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Cysteinyl Leukotriene Receptor 1 Gene variation and Risk of Asthma in Chinese Population

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Abstract

While it has been recognized that genetics plays an important role in the development of asthma, important causal loci remain to be identified. This study was to examine the association of known and novel candidate genes with asthma.

Two independent samples, including 170 asthmatic cases and 347 controls in the initial sample, as well as 202 asthmatic cases and 332 controls in the confirmation sample, were recruited from the same region in China. 129 functional single nucleotide polymorphisms (SNPs) from 105 genes were genotyped using Sequenom MassArray technology and 119 SNPs were used for subsequent analysis.

In the initial sample, three SNPs, rs320995 in the CYSLTR1 gene, rs1047266 in the TNFRSF10B gene and rs40401 in the IL3 gene, were associated with the risk of asthma at $P_{observed}$ <0.01. Notably, under the recessive genetic model, subjects without T-allele in SNP rs320995 had 3.1 times higher risk of asthma ($P_{observed}$ = 0.00004), which remained significant after accounting for multiple testing (P_{global}=0.047). This association was replicated in the confirmation sample (P=0.032) and validated by meta-analysis. Further, gender-specific analysis was performed, while no gender difference was found.

Our study has provided coherent evidence that *CYSLTR1* variation is associated with the risk of asthma.

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Key word

Asthma susceptibility, Cysteinyl Leukotriene Receptor 1 Gene, Single nucleotide polymorphism

Introduction

Asthma, a chronic inflammatory disorder of airways, has been recognized as a serious public health problem worldwide. As estimated by the Global Initiative for Asthma (GINA) in 2006, approximately 300 million individuals of all ages and different ethnicities suffered from asthma, and annual worldwide deaths from asthma were estimated at about 250,000 individuals. Since the burden of this disease has continued increasing worldwide, better understanding of the etiology and underlying biological mechanisms of asthma will be important for effective prevention and treatment of asthma.

It has been well established that the development of asthma is influenced by genetic factors, environmental factors and their interactions. Currently, more than 100 asthma susceptibility genes have been reported in at least one population (1-3). About 33 genes, which replicated by more than 5 studies, have been suggested to be underlying candidate genes of asthma(3). However, important gaps remain in dissecting genetic predispositions of asthma. For example, even a genotype association is replicated, it is often associated with different phenotypes, different polymorphisms in the same gene, different alleles of the same variant, or in different populations (1). Since asthma is a complex disease, genes with different biological functions and involved in various regulatory pathways may simultaneously affect the development of asthma. But most reported genetic studies of asthma examined only one or a few candidate genes in a single study. Only limited studies systematically examined a large number of candidate genes in relation to asthma or asthma-related phenotypes (4-11).

The aim of this study was to systematically examine the association of known or suspected novel candidate genes with asthma and asthma-related phenotypes in two sets of homogeneous samples recruited from the same region in China: one for initial genetic association test and the other for confirmation. To our knowledge, this study is the largest asthma candidate gene study in Chinese population.

Materials and Methods

Subject recruitment and phenotype definition

In this study, 170 unrelated asthmatic cases and 347 unrelated controls (the initial sample) were selected from 3,022 families. A detailed description of the study site, subject recruitment and protocols of data collection has been described previously(12). Briefly, the study subjects were enrolled from the eight counties of Anqing city in China. The following data were available for each participant: 1)information on respiratory symptoms, history of disease, and smoking, collected with a questionnaire; 2) Forced expiratory volume in 1 second (FEV1) and forced vital capacity(FVC), measured via a standardized spirometry test; 3) Airway methacholine challenge test with the following 5 combinations of numbers of breaths and methacholine concentration in sequential order: 1 breath of 1 mg/mL,1 breath of 5 mg/mL, 4 breaths of 5 mg/mL,1 breath of 25 mg/mL, and 4 breaths of 25 mg/mL; the test terminated at the dose that produced a ≥20% drop in FEV1 from the baseline (PD20), or at the final dose if PD20 was not observed; 4) blood eosinophil count (EOS) , performed by use of a coulter counter. The study protocol was approved by the Human Subjects Committee (the institutional review board (IRB)) of Harvard School of Public Health, by the IRB of University of Illinois at Chicago, and by the Ethics Committee of Anhui Medical University. Written informed consent was explained to, read and signed by each participant.

In the initial step of the current study, asthma cases were defined if they 1) had self-reported physician-diagnosed asthma; 2) had an observed PD20 on and before the third dose during the 5-dose test; and 3) were in the top quartile of EOS distribution; The controls was defined if they 1) had no history of self-reported physician-diagnosed asthma; 2) did not have an observed PD20 during the 5-dose test; and 3) were in the bottom quartile of EOS distribution. 170 asthma cases and 347 controls were enrolled for genotyping.

We further selected additional 202 unrelated asthmatic cases and 332 unrelated matched controls (the confirmation sample) from the same study population to independently confirm the significant signal detected in the initial screening. The selection criteria for asthmatic cases and controls in the confirmation sample were almost the same as in the initial sample, except that the criterion based on the EOS distribution was not applied in the enrollment of the confirmation sample. Of note, all the cases and controls in the two data sets were unrelated.

Criteria of candidate genes and SNPs selection

We first identified asthma candidate genes from the GeneCards (http://www.genecards.org/index.shtml). Specifically, we identified candidate genes by typing in the following keywords: "asthma", "bronchial responsiveness", "lung function", "airway smooth muscle", "airway allergy or inflammation", "eosinophil and allergy or inflammation", "mast cell and allergy or inflammation", "IgE", and "atopy". 989 potential asthma candidate genes were identified from the GeneCards database. Next, we selected candidate SNPs in these 989 candidate genes based on the following criteria: 1) allele frequency in Asian populations obtained from the HapMap project (http://www.hapmap.org/cgi-perl/gbrowse/) and Affymetrix (http://www.affymetrix.com/products/reagents/specific/application_specific.affx); 2) BLOSUM80 score (13), which represents the severity of amino acid change caused by a nonsynonymous SNP, a lower score means more severity in amino acid change; and 3) previously reported synonymous SNPs which showed association with asthma. We removed all SNPs with minor allele frequency (MAF) <5% and all nonsynonymous SNPs with BLOSUM80 score ≥0. If complete LD (r^2=1) existed among two or more nonsynonymous SNPs on the same gene, only the SNP with the smallest BLOSUM80 score was included. As a result, a total of 141 SNPs from 113 genes were selected and 129 of them from 105 genes

were successfully genotyped. The detailed information of each genotyped SNP was provided in Table E1 in the Online Repository.

Genotyping

Venous blood sample was obtained from each study participant and genomic DNA was extracted from blood lymphocyte by a standard salting out procedure. Genotyping of 129 SNPs was performed using Sequenom MassArray technology in the Broad Institute Center for Genotyping and Analysis. In the confirmatory stage, SNP rs320995 was genotyped using the TaqMan allelic discrimination method, which has been described previously(14) . A random 5% of the samples were independently repeated to confirm genotyping results. The concordance of these duplicated samples was above 99.5%. Additionally, we randomly picked 50 samples which had been genotyped with Sequenom MassArray technology. SNP rs320995 was re-genotyped with the TaqMan allelic discrimination method in these 50 samples. Our data showed that the concordant rate of these two genotyping methods was 100%.

Data Analysis

The primary outcome was the risk of developing asthma, and the secondary outcomes were continuous variables related with lung function, including FEV_1, FVC, and FEV_1/FVC. For SNPs on the autosomal chromosomes, we examined Hardy-Weinberg equilibrium (HWE) in the control group by a chi-square test and those failed in HWE were removed from further analysis. Under three different genetic models (additive, recessive and dominant), we used multiple logistic and linear regression models to estimate the effect of each SNP on asthma and on lung function, separately, with the adjustment of age, age squared, height, height squared, weight, current smoking status and study sites. All the regression analyses were performed using SAS version 8.2 (SAS institute, Cary, NC). To account for multiple testing, we performed a permutation of 10,000 times for 119 SNPs and 4 phenotypes under three genetic

models to obtain empirical P values. The permutations were performed using R statistical package (http://www.r-project.org/).

HWE test for the SNP on the X-chromosome was performed according to the method suggested by Zheng et al (15). Due to the consideration that most loci on the X chromosome are subject to X chromosome inactivation, we treated males as homozygous females in the initial analysis, as applied previously in a genome-wide association study(16), and then tested the association under the three genetic models. Gender-specific analysis was also performed.

Next, we undertook a meta-analysis to evaluate the overall effect of SNP rs320995 in the *CYSLTR1* gene. Published reports with the terms "*CYSLTR1* asthma", "cysteinyl leukotriene receptor 1 asthma" and "cysteinyl-leukotriene type 1 receptor asthma" were identified by Entrez Pubmed searches (1966-April 2008) by two independent investigators (XH, HJT). From the initial search, six articles were found(17-22). Three articles were excluded due to not case-control study design and no information on sample size(19, 21, 22). Heterogeneity was assessed using a chi-square test, and the meta-analysis was performed using a fix-effect model if no significant heterogeneity existed, or using a random-effect model if huge heterogeneity existed. The pooled OR and 95% CI were calculated as a measure of the genetic effect of rs320995 on asthma in the whole population as well as in gender-specific subgroups. Of note, in gender-specific meta-analyses, the report by Choi JH et al was excluded because they did not report gender-specific results. All the meta-analyses were performed using R statistical package.

Results

Demographic and genotyping characteristics

The phenotypic characteristics of 170 asthmatic cases and 347 controls in the initial sample, as well as 332 asthmatic cases and 202 controls in the confirmation sample were summarized

in Table 1. In the initial sample, asthmatic cases were relatively younger, shorter, had lower BMI and higher percentage of males, when compared with controls. In the confirmation sample, asthmatic cases and controls were comparable in terms of age, gender, weight, height and BMI.

129 SNPs from 105 genes with different biological functions were genotyped in the initial sample (see Table E1 in the Online Repository). Among them, 16 genes have been reported to be associated with asthma or asthma-related phenotypes in at least one published studies. The average genotyping call rate for the 129 SNPs was about 97.5%. Six SNPs with MAF<0.05 were excluded in the subsequent analyses. The average MAF for the rest 123 SNPs was about 0.23. Using P<0.01 as the statistical significance cutoff, four SNPs (rs3732486, rs11584340, rs2071499, rs2274907) were out of HWE and removed from the subsequent analysis. Finally, a total of 119 SNPs from 98 genes, with the genotyping call rate of 97.9%, were used to examine the association with asthma and lung function.

Initial association analysis

In the initial sample, we found suggestive evidence of association with asthma for three SNPs with $P_{observed}$ <0.01, including rs320995 in the cysteinyl leukotriene Receptor 1 (CYSLTR1) gene (OR=3.1, 95%CI=1.8-5.4, $P_{observed}$=0.00004), rs1047266 in the tumor necrosis factor receptor superfamily, member 10b (*TNFRSF10B*) gene (OR=1.9, 95%CI=1.2-3.1, $P_{observed}$=0.007), and rs40401 in the interleukin-3 (*IL3*) gene (OR=0.5, 95%CI=0.3-0.8, $P_{observed}$=0.008) (Table 2). Particularly, only the association between SNP rs320995 and asthma remained significant after accounting for multiple testing by permutation(P_{global}=0.047). Interestingly, this association remained marginally significant, if Bonferroni correction was applied instead of permutation.

The association between each SNP and continuous variables related with lung function were shown in Table E2 in the Online Repository. SNP rs320995 tended to be associated with

FEV_1/FVC ($P_{observed}$=0.0004), but not with FEV_1 or FVC. We also detected suggestive evidence of association with FEV1 for four SNPs with $P_{observed}$ <0.01. However, none of these associations were statistically significant after accounting for multiple testing.

We further did subgroup analyses for the relationship between rs320995 and asthma in subjects aged <25 years old and subjects aged ≥25 years old, and detected similar associations in the younger (OR=5.2, 95%CI=1.7-16.5, $P_{observed}$ =0.005) and in the older group (OR=3.0, 95%CI=1.4-6.3, $P_{observed}$=0.004). There was no significant interaction between age and rs320995 by log likelihood ratio test.

Confirmatory and pooled analyses for SNP rs320995

The association between SNP rs320995 and asthma was successfully validated in another independent sample of 202 asthmatic cases and 332 controls (OR=1.6, 95%CI=1.0-2.4, *P*=0.032), as well as in the pooled sample (OR=2.0, 95%CI=1.4-2.7, *P*=0.00002) (Table 3).

Next, we performed gender-stratified analysis in each independent sample and in the pooled sample, separately. Our results suggested that males without T-allele in SNP rs320995 had significantly higher risk of asthma in the initial sample (OR=3.2, 95%CI=1.5-7.1, *P*=0.004), in the confirmation sample (OR=1.7, 95%CI=1.0-2.9, *P*=0.045), and in the pooled sample (OR=2.1, 95%CI=1.4-3.1, *P*=0.0003) (Table 3). Likewise, under the genetic recessive model, females without T-allele in SNP rs320995 showed significantly higher risk of asthma in the initial sample (OR=3.3, 95%CI=1.5-7.3, *P*=0.003) and in the pooled sample (OR=2.0, 95%CI=1.1-3.4, *P*=0.014). The effect of SNP rs320995 on the risk of asthma was comparable in males and females.

Finally, we examined the association for SNP rs320995 with lung function in the confirmation and pooled samples. We found that in males, the relationship between FEV1/FVC and SNP rs320995 in the confirmation sample and in the pooled samples showed similar trend as that detected in the initial sample (see Table E3 in the Online Repository).

Meta-analysis for SNP rs320995

Meta-analysis was performed to estimate the genetic effect of rs320995 on asthma with our data and data available in previous published reports, for which, a mild heterogeneity was observed (p=0.03). We found significant association between rs320995 and asthma using a fix-effect model (OR=1.3, 95%CI=1.1-1.5) (figure 1) and marginally significant association using a random-effect model (OR=1.2, 95%CI=0.8-1.9). In gender-specific analysis, we did not observe heterogeneity and detected significant association between rs320995 and asthma both in males (OR=2.0, 95%CI=1.4-2.8) and in females (OR=1.4, 95%CI=1.0-2.1) using a fix-effect model.

Discussion

Taken together, this study has contributed to the genetics of asthma in the following aspects. First, we systematically examined the coding SNPs in 98 asthma candidate genes in relation to asthma and lung function. To our knowledge, this is the largest-scale asthma candidate gene study in a homogeneous Chinese population.

Second, we validated a significant association between SNP rs320995 in the *CYSLTR1* gene and the risk of asthma in two independent Chinese samples. While the associations between *CYSLTR1* variations and asthma or asthma-related phenotypes were investigated in Caucasian(17, 19, 20) and Asian populations (18, 21-24) previously, the results were inconsistent. In detail, Arriba-Mendez et al reported that the *CYSLTR1 C927T* (rs320995) variation could predispose male children to asthma and atopic dermatitis (AA-AD) in Caucasian population(17). This positive association was confirmed by San Z et al in adult Caucasian population(20) and by Kim et al in adult Korean population, while Kim et al presented a positive relationship between the promoter variations and the risk of aspirin-intolerant asthma (23, 24). Inconsistent to these findings, two studies in Japanese

population found no association for the promoter variations as well as rs320995 in the CYSLTR1 gene with asthma (21, 22). The study in Caucasian population found that rs320995 was associated with female atopic severity but not with asthma (19). These inconsistent results may be partly caused by limited statistical power in some previous reports with relatively small sample size (<400). Another possibility for the inconsistency is due to the genetic heterogeneity of asthma and ethnicity/geometric difference, which suggests that the association between *CYSLTR1* variations and asthma should be replicated in different population. The results of the meta-analysis using our data and data from three previous reports(17, 18, 20) further supported the genetic effect of SNP rs320995 on asthma.

The association between *CYSLTR1* variation and the risk of asthma is biologically plausible. The *CYSLTR1* gene encodes the G protein-coupled cysteinyl leukotrienes receptor 1. Cysteinyl Leukotrienes (cys-LTs) are biologically active lipid mediators, which can promote human airway smooth muscle contraction, proliferation and migration(25). Cys-LTs also have been reported to involve in generation of Th2 cytokines and expression of some mediators involved in the process of airway inflammation and remodeling, such as tumor necrosis factor (TNF)-a, endothelin-1(26), reactive oxygen intermediate(27) , histamine receptor(28), and interleukin-11(29). These biological actions of cys-LTs occur via binding to cys-LTs receptors: CYSLTR1 and CYSLTR2(30). Activation of CYSLTR1 may result in proliferation and contraction of smooth muscle, edema and eosinophil migration to the lungs, and thus play an important role in the development of asthma. In the current study, we found suggestive evidence that SNP rs320995 may affect asthma and also FEV1/FVC, but not FEV1 or FVC. Given the multiple functions of CYSLTR1 gene, the specific pathways by which SNP rs320995 affects the risk of asthma should be further investigated.

Although SNP rs320995 does not cause any change in amino acid sequence, it is still likely that the nucleotide substitution at SNP rs320995 might affect the efficiency of *CYSLTR1*

mRNA processing and/or stability. Alternatively, SNP rs320995 may be in strong or complete LD with some important functional SNPs in the *CYSLTR1* gene, which are asthma causal SNPs. Therefore, we explored the available SNPs in the *CYSLTR1* gene in Chinese population from HapMap project and found that SNP rs320995 was in strong LD with SNP rs3201029 in the promoter region (r^2=0.87). In addition, published data indicated that SNP rs320995 was in complete LD with SNPs rs3201029, rs2637204 and rs2806489 in the promoter regions(21). These polymorphisms have been reported to significantly change promoter activity and increase the risk in the development of aspirin-intolerant asthma (21, 23, 24). As such, SNP rs320995 may be a surrogate marker for a causal SNP in the promoter region of *CYSLTR*1. In the future, it will be more informative to map the causal SNP(s) in the CYSLTR1 gene by genotyping complete SNP set covering the whole gene.

In addition to SNP rs320995, our findings also suggested that SNP rs40401 in the *IL3* gene should be of particular interest for further investigation. Specifically, our study validated the protective effect of the Pro27 allele in SNP rs40401 in the *IL3* gene on asthma, which has been reported previously in Korean population (31). Similar associations were detected between this SNP and lung function, including FVC and FEV_1/FVC. Although the results did not remain statistically significant after correcting for multiple testing, it is likely due to a modest genetic effect and/or relatively small sample size.

This study has several strengths. First, we carefully defined asthmatic cases based on physician diagnosis plus airway responsiveness to methacholine challenge. This combined definition is more reliable than physician diagnosis alone, since 1) clinical definition of asthma relies on the clinical experience of physicians and thus lacks standardization; and 2) airway responsiveness to methacholine is a reproducible and robust method associated with the diagnosis of asthma (32). Second, all the participated subjects were recruited from a rural area and were homogeneous with respect to ethnicity, lifestyle, occupation, as well as social and

cultural norms, which would minimize potential confounding effects and decrease the degree of false-positive results. Third, we identified and confirmed the association between SNP rs320995 in the *CYLSTR1* gene and asthma in two independent samples. Since false-positive association has been a critical concern in candidate gene association studies, the approach herein would ensure that the observed association was less likely to be a false positive result.

However, there are several limitations that should be considered. In the initial sample, the controls were older than the cases. This was purposely done to ensure that controls would not develop asthma. To minimize the potential confounding effects caused by age, height and weight, we included these three variables as covariates in all the analyses and further confirmed the observed association in an independent set of cases and controls with matched characteristics in terms of age, height and weight. We acknowledged that such study design may be problematic to detect age-related associations. However, in this study, similar associations between rs320995 and asthma were detected in both age groups and no significant interaction was found between age and rs320995, which suggested that age may have minimal effect on the association between rs320995 and asthma. Another limitation is that the cases and controls in the initial sample were selected based on an additional criterion which was not applied in the confirmation sample, that is: the cases/controls in the initial sample had extremely high/low EOS level. Since we could not observe any association between SNP rs320995 and EOS in the confirmation sample and the association between SNP rs320995 and asthma remained significant with additional adjustment of $\log_{10}$-transformed EOS in the pooled sample, we were confident that such sample selection would not lead to a bias in our findings. Finally, total IgE data was not available for the study, which limited our ability to explore the association of rs320995 with atopy.

In conclusion, our study provided strong evidence that SNP rs320995 in the *CYSLTR1* gene was associated with asthma in Chinese population, and such effect was comparable in males

and females. Further investigation on biological functions of the *CYSLTR1* gene in relation to asthma and its related phenotypes may provide new clues for asthma therapeutic and preventive strategies.

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Table 1. The population characteristics of two sample sets

Variable	the initial sample		the confirmation sample	
	Case	Control	Case	Control
n	170	347	202	332
Age, yrs	22.6±14.5**	33.0±13.1	28.0±15.2	29.0±16.0
Weight , kg	40.7±14.9**	51.0±9.3	44.7±15.2	45.4±12.8
Height , cm	145.1±17.4**	157.5±9.6	149.5±17.6	151.9±14.3
BMI, kg/m^2	18.5±3.3**	20.4±2.6	19.2±3.4	19.2±3.0
Asthma-related phenotype				
FEV1 (L)	2.1±0.8**	3.1±0.7	2.3±0.8**	3.0±0.9
FVC (L)	3.1±1.2**	3.7±0.9	3.3±1.2*	3.5±1.1
FEV1/FVC	0.7±0.1**	0.8±0.1	0.7±0.1**	0.9±0.1
LogEOS (cells/mm^3)	2.7±0.2**	1.4±0.1	2.0±0.5**	1.9±0.3
Male, %	87 (51.2)*	145(41.8)	119 (58.9)	196 (59.0)
Children[a] ,%	90 (52.9)**	52 (15.0)	69 (34.2)	123 (37.1)
Current smoker [b],%	17 (21.3)	76 (25.8)	54 (26.7)	85 (25.6)
Study Sites, n (%)				
Huanning County	42 (12.1)	9 (5.3)	49 (14.8)	37 (18.3)
Qianshan County	18 (5.2)	24 (14.1)	53 (16.0)	18 (8.9)
Susong County	16 (4.6)	20 (11.8)	11 (3.3)	12(5.9)
Tongchen County	9 (2.6)	12 (7.1)	5 (1.5)	10 (4.9)
Taihu County	31 (8.9)	23 (13.5)	9(2.7)	24 (11.9)
Wangjiang County	174(50.1)	34 (20.0)	82 (24.7)	50 (24.8)
Yuexi County	8 (2.3)	7 (4.1)	48 (14.5)	10 (5.0)
Zhongyang County	49 (14.1)**	41 (24.2)	75 (22.6) **	41 (20.3)

*,** T-test and chi-square test were performed to compare the difference in distribution of continuous variables and categorical variables between cases and controls, respectively. *p<0.05, ** p<0.01

[a] Age≤18 years old.

[b] Only calculated in adults.

LogEOS: Log_{10}-transformed blood eosinophil count.

Table 2 Association of SNPs with the risk of developing asthma in the initial screening ($P_{observed}$<0.05)

Gene	SNP	Polymor-phism	MAF[a]	Genotypic distribution[b]		Genotypic Analysis		
				Case	Control	Model [c]	OR (95%CI) [d]	$P_{observed}$ [e]
CYSLTR1	Rs320995	Phe309Phe	40.0	89/26/55	204/79/60	recessive	3.1 (1.8-5.4)	**0.00004**[#]
TNFRSF10B	Rs1047266	Ala67Val	26.9	69/77/21	186/128/28	Dominant	1.9 (1.2-3.1)	0.007
IL3	Rs40401	Ser27Pro	49.7	46/94/30	93/163/91	recessive	0.5 (0.3-0.8)	0.008
RNASE3	Rs2073342	Thr124Arg	27.5	75/81/14	182/136/27	dominant	1.8 (1.1-2.9)	0.011
CSF2	Rs25882	Ile117Thr	38.7	64/88/17	136/148/58	recessive	0.4 (0.2-0.9)	0.020
SERPINB2	Rs6104	Ser413Cys	44.0	38/94/37	100/183/59	Additive	1.5 (1.0-2.1)	0.026
TPO	Rs1126799	Val847Ala	37.1	70/61/29	130/163/43	dominant	0.6(0.4-1.0)	0.042
MMP8	Rs3765620	Ile32Thr	39.1	75/71/21	129/161/54	recessive	0.5(0.2-1.0)	0.040

[a] Allele frequency for the minor allele in the control sample.

[b] The three values indicate the number of homozygotes for the major allele, the number of heterozygotes and homozygotes for the minor allele.

[c] After testing for the three possible genetic models (recessive, dominant, additive), the most proximate one was selected for each SNP.

[d] Adjusted by age, age squared, height, height squared, weight, current smoker and gender.

[e] $P_{observed}$: P-value without accounting for multiple testing.

[#] P_{global}=0.047 after accounting for multiple testing by permutation.

Table 3. Association of rs320995 with the risk of developing asthma in Chinese population

Genotype	the initial sample			the confirmation sample			pooled sample		
	Case n=170	Cont n=343	OR[a]	Case n=202	Cont n=332	OR[a]	Case n=372	Cont n=675	OR[a]
Total									
T-allele carrier	67.6	82.5	1.0	69.8	77.4	1.0	69.5	80.9	1.0
Non-T-allele carrier[b]	32.4	17.5	3.1(1.8-5.4)****	30.2	22.6	1.6(1.0-2.4)*	30.5	19.1	2.0 (1.4-2.7)****
Male									
T-allele carrier	56.3	74.7	1.0	60.5	70.4	1.0	58.7	72.2	1.0
Non-T-allele carrier[b]	43.7	25.4	3.2 (1.4-7.1)**	39.5	29.6	1.7 (1.0-2.9)*	41.3	27.8	2.1 (1.4-3.1)***
Female									
T-allele carrier	79.5	88.1	1.0	83.1	87.5	1.0	81.3	87.8	1.0
Non-T-allele carrier[b]	20.5	11.9	3.3 (1.5-7.3)**	16.9	12.5	1.4 (0.6-3.3)	18.7	12.2	2.0 (1.1-3.4)*

[a] Adjusted by age, age squared, height, height squared, current smoker, gender (only for the analysis in total population) and study sites;

[b] Non-T-allele carrier means males with C-allele or females with CC genotype

* $P<0.05$; ** $P<0.01$; *** $P<0.001$; **** $P<0.0001$

Legend

Figure 1. ORs and 95% CI estimated from the studies included in meta-analysis for rs320995 and risk of asthma using fix-effect model.