Z Rheumatol 2017 · 76:622–629
DOI 10.1007/s00393-016-0169-0
Published online: 31 August 2016

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Association between IRAK1 rs3027898 and miRNA-499 rs3746444 polymorphisms and rheumatoid arthritis

A case control study and meta-analysis

Introduction

Genetic factors are believed to be responsible to 50–60 % for the risk of developing rheumatoid arthritis (RA) [1]. Interleukin-1 receptor associated kinase (IRAK1), a serine-threonine protein kinase, is an essential component involved in the signaling cascade of the Toll/interleukin-1 receptor (TIR) family [2]. After Toll-like receptor (TLR) stimulation, IRAK1 is recruited and phosphorylated to induce a critical downstream signaling cascade [3, 4]. The phosphorylation of IRAK1 is associated with the activation of nuclear factor-κB (NF-κB) in inflammatory diseases and the activity of NF-κB is a crucial mediator of the expression of proinflammatory cytokines [5]. Also IRAK1 has been found to play an important role in an autoimmune animal model and in autoimmune diseases patients, including RA patients [6–10]. In addition, genetic variants of IRAK1 were found to be associated with the plasma concentration of C-reactive protein in Caucasian women [11]. It was also reported that the rs3027898 polymorphism of IRAK1 is associated with atherothrombotic cerebral infarction in men [12]. Notably, IRAK1 is subjected to a negative feedback by miR-146a, the expression of which is also NF-κB-dependent, leading to a concerted immunological response [10].

MicroRNAs (miRNAs) consist of approximately 22 nucleotides and participate in transcriptional and translational regulation [13]. They act by binding to the 3′ untranslated region of target mRNA, leading to either degradation of mRNA or translational repression [14]; therefore, miRNAs play a role in cell proliferation, differentiation and apoptosis [15]. The MiRNA known as miR-499 targets IL-17 receptor B and IL-6, which both play key roles in the pathogenesis of inflammatory arthritis [16]. The miR-499 polymorphism rs4746444 is located in the mature miRNA region and may affect target mRNA binding and the pre-miRNA maturation process [17]. The miR-499 polymorphism rs3746444 has been reported to be involved in RA risk [18, 19]. Yang et al. [20] did not find a significant association between miR-499 rs3746444 and the susceptibility to RA but they found the single nucleotide polymorphism (SNP) rs3746444 may affect anti-cyclic citrullinated peptide (anti-CCP) antibody production. Thus, a number of studies have been conducted to investigate the association between the IRAK1 rs3027898 and miR-499 rs3746444 polymorphisms and the risk of RA in diverse populations [10, 18–23]; however, the results were mixed and inconclusive. Therefore, we carried out an additional case control study on the relationship between IRAK1 rs3027898 and miR-499 rs3746444 and RA risk in a Chinese population and present a meta-analysis based on currently available data. We hoped to clarify the association between IRAK1 rs3027898 and miR-499 rs3746444 and RA risk.

Material and methods

Patients and control subjects

A total of 386 patients with RA (54 males and 332 females, mean ± SD age 51.91 ± 13.03 years) were recruited from Anhui Provincial Hospital. All RA patients met the American College of Rheumatology criteria for RA [24]. The age and gender-matched 576 healthy controls (59 males and 517 females, mean ± SD age 50.53 ± 15.73 years) without RA or other autoimmune diseases were also enrolled in the present study. All participants were of Chinese Han origin. Information on demographic data were obtained through reviewing hospital records or by questionnaire. This study was reviewed and approved by the ethics committee of Anhui Medical University. Informed consent was provided by all the participants.

Table 1 The main demographic and clinical characteristics of rheumatoid arthritis patients

Characteristics	RA patients (*N* = 386)
Age (years, M ± SD)	51.91 ± 13.03
Sex (female/male)	332/54
Age of onset (years, M ± SD)	45.18 ± 13.76
Disease duration (years, M ± SD)	6.13 ± 6.64
Treatment duration (years, mean ± SD)	5.57 ± 6.91
RF positive (%)	75.2
Anti-CCP positive (%)	79.3

RA rheumatoid arthritis, *RF* rheumatoid factor, *Anti-CCP* anti-cyclic citrullinated peptide, *M* mean, *SD* standard deviation

Table 2 Allele and genotype frequency distributions of IRAK1 rs3027898 and miRNA-499 rs3746444 in RA patients and controls

Genotype	RA patients (*n* = 386), *n* (%)	Controls (*n* = 576), *n* (%)	χ^2	*P*-value	OR (95% CI)
IRAK1 rs3027898					
CC	290 (75.1)	379 (65.8)	10.462	0.005*	NA
CA	79 (20.5)	171 (29.7)	–	–	–
AA	17 (4.4)	26 (4.5)	–	–	–
C	659 (85.4)	929 (80.6)	7.145	0.008*	1.400 (1.093–1.793)
A	113 (14.6)	223 (19.4)	–	–	–
Dominant model					
CC + CA	369 (95.6)	550 (95.5)	0.007	0.936	1.026 (0.549–1.918)
AA	17 (4.4)	26 (4.5)	–	–	–
Recessive model					
CC	290 (75.1)	379 (65.8)	9.500	0.002*	1.570 (1.177–2.094)
CA + AA	96 (24.9)	197 (34.2)	–		–
miRNA-499 rs3746444					
CC	5 (1.3)	8 (1.4)	2.016	0.365	NA
CT	99 (25.6)	125 (21.7)	–	–	–
TT	282 (73.1)	443 (76.9)	–	–	–
C	109 (14.1)	141 (12.2)	1.444	0.229	1.179 (0.901–1.542)
T	663 (85.9)	1011 (87.8)	–	–	–
Dominant model					
CC + CT	104 (26.9)	133 (23.1)	1.848	0.174	1.228 (0.913–1.653)
TT	282 (73.1)	443 (76.9)	–	–	–
Recessive model					
CC	5 (1.3)	8 (1.4)	–	–	–
CT + TT	381 (98.7)	568 (98.6)	0.015	0.902	0.932 (0.303–2.870)

RA rheumatoid arthritis, *OR* odds ratio, *CI* confidence interval, *NA* not available, *IRAK1* interleukin-1 receptor associated kinase
*Statistically significant ($p < 0.05$)

DNA extraction and genotyping

An anti-coagulated venous EDTA blood sample was collected from each subject enrolled in the current investigation. Genomic DNA was extracted from peripheral blood leukocytes by standard procedures with the Flexi Gene_ DNA kit (Qiagen, Valencia, CA). Genotyping of IRAK1 rs3027898 and miR-499 rs3746444 was performed by TaqMan SNP Genotyping Assay Kit (Applied Bio-systems, USA) (probes assays C__15765198_10 for rs3027898, C___2142612_30 for rs3746444). All genotyping was determined using Fluidigm® 192.24 Dynamic Array IFC (Integrated Fluidic Circuit, Fluidigm, San Francisco, CA) in accordance with the manufacturer's instructions. Each assay (4 µl) comprised 2.0 µl assay loading reagent (Fluidigm, 2×), 1.0 µl SNP genotyping assay mix (40×) (Applied Bio-systems), 0.2 µl ROX (50×) (Invitrogen, Carlsbad, CA), and 0.8 µl DNA-free water. Each sample (5 µl) contained 1.6 µl genomic DNA, 2.0 µl GTX press master mix (2×) (Applied Biosystems, PN 4401892), 0.2 µl Fast GT sample loading reagent (20×) (Fluidigm, PN 100–3065) and 0.2 µl DNA-free water. Non-template controls (NTCs) were included in each run. Each of the assays (4 µl) and samples (5 µl) was pipetted into separate inlets on the frame of the chip. The assays and samples were loaded into the chip and mixed using the Integrated IFC Controller RX software. After load/mix was completed, the IFC was transferred to the EP1 reader for scanning. Genotype calling was performed on the Fluidigm SNP genotyping analysis software.

Statistical analysis

Genotype and allele distributions of cases and controls were analyzed by the χ^2-test or Fisher's exact test. Odds ratios (OR) and 95 % confidence intervals (95 % CI) were also calculated. Hardy-Weinberg equilibrium was evaluated in the control group. If the *p* value was <0.05, the population was considered to show deviations from HWE. All analyses were performed by SPSS 19.0 software. Statistical power was calculated by the free-download software-Power and Sample Size Calculation Software (http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize). A two-sided *P*-value of <0.05 was regarded as statistically significant.

Meta-analysis

To search for eligible studies for the meta-analysis, two of the authors exten-

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Association between IRAK1 rs3027898 and miRNA-499 rs3746444 polymorphisms and rheumatoid arthritis. A case control study and meta-analysis

Abstract

Background. The IRAK1 and miR-499 polymorphisms play an important role in the etiology of rheumatoid arthritis (RA). Several studies have been carried out to estimate the association between IRAK1 rs3027898 and miR-499 rs3746444 and RA risk; however, the results were inconsistent.

Aim. A case control study was carried out to explore the association between IRAK1 rs3027898 and miR-499 rs3746444 and the RA risk in a Chinese population. Meta-analyses combining present with previous studies were conducted to further explore the association.

Material and methods. A total of 386 RA patients were enrolled along with 576 matched healthy controls. Genotyping was performed by using TaqMan genotyping assays on Fluidigm 192.24 system. For the meta-analysis, a systematic literature search was conducted to identify all relevant studies.

Results. This case control study showed that the IRAK1 rs3027898 C allele was associated with increased risk of RA with an odds ratio (OR) = 1.4 and 95 % confidence intervals (CI) = 1.093–1.793, $P = 0.008$ but miR-499 rs3746444 polymorphisms were not significantly associated with the risk for RA. The meta-analyses included a total of 4 case control studies on IRAK1 rs3027898 and 4 studies on miR-499 rs3746444. The IRAK1 rs3027898 C allele had an overall OR of 1.268 (95 % CI = 1.130–1.424, $P < 0.001$). After stratification by ethnicity the C allele had an OR of 1.238 (95 % CI = 1.096–1.398, $P = 0.001$) in Asians. No association between miR-499 rs3746444 polymorphism and RA was found in the overall and Asian populations.

Conclusion. The results from our case control study and the meta-analyses indicate that the IRAK1 rs3027898 C allele is significantly associated with an increased risk of RA, especially in Asians.

Keywords

Signal transduction pathway · MicroRNAs · Chinese · Genotype · Population study

Assoziation zwischen Polymorphismen von IRAK1 rs3027898 und miRNA-499 rs3746444 und rheumatoider Arthritis. Eine Fall-Kontroll-Studie und Metaanalyse

Zusammenfassung

Hintergrund. Die Polymorphismen von IRAK1 und miR-499 spielen eine wichtige Rolle in der Ätiologie der rheumatoiden Arthritis (RA). In mehreren Studien wurde ein Zusammenhang zwischen IRAK1 rs3027898 und miR-499 rs3746444 und dem Risiko einer RA angenommen, die Ergebnisse waren jedoch widersprüchlich.

Ziel. Bei einer chinesischen Population wurde eine Fall-Kontroll-Studie zur Beurteilung der Assoziation zwischen IRAK1 rs3027898 und miR-499 rs3746444 und dem Risiko einer RA durchgeführt. Es erfolgten Metaanalysen, die aktuelle mit früheren Studien kombinierten, um diesen Zusammenhang weiter zu untersuchen.

Materialien und Methoden. Insgesamt 386 RA-Patienten sowie 576 gematchte gesunde Kontrollpersonen wurden eingeschlossen. Die Genotypisierung erfolgte mit Hilfe der TaqMan-Genotyping-Assays mit dem Fluidigm-192.24-System. Für die Metaanalyse wurde eine systematische Literaturrecherche durchgeführt, um alle relevanten Studien zu identifizieren.

Ergebnisse. Die Fall-Kontroll-Studie zeigte, dass das C-Allel von IRAK1-rs3027898 mit einem erhöhten Risiko für RA assoziiert war (Odds-Ratio [OR] = 1,4, 95 % Konfidenzintervall [CI] = 1,093–1,793; $P = 0{,}008$). Polymorphismen von miR-499 rs3746444 waren jedoch nicht signifikant mit dem Risiko für eine RA assoziiert. Die Metaanalyse schloss insgesamt 4 Fall-Kontroll-Studien zu IRAK1 rs3027898 und 4 Studien zu miR-499 rs3746444 ein. Die Gesamt-OR für das C-Allel von IRAK1-rs3027898 betrug 1,268 (95 % CI = 1,130–1,424; $p < 0{,}001$). Nach der Stratifizierung nach Ethnizität betrug die OR für das C-Allel 1,238 (95 % CI = 1,096–1,398; $P = 0{,}001$) bei Asiaten. Es wurde bei der gesamten asiatischen Population kein Zusammenhang zwischen dem Polymorphismus von miR-499 rs3746444 und RA festgestellt.

Schlussfolgerung. Die Ergebnisse dieser Fall-Kontroll-Studie und der Metaanalyse zeigen, dass das C-Allel von IRAK1-rs3027898 signifikant mit einem erhöhten Risiko für RA assoziiert ist, insbesondere bei Asiaten.

Schlüsselwörter

Signaltransduktionsweg · MikroRNAs · Chinesische Population · Genotyp · Populationsstudie

sively reviewed the PubMed, Embase, the China National Knowledge Infrastructure (CNKI), the Chinese Biomedical Literature Database (CBM) and Wanfang (Chinese) by following the key words: "microRNA" "miR-499" "IRAK1" "polymorphism" and "rheumatoid arthritis" or "RA". The final search was carried out on 1 January 2016. The inclusion criteria for the meta-analysis were as follows: (a) evaluation of IRAK1 rs3027898 and hsa-mir-499 rs3746444 polymorphisms and RA risk, (b) case control study design, (c) availability of genotype frequencies or odds ratio and 95 % CI and (d) genotype distribution of the control group was in Hardy-Weinberg equilibrium (HWE). The major reasons for exclusion of the studies were overlapping data, case reports and review articles. For each study, we recorded the first author's surname, year of publication, country of origin, ethnicity of the population tested, the method of genotyping, the number of genotyped samples and controls of the population tested.

The χ^2-test was used to determine if the observed frequencies of the genotypes in control groups conformed to the Hardy-Weinberg equilibrium (HWE). The OR and 95 % CI were determined for each study. The Cochran's (Q) tests was generally used to assess the between-study heterogeneity ($p \leq 0.10$ was considered to be representative of statistically significant heterogeneity). If there was no obvi-

Table 3 Stratified analyses between IRAK1 rs3027898 and miRNA-499 rs3746444 polymorphisms and risk of rheumatoid arthritis

Variable	Genotype frequency, *n* (%)			χ^2	*P*-value	Allele frequency, *n* (%)		χ^2	*P*-value
IRAK1 rs3027898	CC	CA	AA	–	–	C	A	–	–
RF									
Positive	214(73.5)	63(21.7)	14(4.8)	1.256	0.534	491(84.4)	91(15.6)	1.406	0.236
Negative	63(78.7)	15(18.8)	2(2.5)	–	–	141(88.1)	19(11.9)	–	–
Anti-CCP									
Positive	233(75.9)	60(19.5)	14(4.6)	1.261	0.532	526(85.7)	88(14.3)	0.077	0.781
Negative	35(72.9)	12(25.0)	1(2.1)	–	–	82(85.4)	14(14.6)	–	–
miRNA-499 rs3746444	CC	CT	TT	–	–	C	T	–	–
RF									
Positive	5(1.7)	78(26.8)	208(71.5)	–	0.327[a]	88(15.1)	494(84.9)	2.088	0.149
Negative	0(0.0)	17(21.2)	63(78.8)	–	–	17(10.6)	143(89.4)	–	–
Anti-CCP									
Positive	4(1.3)	82(26.7)	221(72.0)	–	0.250[a]	90(14.7)	524(85.3)	2.791	0.095
Negative	0(0.0)	8(16.7)	40(83.3)	–	–	8(8.3)	88(91.7)	–	–

RA rheumatoid arthritis, *RF* rheumatoid factor, *Anti-CCP* anti-cyclic citrullinated peptide, *IRAK1* interleukin-1 receptor associated kinase
[a]Fisher's exact test

Table 4 Characteristics of the studies included in the meta-analysis

Author	Year	Country	Sample size		Genotyping method	*p* HWE for controls
			Case	Control		
IRAK1 rs3027898						
Chatzikyriakidou et al. [21]	2010	Greece	136	147	PCR-RFLP	0.385
Gao et al. [22]	2012	China	123	220	PCR-LDR	0.069
Han et al. [23]	2013	Korea	1158	849	Mass array	0.687
Present study	2016	China	386	576	Taqman	0.238
miRNA-499 rs3746444						
Zhang et al. [10]	2013	China	206	466	MALDI-TOF MS	0.719
El-Shal et al. [18]	2013	China	217	245	T-ARMs-PCR	0.841
Yang et al. [20]	2011	Egypt	208	240	PCR-RFLP	0.624
Present study	2016	China	386	576	Taqman	0.807

PCR-RFLP polymerase chain reaction resection fragment length polymorphism, *PCR-LDR* polymerase chain reaction-ligation detection reaction, *T-ARMs-PCR* tetra amplifcation refractory mutation system-polymerase chain reaction, *MALDI-TOF* matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

ous heterogeneity, the fixed-effects model (the Mantel-Haenszel method) was used to estimate the summary OR. Otherwise, the random-effects model (the DerSimonian and Laird method) was used. The funnel plot was used to assess potential publication bias and the Egger's linear regression test was applied to evaluate the funnel plot asymmetry, using a natural logarithm scale of the OR [25]. A *p*-value less than 0.05 was considered to be significant publication bias. All the statistical analyses were performed by STATA 12.0 software (Stata Corporation, College Station, TX).

Results

Case control study

The distribution of the demographic and clinical characteristics in the patient groups are summarized in **Table 1**. The listed laboratory parameters were rheumatoid factor (RF, 75.2 %) and anti-citrullinated peptide (anti-CCP, 79.3 %). The genotype and allele frequencies of IRAK1 rs3027898 and miRNA-499 rs3746444 in patients and control subjects are shown in **Table 2**.

As shown in **Table 2** the genotypic frequencies of IRAK1 rs3027898 were in HWE in controls (χ^2 = 1.39, P = 0.238). Significant differences were found in the genotype distribution between cases and controls (χ^2 = 10.462, P = 0.005). The allele C was significantly increased in the patient group compared with control subjects (C versus A, OR = 1.400, 95 % CI = 1.093–1.793, P = 0.008). A statistically significant difference was also found in the recessive model (CC versus AA + CA: OR = 1.570, 95 % CI = 1.177–2.094, P = 0.002), whereas no significant differences were discovered in the dominant model (CC + CA versus AA: OR = 1.026, 95 % CI = 0.549–1.918, P = 0.936). No deviation from HWE was observed for miRNA-499 rs3746444 in the control group (χ^2 = 0.06, P = 0.807). No significant differences in genotype or allele frequencies between cases and controls were detected. In addition, no significant association was detected under either the dominant or recessive model.

To evaluate the effects of IRAK1 rs3027898 and hsamir-499 rs3746444 polymorphisms on RA risk according to RF and anti-CCP status, we performed a stratification analyses; however, both IRAK1 rs3027898 and hsamir-499 rs3746444 polymorphisms were found not to be associated with the risk of RA after stratification (all p > 0.05, **Table 3**).

Meta-analysis

Study characteristics

Characteristics of all eligible studies included for meta-analysis are shown in

Table 5 Meta-analysis results of the IRAK1 rs3027898 and miRNA-499 rs3746444 polymorphisms with genetic susceptibility to rheumatoid arthritis

Polymorphisms	Population	Number of studies	Sample size		Test of association			Model	Test of heterogeneity			Egger's test (*P*-value)
			Case	Control	OR	95% CI	*P*-value		Q	*P*-value	I2 (%)	
IRAK1 rs3027898												
C vs. A	Overall	4	1803	1792	1.268	1.130–1.424	0.000	F	3.57	0.312	15.9	0.754
	Asian	3	1667	1645	1.238	1.096–1.398	0.001	F	1.92	0.382	0.0	0.848
	European	1	136	147	1.601	1.099–2.333	0.014	F	0.00	NA	NA	NA
CC + CA vs. AA	Overall	4	1803	1792	1.274	0.977–1.660	0.073	F	0.93	0.818	0.0	0.963
	Asian	3	1667	1645	1.187	0.862–1.634	0.295	F	0.33	0.849	0.0	0.955
	European	1	136	147	1.488	0.927–2.388	0.100	F	0.00	NA	NA	NA
CC vs. CA + AA	Overall	4	1803	1792	1.339	1.160–1.545	0.000	F	6.01	0.111	50.1	0.642
	Asian	3	1667	1645	1.309	1.132–1.513	0.000	F	3.29	0.193	39.2	0.808
	European	1	136	147	2.644	1.159–6.030	0.021	F	0.00	NA	NA	NA
miRNA-499 rs3746444												
C vs. T	Overall	4	1017	1527	1.162	0.878–1.538	0.295	R	8.85	0.031	66.1	0.627
	Asian	3	800	1282	1.039	0.863–1.251	0.686	F	2.09	0.351	4.4	0.500
	Egyptian	1	208	240	1.694	1.235–2.322	0.001	F	0.00	NA	NA	NA
CC + CT vs. TT	Overall	4	1017	1527	1.196	0.845–1.693	0.312	R	10.57	0.014	71.6	0.793
	Asian	3	800	1282	1.046	0.851–1.286	0.670	F	2.33	0.312	14.2	0.376
	Egyptian	1	208	240	1.971	1.350–2.877	0.000	F	0.00	NA	NA	NA
CC vs. CT + TT	Overall	4	1017	1527	1.192	0.695–2.043	0.523	F	1.56	0.668	0.0	0.279
	Asian	3	800	1282	1.027	0.526–2.006	0.938	F	1.05	0.592	0.0	0.608
	Egyptian	1	208	240	1.582	0.624–4.008	0.334	F	0.00	NA	NA	NA

R random-effects model; *F* fixed-effects model

Table 4: three reports [21–23] which fulfilled the inclusion criteria were included for meta-analysis of IRAK1 rs3027898 polymorphism and three studies for miRNA-499 rs3746444 [10, 18, 20] were found to be suitable. In addition, the data of the present study were also included. The evaluation included 4 studies for IRAK1 rs3027898 with 1803 RA patients and 1792 healthy controls. For miRNA-499 rs3746444, a total of 4 studies involving 1017 patients and 1527 controls were analyzed.

Meta-analysis of the association between IRAK1 rs3027898 polymorphism and RA

The meta-analysis revealed significant association of IRAK1 rs3027898 C allele, CC genotype with genetic susceptibility to RA (C vs. A: OR = 1.268, 95% CI = 1.130–1.424, $P < 0.001$; CC vs. CA + AA: OR = 1.339, 95 % CI = 1.160–1.545, $P < 0.001$). After stratification by ethnicity a significant association between IRAK1 rs3027898 C allele, CC genotype and genetic susceptibility to RA was identified in Asians (C vs. A: OR = 1.238, 95% CI = 1.096–1.398, $P = 0.001$; CC vs. CA + AA: OR = 1.309, 95 % CI = 1.132–1.513, $P < 0.001$) (**Table 5**; **Fig. 1a–c**).

Meta-analysis of the association between miRNA-499 rs3746444 polymorphism and RA

The meta-analysis found no significant association between miRNA-499 rs3746444 C allele, CC + CT genotype and CC genotype and genetic susceptibility to RA (C vs. T: OR = 1.162, 95 % CI = 0.878–1.538, $P = 0.295$; CC + CT vs. TT: OR = 1.196, 95 % CI = 0.845–1.693, $P = 0.312$; CC vs. CT + TT: OR = 1.192, 95 % CI = 0.695–2.043, $P = 0.523$). When stratified by ethnicity we also failed to detect associations in Asians (C vs. T: OR = 1.039, 95 % CI = 0.863–1.251, $P = 0.686$; CC + CT vs. TT: OR = 1.046, 95 % CI = 0.851–1.286, $P = 0.670$; CC vs. CT + TT: OR = 1.027, 95 % CI = 0.526–2.006, $P = 0.938$). The single Egyptian study on the miRNA-499 rs3746444 polymorphism revealed a significant association with RA (**Table 5**).

Heterogeneity and publication bias

The heterogeneity of the included studies is presented in **Table 5**. Heterogeneity was found for the miRNA-499 rs3746444 C-allele, CC + CT genotype and RA in the overall populations. Thus, these meta-analysis were performed using the random-effects model. No evidence of obvious asymmetry was detected after checking the shapes of the funnel plots (data not shown). Egger's linear regression test was used to assess the funnel plot asymmetry and the results indicated no publication bias (**Table 5**).

Discussion

In the present study we investigated the association between the IRAK1 rs3027898 and miR-499 rs3746444 polymorphisms and the risk of RA in a Chinese population. The results demonstrate

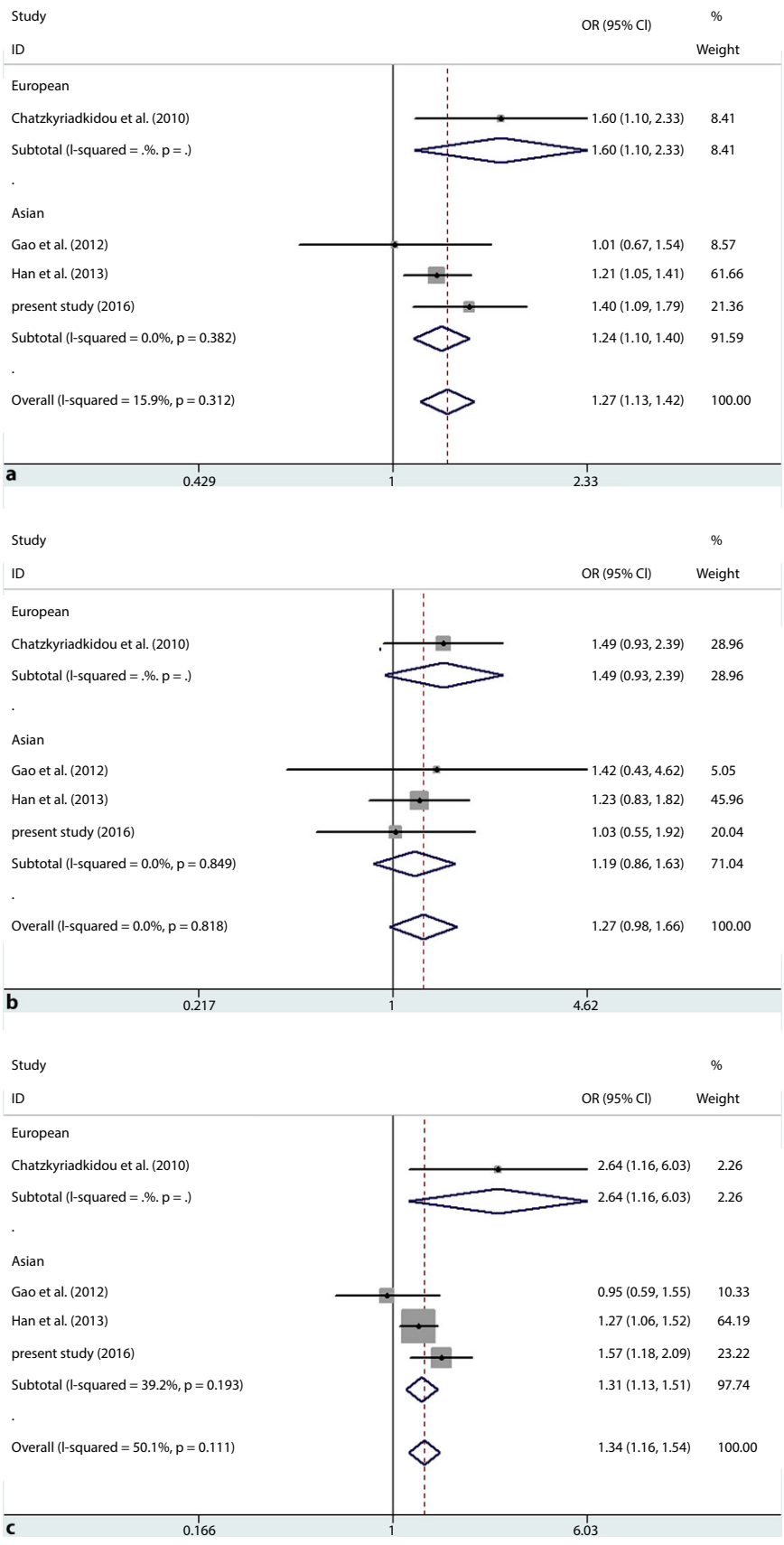

Fig. 1 ▲ Odds ratios and 95 % confidence intervals of individual studies and pooled data for the association between IRAK1 rs3027898 polymorphism and RA. **a** C vs. A, **b** CC + CA vs. AA, **c** CC vs. CA + AA

a significant association between IRAK1 rs3027898 C allele and the risk of RA in a Chinese population (OR = 1.400, $P = 0.008$). We further confirmed this association with a meta-analysis (OR = 1.268, $P < 0.001$).

The MiR-146a is involved in modulating the expression of inflammatory cytokines and regulates tumor necrosis factor alpha (TNF-alpha) via TRAF-6/IRAK1 and IRAK1 is a target of miR-146a. Both are associated with RA pathogenicity [17, 26]. Chatzikyriakidou et al. [21] investigated the potential association of the miR-146a variant rs2910164 and the two polymorphisms located in the 3′-UTR of the IRAK1 gene (rs3027898 and rs1059703) with RA susceptibility in Greek population. They found a significant difference in the distribution of IRAK1 rs3027898 A/C genotypes between RA patients and healthy controls but no difference was observed in the distribution of IRAK1 rs1059703 and miR-146a rs2910164 variants between cases and controls. It has also been suggested that the IRAK1 rs3027898 polymorphism is significantly associated with RA in Chinese [10] and Korean [23] populations. The same association was recently reported for other autoimmune diseases (AD), such as systemic lupus erythematosus (SLE) [2], psoriatic arthritis (PsA) [27] and ankylosing spondylitis (AS) [27]. The SNP hsa-mir-499 rs3746444 has been investigated in SLE [28] but no association has been identified. Previous studies [10, 20] showed that the miR-499 rs3746444 polymorphism was not associated with RA susceptibility. Similarly, we found no associations of rs3746444 with RA both in our case control study and the current meta-analyses. In this meta-analysis we excluded studies in which HWE was absent in the control population. As several studies have reported significant associations between genetic polymorphisms and diseases when the genotype distribution of the control group deviated from HWE but deviation from HWE in the control group might imply potential selection biases of controls or genotype errors. Thus, our meta-analysis might result in a more reliable conclusion. Song et al. [17] performed a meta-

analysis to indicate the potential role of IRAK1 rs3027898 polymorphism in the pathogenesis of ADs, while Li et al. [29] demonstrated the association between IRAK1 rs3027898 polymorphism and inflammatory arthritis; however, both did not investigated whether IRAK1 rs3027898 polymorphism is associated with RA susceptibility. Thus, our meta-analysis is the first to confirm the relationships between IRAK1 rs3027898 polymorphism and RA. In addition, our results for miR-499 rs3746444 polymorphism were very different to those previous reported meta-analyses [30–32].

There are some limitations in this study. Firstly, the sample size of our case-control study is not large enough, resulting in moderate statistical powers (0.591) for miR-499 rs3746444 and (0.736) for IRAK1 rs3027898; however, the allele frequency in our study is similar to other Chinese population-based results [10, 20, 22]. Secondly, for the meta-analysis the number of included papers is small and the pooled results can be influenced by the large sample size designed study.

In conclusion, our study provides strong evidence that IRAK1 rs3027898 functional polymorphisms may contribute to the risk of RA, especially in Asians. The MiR-499 rs3746444 polymorphism is not be associated with RA susceptibility. Further studies with a large sample size are needed to evaluate the association with these diseases in different ethnic populations.

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Acknowledgements. This work was supported by grants from the National Natural Science Foundation of China (81473058).

Compliance with ethical guidelines

Conflict of interests. X.-K. Yang, P. Li, C. Zhang, R.-X. Leng, S. Li, J. Liu, B.-Z. Li, H.-F. Pan and D.-Q. Ye declare that they have no conflict of interests.

All studies on humans described in the present manuscript were carried out with the approval of the responsible ethics committee and in accordance with national law and the Helsinki Declaration of 1975 (in its current revised form). Informed consent was obtained from all participants included in the study.

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