Association between peptidyl arginine deiminase 4 (PADI4)-104C/T polymorphism and rheumatoid arthritis: a meta-analysis in the Chinese population

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ABSTRACT. The correlation between the -104C/T polymorphism in the peptidyl arginine deiminase 4 (*PADI4*) gene and rheumatoid arthritis (RA) risk has been analyzed in several studies. However, the results are inconclusive and remain to be confirmed in several ethnic groups. The effect of the *PADI4*-104C/T polymorphism on RA risk in the Chinese population was evaluated in a meta-analysis. Studies with dates of publication up to July 2015 conforming to the inclusion criteria were retrieved from PubMed and Chinese databases. The associations were assessed with pooled odds ratios (ORs) and 95% confidence intervals (CIs). Ten studies, including 2119 RA cases and 1962 controls, that conformed to the study criteria were included in this analysis. The overall analysis indicated a significant association between the *PADI4*-

104C/T polymorphism and RA risk in the Chinese population (T *vs* C: OR = 1.45, 95%CI = 1.18-1.78; TT *vs* CC: OR = 1.49, 95%CI = 1.24-1.80; TT *vs* CC+CT: OR = 1.28, 95%CI = 1.08-1.51; TT+CT *vs* CC: OR = 1.75, 95%CI = 1.30-2.37). Analysis of data stratified by the geographic area and source of controls revealed that the *PADI4*-104C/T polymorphism was significantly associated with RA risk in a North Chinese population. In conclusion, the results of this meta-analysis indicated that the *PADI4*-104C/T variants could influence the risk of RA in the Chinese population; further studies in other ethnic groups are required to draw definite conclusions.

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

INTRODUCTION

Arthritis and other rheumatic conditions are prevalent worldwide. However, there are several regional and racial differences in the prevalence of different types of rheumatic diseases (Johnsen et al., 1992; Sangha, 2000; Dai et al., 2003). Rheumatoid arthritis (RA) affects approximately 1% of the global population, with genetic factors accounting for ~60% of the disease risk (Turesson and Matteson, 2006). Many candidate genes have been identified as potential RA susceptibility loci. One such gene is the peptidyl arginine deiminase 4 (*PADI4*) gene, a member of the *PADI* gene family that codes for enzymes involved in the posttranslational conversion of arginine to citrulline. Several polymorphisms have been identified in the promoter region of *PADI4*, which is located on chromosome 1p36. Of these, the -104C/T (or rs1748033) single nucleotide polymorphism (SNP) has been extensively studied. In 2003, Suzuki et al. (2003) identified an association between the *PADI4* -104C/T polymorphism and rheumatoid arthritis in a Japanese population. Consequently, several studies have attempted to clarify this relationship; however, there has been no definite consensus to date. Differences in the results of these studies may be attributed to the ethnic and clinical heterogeneity of the patients studied or the relatively small number of patients included in the studies.

Meta-analyses, which combine the results of several studies, have greater statistical power. Several meta-analyses have been previously conducted on RA in the Asian and Caucasian populations, with conflicting results (Lee et al., 2007; Hou et al., 2013; Lee and Bae, 2015; Yang et al., 2015). This meta-analysis was performed to filter out the influence of different genetic backgrounds on the relationship between the *PADI4*-104C/T polymorphism and risk of RA in a Chinese population. The data was also subjected to a subgroup analysis by stratifying the data according to the geographic location and source of control population, to explore the possible effects of gene-environment interactions on RA risk.

MATERIAL AND METHODS

Search strategy and selection criteria

Studies reporting on the relationship between the *PADI4*-104C/T polymorphism and RA up to July 25, 2015 were retrieved from PubMed and Chinese databases using the

search keywords "PADI4", "peptidyl arginine deiminase 4", "-104C/T", "rheumatoid arthritis", "Chinese", "China", and "Taiwan". The search was performed with no language restrictions, and focused on human studies only. The references cited in the retrieved articles were also searched.

The inclusion criteria for study selection were as follows: 1) case-control or cohort studies describing the association between the *PADI4*-104C/T polymorphism and RA, 2) patients diagnosed with RA based on the classification criteria proposed by the American College of Rheumatology for RA, 1987, 3) clear description of the *PADI4*-104C/T polymorphism in RA patients and controls, and 4) Chinese subjects only. Duplicate publications, studies with incomplete data, those without controls, and meta-analyses, letters, meeting abstracts, reviews, and editorial articles were excluded from this analysis.

Data extraction

Data was retrieved independently by two authors from all included publications. Disagreements were resolved by discussion. The titles and abstracts of all potentially relevant articles were screened to determine their relevance. Full articles were scrutinized in case of ambiguity in the title and abstract. The following data were extracted from the identified studies: name of the first author, publication year, source of controls, geographic area, sample size, and number of subjects expressing the *PADI4*-104C/T genotype.

Statistical analysis

The data was analyzed using the STATA v.10 software package (STATA, College Station, TX, USA). The genotype distribution in the controls was tested for conformance with the Hardy-Weinberg equilibrium (HWE) using the chi-square test, and deviations were indicated by P values < 0.05. The association between the *PADI4*-104C/T polymorphism and RA risk was estimated by the odds ratios (ORs) with their 95% confidence intervals (CIs). Heterogeneity was tested by Q-statistic with P values < 0.10. In case of heterogeneity, the random-effect model was used to pool the ORs (with their corresponding 95%CIs); the fixed-effect model was used in other cases. The significance of the pooled OR was determined by a *Z*-test. A sensitivity analysis was conducted to illustrate the accuracy and stability of the analytical results. Sensitivity analyses were conducted by deleting individual studies included in the meta-analysis. Publication bias was assessed by Begg's funnel plot and the Egger linear regression test. The data was stratified according to the geographic area and source of controls and subsequently analyzed. Two-sided P values less than 0.05 were considered to be statistically significant.

RESULTS

Description of included studies

The literature search strategy and the study selection criteria are depicted in Figure 1. A total of 57 articles that analyzed the association between *PADI4* polymorphisms and risk of RA were identified after the removal of duplicate studies obtained from different databases. A thorough screening of the titles and abstracts resulted in the exclusion of 44 reviews or meeting abstracts, studies conducted in a non-Chinese population or those irrelevant to this

study. Of the 13 potentially relevant articles (Cui et al., 2007; Lu, 2007; Fan et al., 2008, 2009; Feng et al., 2010; Shi, 2010; Shi et al., 2010; Wei, 2010; Chen et al., 2011; Xu et al., 2011; Li et al., 2012; Liu et al., 2012; Du et al., 2014), three duplicate studies were excluded (Fan et al., 2009; Shi, 2010; Wei, 2010). Finally, 10 case-control studies that met the inclusion criteria (Cui et al., 2007; Lu, 2007; Fan et al., 2008; Feng et al., 2010; Shi et al., 2010; Chen et al., 2011; Xu et al., 2011; Li et al., 2012; Liu et al., 2012; Du et al., 2014) were included in this study. The included studies were published between 2007 and 2014. In total, 2119 RA cases and 1962 controls were included in this meta-analysis. The source of controls was population-based in nine studies. The characteristics of included studies are summarized in Table 1.

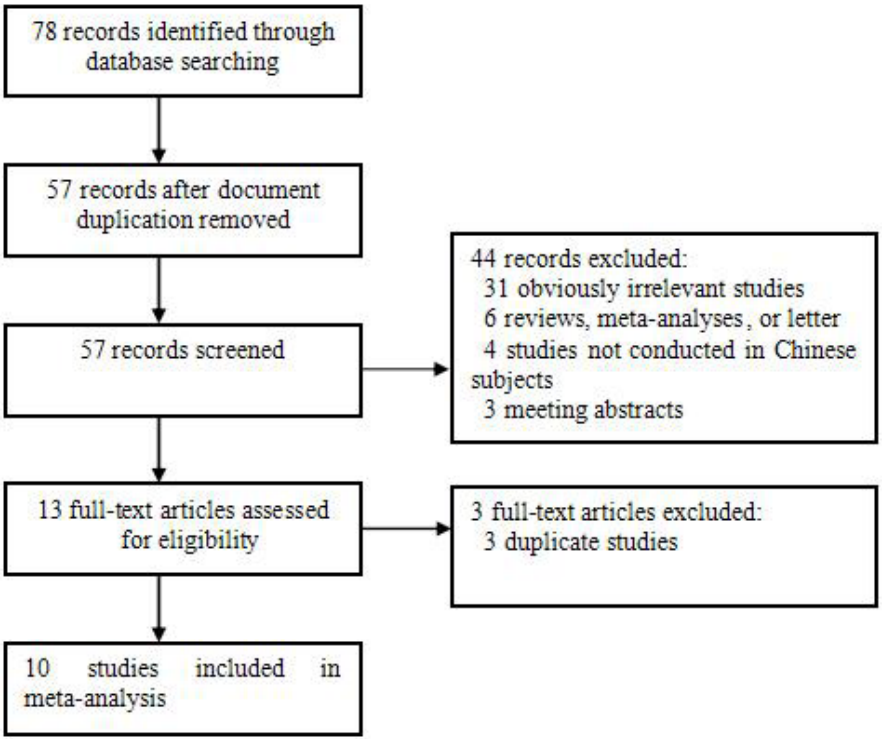

Figure 1. Flow diagram depicting the literature search strategy.

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

First author and year of publication	Source of controls	Geographic location	Number of cases	Number of controls	Cases			Controls			HWE	
					CC	CT	TT	CC	CT	TT	χ^2	P
Cui 2007	PB	Hebei	92	116	25	56	11	50	53	13	0.03	0.852
Lu 2007	PB	Shanghai	41	56	7	34	0	41	15	0	1.34	0.247
Fan 2008	PB	Shanghai	70	81	22	30	18	48	22	11	7.96	0.005
Shi 2010	PB	Anhui	112	97	36	59	17	44	39	14	1.19	0.275
Feng 2010	PB	Hebei	115	106	41	50	24	43	47	16	0.28	0.595
Chen 2011	PB	Shanghai	378	204	137	183	58	77	92	35	0.69	0.407
Xu 2011	PB	Henan	130	130	43	63	24	57	56	17	0.30	0.581
Liu 2012	PB	Qinghai	90	90	33	43	14	33	41	16	0.27	0.601
Li 2012	HB	Inner Mongolia	53	42	14	24	15	20	19	3	0.28	0.595
Du 2014	PB	Beijing	1038	1040	368	479	191	437	450	153	4.38	0.036

PB: population-based; HB: hospital-based; HWE, Hardy-Weinberg equilibrium.

Meta-analysis

The primary results of the meta-analysis are summarized in Table 2. Overall, all variants of the *PADI4*-104C/T SNP were associated with a significantly elevated risk of RA (TT *vs* CC: OR = 1.49, 95%CI = 1.24-1.80; TT *vs* CC+CT: OR = 1.28, 95%CI = 1.08-1.51; TT+CT *vs* CC: OR = 1.75, 95%CI = 1.30-2.37). The pooled OR of the allele distribution at this SNP site (T *vs* C) was 1.45 (95%CI = 1.18-1.78) (Figure 2).

Table 2. Association of *PADI4* -104C/T polymorphism with rheumatoid arthritis susceptibility.

Analytical model		N	ORr (95%CI)	ORf (95%CI)	P_h
T *vs* C	Total analysis	10	1.45 (1.18-1.78)	1.29 (1.18-1.41)	0.000
	Population-based	9	1.39 (1.14-1.70)	1.27 (1.16-1.39)	0.001
	South China	3	2.12 (0.87-5.15)	1.36(1.10-1.67)	0.000
	North China	7	1.30 (1.14-1.48)	1.28 (1.16-1.41)	0.305
TT *vs* CC	Total analysis	9	1.56 (1.10-2.16)	1.49 (1.24-1.80)	0.094
	Population-based	8	1.45 (1.13-1.85)	1.44 (1.20-1.74)	0.273
	South China	2	1.73 (0.46-6.42)	1.29 (0.83-1.99)	0.011
	North China	7	1.55 (1.21-1.98)	1.54 (1.26-1.89)	0.363
TT *vs* CC+CT	Total analysis	9	1.27 (1.01-1.60)	1.28 (1.08-1.51)	0.240
	Population-based	8	1.24 (1.04-1.47)	1.24 (1.04-1.47)	0.545
	South China	2	1.30 (0.53-3.18)	1.09 (0.73-1.63)	0.057
	North China	7	1.31 (1.08-1.59)	1.32 (1.09-1.59)	0.416
TT+CT *vs* CC	Total analysis	10	1.75 (1.30-2.37)	1.45 (1.28-1.65)	0.000
	Population-based	9	1.71 (1.25-2.33)	1.44 (1.26-1.63)	0.000
	South China	3	3.32 (0.87-12.62)	1.72 (1.29-2.28)	0.000
	North China	7	1.40 (1.21-1.61)	1.40 (1.21-1.61)	0.422

ORr: odds ratio for random-effect model; ORf: odds ratio for fixed-effect model; CI, confidence interval; P_h: P value for heterogeneity test. The North China region included the Hebei, Anhui, Henan, Qinghai, Inner Mongolia, and Beijing provinces. Only Shanghai was included from the South China region.

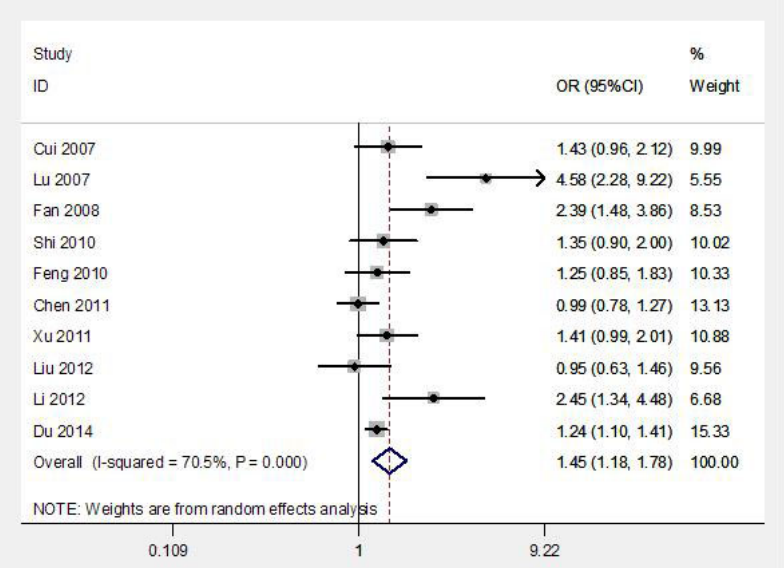

Figure 2. Forest plots of all selected studies showing the association between the *PADI4 -104C/T* polymorphism and RA risk in a Chinese population (allelic model: T *vs* C).

The results of the subgroup analysis based on source of controls revealed a significant correlation between the *PADI4*-104C/T polymorphism and RA risk in the studies with population-based controls (T *vs* C: OR = 1.39, 95%CI = 1.14-1.70; TT *vs* CC: OR = 1.44, 95%CI = 1.20-1.74; TT *vs* CC+CT: OR = 1.24, 95%CI = 1.04-1.47; TT+CT *vs* CC: OR = 1.75, 95%CI = 1.30-2.37) (Table 2). Analysis of the data stratified according to the geographic area revealed that these polymorphisms induced a significantly higher risk of RA in the Northern Chinese population (T *vs* C: OR = 1.28, 95%CI = 1.16-1.41; TT *vs* CC: OR = 1.54, 95%CI = 1.26-1.89; TT *vs* CC+CT: OR = 1.32, 95%CI = 1.09-1.59; TT+CT *vs* CC: OR = 1.40, 95%CI = 1.21-1.61) (Table 2).

Sensitivity and bias diagnosis

The differences between studies were compared and the sensitivity of the meta-analysis was evaluated by analyzing the overall data after deleting individual studies to reflect the influence of the individual data-set on the pooled OR. The pooled OR was not altered significantly in the overall analysis (Figure 3). Therefore, the conclusions of this meta-analysis are relatively stable and credible for the overall analysis. The publication bias in the included studies was assessed by the Begg's funnel plot and the Egger test. The shape of the funnel plots did not reveal obvious asymmetry (Figure 4). Similarly, the Egger test indicated no publication bias in the reviewed studies (t = 0.52, P = 0.619).

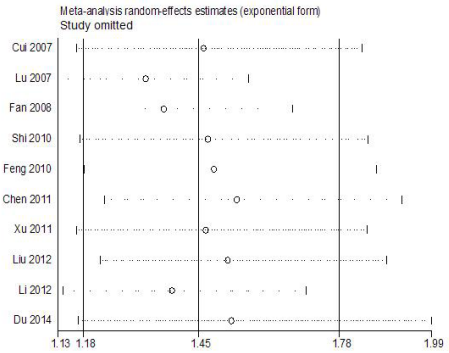

Figure 3. Sensitivity analysis of the association between the *PADI4*-104C/T polymorphism and RA risk in as Chinese population (allelic model: T *vs* C).

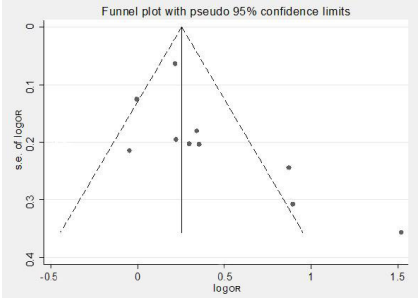

Figure 4. Evaluation of the publication bias in all included studies for allele contrast (T *vs* C) of the *PADI4*-104C/T polymorphism.

DISCUSSION

Despite the well-known multifactorial nature of RA, genetic factors are considered to be the strongest determinants of this disease, encouraging researchers to search for the responsible genes (Turesson and Matteson, 2006; Yang et al., 2015). The first positive association between *PADI4*-104C/T and RA was reported in a Japanese population (Suzuki et al., 2003); several studies have attempted to replicate this association since then. However, the results of individual studies remained inconclusive. A previous meta-analysis reported a significant association between the *PADI4*-104C/T polymorphism and RA risk in Asian and European populations (Lee and Bae, 2015), while other meta-analyses reported a significant association between this polymorphism and RA in Asian individuals only (Lee et al., 2007; Hou et al., 2013). These conflicting results could be attributed to various regional and racial differences. Therefore, this meta-analysis was conducted to provide a precise estimate of the association between the *PADI4*-104C/T SNP and RA susceptibility in the Chinese population, in order to lessen the impact of regional and racial differences. Ten case-control studies, comprising 2119 RA cases and 1962 controls, were included in this meta-analysis. The results of this study showed a significant overall association between the *PADI4*-104C/T polymorphism and RA. To our knowledge, this is the first meta-analysis evaluating the association between the *PADI4*-104C/T variants and RA in a Chinese population (with a large sample size).

In the subgroup analyses, wherein the cases and controls were stratified according to the geographic area and source of controls, we identified a significant association between this polymorphism and RA risk in the North Chinese population, and not in the South Chinese population. This showed that Northern Chinese subjects expressing the T variant of *PADI4*-104C/T were more susceptible to RA compared to Southern Chinese subjects. These suggest the possible effect of the geographic differences and environment on the genetic background of the population. However, we were unable to stratify the data based on the differences in ethnicity and other environmental factors because of insufficient data. Therefore, studies on RA-related genes should be based on the region and nationality; additionally, a larger number of associated genes must be simultaneously tested in a greater number of people (larger sample size) in the future.

This study is subject to several limitations. First, this meta-analysis only included data from Chinese patients with RA; therefore, our results are only applicable to this ethnic group. Secondly, as this meta-analysis was primarily based on unadjusted effect estimates and CIs, potential confounding factors were not controlled. Thirdly, differences in the genotyping methods and disease statuses might affect the interpretation of the data of included studies. However, our meta-analysis also has several strong points. Firstly, the inclusion and exclusion criteria were meticulously adhered to, in order to reduce possible selection bias. In addition, the conformance of the genotype distribution in the control groups to the HWE suggested the lack of significant differences in the genetic backgrounds among the participants. Thirdly, the sensitivity analysis confirmed the reliability and stability of this meta-analysis, and the Egger test indicated no publication bias among the included studies. Finally, the effect of different genetic backgrounds was minimized by including only studies conducted in the Chinese population. Therefore, the findings of this meta-analysis provide strong evidence regarding the association between the *PADI4*-104C/T polymorphism and RA in the Chinese population.

In conclusion, this meta-analysis demonstrated that the *PADI4*-104C/T polymorphism might be a significant risk factor for RA in the Chinese population; in particular, this polymorphism appeared to play a larger role in RA susceptibility in the northern Chinese

population, compared to the southern Chinese population. The effect of the *PADI4*-104C/T polymorphism on RA risk in other ethnic groups must be analyzed in future studies.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

Chen R, Wei Y, Cai Q, Duan S, et al. (2011). The PADI4 gene does not contribute to genetic susceptibility to rheumatoid arthritis in Chinese Han population. *Rheumatol. Int.* 31: 1631-1634. http://dx.doi.org/10.1007/s00296-010-1519-x

Cui LF, Yang WH, Song HC, Shu R, et al. (2007). Association of polymorphism of peptidylarginine deiminase 4 gene and rheumatoid arthritis in Han Population. *Chin. J. Allergy Clin. Immunol.* 1: 158-162.

Dai SM, Han XH, Zhao DB, Shi YQ, et al. (2003). Prevalence of rheumatic symptoms, rheumatoid arthritis, ankylosing spondylitis, and gout in Shanghai, China: a COPCORD study. *J. Rheumatol.* 30: 2245-2251.

Du Y, Liu X, Guo JP, Liu X, et al. (2014). Association between PADI4 gene polymorphisms and anti-cyclic citrullinated peptide antibody positive rheumatoid arthritis in a large Chinese Han cohort. *Clin. Exp. Rheumatol.* 32: 377-382.

Fan LY, Wang WJ, Wang Q, Zong M, et al. (2008). A functional haplotype and expression of the PADI4 gene associated with increased rheumatoid arthritis susceptibility in Chinese. *Tissue Antigens* 72: 469-473. http://dx.doi.org/10.1111/j.1399-0039.2008.01119.x

Fan LY, Zong M, Lu TB, Yang L, et al. (2009). [Association of the PADI4 gene polymorphism and HLA-DRB1 shared epitope alleles with rheumatoid arthritis]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 26: 57-61.

Feng ZJ, Niu HZ, Liang Y, Niu YL, et al. (2010). Association between rheumatoid arthritis and single nucleotide polymorphisms of padi4-104 in Han population in Hebei province. *J. Mol. Diagn. Ther.* 2: 244-247.

Hou S, Gao GP, Zhang XJ, Sun L, et al. (2013). PADI4 polymorphisms and susceptibility to rheumatoid arthritis: a meta-analysis. *Mod. Rheumatol.* 23: 50-60. http://dx.doi.org/10.3109/s10165-012-0639-4

Johnsen K, Gran JT, Dale K and Husby G (1992). The prevalence of ankylosing spondylitis among Norwegian Samis (Lapps). *J. Rheumatol.* 19: 1591-1594.

Liu J, Yang FM, Zhang PL, Li R, et al. (2012). Association of peptidylarginine deiminase 4 single nucleotide polymorphisms with rheumatoid arthritis in Chinese Han, Zang and Hui populations. *Chin. J. Gerontol.* 32: 3881-3883.

Lee YH and Bae SC (2015). Association between susceptibility to rheumatoid arthritis and PADI4 polymorphisms: a meta-analysis. *Clin. Rheumatol.* 35: 961-971. http://dx.doi.org/10.1007/s10067-015-2904-3

Lee YH, Rho YH, Choi SJ, Ji JD, et al. (2007). PADI4 polymorphisms and rheumatoid arthritis susceptibility: a meta-analysis. *Rheumatol. Int.* 27: 827-833. http://dx.doi.org/10.1007/s00296-007-0320-y

Li HB, Tie N, Jia YF, Shi L, et al. (2012). [Association between the synovial expression of cyclic citrullinated peptide and susceptibility variants of HLA-DRB1 shared epitope alleles and PADI 4 gene single nucleotide polymorphisms in patients with rheumatoid arthritis]. *Zhonghua Yi Xue Za Zhi* 92: 1607-1611.

Lu TB (2007). Study of the effect of peptidylarginine deiminase 4 on rheumatoid arthritis. Master's Thesis, Tongji University, Shanghai.

Sangha O (2000). Epidemiology of rheumatic diseases. *Rheumatology* 39 (Suppl 2): 3-12. http://dx.doi.org/10.1093/rheumatology/39.suppl_2.3

Shi HX (2010). The significance of peptidylarginine deaminase 4 gene and its protein, antibody in rheumatoid arthritis. Master's Thesis, Anhui Medical University, Anhui.

Shi HX, Qian L, Li XP, Li XM, et al. (2010). Association of polymorphism of peptidylarginine deaminase-4 gene and rheumatoid arthritis. *Chin. J. Rheumatol.* 14: 336-339.

Suzuki A, Yamada R, Chang X, Tokuhiro S, et al. (2003). Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nat. Genet.* 34: 395-402. http://dx.doi.org/10.1038/ng1206

Turesson C and Matteson EL (2006). Genetics of rheumatoid arthritis. *Mayo Clin. Proc.* 81: 94-101. http://dx.doi.org/10.4065/81.1.94

Wei YB (2010). Association analysis of PADI4 gene for ankylosing spondylitis and rheumatoid arthritis. Master's Thesis, Second Military Medical University, Shanghai.

Xu BS, Shao FM, Sun CY, Qin WS, et al. (2011). Association of peptidylarginine deiminase 4-94 and 104 single nucleotide polymorphisms with rheumatoid arthritis. *Chin. J. Prev. Contr. Chron. Dis.* 19: 489-491.

Yang XK, Liu J, Liu J, Liang Y, et al. (2015). Associations between PADI4 gene polymorphisms and rheumatoid arthritis: an updated meta-analysis. *Arch. Med. Res.* 46: 317-325. http://dx.doi.org/10.1016/j.arcmed.2015.05.011