

ORIGINAL ARTICLE

IFNG+874A/T polymorphisms and IFNG CA repeat polymorphism associated with asthma in Asian – a meta-analysisYuee Huang, MD^{1,2*}, Yongjun Zhang, MD^{3*}, Wenjie Sun, MD, PhD^{4*}, Kayllyn Germ, PhD⁵, Yuxin Jiang, PhD⁶, Wei Guo, MD⁶, Chao Xu, MS⁷, and Chaopin Li, PhD^{1,6}

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Abstract

Introduction: Asthma is an etiologically complex disease and develops by the interaction of multiple genes and environmental factors. Previous studies suggested a linkage between the interferon-gamma (*IFNG*) gene cluster in the chromosome 12q region and susceptibility to asthma. **Methods:** This meta-analysis was undertaken to examine the relationship between +874A/T and CA-repeat polymorphisms in *IFNG* gene and asthma. Eligible articles were accumulated via online databases in October 2013. A total of nine articles were included that were associated with the +874A/T polymorphism, involving 470 cases and 574 controls, as well as articles involving CA-repeat polymorphisms, which included 365 cases and 517 controls. The meta-analysis was performed for heterogeneity and calculation of pooled odds ratio and evaluation of publication bias by Egger's linear regression test. **Results:** The differences between *IFNG* polymorphism and the susceptibility to asthma may vary across ethnicities and ages, and also that the allelic frequencies of the AA genotypes in *IFNG* at +874 is associated with an increased risk of asthma in the Chinese Han adult population; additionally, CA14 occurrence in the first intron can increase the risk of asthma in the Japanese population. **Conclusion:** Our findings suggested that some polymorphisms in the *IFNG* gene could act as high prevalence-susceptibility markers of asthma. Larger scale studies are warranted to address these associations among different ethnic populations, ages and the severity of asthma prognosis.

KeywordsAsthma, *IFNG*+874A/T, *IFNG* CA repeat, polymorphism**History**

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Introduction

Asthma is a common, chronic respiratory disease in children and adults, which is characterized by bronchial hyperresponsiveness (BHR) and chronic bronchial inflammation [1]. An imbalance between Th1 and Th2 cytokines leads to an inflammatory response in the immune system [2–4]. Interferon-gamma (*IFNG*) is an important Th1 cytokine and regulates multiple features of asthma pathogenesis, such as suppression of Th2 cytokine release, inhibition of effectors cell recruitment to the site of inflammation, induction of apoptosis in T cells, eosinophils and mucus-storing cells and blockage of Ig E isotype switching in B cells, etc. [5].

Asthma is an etiologically complex disease, which develops by interaction between multiple genes and environmental

factors [6,7]. These studies reported by Hakonarson et al. [8], Haagerup et al. [9] and Yokouchi et al. [10], which focused on a single chromosome [11,12] suggest a linkage between chromosome 12q regions to asthma or related phenotypes. These are the strongest candidate regions for human asthma, and the *IFNG* gene cluster is found to be a significant locus [13]. Genome-wide linkage studies have identified a number of autosomal regions providing evidence of a link to asthma [9,14]. Like most cytokines, the *IFNG* gene-coding region has been found to be invariant [15]. Some polymorphisms, such as a CA repeat in the first intron and single nucleotide polymorphism (T to A) at position +874A/T (*rs2430561*), have been identified in the non-coding region of this gene and are thought to be associated with pathological features of asthma [16]. However, these results were not consistent across diverse populations, age ranges, etc., which generated considerable controversy as a result [17,18].

In this meta-analysis, an attempt was made to investigate the association between *IFNG* + 874A/T and CA-repeat polymorphisms in the first intron of *IFNG* gene, with the occurrence of asthma.

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Methods

Data sources

Data were collected from the following electronic databases: PubMed, Web of Science, the Chinese Biomedical Database, Chinese National Knowledge Infrastructure and the Wan Fang (Chinese) Databases, all updated through October 2013. Publications were not excluded based on a limiting date. Keywords included in our search were: “asthma” or “asthmatic”, “*IFNG*” or “*IFN- γ* ” and “polymorphism” or “mutation” or “variant”.

Study selection

We recruited data exclusively from publications with full text provided, rather than meeting reports or conference abstracts. Retrieved abstracts were reviewed to identify whether the studies were associated with the genotype between SNPs within the *IFNG* gene and asthma. Additional inclusion criteria for the literature selection included the following: (1) case–control studies were used to explore associations between *IFNG* polymorphisms and asthma; (2) the papers must present sample size, distribution of alleles, genotypes or other information that contributes to estimating an odds ratio (OR) with a 95% confidence interval (CI) and a *p* value; and (3) literature is published in either English or Chinese. The latest studies with the largest sample sizes were selected in case of articles with overlapping data from the same population resources.

Data extraction

All data were independently extracted from each study by two reviewers according to the selection criteria described previously. The following information was collected from each study: the first author, year of publication, type of study design, country of origin, racial ancestry of study population, age of asthma patients and controls, definition of cases, selection of control group, total sample size and the number of asthma cases and control subjects for each *IFNG* + 874A/T (*rs2430561*) and *IFNG* CA-repeat polymorphisms (Figure 1).

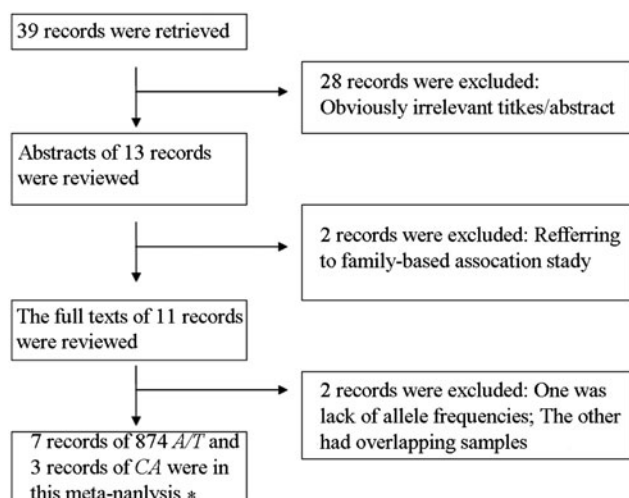


Figure 1. Flow chart of selection of studies in the meta-analysis. *One record had overlapping samples.

The meta-analysis was performed to examine the comparison between patients with asthma and normal controls, focusing on the relationship between gene polymorphisms at *IFNG* + 874 A/T and *IFNG* CA-repeat genotypes, and risk factors for the development of asthmatic diseases. The *IFNG* + 874 A/T genotype contrasts were identified as four models, such as T1/A1 vs. T2/A2, TT1/AA1 vs. TT2/AA2, TT1/AA1 + AT1 vs. TT2/AA2 + AT2 and AA1/TT1 + AT1 vs. AA2/TT2 + AT2. In addition, we examined the relationships between CA₁₂, CA₁₃, CA₁₄, CA₁₅, CA₁₆, CA₁₇ and CA₁₈ alleles and their susceptibility to the causation of asthma. The crude OR and its corresponding 95% CI were estimated in each study. Subgroup analysis was also performed with regard to ethnicity for *IFNG* + 874 polymorphisms. In this study, the meta-analysis of *IFNG* gene polymorphisms was applied for dichotomous outcomes according to the results of heterogeneity testing among individual studies. These data were compiled in Stata software version 10.0 (College Station, TX) using the fixed-effects model [19] or the random-effects model [20]. Heterogeneity was evaluated using the *Q*-statistic and *I*² test [21]. Recognizing that the *Q*-test is often underpowered for detecting statistically significant heterogeneity, particularly when there are few trials in the analysis, a relatively conservative threshold of *p* < 0.10 was chosen to suggest significant heterogeneity across trials (Paul and Donner 1992) [22]. Heterogeneity was considered statistically significant if *p* < 0.10. The random-effects model (if *p* < 0.10) or the fixed-effects model (if *p* > 0.10) was used to summarize the combined OR and its 95% CI. The significance of the pooled OR was determined by the Z-test, and a *p* value < 0.05 was considered significant.

Begg's funnel plot and Egger's linear regression tests were used to evaluate publication bias, and a *p* value < 0.05 was considered significant. Statistical analyses were conducted by Stata software version 10.0, and *p* values were two-tailed.

Results

Characteristics of eligible studies

In this meta-analysis, seven studies held data comparing the association between *IFNG* + 874 polymorphisms and susceptibility to asthma (Table 1); three studies reported *IFNG* CA-repeat polymorphisms (Table 2). Of the seven eligible studies pertinent to *IFNG* + 874 A/T polymorphisms, six were conducted in Asian populations, where four were done in the Chinese Han population. In total, 470 patients with asthma and 574 normal controls were included to investigate the relationship between *IFNG* + 874 A/T polymorphisms and susceptibility to asthma. While 365 asthma patients and 517 healthy controls were examined for a relationship between CA-repeat polymorphism in the first intron of *IFNG* and asthma. Physicians diagnosed asthma based on clinical signs and symptoms consistent with asthma, in addition to a positive result on a skin-prick test, and/or an increase of more than 15% in PEF (forced expiratory volume) of 1 s with a β_2 -agonist, etc. The two groups, asthma and normal controls, were not significantly different in terms of gender or age distribution (*p* > 0.05). Genes were typed using the amplification refractory mutation system polymerase chain reaction (PCR) or PCR-restriction fragment length polymorphism. The

Table 1. Characteristics of case-control studies with inclusion of *INFG* + 874A/T polymorphism and asthma.

References	Year	Country (ethnicity)	Age	Subjects	Total	Allele		Genotype		
						T	A	TT	TA	AA
Li et al. [17]	2006	Chinese	Children	Case	30	9	51	1	7	22
				Control	26	25	27	7	11	8
Li et al. [24]	2007	Chinese	Adults	Case	95	102	88	34	34	27
				Control	95	136	54	53	30	12
Hussein et al. [25]	2009	Egyptians	Age-matched	Case	25	30	20	11	8	6
				Control	25	46	4	21	4	0
Rad et al. [18]	2010	Iran	Adults	Case	64	63	65	16	31	17
				Control	109	107	111	24	59	26
Huang et al. [27]	2011	Chinese	Children	Case	100	37	163	2	33	65
				Control	122	51	193	4	43	75
Jiao et al. [26]	2011	Chinese	Adults	Case	75	12	138	0	12	63
				Control	73	28	118	0	28	45
Daneshmandi et al. [30]	2012	Iran	Adults	Case	81	69	93	19	31	31
				Control	124	111	137	29	53	42

Table 2. Characteristics of case-control studies with inclusion of *IFNG* CA-repeat polymorphism.

References	Year	Country (ethnicity)	Age	Total	Asthma				Normal control	
					n1	CA ₁₂ (+)	CA ₁₂ (–)	n2	CA ₁₂ (+)	CA ₁₂ (–)
Nakao et al. [16]	2001	Japan	Children	376	158	39	277	218	40	396
Shao et al. [33]	2004	Japan	Adults	291	113	24	202	178	39	317
Huang et al. [27]	2011	China	Children	215	94	29	159	121	41	201

Table 3. Summary ORs for the association of *IFNG* + 874A/T polymorphism and asthma.

Analysis model	Comparisons (study no.)	OR	95% CI	p ^a	p ^b
<i>T</i> vs. <i>A</i>	Asthma vs. controls (7)	0.515 ^c	0.327–0.812	0.000	0.004
	Asian descent (6)	0.596 ^c	0.391–0.908	0.001	0.016
	China Han descent (4)	0.444 ^c	0.256–0.770	0.016	0.004
<i>TT</i> vs. <i>AA</i>	Asthma vs. controls (7)	0.420 ^c	0.185–0.953	0.002	0.038
	Asian descent (6)	0.500 ^c	0.229–1.091	0.035	0.082
	China Han descent (4)	0.260	0.132–0.513	0.242	0.000
<i>TT</i> vs. <i>AA</i> + <i>AT</i>	Asthma vs. controls (7)	0.530 ^c	0.274–1.023	0.018	0.059
	Asian descent (6)	0.666 ^c	0.362–1.227	0.064	0.193
	China Han descent (4)	0.398	0.235–0.674	0.359	0.001
<i>AA</i> vs. <i>TT</i> + <i>AT</i>	Asthma vs. controls (7)	2.021 ^c	1.232–3.316	0.015	0.005
	Asian descent (6)	2.571 ^c	1.369–4.830	0.000	0.003
	China Han descent (4)	2.563 ^c	1.287–5.104	0.023	0.007

^ap Value for heterogeneity test.^bp Value for test of OR.^cEstimates for random effects model.

characteristics of the studies (first author, year of publication, country of origin, racial ancestry of study population, age of asthma patients and controls, total sample size and the number of asthma cases and control subjects, etc.) included in the meta-analysis are listed in Tables 1 and 2.

Meta-analysis of *INFG* + 874A/T polymorphism and asthma incidence

The main results of *INFG* + 874A/T polymorphism and susceptibility to asthma *via* meta-analysis for estimating random or fixed effects models are listed in Table 3; *p* value for heterogeneity and for test of OR, OR with 95% CI are also shown. The findings suggest a significant association of overall effects between AA of *INFG* + 874A/T polymorphism with the risk for asthma (*AA* vs. *TT* + *AT*: OR = 2.021, 95% CI = 1.232–3.316, *Z* = 2.79, *p* = 0.005; *TT* vs. *AA*:

OR = 0.420, 95% CI = 0.185–0.953, *Z* = 2.07, *p* = 0.038; *T* vs. *A*: OR = 0.515, 95% CI = 0.327–0.812, *Z* = 2.86, *p* = 0.004) (Table 3; Figure 2).

Ethnic subgroup analysis was performed concerning Asian population and the Chinese Han population, and two genetic models (*AA* vs. *TT* + *AT* and *T* vs. *A*) for *INFG* + 874A/T were found have significant risks for development of asthma in the two population, whereas no significant association was seen with asthma risks in the two genetic models of the *INFG* + 874A/T in Chinese Han population (*TT* vs. *AA*: OR = 0.500, 95% CI = 0.229–1.091, *Z* = 1.74, *p* = 0.082; *TT* vs. *AA* + *AT*: OR = 0.666, 95% CI = 0.362–1.227, *Z* = 1.30, *p* = 0.193, respectively).

By analysis of the ages of children and adult subgroups, we determined no significant association in the genetic models of *T* vs. *A* of the *INFG* + 874A/T with asthma risks for the two groups (*T* vs. *A*: OR = 0.425, 95% CI = 0.097–1.855,

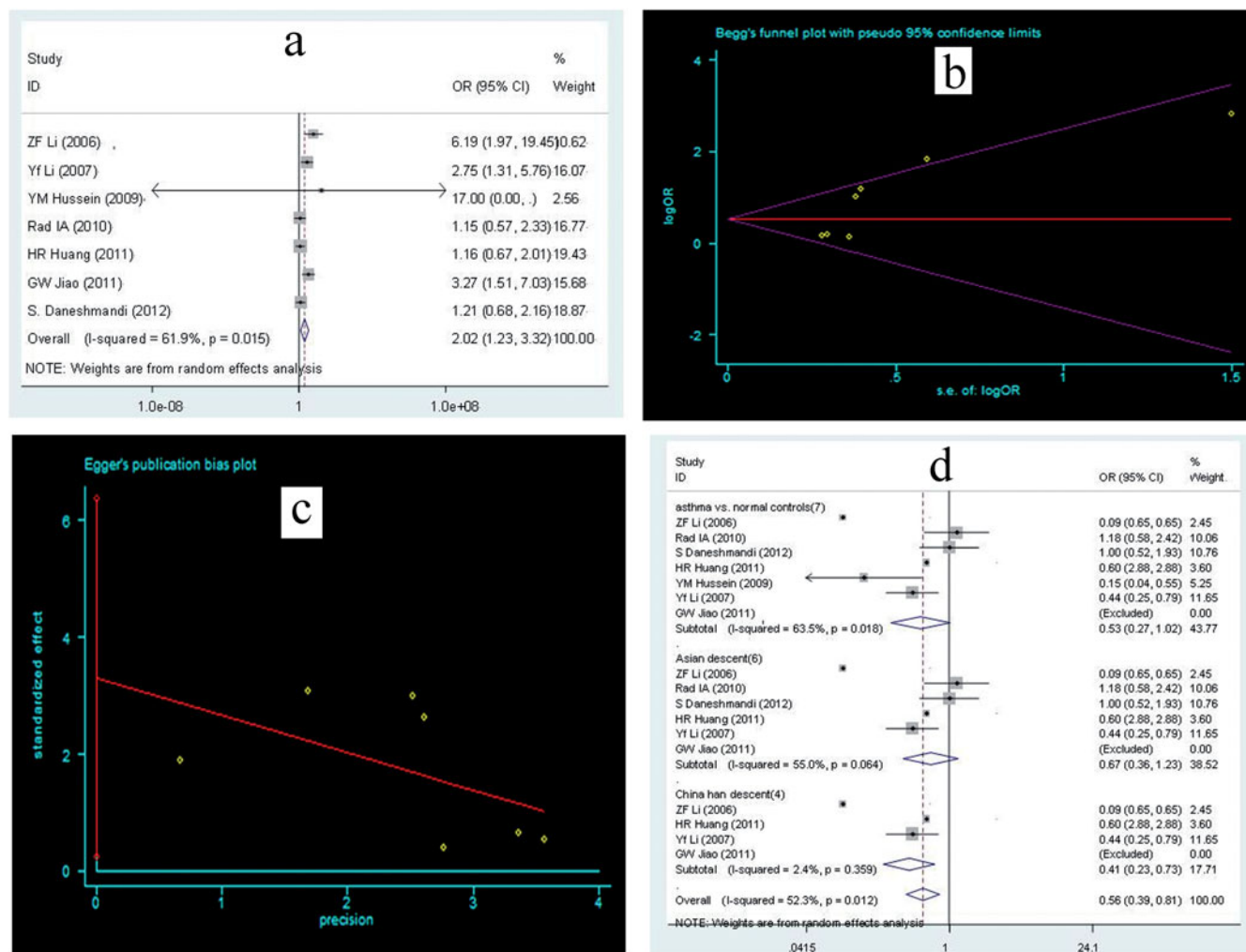


Figure 2. Forest plot, Begg's funnel plot and Egger's publication bias plot for the association between genetic model (AA vs. TT+AT) of *IFNG* +874A/T polymorphism and AD. In the Forest plot represents the point effect estimate for the OR and 95% CI for the OR. (a) Forest plot displaying the overall population; (b) Begg's funnel plot for overall population; (c) Egger's publication bias plot analysis for the overall population; and (d) Forest plot analysis in ethnic subgroup population.

Table 4. Summary ORs for the association of allele frequencies of *IFNG* gene CA-repeat polymorphism and asthma.

CA repeat genotypic	Asthma		Controls		OR	OR 95% CI	p^a	p^b
	CA (+)	CA (–)	CA (+)	CA (–)				
CA ₁₂	92	638	120	914	1.086	0.812–1.452	0.403	0.578
CA ₁₃	285	257	460	332	0.798	0.641–0.995	0.164	0.045
CA ₁₄	16	526	11	781	2.179	1.002–4.740	0.702	0.049
CA ₁₅	156	386	207	585	1.172 ^c	0.698–1.968	0.035	0.549
CA ₁₆	7	535	28	764	0.352 ^c	0.153–0.809	0.014	0.014
CA ₁₇	3	539	0	792	5.884	0.652–53.056	0.865	0.114
CA ₁₈	12	530	7	785	2.537	0.988–6.516	0.211	0.053

^a p Value for heterogeneity test.

^b p Value for test of OR.

^cEstimates for random effects model.

$p = 0.003$ (value for heterogeneity), $p = 0.255$ (value for test of OR); OR = 0.657, 95% CI = 0.415–1.309, $p = 0.013$ (value for heterogeneity), $p = 0.073$ (value for test of OR, respectively). Although the association between the genetic models of AA vs. TT+AT and the development of asthma in children was not significant, there were significant differences among the adults in the subgroups (AA vs. TT+AT: OR = 2.474, 95% CI = 0.485–12.626, $p = 0.011$ (value for heterogeneity), $p = 0.276$ (value for test of OR);

OR = 1.816, 95% CI = 1.076–3.067, $p = 0.081$ (value for heterogeneity) and $p = 0.026$ (value for test of OR, respectively).

Meta-analysis of *IFNG* gene CA-repeat polymorphism in the first intron and asthma incidence

The dinucleotide CA repeat polymorphisms with the repeat number varying from 12 to 18 were extracted from three

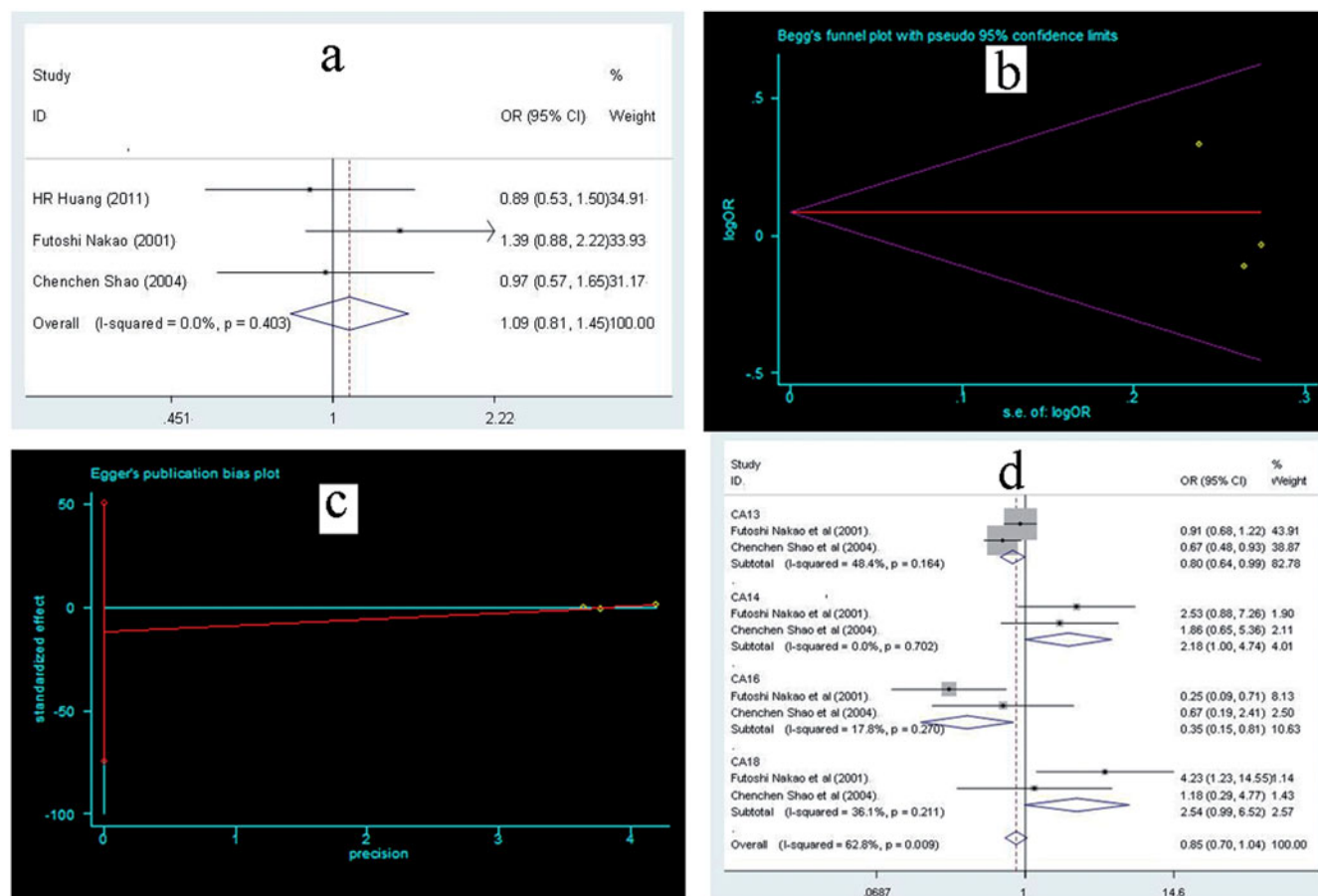


Figure 3. Forest plot, Begg's funnel plot and Egger's publication bias plot for the association between genetic model *INFG* CA-repeat polymorphism and AD. In the Forest plot represents the point effect estimate for the OR and 95% CI for the OR. (a) Forest plot analysis for CA12 polymorphism; (b): Begg's funnel plot for CA12 polymorphism; (c): Egger's publication bias plot for CA12 polymorphism; and (d): Forest plot analysis for CA13, CA14, CA16 and CA18 polymorphism.

studies (Table 4; Figure 2). The main results of CA-repeat polymorphism susceptibility and asthma *via* meta-analysis for OR, OR with 95% CI and *p* values of the Z-test of OR are listed in Table 4. Comparison of the CA-repeat frequencies between patients with atopic asthma and control subjects showed a significant difference in the whole-allele distribution. After comparing the frequency of different CA-repeats between patients and control subjects, CA13 and CA16 were significantly less frequent in patients with asthma (OR = 0.798, *p* = 0.045; OR = 0.352, *p* = 0.014, respectively; Table 4, Figure 3), whereas CA14 presented a higher frequency in asthmatics (OR = 2.179, *p* = 0.702; Table 4, Figure 3).

Evaluation of study quality and heterogeneity

Distribution of the genotypes in the control group pertinent to *INFG* + 874A/T polymorphism were summed up from the studies in compliance with Hardy-Weinberg equilibrium (HWE), except for Li et al. and Jiao et al. (Table 3, Table 4). Deviation from H to W equilibrium among controls could imply some potential biases in the selection of control or genotyping errors. Excluding studies in conformity HWE, sensitivity analyses produced different results between the genetic models of AA vs. TT + AT with of asthma disease risk (AA vs. TT + AT: OR = 1.681, 95% CI = 0.926–3.252, *p* = 0.039 (heterogeneity test), *p* = 0.088 (test of OR). The

corresponding pooled OR compared with the overall effect (Table 3, the genetic models of AA vs. TT + AT) was materially altered. The two studies not being in accordance with HWE involved in this meta-analysis reflect the influence of the individual data.

Evaluation of publication bias

Egger's linear regression test was used to measure the asymmetry of the funnel plot [23] (Table 5). If there is asymmetry, with smaller studies showing effects that differ systematically from larger studies, the regression line will not run through the origin. The intercept *a* provides a measure of asymmetry the larger its deviation from the zero, the more pronounced the asymmetry. After Egger's test was performed, Table 5 shows the publication bias existing in some comparisons of *INFG* + 874A/T polymorphism in this meta-analysis. The results suggested publication bias evidence with the *INFG* + 874A/T in the dominant model (AA vs. TT + AT: *t* = 2.78, *p* = 0.039; Table 5; Figure 2) and allelic model (T vs. A: *t* = -3.26, *p* = 0.023). The publication bias could not be found concerning CA12 polymorphism of *INFG* (CA12 (+) vs. CA12 (-): *t* = -2.40, *p* = 0.252; Figure 3).

Discussion

In this study, we were able to make an association between the *INFG* polymorphism with asthma. The results indicate that

Table 5. Egger's linear regression test to measure the funnel plot asymmetric regarding *IFNG* + 874A/T polymorphism and asthma.

Analysis model	Comparisons (study no.)	Y axle intercept: <i>a</i>	95% CI	<i>t</i>	<i>p</i>
<i>T</i> vs. <i>A</i>	Asthma vs. controls (7)	−5.049354	−9.034999–1.06371	−3.26	0.023
	Asian descent (6)	−5.369057	−5.8705–1.380095	−2.28	0.085
	China Han descent (4)	−4.072032	−6.210855–2.372854	−1.40	0.295
<i>TT</i> vs. <i>AA</i>	Asthma vs. controls (7)	−2.245203	0.2511329–6.37009	−1.72	0.161
	Asian descent (6)	−2.048224	−11.91633–1.178215	−1.04	0.375
	China Han descent (4)	−0.889918	−8.318024–4.221576	−0.42	0.749
<i>TT</i> vs. <i>AA</i> + <i>AT</i>	Asthma vs. controls (7)	−1.919001	−6.726374–4.280232	−1.24	0.282
	Asian descent (6)	−1.223071	1.411998–10.60612	−0.71	0.530
	China Han descent (4)	−0.850354	−16.55187–8.407811	−0.64	0.635
<i>AA</i> vs. <i>TT</i> + <i>AT</i>	Asthma vs. controls (7)	3.310611	−28.05076–26.27092	2.78	0.039
	Asian descent (6)	6.009058	−17.60954–15.90883	3.63	0.022
	China Han descent (4)	5.909748	0.2131778–11.60632	4.46	0.047

not only *IFNG* + 874A/T genotype distribution was significantly associated, but also *CA-repeat* polymorphism of *IFNG* allele frequency was significantly associated with susceptibility to asthma disease. Our analyses of *IFNG* + 874A/T indicated that the quantitative synthesis affecting rs2430561 A > T mutation was associated with an increased risk of asthma in the overall population. These findings indicate that the frequency of allele A874 was greater in asthma patients than in control patients. This is consistent with those of Li et al. [17], Li et al. [24], Hussein et al. [25] and Jiao et al. [26]. The largest sample size on *IFNG* + 874A/T included in our meta-analysis was Huang et al. [27], which described the distribution frequency of the AA, AT and TT genotypes at +874 with little difference between the asthmatic group and the control group. Pravica et al. [28] reported that the presence of the A874 allele was correlated with the level of *IFNG* production. Huang et al. [27] reported comparisons between *IFNG* levels among different genotypes and demonstrated that *IFNG* gene expression was significantly lower in the AA group than in the TT group, suggesting that polymorphism at 874 was correlated with *IFNG* expression. Hussein et al. [24] reported that the serum *IFNG* level was significantly lower in the atopic asthma group than in the control group, and the significant correlation between serum *IFNG* levels and severity of asthma is consistent with the results of Nurse et al. [29] in South African patients. However, our meta-analysis cannot be analyzed according to disease classification due to the severity information of asthma cannot be obtained. Thus, further studies with large number of samples are still necessary to differentiate the correlation of *IFNG* gene (+874T/A) and asthma on the basis of the severity or the serum *IFNG* level of asthma.

After stratified analysis by ethnicity and age, heterogeneity was substantially reduced or removed from the Chinese or adult population data. These results indicate that there is varied association with *IFNG* + 874A/T polymorphism and susceptibility to asthma, which may exist among different ethnicities or ages, accounting for heterogeneity of this meta-analysis. In the models for *T* vs. *A*, *TT* vs. *AA*, *TT* vs. *AA* + *AT* and *AA* vs. *TT* + *AT* of the *IFNG* gene (+874T/A), authors found significant heterogeneity throughout. The frequency of allele A874 was greater in atopic patients than in control patients in the overall population. However, in subgroup analysis, divided by ethnicity between Asian and Chinese Han populations, *TT* vs. *AA* and *TT* vs. *AA* + *AT* of *IFNG* + 874A/T

were not significantly different in Asians but was found to be significant in its association with susceptibility to asthma in the Chinese Han population. In subgroup analysis by age, classified as children vs. adult groups, results found no significant association in the genetic models of *T* vs. *A* of the *IFNG* + 874A/T with asthma risks between the groups. Although the association between the genetic models of *AA* vs. *TT* + *AT* with the development of asthma in children was not significant, it was statistically significant in adults. This result is comparable with those of Huang et al. [27], Li et al. [24] and Jiao et al. [26]. The largest sample size on *IFNG* + 874A/T included in our meta-analysis was from Huang et al. [27] who reported that the polymorphisms of the *IFN-γ* gene at +874A/T was not correlated with susceptibility to childhood asthma. Li et al. [17], having a sample size of only 30 cases, reported that the polymorphism in the promoter of the *IFNG* + 874A/T gene conferred a susceptibility to the development of childhood asthma. Hence, we predict that the *IFN-γ* gene at +874A/T was not truly correlated with susceptibility to childhood asthma. Li et al. [24] and Jiao et al. [26], who studied the Chinese Han population, reported that the *IFNG* + 874A/T gene was correlated to the susceptibility of adults to asthma, yet there was no association between the genetic models of *AA* vs. *TT* + *AT* and the development of asthma in adults, which was reported by Rad et al. [18] and Daneshmandi et al. [30] who studied the Iranian population. With these data, we think the *IFN-γ* gene at +874A/T is correlated to the susceptibility of adults to asthma in the Chinese Han population. This suggests that different genetic backgrounds, environmental factors and population features are potential influence factors. For example, Dagan et al. [31] reported *IFNG* genotype and sex interact to influence risk of childhood asthma in Chicago by using a birth cohort study. The need for further research larger sample sizes remains, in order to differentiate the correlation of *IFNG* gene (+874T/A) and asthma on the basis of different population, age and sex and so on.

Regarding the *CA-repeat* polymorphism of *IFNG*, the findings reveal that *CA14* had a strong association with an increased risk of asthma in the Japanese population, whereas *CA13* and *CA16* genotypes would reduce the risks of asthma in the overall population. Nagarkatti et al. [32] examined an Indian population *via* case-control study, and reported an association between the *CA-repeat* to asthma and to serum total IgE levels. Pravica et al. [28] described that the single

nucleotide polymorphism, *T* to *A* at the 5' end of the *CA-repeat* of the human *IFNG* gene, directly affected the level of *IFNG* production. Huang et al. [27] reported that the *CA-repeat* of the *IFN-γ* gene polymorphism was not correlated with susceptibility to childhood asthma in Chinese Han population. In our meta-analysis, the study of the *CA14* polymorphism was only from the Japanese population, the other population was not reported about the *CA14* polymorphism; with this information, our results of this study may only indicate that the *CA14* could increase the risk of asthma in those of Japanese ethnicity. The authors feel that further studies should be carried out to differentiate the correlation effects of *CA-repeat* polymorphism of *IFNG* and asthma on the basis of different population.

Meta-analyses have been recognized as effective methods to solve a variety of clinical dilemmas by quantitatively summarizing and reviewing previous literature relevant to studies in each field. However, limitations of meta-analyses should be considered when interpreting the results. First, sample sizes in the meta-analyses tend to be insufficient. This meta-analysis employs only nine eligible studies, seven of which study the data on comparing an association between *IFNG* + 874 polymorphisms and susceptibility to asthma and three on *IFNG CA-repeat* polymorphisms. The largest sample size included in our study is 100, while the minimum is 24. The study collected data in Asia as extensively as possible. Second, heterogeneity is a major concern in our meta-analysis, for it affects interpretation of the results. Most analysis models found significant heterogeneity in this meta-analysis. Heterogeneity was explored using the *Q*-statistic and *I*² test [21]. Recognizing that the *Q*-test is often underpowered to detect statistically significant heterogeneity, particularly when there are few trials in the analysis, the relatively conservative threshold of *p* < 0.10 was chosen to suggest statistically significant heterogeneity across trials [22]. The heterogeneity was considered statistically significant if *p* < 0.10. The random-effects model (if *p* < 0.10) or the fixed-effects model (if *p* > 0.10) was used to summarize the combined OR and its 95% CI. Not all controls in our included studies were in HWE, so, we studied the sensitivity analyses excluding studies in conformity HWE, which implied some potential biases. Third, this meta-analysis was based on unadjusted estimates, although we hope stratified analyses on parameters such as age, sex, severity of asthma, environmental exposure and other factors, more precise analysis could not be performed because the clinical information for individual patients was insufficient. Finally, publication bias was observed by Egger's linear regression analysis, which shows publication bias existing in some comparisons of the *IFNG* + 874A/T in the dominant model (*AA* vs. *TT* + *AT*) and allelic model (*T* vs. *A*). The published studies included small sample sizes, insufficient sample numbers, etc., therefore our meta-analysis may lead to additional bias. Moreover, articles of unpublished articles or conference material were not included. Language bias may also have occurred, although our statistical tests were unable to show this; articles we researched are published only in English or Chinese language.

In conclusion, based on these data, the comprehensive meta-analysis provides evidence in the association between

IFNG + 874A/T polymorphism, *CA-repeat* polymorphism of *IFNG* allele frequency and asthma. The *IFNG* polymorphism susceptibility to the development of asthma has been associated with different ethnic population, age, etc. The frequency of allele A874 is associated with an increased risk of asthma in the Chinese Han population and in the adult subgroup. *CA14* in the first intron can increase the risk of asthma in the Japanese population. This finding suggests that some polymorphisms in *IFNG* gene could act as effective susceptibility markers of asthma.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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