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Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem*IL-13* polymorphisms contribute to the risk of asthma: A meta-analysisLin Cui^{a,1}, Jing Jia^{b,1}, Chun-Fen Ma^{c,*}, Su-Yun Li^a, You-Ping Wang^a, Xiao-Min Guo^d, Qiang Li^a, Hai-Bin Yu^a, Wei-Hong Liu^a, Lin-Bo Gao^{e,f,*}^a Central Laboratory, the First Affiliated Hospital of Henan College of Traditional Chinese Medicine, Zhengzhou, 450000, People's Republic of China^b Molecular Medicine Centre, Zhejiang Academy of Medical Sciences, Hangzhou, Zhejiang, 310013, People's Republic of China^c Department of Gynecology, the Second Affiliated Hospital of Henan College of Traditional Chinese Medicine, Zhengzhou, 450003, Henan, People's Republic of China^d Department of Otolaryngology, Third Affiliated Hospital of Henan University of Science and Technology and Luo Yang East Hospital, Luoyang, Henan 471003, People's Republic of China^e Laboratory of Molecular and Translational Medicine, West China Institute of Women and Children's Health, West China Second University Hospital, Sichuan University, Chengdu, Sichuan 610041, People's Republic of China^f Key Laboratory of Obstetric & Gynecologic and Pediatric Diseases and Birth Defects of Ministry of Education, Chengdu, Sichuan 610041, People's Republic of China

ARTICLE INFO

Article history:

Received 17 August 2011

received in revised form 30 November 2011

accepted 10 December 2011

Available online xxxx

Keywords:

Asthma

IL-13

Meta-analysis

Polymorphism

ABSTRACT

Objectives: The purpose of this study was to evaluate the effects of interleukin-13 (*IL-13*) polymorphisms on the risk of asthma using a meta-analysis.**Design and methods:** Fifteen publications were identified by searching Pubmed, Embase, ISI, OVID, and EBSCO databases. Odds ratios with corresponding 95% confidence intervals were computed to estimate the association between *IL-13* polymorphisms and risk of asthma.**Results:** The polymorphisms of R130Q (rs20541) and -1112C/T (rs1800925) in *IL-13* gene were associated with significantly increased risks of asthma in overall analyses. Subgroup analyses showed that the elevated risks occurred in adult-onset asthma, Caucasians, and high quality studies.**Conclusions:** This meta-analysis provides evidence that the R130Q and -1112C/T polymorphisms in *IL-13* are risk factors for asthma.

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Introduction

Asthma is a complex disorder caused by interaction of multiple genetic and environmental factors, and the suggested overall genetic contribution is around 50–60% [1]. Until now more than 100 genes have been reported to relate to asthma, and these genes always contain polymorphisms that modify their function.

Interleukin-13 (*IL-13*) is a critical cytokine in the pathogenesis of allergic asthma, with a crucial function in determination of bronchial hyper responsiveness in animal models of allergic asthma [2]. In human beings, *IL-13* is produced at high levels by Th2-like cells, Th1-like and CD8+ cells, such as CD4 T cells, eosinophils, mast cells, basophils, and natural killer T cells. *IL-13* could induce IgE synthesis in cultured B cells. Levels of *IL-13* mRNA and protein are increased in sputum of subjects with asthma [3–5].

IL-13 locates on chromosome 5q, a region frequently linked to asthma, total serum IgE, airway responsiveness, and other asthma related phenotypes [6]. Some common polymorphisms in *IL-13* have been reported previously. For example, R130Q (rs20541), a

coding SNP in exon 4 (Arg130Gln), and the 130Gln substitution results in signal transducer and activator of transcription phosphorylation in monocytes, decreased affinity of *IL-13* for *IL-13* receptor, and increased expression of *IL-13* in patients with asthma. The 130Q variant allele is more active than the wild-type allele in stimulating *IL4RA*-dependent STAT6 phosphorylation and up-regulation of CD23 molecules, and promoting IgE production [7–9]. Another polymorphism is -1112C/T (rs1800925) which is located within the extended matrix of a STAT binding site in the *IL-13* promoter. The polymorphism may change the binding of STAT transcription factors and influence the expression of *IL-13* in T cells [10,11]. Until recently, a number of studies have been carried out to assess the association between *IL-13* polymorphisms and risk of asthma in different populations; however, the results are inconsistent and inconclusive [7,12,13].

Different methodologies have been used for evaluation, but most of the studies used a small sample size, and thus there has been a lack of replication between the various studies. By using all the available published data to increase the statistical power, we guess that a meta-analysis might allow plausible candidate genes to be excluded and causative genes to be identified. We therefore performed a meta-analysis of all of the published case-control studies until 30 April, 2011 to ascertain whether the polymorphisms of R130Q (rs20541) and -1112C/T (rs1800925) in *IL-13* gene increased the risk of asthma.

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Materials and methods

Selection of published studies

The study protocol followed the guidelines of meta-analysis of observational studies in epidemiology (MOOSE) group [14]. The databases searched were as following: Pubmed, Embase (1966–2011), ISI (1986–2011), OVID (1948–2011), EBSCO (1999–2011) databases. The following terms were used in this search: “IL-13 or interleukin 13” and “polymorphism* or variant” and “asthma” (last search update 30 April, 2011). We also manually searched the reference lists of original and review articles for additional relevant publications. For inclusion, a study had to meet the following criteria: (a) the design had to be a case-control and case-cohort study; (b) the study evaluated the association between R130Q (rs20541) and -1112C/T (rs1800925) polymorphisms in *IL-13* gene and asthma; (c) original data for odds ratios (ORs) calculation were reported; and (d) records were published in English. We excluded the publications that were a deviation from Hardy–Weinberg equilibrium.

Seven hundred and ten articles were identified by searching Pubmed, Embase, ISI, OVID, and EBSCO databases, and manual search. 453 articles were excluded for duplicate publication. After reading the title and abstract, 175 articles were excluded (78 articles were not *IL-13* polymorphisms; 73 articles were not relevant to asthma; 17 articles were review articles; and 7 articles were not performed in human). After the full-text evaluation, sixty-three were excluded with reasons for no available data ($n=26$), no *IL-13* polymorphism ($n=24$), review articles ($n=7$), no human study ($n=2$), meta-analysis ($n=2$), and not case-control studies ($n=2$). Additionally, we excluded the overlapped data reported by the same research group [12,15–18]. Finally, 15 studies were included in this meta-analysis (Supplementary Fig. 1), among which, 12 articles studied the association between the *IL-13* R130Q polymorphism and asthma; and 10 articles studied the association between the *IL-13* -1112C/T polymorphism and asthma.

Data extraction

According to inclusion criteria, two investigators (Cui and Jia) extracted data independently. The data extracted were as follows: the surname of the first author, date of publication, country of origin, ethnicity, study design, characteristics of cases and controls, matching variables, mean age of the case group, number of cases and controls, genotyping method, genotype distribution of cases and controls, quality control for genotyping assay and Hardy–Weinberg equilibrium (HWE). To ensure the accuracy of the extracted data, a third investigator (Gao) checked the data further and solved the inconsistent problem through discussion.

Quality score evaluation

The included articles were scored according to the grading standard cited from literature [19]. Briefly, the evaluation was carried out according to the following variables: representativeness of cases and controls, ascertainment of asthma and controls, genotyping examination, HWE, association assessment, and response rate. Full mark is 15 points. The higher the publication scored, the better quality it is, and vice versa. The study scored >6 was defined as high quality study, while the study scored ≤ 6 was low quality study.

Statistical analysis

ORs and 95% CI were used to evaluate the association between the *IL-13* polymorphisms and risk of asthma. We used fixed-effects model for evaluation firstly, and then random-effects model would be used to estimate summary OR value when P value ≤ 0.10 in heterogeneity

analysis, which demonstrated apparent heterogeneity. The association between asthma and the 2 polymorphisms in *IL-13* was evaluated using heterozygote comparison, homozygote comparison, dominant model, and recessive model, respectively.

The subgroup analyses were carried out according to ethnicity, age of disease onset (adult versus children), study quality (high versus low). If there is apparent heterogeneity that existed, logistic meta-regression would be used to explore the sources of heterogeneity: the age of onset, ethnicities, genotyping methods, quality control, and sample size. Publication bias was evaluated with Funnel plot and Egger's regression asymmetry test. All analyses were done by STATA software, version 10.0 (STATA Corp., College Station, TX, USA).

Results

The baseline characteristics of the included studies are shown in Supplementary Table 1.

Overall, there was evidence of an association between the *IL-13* R130Q polymorphism and the risk of asthma when all the eligible publications were pooled into the meta-analysis (Fig. 1, Supplementary Table 2) (heterozygote comparison: OR = 1.20, 95%CI, 1.01–1.42; homozygote comparison: OR = 1.21, 95%CI, 1.01–1.46; and dominant model: OR = 1.22, 95%CI, 1.03–1.44, respectively). Similar findings were observed in adult-onset asthma (heterozygote comparison: OR = 1.31, 95%CI, 1.05–1.64; and dominant model: OR = 1.32, 95%CI, 1.06–1.63, respectively) but not in child-onset asthma. In the subgroup analysis by ethnicity, the significant association was also found in Caucasians (heterozygote comparison: OR = 1.37, 95%CI, 1.01–1.85; and dominant model: OR = 1.38, 95%CI, 1.04–1.83, respectively) but not in Asians. When stratified according to quality score, the excess risk of the *IL-13* R130Q polymorphism occurred in high quality studies (dominant model: OR = 1.34, 95%CI, 1.00–1.80) but not in low quality studies.

The evaluations of the association between the *IL-13* -1112 C/T polymorphism and the risk of asthma are summarized in Supplementary Table 3. The CT and TT/CT genotypes were associated with a significantly increased risk of asthma when compared with CC genotype (CT versus CC: OR = 1.24, 95%CI, 1.03–1.48; and TT/CT versus CC: OR = 1.28, 95%CI, 1.06–1.56, respectively) (Fig. 2). Similar elevated risk was detected in adult-onset asthma (CT versus CC: OR = 1.34,

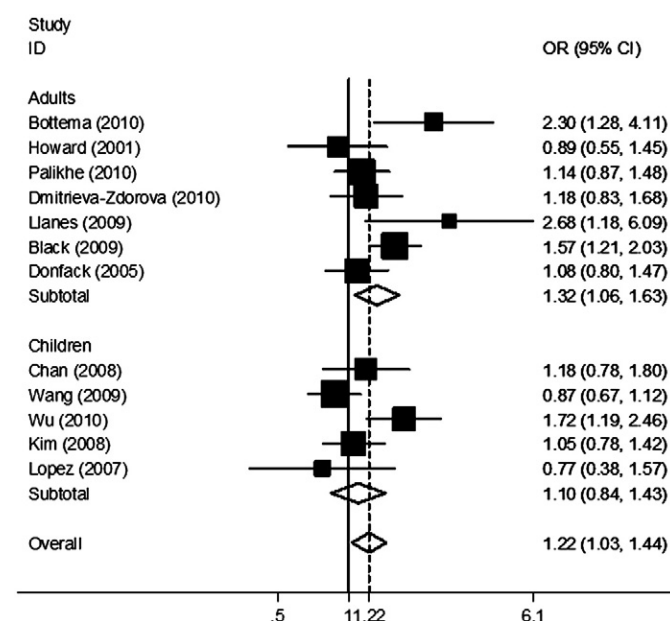


Fig. 1. Meta-analysis of the association between the *IL-13* R130Q polymorphism and risk of asthma (TT/CT versus CC). The random-effects model was used.

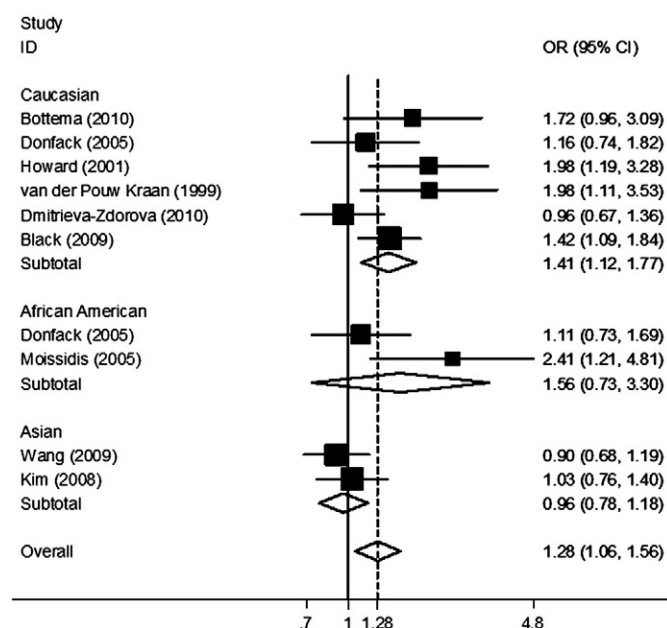


Fig. 2. Meta-analysis of the association between the *IL-13* -1112C/T polymorphism and risk of asthma (TT/CT versus CC). The random-effects model was used.

95%CI, 1.14–1.58; and TT/CT versus CC: OR = 1.34, 95%CI, 1.15–1.57, respectively) but not in child-onset asthma. In the subgroup analysis by ethnicity, significantly elevated asthma risk was observed in Caucasians (CT versus CC: OR = 1.34, 95%CI, 1.13–1.58; and TT/CT versus CC: OR = 1.37, 95%CI, 1.17–1.61, respectively) rather than in Asians and African Americans. When stratified according to quality score, significantly increased risk was found in high quality studies (CT versus CC: OR = 1.28, 95%CI, 1.08–1.51; and dominant model: OR = 1.29, 95%CI, 1.10–1.51, respectively) rather than in low quality studies.

Heterogeneity analysis

There is heterogeneity existed in overall comparisons and subgroup analyses. To explore the sources of heterogeneity, we evaluated the following variables: the age of onset, ethnicities, genotyping methods, study quality, and sample size (≤ 500 , and > 500 subjects). For the *IL-13* -1112C/T polymorphism, we found that sample size could influence the initial heterogeneity (CT vs. CC: $P = 0.036$; TT vs. CC: $P = 0.035$; TT/CT vs. CC: $P = 0.015$; and TT vs. CC/CT: $P = 0.047$). Meta-regression analyses indicated that the sample size could explain 63.5% (CT vs. CC), 78.1% (TT vs. CC), 75.8% (TT/CT vs. CC), and 71.5% (TT vs. CC/CT) of the τ^2 . For the *IL-13* R130Q polymorphism, however, none of the possible variables could explain the heterogeneity between studies.

Publication bias

The publication bias of literatures included was evaluated by Funnel plot and Egger's test. There was a marginal significance for dominant model of the *IL-13* -1112C/T polymorphism ($P = 0.042$). The publication bias disappeared after exclusion of the studies that the quality score is the lowest ($P = 0.12$) [20,21]. No evidence of publication bias for the *IL-13* R130Q polymorphism was detected in all comparison models.

Discussion

Allergic asthma is a complex disease that results from maladaptive inflammatory responses to ubiquitous environmental proteins in genetically susceptible persons. Allergic asthma is a chronic inflammatory

disorder of the airways mediated by polarizations of CD4+ T cells to a type 2 helper (Th2) phenotype. The cardinal features of asthma, such as pulmonary eosinophilia, elevated concentrations of serum IgE, airway hyperresponsiveness, and excessive production of mucus in the airways, are thought to be induced by Th2 cytokines, including IL-14, IL-15, and IL-13. In particular, IL-13 plays a critical role in mediating airway hyperresponsiveness and mucous metaplasia, the elements of allergic asthma that are most closely linked to disease symptoms. Animal model studies have shown that IL-13 is sufficient to mediate all the cardinal features of Th2 inflammation in the lung [22,23]. In humans, IL-13 and its receptors are found to be highly expressed in the respiratory tract of patients with asthma. Th2 cells, as well as eosinophils, mast cells, and basophils all produce IL-13. IL-13 stimulates IgE synthesis by human B cells, which is a key effector in Th2-mediated disease.

Given the key roles of IL-13 playing in the development of asthma, a large number of genetic studies have been performed to investigate the association between the *IL-13* polymorphisms and risk of asthma [13,20,21,24–35]. The results, however, remain conflicting, and thus a systematic review and meta-analysis of association between the *IL-13* polymorphisms and asthma were of great value. In this study, we collected 15 case-control studies with complete data and information, and performed a meta-analysis to evaluate the association between the polymorphisms of R130Q (rs20541) and -1112C/T (rs1800925) in *IL-13* gene and asthma. Our meta-analysis provided evidence that the two polymorphisms were associated with significantly increased risks of asthma in overall analyses and subgroup analyses. Our findings were in agreement with the genome-wide linkage studies that have identified several susceptibility loci of asthma [36]. Some of the gene groups located on chromosome 5q contain the Th2 cytokine *IL-13*. Taken together, these findings indicate that *IL-13* is a candidate gene for asthma.

When stratified according to age of onset, the R130Q and -1112C/T polymorphisms in *IL-13* were found to be associated with an elevated risk in adult-onset asthma but not in child-onset asthma. In the subgroup analysis by ethnicity, significantly elevated risk was found in Caucasians rather than in Asians and African Americans. Although the reason for these discrepancies is not well known, some possibilities should be considered. Sample sizes differed significantly. The sample size of the *IL-13* -1112C/T polymorphism in African Americans was comparatively small, which may contribute to the discrepancy of the *IL-13* -1112C/T polymorphism in ethnicity subgroup analysis. Moreover, interaction between gene and environment would finally induce genesis of extremely complex diseases. Different environmental factors for asthma in children compared with adults were found by epidemiological surveys, such as cigarette smoking and occupational chemical exposure, which may exert significant influences on the pathogenesis. Therefore, environment factor may be another reason for this phenomenon of asthma in adults. The results provide new direction for future research: it is necessary to conduct multi-centric, multi-ethnicity, cooperative studies with large sample size to confirm the results.

After stratified based on the quality of the literatures, we found that the excess risk of the two polymorphisms in *IL-13* occurred in high quality studies but not in low quality studies. Several possibilities should be considered to explain these findings. Genotyping assays without quality control in lower quality studies may have a high risk of genotyping error, which may be eventually responsible for these inconsistent results. Representativeness of cases and controls may be another reason for the deviation of the results. Cases/controls that were not randomly drawn from the same sampling frame in lower quality studies are liable to have a selection bias. These possible reasons strengthen the importance of methodological design in observational studies.

Publication bias is one of major problems that a sound meta-analysis should concentrate on. In the current study, Funnel plot

and Egger's test were done to assess publication bias. No evidence of publication bias for the two polymorphisms was examined in all comparison models except for a dominant model of the *IL-13* -1112C/T polymorphism. The bias disappeared after exclusion of the lowest quality studies [20,21]. Another important concern for meta-analysis is the existence of heterogeneity between the individual component studies and the degree of it, because non-homogeneous data is liable to result in misleading results. Heterogeneity existed in the two polymorphisms in overall comparisons and subgroup analyses. Thus, the sources of heterogeneity were explored by meta-regression analysis, and it was found that the heterogeneity between studies could be explained by sample size for the *IL-13* -1112C/T polymorphism. However, none of the possible variables could explain the heterogeneity between studies for the *IL-13* R130Q polymorphism. There may be other reasons for accounting the heterogeneity.

In conclusion, this meta-analysis provides evidence that the R130Q (rs20541) and -1112C/T (rs1800925) polymorphisms in *IL-13* are risk factors for asthma. It is necessary to conduct well-designed, multicentre, unbiased prospective studies with larger sample size to provide further evidence of these results. Moreover, further studies evaluating the effects of gene–gene and gene–environment interactions may eventually provide a comprehensive understanding of the association between the *IL-13* polymorphisms and asthma risk.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.clinbiochem.2011.12.012.

References

- [1] Los H, Koppelman GH, Postma DS. The importance of genetic influences in asthma. *Eur Respir J* 1999;14:1210–27.
- [2] Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998;282:2258–61.
- [3] de Waal Malefyt R, Abrams JS, Zurawski SM, Lecron JC, Mohan-Peterson S, Sanjanwala B, et al. Differential regulation of IL-13 and IL-4 production by human CD8+ and CD4+ Th0, Th1 and Th2 T cell clones and EBV-transformed B cells. *Int Immunol* 1995;7:1405–16.
- [4] Jung T, Wijdenes J, Neumann C, de Vries JE, Yssel H. Interleukin-13 is produced by activated human CD45RA+ and CD45RO+ T cells: modulation by interleukin-4 and interleukin-12. *Eur J Immunol* 1996;26:571–7.
- [5] Van der Pouw Kraan TC, Van der Zee JS, Boeijs LC, De Groot ER, Stapel SO, Aarden LA. The role of IL-13 in IgE synthesis by allergic asthma patients. *Clin Exp Immunol* 1998;111:129–35.
- [6] Liu X, Nickel R, Beyer K, Wahn U, Ehrlich E, Freidhoff LR, et al. An IL13 coding region variant is associated with a high total serum IgE level and atopic dermatitis in the German multicenter atopy study (MAS-90). *J Allergy Clin Immunol* 2000;106:167–70.
- [7] Heinzmann A, Mao XQ, Akaiwa M, Kremer RT, Gao PS, Ohshima K, et al. Genetic variants of IL-13 signalling and human asthma and atopy. *Hum Mol Genet* 2000;9:549–59.
- [8] Chen W, Ericksen MB, Levin LS, Khurana Hershey GK. Functional effect of the R110Q IL13 genetic variant alone and in combination with IL4RA genetic variants. *J Allergy Clin Immunol* 2004;114:553–60.
- [9] Vladich FD, Brazille SM, Stern D, Peck ML, Ghittoni R, Vercelli D. IL-13 R130Q, a common variant associated with allergy and asthma, enhances effector mechanisms essential for human allergic inflammation. *J Clin Invest* 2005;115:747–54.
- [10] Graves PE, Kabisch M, Halonen M, Holberg CJ, Baldini M, Fritzsche C, et al. A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children. *J Allergy Clin Immunol* 2000;105:506–13.
- [11] Vercelli D. Genetics of IL-13 and functional relevance of IL-13 variants. *Curr Opin Allergy Clin Immunol* 2002;2:389–93.
- [12] Leung TF, Tang NL, Chan IH, Li AM, Ha G, Lam CW. A polymorphism in the coding region of interleukin-13 gene is associated with atopy but not asthma in Chinese children. *Clin Exp Allergy* 2001;31:1515–21.
- [13] Howard TD, Whittaker PA, Zaiman AL, Koppelman GH, Xu J, Hanley MT, et al. Identification and association of polymorphisms in the interleukin-13 gene with asthma and atopy in a Dutch population. *Am J Respir Cell Mol Biol* 2001;25:377–84.
- [14] Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000;283:2008–12.
- [15] Chan IH, Leung TF, Tang NL, Li CY, Sung YM, Wong GW, et al. Gene–gene interactions for asthma and plasma total IgE concentration in Chinese children. *J Allergy Clin Immunol* 2006;117:127–33.
- [16] Kim HB, Lee YC, Lee SY, Jung J, Jin HS, Kim JH, et al. Gene–gene interaction between IL-13 and IL-13Ralpha1 is associated with total IgE in Korean children with atopic asthma. *J Hum Genet* 2006;51:1055–62.
- [17] Kang MJ, Lee SY, Kim HB, Yu J, Kim BJ, Choi WA, et al. Association of IL-13 polymorphisms with leukotriene receptor antagonist drug responsiveness in Korean children with exercise-induced bronchoconstriction. *Pharmacogenet Genomics* 2008;18:551–8.
- [18] Larocca NE, Moreno D, Toro F, Garmendia JV, De Sanctis JB. IL-4, intron 3 variable number tandem repeats analysis (VNTR), and IL-13-1055 gene polymorphism in patients with asthma and COPD in Venezuela. *J Allergy Clin Immunol* 2008;121:S77.
- [19] Thakkinstant A, McEvoy M, Minelli C, Gibson P, Hancox B, Duffy D, et al. Systematic review and meta-analysis of the association between (beta)2-adrenoceptor polymorphisms and asthma: a HuGe review. *Am J Epidemiol* 2005;162:201–11.
- [20] Larocca N, Moreno D, Garmendia J, Toro F, De Sanctis J, et al. IL-4, intron 3 variable number tandem repeats analysis (VNTR), and IL-13-1055 SNP gene polymorphism in patients with asthma and chronic obstructive pulmonary disease in Venezuela. *Allergy Eur J Allergy Clin Immunol* 2010;65:338.
- [21] Moissidis I, Chinoy B, Yanamandra K, Napper D, Thurmon T, Bocchini Jr T, et al. Association of IL-13, RANTES, and leukotriene C4 synthase gene promoter polymorphisms with asthma and/or atopy in African Americans. *Genet Med* 2005;7:406–10.
- [22] Yang G, Li L, Volk A, Emmell E, Petley T, Giles-Komar J, et al. Therapeutic dosing with anti-interleukin-13 monoclonal antibody inhibits asthma progression in mice. *J Pharmacol Exp Ther* 2005;313:8–15.
- [23] Yang G, Volk A, Petley T, Emmell E, Giles-Komar J, Shang X, et al. Anti-IL-13 monoclonal antibody inhibits airway hyperresponsiveness, inflammation and airway remodeling. *Cytokine* 2004;28:224–32.
- [24] Black S, Teixeira AS, Loh AX, Vinall L, Holloway JW, Hardy R, et al. Contribution of functional variation in the IL13 gene to allergy, hay fever and asthma in the NSHD longitudinal 1946 birth cohort. *Allergy* 2009;64:1172–8.
- [25] Bottema RW, Nolte IM, Howard TD, Koppelman GH, Dubois AE, de Meer G, et al. Interleukin 13 and interleukin 4 receptor-alpha polymorphisms in rhinitis and asthma. *Int Arch Allergy Immunol* 2010;153:259–67.
- [26] Chan IH, Tang NL, Leung TF, Huang W, Lam YY, Li CY, et al. Study of gene–gene interactions for endophenotypic quantitative traits in Chinese asthmatic children. *Allergy* 2008;63:1031–9.
- [27] Dmitrieva-Zdorova EV, Voronko OE, Aksenova MG, Bodoev NV. Association of interleukin-13 gene polymorphisms with atopic bronchial asthma. *Russ J Genet* 2010;46:99–104.
- [28] Donfack J, Schneider DH, Tan Z, Kurz T, Dubchak I, Frazer KA, et al. Variation in conserved non-coding sequences on chromosome 5q and susceptibility to asthma and atopy. *Respir Res* 2005;6:145.
- [29] Kim HB, Kang MJ, Lee SY, Jin HS, Kim JH, Kim BS, et al. Combined effect of tumour necrosis factor-alpha and interleukin-13 polymorphisms on bronchial hyperresponsiveness in Korean children with asthma. *Clin Exp Allergy* 2008;38:774–80.
- [30] Llanes E, Quiralte J, Lopez E, Sastre B, Chacartegui M, del Pozo V, et al. Analysis of polymorphisms in olive pollen allergy: IL13, IL4RA, IL5 and ADRB2 genes. *Int Arch Allergy Immunol* 2009;148:228–38.
- [31] Lopez KI, Martinez SE, Moguel MC, Romero LT, Figueroa CS, Pacheco GV, et al. Genetic diversity of the IL-4, IL-4 receptor and IL-13 loci in mestizos in the general population and in patients with asthma from three subpopulations in Mexico. *Int J Immunogenet* 2007;34:27–33.
- [32] Palikhe NS, Kim SH, Cho BY, Choi GS, Kim JH, Ye YM, et al. IL-13 gene polymorphisms are associated with rhinosinusitis and eosinophilic inflammation in aspirin intolerant asthma. *Allergy Asthma Immunol Res* 2010;2:134–40.
- [33] van der Pouw Kraan TC, van Veen A, Boeijs LC, van Tuyt SA, de Groot ER, Stapel SO, et al. An IL-13 promoter polymorphism associated with increased risk of allergic asthma. *Genes Immun* 1999;1:61–5.
- [34] Wang J, Liou Y, Wu Y, Hsiao Y, Wu L. An association study of 13 SNPs from seven candidate genes with pediatric asthma and a preliminary study for genetic testing by multiple variants in Taiwanese population. *J Clin Immunol* 2009;29:205–9.
- [35] Wu X, Li Y, Chen Q, Chen F, Cai P, Wang L, et al. Association and gene–gene interactions of eight common single-nucleotide polymorphisms with pediatric asthma in middle china. *J Asthma* 2010;47:238–44.
- [36] Denham S, Koppelman GH, Blakey J, Wjst M, Ferreira MA, Hall IP, et al. Meta-analysis of genome-wide linkage studies of asthma and related traits. *Respir Res* 2008;9:38.