGEN biochemical Co. Ltd., according to the manufacturer's instructions.

The SNP at position -1112C>T (rs1800925) in the promoter region and 2044G>A (rs2044) in the common coding region of the human *IL-13* gene was amplified by PCR using published forward and reverse primers [16, 17]. The two SNP primer sequences and the necessary reaction condition are listed in **Table 1**. To perform PCR, AccuPower PCR PreMix tubes (BIONEER,

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Table 2. Genotype and allele distribution comparison of *IL-13* gene rs1800925 and rs20541 polymorphisms

| Genotype/Allele | Control n=125 (%) | Case n=103 (%) | χ^2 | P | OR (95% CI) |
|------------------|-------------------|----------------|----------|-------|---------------------|
| Rs1800925 | | | | | |
| Genotype | | | | | |
| CC | 80 (64.0) | 48 (46.6) | - | - | 1.000 |
| CT | 38 (30.4) | 42 (40.8) | 4.513 | 0.044 | 1.842 (1.046-3.245) |
| TT | 7 (5.6) | 13 (12.6) | 5.399 | 0.027 | 3.095 (1.155-8.297) |
| Allele | | | | | |
| C | 198 (79.2) | 138 (67.0) | - | - | 1.000 |
| T | 52 (20.8) | 68 (33.0) | 8.683 | 0.004 | 1.876 (1.231-2.860) |
| Dominant mode | | | | | |
| CC | 80 (64.0) | 48 (46.6) | - | - | 1.000 |
| CT+TT | 45 (36.0) | 55 (53.4) | 6.942 | 0.011 | 2.037 (1.196-3.468) |
| Rs20541 | | | | | |
| Genotype | | | | | |
| GG | 71 (56.8) | 43 (41.7) | - | - | 1.000 |
| GA | 43 (34.4) | 45 (43.7) | 3.636 | 0.064 | 1.728 (0.983-3.037) |
| AA | 11 (8.8) | 15 (14.6) | 3.481 | 0.078 | 2.252 (0.948-5.349) |
| Allele | | | | | |
| G | 170 (68.0) | 131 (63.59) | - | - | 1.000 |
| A | 80 (32.0) | 75 (36.41) | 0.978 | 0.371 | 1.217 (0.825-1.795) |
| Dominant mode | | | | | |
| GG | 71 (56.8) | 43 (46.6) | - | - | 1.000 |
| GA+AA | 54 (43.2) | 60 (53.4) | 5.118 | 0.033 | 1.835 (1.082-3.110) |

Daejeon, Republic of Korea) preloaded with 1U of Taq DNA polymerase, 250 μ M dNTPs, 10 mM Tris-HCl (pH 9.0), 40 mM KCl, 1.5 mM MgCl₂, stabilizer, and tracking dye were used. Total reaction volume was 20 μ L, and this contained 1 μ L of template DNA, 1 μ L of each primer (50 pmol/ μ L), and 17 μ L ddH₂O. Polymerase chain reaction was performed using a PTC-100 Peltierthermal Cycler (MJ Research, StWaltham, MA, USA) using the following cycling conditions: 95°C pre-denaturation for 3 min; and then 35 cycles of 94°C denaturation for 30 s, 66°C (rs1800925) and 62°C (rs20541) annealing for 35 s, and 72°C extension for 90 s; 72°C final extension for 8 min and preserved at 4°C. The PCR products were checked using 1.5% agarose gel electrophoresis. The PCR products were purified using a commercial kit (GENOMED, Bad Oyenhausen, Germany).

Genotyping based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

The analysis of *IL-13* polymorphisms rs1800925 and rs20541 were performed by PCR-RFLP method. The restriction enzymes Hpy8I (rs1800925) and NlaIV (rs20541) were used to

digest the PCR products. The enzyme digestion system was a total of 20 μ L solution, including the above-mentioned 0.2 μ L restriction enzyme, 10 μ L PCR products, 2 μ L 10 \times buffer solution, and 7.8 μ L sterilization double distilled water. The enzyme digestion products were separated by 2% agarose gel electrophoresis (AGE) and stained with GoldView. The accuracy of the genotyping results was assessed using the ABI PRISM 3730 to examine the representative PCR-amplified DNA samples.

Statistical analysis

Statistical analyses were carried out using the Statistical Package for Social Sciences version 19.0 (SPSS Inc, Chicago, IL, USA). Statistical differences between genotype and allele frequencies were compared using Chi-square test (χ^2 -test). Chi-square test was performed to determine if the association between genotype and allele frequencies of patients and control subjects was statistically significant. Allele and genotype frequency data was also subjected to the Hardy-Weinberg equilibrium test (data not shown). The strength of the relationship in the genotypic and allelic distribution between

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Table 3. Analyses of LD and haplotypes in alleles of *IL-13* gene rs1800925 and rs20541 polymorphisms

| Haplotype SNP1-SNP2 | C-G | C-A | T-G | T-A |
|---------------------|------------|---------------------|---------------------|---------------------|
| Control 2n=250 (%) | 141 (56.4) | 78 (31.2) | 26 (10.4) | 5 (2) |
| Case 2n=206 (%) | 97 (47.1) | 69 (33.5) | 33 (16.0) | 7 (3.4) |
| χ^2 | - | 1.416 | 4.424 | 1.453 |
| <i>P</i> | - | 0.245 | 0.041 | 0.245 |
| OR (95% CI) | 1.000 | 1.286 (0.850-1.946) | 1.845 (1.038-3.280) | 2.035 (0.628-6.599) |

Note: SNP1: rs1800925; SNP2: rs20541.

patients and controls was assessed by the odds ratios (OR) and 95% confidence intervals (95% CI), and the *P* value was tested by the Chi-square test. Differences were deemed to be significant at *P*<0.05. Besides, the linkage disequilibrium (LD) and haplotype were also analyzed according to haploview software.

Results

Clinically detailed information of all subjects

This study totally covered 103 cases diagnosed by two independent experienced pathologists, including 54 males and 49 females; their age range was 0~28 days with the mean age of 10.32±8.23. At the same time, 53.6% of 125 controls recorded in this study were boys and only 46.4% were girls, their mean age was 10.09±9.16 with the age range of 0~28. According to χ^2 test, there was no obvious distribution difference between the cases and controls in terms of age and gender (*P*>0.05). Furthermore, we tested the Hardy-Weinberg equilibrium (HWE) for the two selected SNPs. The distribution of the rs1800925 and rs20541 SNPs among the controls was consistent with the HWE test (*P*=0.39 and *P*=0.24, respectively).

Correlation between IL-13 gene polymorphisms and susceptibility of neonatal asthma

The genotype frequencies of each of the *IL-13* gene polymorphisms were categorized into groups, as shown in **Table 2**. In the neonatal asthma patients, the frequencies of the CC, CT, and TT genotypes of rs1800925 were 46.6%, 40.8%, and 12.6%, respectively, and in the healthy controls, they were 64%, 30.4%, and 5.6%, respectively. Using subjects with the CC genotype as a reference group, the results showed that there was a significant difference

between the genotype and allele frequencies of the *IL-13* gene rs1800925 polymorphisms and neonatal asthma risk after adjusting for gender and age using binary logistic regression analyses. CT genotype and TT genotype had higher frequency in cases than controls and its carriers were high risk to suffer from neonatal asthma, compared with CC genotype carriers (OR=1.842, 95% CI=1.046-3.245 and OR=3.095, 95% CI=1.155-8.297, respectively). T allele of rs1800925 might increase the susceptibility of neonatal asthma (*P*=0.004, OR=1.876, 95% CI=1.231-2.860). Besides, in the neonatal asthma patients, the frequencies of the GG, GA, and AA genotype of rs20541 were 41.7%, 43.7%, and 14.6%, respectively, and in the healthy controls, they were 56.8%, 34.4%, and 8.8%, respectively. Compared with the GG genotype, the GA genotype and the AA genotype had no significant association with the risk of neonatal asthma (*P*=0.064 and 0.078, respectively). In contrast, GA and AA genotypes of rs20541 obviously related to the occurrence of neonatal asthma (*P*=0.033, OR=1.835, 95% CI=1.082-3.110). However, we found that there was no significant difference between the cases and controls based on allele (*P*=0.371).

Haplotype analysis of IL-13 gene

Between *IL-13* rs1800925 and rs20541 polymorphisms was identified the linkage disequilibrium (LD). A total of four haplotypes for rs1800925-rs20541 were identified in our study population, namely C-G, C-A, T-G, and T-A haplotypes. Among them, only the frequency of haplotype T-G had statistically significant difference between the two groups (*P*=0.041) (**Table 3**). People who carried T-G haplotype had a 1.845 times risk to suffer from neonatal asthma, compared with C-G haplotype carriers (OR=1.845, 95% CI=1.038-3.280).

Discussion

Asthma exacerbations are among the most frequent causes of hospitalization during newborn and childhood and are responsible for large health-care expenditures [18-21]. Available treatment options for prevention and treatment of asthma exacerbations are inadequate [22], suggesting that asthma with severe exacerbations may represent a distinct sub-type of disease and demonstrating a need for improved understanding of its pathogenesis. Asthma heritability is estimated to be 70-90% [5, 23]. Asthma susceptibility genes are mapped to a region on human chromosome 5q31-q33, which contains a cluster of pro-inflammatory cytokine genes such as interleukin-13 (*IL-13*), which is associated with asthma.

IL-13, well known as a Th2 anti-inflammatory cytokine, is involved in mediating B cell and mast cell proliferation and correlates with IgE synthesis, which is a major regulator in Th2-mediated disease [24]. To date, many epidemiological studies have been carried out to evaluate whether polymorphisms in *IL-13* contribute to an individual's susceptibility to cancer. The *IL-13* rs20541 [25] and rs1800925 [26] polymorphisms were significantly associated with decreased a risk of glioma. Sainz J et al. [27], reported that patients harboring the *IL-13* rs20541 T allele had a reduced risk of colorectal cancer. In addition, a great deal of genetic studies also have focused on the contribution of *IL-13* polymorphisms to the risk of allergic rhinitis and asthma. Bottema et al. [28] investigated *IL-13* polymorphisms in rhinitis and asthma populations in Dutch. Their results showed that *IL-13* rs1800925 was significantly associated with rhinitis in Dutch population. Besides, they also proved that the polymorphisms of rs20541 and rs1295685 were consistently associated with asthma, which were consistent with the results of haplotypes. At the same time, Ying et al. [29] reported that the *IL-13* rs20541 SNP is associated with an increased risk of allergic rhinitis. So the aim of our study is to investigate the association of *IL-13* gene polymorphisms (rs20541 and rs1800925) and the risk of neonatal asthma. To the best of our knowledge, this is the first study documenting the relationship between *IL-13* genetic variants and neonatal asthma.

In this article, two polymorphisms of *IL-13* gene (rs20541 and rs1800925) in encoding and pro-

motor region were amplified to analyze the susceptibility to neonatal asthma based on the different genotypes and alleles. In our study population, both the rs1800925 CT genotype and TT genotype were significantly associated with risk in neonatal asthma. Meanwhile, T allele of rs1800925 polymorphism showed a higher frequency in cases than controls and increased the risk of neonatal asthma, compared with C allele. In rs20541 polymorphism, GA and AA genotypes were found to increase the risk of neonatal asthma development, compared with GG genotype. However, A allele of rs20541 polymorphism showed a higher frequency in cases than controls, but did not increase the risk of neonatal asthma, compared with G allele. In addition, these two SNPs presented the LD and haplotype T-G significantly decreased the susceptibility to neonatal asthma, compared with C-G haplotype.

In conclusion, the *IL-13* rs1800925 and rs20541 polymorphisms were relate to enhance the risk of neonatal asthma. The haplotype of *IL-13* gene polymorphisms also associated with the susceptibility of neonatal asthma. Although the cases and controls were in accordance with HWE, there still many limitations existed in our study, such as the small sample size and the unadjusted results. That was insufficient to understand the occurrence of neonatal asthma. With rapid advances of high throughput gene sequencing, sample size not of hundreds but thousands of patients. In addition, potential confounding factors has seen implementation of analytic techniques should be considered in the future. A well designed study was necessary, so as to get a sufficient evidence to certify the etiology of neonatal asthma.

Disclosure of conflict of interest

None.

Address correspondence to: Aiyang Wan, Department of Paediatrics, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei, China. E-mail: jiechuasr@yeah.net

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