Association between the PADI4 -94G/A polymorphism and rheumatoid arthritis: a meta-analysis in the Chinese population

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ABSTRACT. Although a number of studies have been conducted on the association between the peptidylarginine deiminase (PADI4) -94G/A polymorphism and rheumatoid arthritis (RA) in the Chinese population, the association remains elusive and controversial. To clarify the impact of the PADI4 -94G/A polymorphism on the risk of RA, a meta-analysis was performed in the Chinese population. Related studies were identified from databases such as, Springer Link, Ovid, Chinese Wanfang Data Knowledge Service Platform, Chinese National Knowledge Infrastructure (CNKI), and Chinese Biology Medicine (CBM) up to May 21, 2015. Pooled odds ratios (ORs) and 95% confidence intervals (CIs) were used to assess the strength of associations. A total of 10 studies with 2783 RA cases and 2887 controls were included in this meta-analysis. Overall, a significantly elevated risk of RA was associated with all variants of PADI4 -94G/A (A *vs* G: OR = 1.24, 95%CI = 1.15-1.34; AA + GA *vs* GG: OR = 1.45, 95%CI = 1.29-1.62; AA *vs* GG: OR = 1.49, 95%CI = 1.28-1.73; AA *vs* GG + GA: OR = 1.19, 95%CI = 1.04-1.35). Subgroup analyses stratified by geographic areas and source of controls revealed significant results in the population-based studies in North and South China. In conclusion, this meta-analysis

showed that the PADI4 -94G/A variants may influence RA risk in the Chinese population. However, further studies with gene-gene and gene-environment interactions are required for definite conclusions.

Key words: Meta-analysis; Peptidylarginine deiminase 4; Polymorphism; Rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis (RA) is the most common autoimmune disease, characterized by diffuse synovial inflammation and destruction and affecting approximately 0.5 to 1% of the adult population worldwide (Begovich et al., 2004; Alamanos and Drosos, 2005; Carmona et al., 2010). Although the etiology of RA is not completely understood, it is believed to arise from complex genetic and environmental factors, which trigger and maintain synovial inflammation in affected individuals (Dieudé and Cornélis, 2005). Thus, genetic factors such as single nucleotide polymorphisms might play important roles in RA pathogenesis (Kochi et al., 2014). In recent years, a number of candidate genes have been identified as potential RA susceptibility loci. An important gene among these is peptidylarginine deiminase 4 (PADI4), a member of the PADI gene family that codes for enzymes involved in the posttranslational conversion of arginine to citrulline in peptides. The PADI4 gene is located on chromosome 1p36, and several polymorphisms have been identified in its promoter. Of these, the -94G/A (or rs2240340) single nucleotide polymorphism has been extensively studied. An association between the PADI4 -94G/A polymorphism and RA was first reported by Suzuki et al. (2003) in Japan. As a consequence, many studies analyzed the influence of the PADI4 -94G/A polymorphism on RA risk; however, no clear consensus was attained. Meta-analyses of studies on this gene in other ethnic groups have been reported elsewhere and have produced conflicting results (Iwamoto et al., 2006; Lee et al., 2007; Takata et al., 2008; Hou et al., 2013). Given the differences in genetic backgrounds between the Chinese and other populations, it is necessary to investigate this association in the Chinese population. In addition, we performed subgroup analysis stratified by geographic area and the source of control population to explore the possible effects of the gene-environment interactions with respect to RA risk.

MATERIAL AND METHODS

Search strategy and selection criteria

A computerized literature search was carried out in the, Springer Link, Ovid, Chinese Wanfang Data Knowledge Service Platform, Chinese National Knowledge Infrastructure (CNKI), and Chinese Biology Medicine (CBM) databases up to May 21, 2015. The following search key words were used: (PADI4 or peptidylarginine deiminase 4 or -94G/A) and rheumatoid arthritis and (Chinese or China or Taiwan). The search was performed without any restrictions on language and was focused on studies conducted in humans. In addition, the references from retrieved articles were also searched. Studies for the meta-analysis were selected if they were 1) independent cohort or case-control studies in humans, 2) if all patients in each study fulfilled the classification criteria for RA proposed by the American College of Rheumatology in 1987, 3) if they examined the association of PADI4 -94G/A gene polymorphisms with RA, 4) provided the distribution of PADI4

-94G/A polymorphism in patients and controls, 5) and if all the participants were Chinese. Studies were excluded if they 1) were duplicate publications; 2) were meta-analyses, letters, meeting abstracts, reviews, or editorial articles, 3) had incomplete data, 4) or lacked controls.

Data extraction

All the data were extracted independently by two reviewers based on the selection criteria. Disagreements were resolved by discussion. The title and abstract of all potentially relevant articles were screened to determine their relevance. In case these were ambiguous, complete articles were also scrutinized. The following data were extracted from the identified studies: the first author, year of publication, source of controls, geographic locations, sample size, and the number of subjects with PADI4 -94G/A genotypes.

Statistical analyses

Statistical analyses were conducted using the STATA statistical package (version 10, STATA, College Station, TX, USA). The χ^2 test was used for determining the Hardy-Weinberg equilibrium (HWE) of genotypes and the heterogeneity of rare allele frequencies in the control groups of each study reviewed. Crude odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the strength of association between the PADI4 -94G/A polymorphism and RA risk. The significance of the pooled ORs was determined by the Z-test. Depending on the results of the heterogeneity test among individual studies, the fixed-effect model (Mantel-Haenszel) or random-effect model (DerSimonian and Laird) was selected to summarize the combined ORs and their 95%CIs. Sensitivity analysis was performed to verify the stability of the meta-analysis using both models (the fixed-effect model and random-effect model). Begg's funnel plots and the Egger linear regression test were used to assess publication bias. In addition to the comparison among all subjects, we also performed stratification analyses by geographical locations and source of controls. All the P values were two-sided, and $P < 0.05$ was considered statistically significant.

RESULTS

Description of included studies

We identified 61 articles that examined the association between PADI4 polymorphisms and the risk of RA. However, after screening the titles and abstracts of all 61 articles, 49 were excluded. Of the 12 potentially relevant articles (Feng et al., 2009; Shi, 2010; Shi et al., 2010; Wei, 2010; Chen et al., 2011; Cui et al., 2007, 2011; Xu et al., 2011; Cheng et al., 2012; Liu et al., 2012; Li et al., 2013; Du et al., 2014) identified for full study retrieval, two (Shi, 2010; Wei, 2010) were excluded because they concerned subjects included in an expanded series (Shi et al., 2010; Chen et al., 2011). Finally, 10 case-control studies (Feng et al., 2009; Shi et al., 2010; Chen et al., 2011; Cui et al., 2007, 2011; Xu et al., 2011; Cheng et al., 2012; Liu et al., 2012; Li et al., 2013; Du et al., 2014) met the inclusion criteria. The studies were published between 2007 and 2014. The flow chart of the study selection process is shown in Figure 1. In total, 2783 RA cases and 2887 controls were included in this meta-analysis to evaluate the relationship between the PADI4 -94G/A polymorphism and RA risk in the Chinese population. The source of the controls was mainly derived from population-based studies. The characteristics of the studies included are summarized in Table 1.

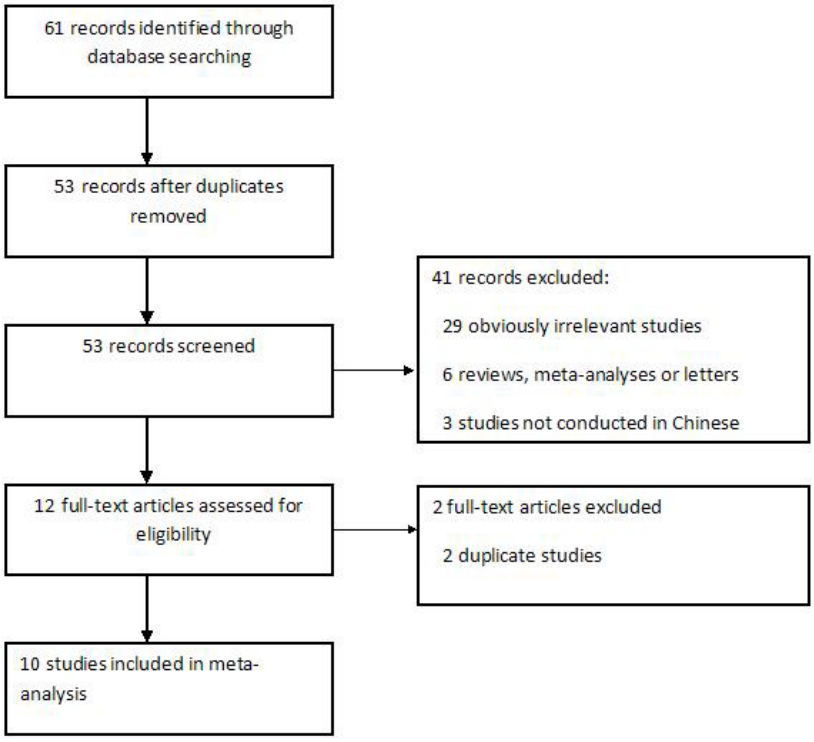

Figure 1. Flow diagram of the literature search.

Table 1. Characteristics of studies included in the meta-analysis.

References (first author)	Source of controls	Geographic locations	Case number	Control number	Cases			Controls			HWE	
					GG	GA	AA	GG	GA	AA	χ^2	P
Cui (2007)	PB	Hebei	92	116	20	55	17	47	49	20	1.32	0.250
Feng (2009)	PB	Hebei	115	106	31	52	32	39	43	24	3.14	0.076
Shi (2010)	PB	Anhui	112	97	31	58	23	39	40	18	1.76	0.185
Chen (2011)	PB	Shanghai	378	204	108	192	78	66	94	44	0.93	0.334
Cui (2011)	PB	Hebei	134	140	34	72	28	56	59	25	1.81	0.179
Xu (2011)	PB	Henan	130	130	34	66	30	53	54	23	1.95	0.162
Cheng (2012)	HB	Jiangsu	324	695	102	152	70	244	339	112	0.10	0.751
Liu (2012)	PB	Qinghai	90	90	33	43	14	26	47	17	0.27	0.602
Li (2013)	PB	Yunnan	192	288	28	123	41	80	153	55	1.43	0.231
Du (2014)	PB	Beijing	1216	1021	340	609	267	361	464	196	4.54	0.033

PB = population-based; HB = hospital-based.

Overall analysis

There was no evidence of between-study heterogeneity among all the included studies (Table 2). Therefore, the fixed-effect model was used in the overall analysis. The combined results based on all studies showed that the A variant of the PADI4 -94G/A gene polymorphism was significantly associated with an increased risk of RA in the Chinese population (A *vs* G: OR = 1.24, 95%CI = 1.15-1.34; AA + GA *vs* GG: OR = 1.45, 95%CI = 1.29-1.62; AA *vs* GG: OR = 1.49, 95%CI = 1.28-1.73; AA *vs* GG + GA: OR = 1.19, 95%CI = 1.04-1.35; Figure 2 and Table 2).

Table 2. Association of the PADI4 -94G/A gene polymorphism with RA susceptibility.

Analysis model		N	ORr (95%CI)	ORf (95%CI)	P_h
A *vs* G	Total analysis	10	**1.24 (1.14-1.35)**	**1.24 (1.15-1.34)**	0.371
	Population-based	9	**1.25 (1.13-1.39)**	**1.25 (1.15-1.35)**	0.291
	South China	3	**1.20 (1.05-1.36)**	**1.20 (1.05-1.36)**	0.377
	North China	7	**1.28 (1.13-1.45)**	**1.26 (1.15-1.39)**	0.287
AA *vs* GG	Total analysis	10	**1.49 (1.28-1.73)**	**1.49 (1.28-1.73)**	0.495
	Population-based	9	**1.49 (1.25-1.78)**	**1.48 (1.26-1.75)**	0.396
	South China	3	**1.47 (1.05-2.06)**	**1.45 (1.12-1.90)**	0.215
	North China	7	**1.50 (1.25-1.81)**	**1.50 (1.25-1.81)**	0.507
AA *vs* GG+GA	Total analysis	10	**1.19 (1.04-1.36)**	**1.19 (1.04-1.35)**	0.916
	Population-based	9	1.15 (0.99-1.32)	1.15 (0.99-1.32)	0.964
	South China	3	1.19 (0.93-1.53)	1.20 (0.96-1.50)	0.302
	North China	7	1.18 (1.00-1.39)	1.18 (1.00-1.39)	0.959
AA+GA *vs* GG	Total analysis	10	**1.50 (1.25-1.81)**	**1.45 (1.29-1.62)**	0.042
	Population-based	9	**1.57 (1.28-1.94)**	**1.51 (1.33-1.71)**	0.057
	South China	3	1.41 (0.98-2.03)	**1.34 (1.10-1.64)**	0.057
	North China	7	**1.58 (1.24-2.00)**	**1.50 (1.30-1.73)**	0.093

ORr = odds ratio for random-effect model; ORf = odds ratio for fixed-effect model; P_h = P value for heterogeneity test; North China including Hebei, Anhui, Henan, Qinghai, and Beijing; South China including Yunnan, Jiangsu, and Shanghai.

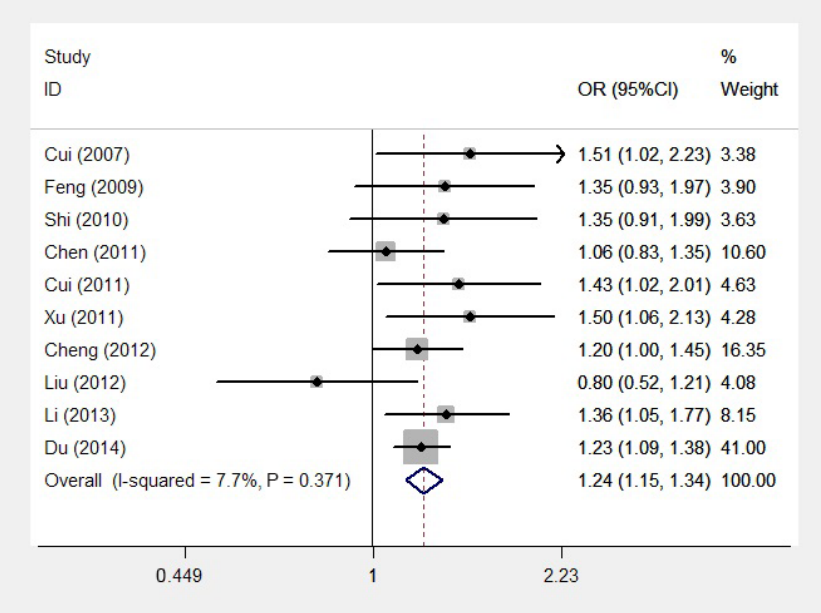

Figure 2. Forest plots of all selected studies on the association between the PADI4 -94G/A polymorphism and RA risk in the Chinese population (for allele model A *vs* G).

Subgroup analysis

In the subgroup analysis based on the source of controls, the results showed that the PADI4 -94G/A polymorphism was significantly associated with RA in population-based analysis (A *vs* G, OR = 1.25, 95%CI = 1.15-1.35; AA *vs* GG, OR = 1.48, 95%CI = 1.26-1.75; AA + GA *vs* GG, OR = 1.51, 95%CI = 1.33-1.71, Table 2). In addition, stratified analyses based on the geographic

area revealed significant differences between South China (A *vs* G, OR = 1.20, 95%CI = 1.05-1.36; AA *vs* GG, OR = 1.45, 95%CI = 1.12-1.90; AA + GA *vs* GG, OR = 1.34, 95%CI = 1.10-1.64) and North China (A *vs* G, OR = 1.26, 95%CI = 1.15-1.39; AA *vs* GG, OR = 1.50, 95%CI = 1.25-1.81; AA + GA *vs* GG, OR = 1.50, 95%CI = 1.30-1.73) (Table 2).

Sensitivity analysis and bias diagnosis

In order to compare the differences and evaluate the sensitivity of the meta-analysis, we used the fixed-effect and random-effect models to evaluate the stability of the meta-analysis. The results were not materially altered in the overall and subgroup analyses (Table 2). Hence, the results of the sensitivity analysis suggest that the data in this meta-analysis are relatively stable and credible.

The Begg's funnel plot and the Egger test were performed to access the publication bias of the literature. As showed in Figure 3A, the shape of the funnel plots revealed obvious asymmetry. However, the Egger test indicated that there was no evidence of obvious publication bias in the 10 reviewed studies (t = 0.38, P = 0.717; Figure 3B).

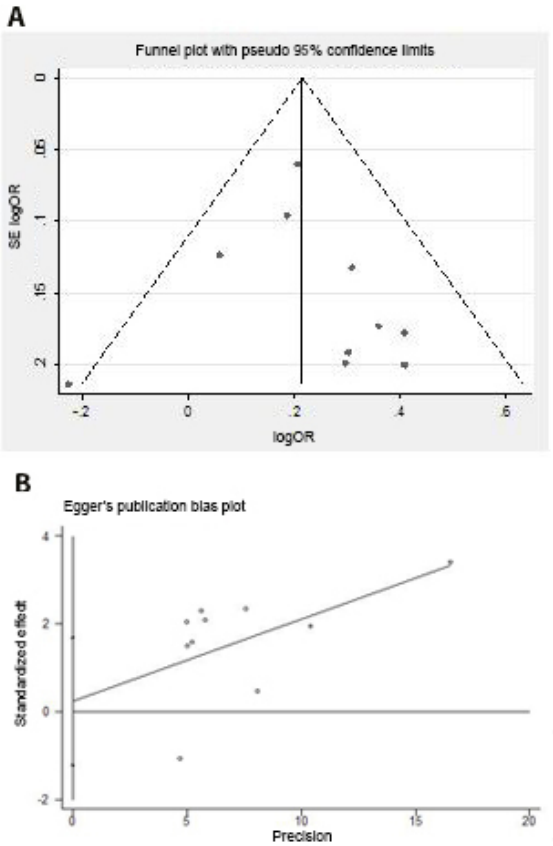

Figure 3. Evaluation of publication bias for allele contrast (A *vs* G) of the PADI4 -94G/A polymorphism in the overall analysis (**A.** funnel plot, **B.** Egger test).

DISCUSSION

Although many studies have analyzed the research regarding the PADI4 -94G/A polymorphism and its association with RA, definite conclusions cannot be drawn. Up to the present time, there are five published meta-analyses regarding the PADI4 -94G/A polymorphism and RA risk (Iwamoto et al., 2006; Lee et al., 2007; Takata et al., 2008; Hou et al., 2013; Yang et al., 2015). Of these, two meta-analyses reported that there was significant association between the PADI4 -94G/A polymorphism and RA risk both in the Asian and European populations (Iwamoto et al., 2006; Takata et al., 2008), while two meta-analyses reported that there was significant association only in Asian individuals (Hou et al., 2013; Yang et al., 2015). Meta-analysis is a powerful statistical method that could improve the reliability of the conflicting results regarding the same topic and could identify the reason for the variation. Therefore, we conducted this meta-analysis to assess the effect of the PADI4 -94G/A polymorphism on risk for RA in the Chinese population specifically, in order to reduce the impact of genetic background. Our meta-analysis included 10 case-control studies with 2783 RA cases and 2887 controls. The results showed that a significantly elevated risk of RA was associated with all variants of PADI4 -94G/A in the overall analysis. In the subgroup analyses, by dividing the samples into subgroups according to geographic area and the source of controls, significant association was found in population-based studies, in South China and North China.

Compared with the previous meta-analyses (Iwamoto et al., 2006; Lee et al., 2007; Takata et al., 2008; Hou et al., 2013; Yang et al., 2015), the current study included more research studies on the Chinese population. The effects of gene-environment interactions with respect to RA risk were also studied by subgroup analyses. To our knowledge, this is the first meta-analysis to investigate the association between the PADI4 -94G/A polymorphism and RA susceptibility in the Chinese population. In addition, testing of the HWE for distribution of genotypes in control groups suggested that there was no significant difference in the genetic background among the participants. The sensitivity analysis confirmed the reliability and stability of the meta-analysis and the Egger test revealed no publication bias among the studies. Therefore, the findings from our meta-analysis provide strong evidence for the association between the PADI4 -94G/A polymorphism and RA in the Chinese population.

Nevertheless, there are several limitations to this meta-analysis. First, observational studies are susceptible to various biases (e.g., recall bias in case-control studies) because of their retrospective nature. Therefore, recall bias could invalidate the results from this meta-analysis. Another potential limitation was that our results were based on unadjusted estimates. More precise analyses can be conducted if individual data were available, which would allow for adjustment by other covariates including age, gender, race, and other factors. Third, the etiology of RA is complex and is mediated by the activities of multiple genes. The effect of any single gene might have a limited impact on RA risk than have been anticipated so far. In this meta-analysis, we only investigated one gene locus.

In conclusion, this meta-analysis demonstrates that PADI4 -94G might be a risk allele for RA susceptibility in the Chinese population. However, further studies are needed to determine if the PADI4 -94G/A polymorphism confers a risk of RA in other ethnic groups. RA is a multifactorial disease caused not only by genetic factors but also by environmental factors, and studies analyzing gene-gene and gene-environment interactions are required to confirm our results. Such studies may eventually lead to a better and comprehensive understanding of the association between the PADI4 -94G/A polymorphism and RA risk.

Conflicts of interest

The authors declare no conflict of interest.

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