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The association between susceptibility to inflammatory arthritis and *miR-146a*, *miR-499* and *IRAK1* polymorphisms

A meta-analysis

Inflammatory arthritis comprises a group of relatively common complex diseases that are known to be caused by interactions between genetic and environmental factors. Although the etiologies of these diseases have not been determined, genetic studies have established that susceptibility to them has a genetic component [1].

MicroRNAs (miRNAs) are noncoding RNA molecules approximately 22 nucleotides in length that participate in transcriptional and translational regulation [2]. They act by binding to the 3′ untranslated region of target mRNA, leading to either degradation of mRNA or repression of protein translation [3]. Therefore, miRNAs play a role in cell proliferation, differentiation and apoptosis [4]. *miR-146a* is involved in modulating the expression of inflammatory cytokines and the *miR-146a* polymorphism *rs2910164* has been associated with several diseases, such as cancer and autoimmune diseases [5, 6]. The *miR-146a rs2910164* polymorphism involves a G→C nucleotide substitution, which leads to a change from a G:U pair to a C:U mismatch in the structure of the miR-146a precursor. The *CC* genotype results in a lower expression level of mature miR-146a and promotes proliferation of cancer cells [7]. In addition, the minor *C* allele of the *miR-146a* polymorphism causes mispairing within the miR-146a hairpin and decreases the expression of its mature form, leading to diverse functional alterations [8]. Single nucleotide polymorphisms (SNPs) in miRNA sequences may alter miRNA expression. miR-146a regulates TNF-α via TRAF-6/IRAK1. IRAK1 is a target of miR-146a and plays an important role in the activation of NF-kB [9]. The most commonly studied polymorphisms of the *IRAK1* gene in inflammatory arthritis are the *rs3027898* (in the 3′ untranslated region) and *rs1059703* (exon 12) polymorphisms. The *miR-499 rs4746444* polymorphism is located in the mature miRNA region and may affect target mRNA binding and the pre-miRNA maturation process. The latter polymorphism has been associated with susceptibility to rheumatoid arthritis (RA) [10].

» miRNAs play a role in cell proliferation, differentiation and apoptosis

Some studies have shown that the polymorphisms in *miR-146a*, *miR-499* and *IRAK1* are associated with inflammatory arthritis, but other reports have found no such associations [11, 12, 13, 14, 15, 16, 17, 18]. These disparities are probably caused by small sample sizes, low statistical power and/or clinical heterogeneity. Therefore, to overcome the limitations of individual studies, resolve inconsistencies and reduce the likelihood that random errors are responsible for false-positive or false-negative associations, we conducted a meta-analysis [19, 20, 21]. The aim of the present study was to determine whether the *miR-146a*, *miR-499* and *IRAK1* polymorphisms are associated with susceptibility to inflammatory arthritis.

Methods

Identification of eligible studies and data extraction

We performed searches for studies that examined associations between the *miR-146a*, *miR-499* and *IRAK1* polymorphisms and inflammatory arthritis. The MEDLINE and EMBASE citation databases were used to identify published articles in which the *miR-146a*, *miR-499* and/or *IRAK1* polymorphisms were analyzed in patients with inflammatory arthritis. Combinations of keywords such as 'microRNA,' 'miR-146a,' 'miR-499,' 'IRAK1,' 'polymorphism,' 'arthritis' and the names of individual diseases were entered as Medical Subject Headings (MeSH) and text words. References in the identified studies were also investigated to identify additional studies not indexed by MEDLINE and EMBASE. No restrictions were placed on language, race, ethnicity or geographic area. Studies were included if they: (1) were published before June 2014, (2) contained original data and (3) provided sufficient genotypic data to calcu-

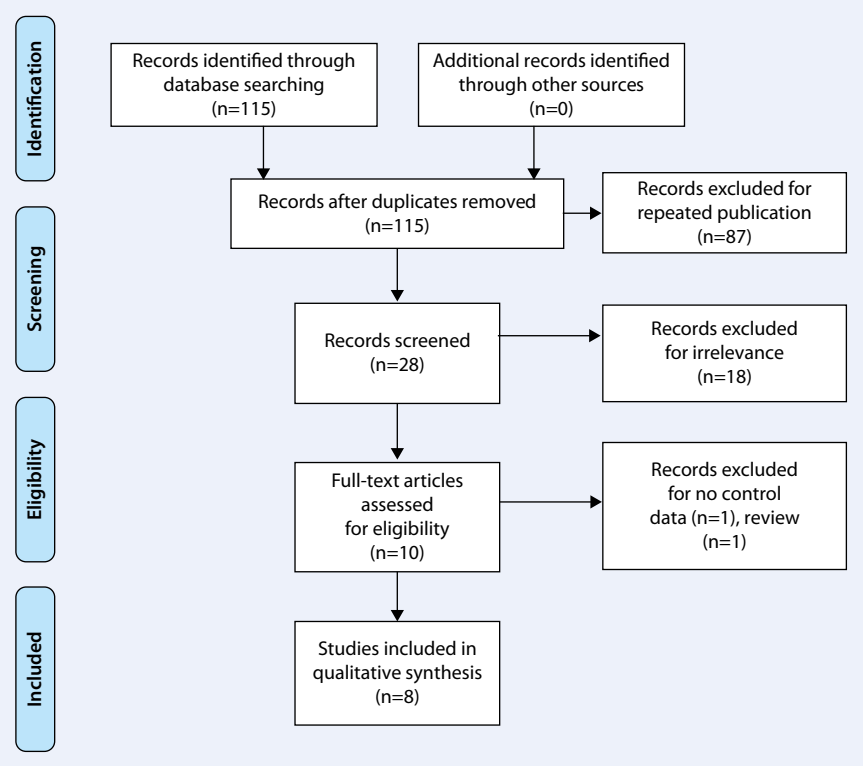

Fig. 1 ▲ Study flow chart

late odds ratios (ORs). The following were excluded: (1) studies containing overlapping data, (2) studies in which numbers of null and wild-type genotypes/alleles could not be ascertained and (3) studies in which family members had been studied (for example, a transmission disequilibrium test) because these analyses are based on linkage considerations. Data on methods and results were extracted from original studies by two independent reviewers. Discrepancy between the reviewers was resolved by consensus or a third reviewer. The following information was obtained for each study: author, year of publication, ethnicity of the study population, demographics and numbers of cases and controls. Frequencies of alleles were calculated from genotype distributions.

Evaluation of publication bias

Funnel plots are often used to detect publication bias. However, due to the limitations of funnel plotting—which requires a range of studies of varying sizes involving subjective judgments—publication bias was evaluated using Egger's linear regression test [22], which measures funnel plot asymmetry using a natural logarithm scale of ORs.

Evaluations of statistical associations

A χ-square test was used to determine whether the observed genotype frequencies conformed to Hardy–Weinberg (HW) expectations (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Meta-analyses were performed using: (1) allelic contrast, (2) homozygote contrast, (3) recessive and (4) dominant models. Subgroup analyses were performed according to ethnicity, disease type and Hardy-Weinberg equilibrium (HWE) status to evaluate ethnic- and disease-specific effects. Point estimates of risks, ORs and 95% confidence intervals (CIs) were estimated for each study. Cochran's Q statistic was used to assess intra- and interstudy variations and heterogeneities. This heterogeneity test assesses the null hypothesis that all studies evaluated the same effect. I^2 values were used to quantify the effect of heterogeneity. I^2 values range between 0 and 100% and represent the proportion of interstudy variability attributable to heterogeneity rather than chance [23]. I^2 values of 25, 50 and 75% were nominally defined as low, moderate and high estimates, respectively. The fixed effects model assumes that a genetic factor has the same effect on disease susceptibility across all studies investigated and that observed variations between studies are caused by chance alone. The random effects model assumes that different studies show substantial diversity and assesses both intrastudy sampling error and interstudy variance. When study groups are homogeneous, the two models are similar; but where this is not the case, the random effects model usually provides wider CIs than the fixed effects model. Furthermore, the random effects model is used in the presence of significant interstudy heterogeneity [24]. Statistical manipulations were undertaken using the Comprehensive Meta-Analysis computer program (Biostat, Englewood, NJ, USA).

Results

Studies included in the meta-analysis

Electronic and manual searching identified 115 reports and 10 were selected for full-text review based on title and abstract details. Two reports were excluded (one had no control data; the other was a review) and eight reports thus met the inclusion criteria [11, 12, 13, 14, 15, 16, 17, 18]. One of these reports contained data on two different groups [17] and we analyzed these reports independently. Therefore, a total of nine separate studies were considered in the meta-analysis, which contained, in total, 1224 patients and 1841 controls. Three studies were based on a European population, two on Middle Eastern populations, two on East Asian populations, one on a South Asian population and one on a South American population (**Fig. 1**, **Tab. 1**). An ethnicity-specific meta-analysis was conducted on European, Middle Eastern and East Asian populations. These studies included patients with RA (n=5), juvenile inflammatory arthritis (JIA; n=2), psoriatic arthritis (PsA; n=1) and ankylosing spondylitis (AS; n=1). A disease-specific meta-analysis was performed on RA and

Abstract · Zusammenfassung

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The association between susceptibility to inflammatory arthritis and *miR-146a*, *miR-499* and *IRAK1* polymorphisms. A meta-analysis

Abstract

Objective. The aim of this study was to explore whether polymorphisms in *miR-146a*, *miR-499* and *IRAK1* are associated with susceptibility to inflammatory arthritis.
Methods. Manual searches performed in the MEDLINE and EMBASE databases were used to identify published articles in which the roles of microRNA (miRNA) and *IRAK1* polymorphisms in inflammatory arthritis were determined. A meta-analysis was conducted to investigate associations of the *miR-146a rs2910164*, *miR-499 rs3746444*, *IRAK1 rs3027898 and IRAK1 rs1059703* polymorphisms with susceptibility to inflammatory arthritis.
Results. Nine studies containing 1224 patients and 1841 controls were included in the meta-analysis. The meta-analysis revealed no association between inflammatory arthritis and the *rs2910164 C* allele of *miR-146a* (odds ratio, OR =0.974; 95% confidence interval, CI =0.810–1.091; p=0.650). Stratification by ethnicity or disease type revealed no association between the *miR-146a C* allele and inflammatory arthritis in European, Middle Eastern or Asian patients with rheumatoid arthritis (RA) or juvenile idiopathic arthritis (JIA). However, the meta-analysis revealed an overall association between RA and the *miR-499 rs374644 C* (OR =1.123, 95% CI =1.019–2.586, p=0.041); stratification by ethnicity revealed a particular association in Middle Eastern populations (OR =1.943, 95% CI =1.508–2.504, $p=2.7\times10^{-8}$). The meta-analysis of *IRAK1* polymorphisms revealed an association between inflammatory arthritis and the *rs3027898 CC* genotype (OR =2.602, 95% CI =1.387–4.879, p=0.003). An analysis using the homozygote contrast showed the same pattern for the *rs3027898 CC* genotype (OR =2.472, 95% CI =1.300–4.700, p=0.006). No association between inflammatory arthritis and the *rs1059703* polymorphism was found.
Conclusion. This meta-analysis suggests that the *miR-499 rs374644* and *IRAKI rs3027898* polymorphisms are associated with susceptibility to inflammatory arthritis.

Keywords

Rheumatoid arthritis · MicroRNA · MEDLINE · Ethnicity · Genotype

Suszeptibilität für inflammatorische Arthritis und *miR-146a, miR-499* and *IRAK1*-Polymorphismen. Eine Metaanalyse

Zusammenfassung

Ziel. Ziel der Studie war die Erforschung möglicher Zusammenhänge zwischen Polymorphismen in den Genen *miR-146a*, *miR-499* und *IRAK1* mit der Suszeptibilität für inflammatorische Arthritiden.
Methoden. Manuell wurde in den Datenbanken MEDLINE und EMBASE nach Veröffentlichungen zur Rolle von microRNA(miRNA)- und *IRAK1*-Polymorphismen bei inflammatorischer Arthritis gesucht. Zur Überprüfung von Assoziationen zwischen *miR-146a rs2910164-*, *miR-499 rs3746444-*, *IRAK1 rs3027898- und IRAK1 rs1059703*-Polymorphismen und der Suszeptibilität für inflammatorische Arthritis wurde eine Metaanalyse durchgeführt.
Ergebnisse. Neun Studien mit 1224 Patienten und 1841 Kontrollen wurden in die Metaanalyse aufgenommen. Die Metaanalyse zeigte keine Assoziation zwischen inflammatorischer Arthritis und dem *rs2910164 C*-Allel von *miR-146a* (Odds Ratio, OR,=0,974; 95%-Konfidenzintervall, 95%-KI =0,810–1,091; p=0,650). Die Stratifizierung nach Ethnizität bzw. Erkrankungstyp ergab keine Assoziation zwischen dem *miR-146a C*-Allel und inflammatorischer Arthritis bei Patienten aus Europa, dem nahen Osten und Asien mit rheumatoider Arthritis (RA) bzw. juveniler idiopathischer Arthritis (JIA). Doch die Metaanalyse deckte einen Zusammenhang insgesamt auf zwischen RA und *miR-499 rs374644 C* (OR =1,123, 95%-KI =1,019–2,586, p=0,041) und die Stratifizierung nach Ethnizität eine besondere Assoziation im Nahostkollektiven (OR =1,943, 95%-KI =1,508–2,504, $p=2,7\times10^{-8}$). Die Metaanalyse von *IRAK1* –Polymorphismen zeigte eine Assoziation zwischen inflammatorischer Arthritis und dem *rs3027898 CC*-Genotyp (OR =2,602, 95%-KI =1,387–4,879, p=0,003). Eine Analyse unter Verwendung von „homozygote contrast" ergab das gleiche Muster für den *rs3027898 CC*-Genotyp (OR =2,472, 95%-KI =1,300–4,700, p=0,006). Keine Assoziation fand sich zwischen inflammatorischer Arthritis und dem *rs1059703*-Polymorphismus.
Fazit. Die Metaanalyse verweist auf einen Zusammenhang zwischen den *miR-499 rs374644-* und *IRAKI rs3027898*-Polymorphismen und der Suszeptibilität für inflammatorische Arthritis.

Schlüsselwörter

Rheumatoide Arthritis · Micro-RNA · MEDLINE · Ethnizität · Genotyp

JIA. All the studies except for one (which showed only allelic data of the polymorphisms) provided genotypic data of the polymorphisms. Eight studies examined the *rs2910164* (*miR-146a*) polymorphism, three the *rs3746444* (*miR-499*) polymorphism, four the *rs3027898* (*IRAK1*) polymorphism and three studies examined the *rs1059703* (*IRAK1*) polymorphism. A meta-analysis was thus performed on the *rs2910164*, *rs3746444*, *rs3027898* and *rs1059703* polymorphisms. Selected characteristics of these studies related to the association between *miR-146a*, *miR-499* and *IRAK1* polymorphisms and inflammatory arthritis diseases are summarized in **Tab. 1**.

Meta-analysis of the *miR-146a* and *miR-499* polymorphisms in inflammatory arthritis

A summary of the meta-analysis findings concerning the associations between the *miR-146a rs2910164* polymorphism and inflammatory arthritis is provided in **Tab. 2**. The meta-analysis revealed no association between inflammatory arthri-

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

Author (reference)	Country	Ethnicity	Disease	Subjects		Polymorphisms studied	Association findings
				Cases	Controls		
Singh, 2014 [11]	India	South Asia	JIA	150	216	*rs2910164 (miR-146a), rs3027898 (IRAK1), rs1059703 (IRAK1)*	*rs2910164 (p=0.042), rs3027898, rs1059703 (NS)*
Hashemi, 2013 [12]	Iran	Middle East	RA	104	110	*rs2910164 (miR-146a), rs3746444 (miR-499)*	*rs2910164 (NS), rs3746444 (p<0.0001)*
El-Shal, 2013 [13]	Egypt	Middle East	RA	217	245	*rs2910164 (miR-146a), rs3746444 (miR-499)*	*rs2910164 (NS), rs3746444 (p<0.001)*
Jimenez-Morales, 2012 [14]	Mexico	South America	JIA	208	531	*rs2910164 (miR-146a)*	NS
Qian, 2012 [18]	China	East Asia	RA	123	220	*rs2910164 (miR-146a)*	NS
Yang, 2011 [15]	China	East Asia	RA	208	240	*rs2910164 (miR-146a), rs3746444 (miR-499)*	NS
Chatzkyriakidou-1, 2010 [16]	Greece	Europe	RA	136	147	*rs2910164 (miR-146a), rs3027898 (IRAK1), rs1059703 (IRAK1)*	*rs2910164 (NS), rs3027898 (p=0.017), rs1059703 (NS)*
Chatzkyriakidou-2, 2010 [17]	Greece	Europe	PsA	29	66	*rs2910164 (miR-146a), rs3027898 (IRAK1), rs1059703 (IRAK1)*	*rs2910164 (NS), rs3027898 (p=0.003), rs1059703 (NS)*
Chatzkyriakidou-3, 2010 [17]	Greece	Europe	AS	49	66	*rs3027898 (IRAK1)*	*rs3027898 (p<0.001)*

JIA juvenile idiopathic arthritis, *RA* rheumatoid arthritis, *PsA* psoriatic arthritis, *AS* ankylosing spondylitis, *NS* not significant.

Tab. 2 Meta-analysis of the association between the *miR-146a rs2910164* polymorphism and inflammatory arthritis

Polymorphism	Population	No. of studies	Test of association			Test of heterogeneity		
			OR	95% CI	p-value	Model	p-value	I^2
rs2910164 C vs. G	Overall	8	0.974	0.810–1.091	0.650	F	0.391	5.03
	Europe	2	1.015	0.739–1.404	0.930	F	0.579	0
	Middle East	2	0.813	0.645–1.024	0.079	F	0.131	56.1
	East Asia	2	1.030	0.839–1.269	0.768	F	0.866	0
	RA	5	0.933	0.809–1.269	0.241	F	0.330	13.2
	JIA	2	1.037	0.854–1.258	0.716	F	0.189	39.4
CC vs. CG + GG (recessive)	Overall	8	0.990	0.820–1.196	0.919	F	0.363	8.67
	Europe	2	0.748	0.363–1.540	0.430	F	0.970	0
	Middle East	2	0.782	0.552–1.107	0.165	F	0.706	0
	East Asia	2	1.036	0.772–1.390	0.813	F	0.772	0
	RA	5	0.909	0.732–1.130	0.391	F	0.755	0
	JIA	2	1.355	0.909-0.019	0.136	F	0.125	61.9
CC + CG vs. GG (dominant)	Overall	8	0.951	0.799–1.132	0.572	F	0.200	28.6
	Europe	2	1.127	0.746–1.704	0.569	F	0.423	0
	Middle East	2	0.694	0.251–1.917	0.481	R	0.016	82.7
	East Asia	2	1.054	0.694–1.59	0.807	F	0.457	0
	RA	5	0.909	0.638–1.296	0.598	r	0.095	49.3
	JIA	2	0.938	0.727–1.210	0.622	F	0.412	0
CC vs. GG	Overall	8	0.952	0.670–1.353	0.784	R	0.092	42.9
	Europe	2	0.819	0.389–1.727	0.601	F	0.843	0
	Middle East	2	0.511	0.290–0.900	0.020	F	0.120	58.5
	Middle East in HWE	1	0.998	0.361–2.760	0.997	NA	NA	NA
	East Asia	2	1.062	0.676–1.671	0.793	F	0.637	0
	RA	5	0.796	0.574–1.125	0.173	F	0.162	38.9
	JIA	2	1.398	0.657–2.977	0.385	R	0.094	64.2

F fixed effects model, *R* random effects model, *OR* odds ratio, *CI* confidence interval, *RA* Rheumatoid arthritis, *JIA* Juvenile inflammatory arthritis, *HWE* Hardy–Weinberg equilibrium.

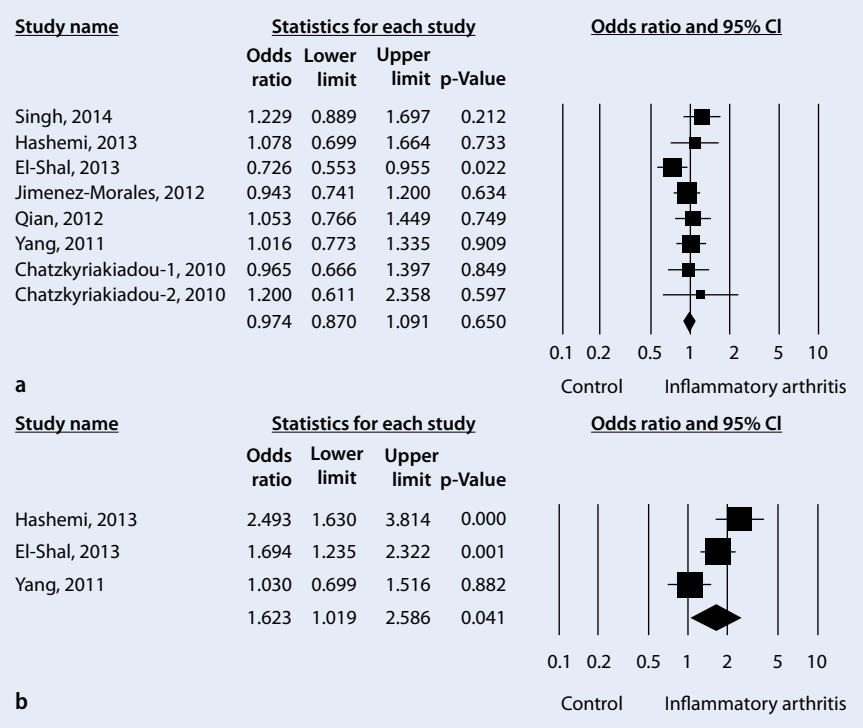

Fig. 2 ▲ Odds ratios and 95% confidence intervals (Cis) of individual studies and pooled data for the allelic association between the *miR-146ars2910614* (**a**) and *rs3746444* (**b**) polymorphisms and inflammatory arthritis in all subjects

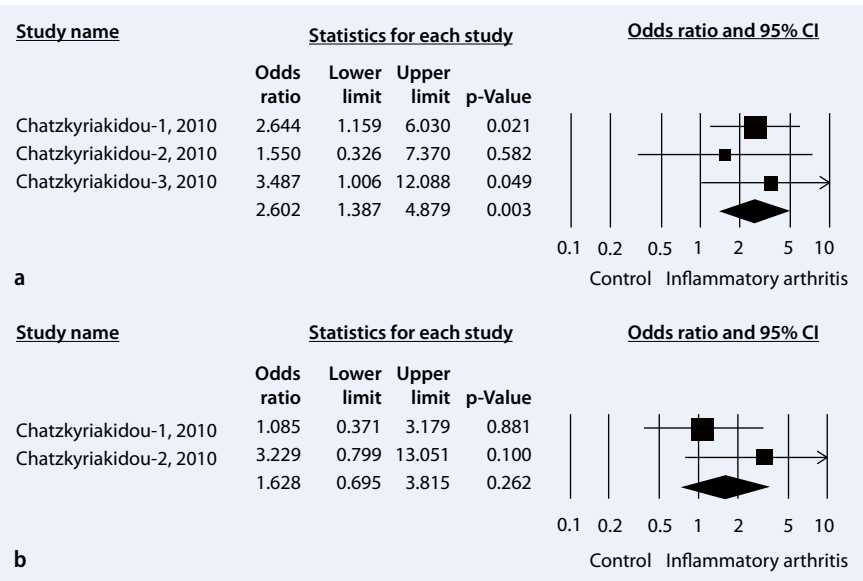

Fig. 3 ▲ Odds ratios and 95% confidence intervals (CIs) of individual studies and pooled data for the association between the *rs3027898 CC* (**a**) and *rs1059703 CC* (**b**) genotypes of the *IRAK1* polymorphism and inflammatory arthritis in a European population

tis and the *rs2910164 C* allele (OR =0.974, 95% CI =0.810–1.091, p=0.650; **Fig. 2**, **Tab. 2**). Stratification by ethnicity indicated no association between the *miR-146a C allele* and inflammatory arthritis in European, Middle Eastern or Asian populations (**Tab. 2**). The disease-specific meta-analysis revealed no association between the *miR-146a C* allele and RA or JIA (**Tab. 2**). Furthermore, using the homozygote contrast, no association was found between the *miR-146a* polymorphism and inflammatory arthritis (**Tab. 2**). The meta-analysis revealed an association between RA and the *miR-499 rs374644 C* allele overall (OR =1.123, 95% CI =1.019–2.586, p=0.041; **Fig. 2**, **Tab. 3**). Stratification by ethnicity revealed an association between and the *rs374644 C* allele and RA in Middle Eastern populations (OR =1.943, 95% CI =1.508–2.504, $p=2.7\times10^{-8}$; **Tab. 3**). Analysis using the recessive model and homozygote contrast showed the same pattern for the *rs374644 C* allele (**Tab. 3**).

Meta-analysis of the *rs3027898* and *rs1059703 IRAK1* polymorphisms and inflammatory arthritis

Meta-analysis revealed an association between inflammatory arthritis and the *rs3027898 CC* genotype of *IRAK1* (OR =2.602, 95% CI =1.387–4.879, p=0.003; **Fig. 3**, **Tab. 4**). Analysis using the homozygote contrast showed the same pattern for the *rs3027898 CC* genotype (OR =2.472, 95% CI =1.300–4.700, p=0.006; **Tab. 4**). However, an association between inflammatory arthritis and the *rs1059703* polymorphism was not found by meta-analysis using allele contrast, the recessive or dominant models, or homozygote contrast (**Tab. 4**).

Heterogeneity and publication bias

Interstudy heterogeneity was found regarding the relationship between the *miR-146a* and *IRAK1* polymorphisms; however, there was no heterogeneity within each ethnic group. The distribution of miR-146a polymorphism genotypes within the control groups were consistent with HW expectations, except for in two studies on the *rs2910164* [13, 17] and one study on the *rs374644* polymorphism [12], which suggests there may have been bias in terms of control selection or genotyping errors. When the studies in which controls were not in equilibrium were removed from the analyses, there was no association between the *rs2910164* polymorphism and inflammatory arthritis in Middle Eastern populations under homozygote contrast (**Tab. 2**). However, one study on *rs3746444* in which the controls were not out of HWE showed a significant associa-

Tab. 3 Meta-analysis of the association between the *miR-146a rs3746444* polymorphism and rheumatoid arthritis

Polymorphism	Population	No. of studies	Test of association			Test of heterogeneity		
			OR	95% CI	*p*-value	Model	p-value	I^2
rs3746444 C vs. T	Overall	3	1.123	1.019–2.586	0.041	R	0.010	78.5
	Overall in HWE	2	1.338	0.822–2.177	0.241	R	0.051	73.8
	Middle East	2	1.943	1.508–2.504	2.7×10^{-8}	F	0.152	51.2
	Middle East in HWE	1	1.694	1.235–2.322	0.001	NA	NA	NA
CC vs. CT + TT (recessive)	Overall	3	2.158	1.274–3.653	0.004	F	0.507	0
	Overall in HWE	2	1.603	0.776–3.314	0.203	F	0.964	0
	Middle East	2	2.317	1.284–4.183	0.005	F	0.297	7.88
	Middle East in HWE	1	1.582	0.624–4.008	0.334	NA	NA	NA
CC + CT vs. TT (dominant)	Overall	3	1.680	0.962–2.934	0.068	R	0.010	78.1
	Overall in HWE	2	1.392	0.6932.796	0.352	R	0.016	82.8
	Middle East	2	2.149	1.572–2.938	1.6×10^{-7}	F	0.424	0
	Middle East in HWE	1	1.971	1.350–2.877	4.4×10^{-5}	NA	NA	NA
CC vs. TT	Overall	3	2.576	1.503–4.415	0.001	F	0.407	0
	Overall in HWE	2	1.830	0.889–13.85	0.100	R	0.756	0
	Middle East	2	2.926	1.596–5.378	0.001	F	0.319	0
	Middle East in HWE	1	2.032	0.793–5.210	0.140	NA	NA	NA

F fixed effects model, *R* random effects model, *OR* odds ratio, *CI* confidence interval, *HWE* Hardy–Weinberg equilibrium, *NA* not applicable.

Tab. 4 Meta-analysis of the association between the *IRAK1* polymorphism and inflammatory arthritis

Polymorphism	Population	No. of studies	Test of association			Test of heterogeneity		
			OR	95% CI	p-value	Model	p-value	I^2
rs3027898 C vs. A	Overall	4	1.071	0.713–1.609	0.742	R	0.003	65.5
	Europe	3	1.336	0.937–1.791	0.053	F	0.209	36.1
CC vs. CA + AA (recessive)	Overall	3	2.602	1.387–4.879	0.003	F	0.727	0
	Europe	3	2.602	1.387–4.879	0.003	F	0.727	0
CC + CA vs. AA (dominant)	Overall	3	0.955	0.436–2.092	0.908	R	0.027	72.3
	Europe	3	0.955	0.436–2.092	0.908	R	0.027	72.3
CC vs. AA	Overall	3	2.472	1.300–4.700	0.006	F	0.838	0
	Europe	3	2.472	1.300–4.700	0.006	F	0.838	0
rs1059703 C vs. T	Overall	3	1.057	0.837–1.335	0.643	F	0.560	0
	Europe	2	1.209	0.853–1.712	0.286	F	0.733	0
CC vs. CT + TT (recessive)	Overall	2	1.628	0.695–3.815	0.262	F	0.225	32.0
	Europe	2	1.628	0.695–3.815	0.262	F	0.225	32.0
CC + CT vs. TT (dominant)	Overall	2	1.180	0.772–1.805	0.445	F	0.206	37.5
	Europe	2	1.180	0.772–1.805	0.445	F	0.206	37.5
CC vs. TT	Overall	2	1.59	0.671–3.781	0.292	F	0.433	0
	Europe	2	1.59	0.671–3.781	0.292	F	0.433	0

F fixed effects model, *R* random effects model, *OR* odds ratio, *CI* confidence interval.

tion in Middle Eastern populations under the allele contrast and dominant models (after excluding a study in which controls were not in HWE; **Tab. 3**). It was difficult to correlate the funnel plot, which is usually used to detect publication bias, because the number of studies included in the analysis was relatively small. Egger's regression test showed no evidence of publication bias (Egger's regression test p-values >0.1).

Discussion

Genetic factors are thought to contribute to inflammatory arthritis and this has encouraged researchers to search for the genes responsible for these diseases. By regulating the activity of immunoregulatory cells and decreasing immune responses, miRNAs play important roles in the pathogenesis of inflammatory diseases [4]. Therefore,

polymorphisms in miRNAs or their target genes could affect immune responses and lead to autoimmune and inflammatory diseases.

Many genes have been studied in this context and studying miRNAs and their target genes will help us to understand the genetic basis of inflammatory arthritis [25].

The present study addresses the association between polymorphisms in *miR-146a*, *miR-499* and *IRAK1* and susceptibil-

ity to inflammatory arthritis. This meta-analysis revealed no association between inflammatory arthritis and the *miR-146a rs2910164* polymorphism. Stratification by ethnicity or disease type revealed no association between the *rs2910164* polymorphism and inflammatory arthritis in European, Middle Eastern or Asian populations, or between this polymorphism and RA or JIA. There was also no association found between inflammatory arthritis and the *IRAK1 rs1059703* polymorphism. However, meta-analysis revealed an association between RA and the *miR-499 rs374644* polymorphism. In addition, the meta-analysis revealed an association between inflammatory arthritis and the *IRAK1 rs3027898* polymorphism.

Our meta-analysis indicated associations between the *miR-499 rs374644* and the *IRAK1 rs3027898* polymorphisms and inflammatory arthritis.

miR-499 targets IL-17 receptor B and IL-6, which both play key roles in the pathogenesis of inflammatory arthritis [26]. IL-17 is a proinflammatory cytokine which induces expression of *TNF-α* and *IL-6* and is overexpressed in the synovium of patients with inflammatory arthritis [26]. Therefore, there is a possibility that the *miR-499 rs374644* polymorphism increases susceptibility to inflammatory arthritis by affecting *IL-17* expression: *rs374644* is located in the mature miRNA region and may affect target mRNA binding, which could alter protein expression. miR499 is not only expressed in immune cells, but also by medullary thymic epithelial cells and could, as such, have an impact on central T cell tolerance and subsequently on development of disease [27]. Fekete et al. [28] showed that IRAK1 plays a crucial role in chronic inflammation and the *IRAK1 rs3027898* polymorphism lies in the 3′ untranslated region where miRNAs act; however, the functional significance of the polymorphism is unknown. There is also a possibility that the polymorphisms may be in linkage disequilibrium (LD) with nearby causal variants.

Our meta-analysis failed to find an association between the risk of developing inflammatory arthritis and the *miR-146a rs2910164* polymorphism. Jazdzewski et al. [8] showed that the *miR-146a rs2910164* polymorphism could reduce mature *miR-146a* expression and affect target mRNA binding. The *GG* genotype of the *miR-146a* polymorphism confers a higher expression level of mature miR-146a. Our finding is not consistent with the functional study. However, it remains unknown whether *rs2910164* has a functional significance, because mixed results have been reported. We also cannot rule out the possibility that the lack of association may be due the small number of studies used, their low statistical power, or type II errors.

The present study has some limitations that require consideration. Firstly, heterogeneity and confounding factors may have distorted the analysis. In particular, publication bias could have affected our findings, because studies that produced negative results may not have been published or may have been missed. Secondly, our ethnicity-specific meta-analysis included data from European, Middle Eastern and East Asian patients, and our results are thus only applicable to these ethnic groups. Further studies are required in different ethnic populations. Thirdly, we did not stratify and analyze factors such as gender or clinical and environmental variables due to lack of data and the polymorphisms may be associated with clinical manifestations in addition to disease susceptibility. Fourthly, study and subject numbers in the disease-type subgroup analysis were relatively small and, as such, our analysis may have been underpowered. Therefore, additional studies are warranted to explore the associations between inflammatory arthritis and the miRNA polymorphisms.

Conclusion

This meta-analysis of published studies suggests that the *miR-499 rs3746444* and *IRAK1 rs3027898* polymorphisms are associated with susceptibility to inflammatory arthritis. However, the *miR-146a rs2910164* and *IRAK1 rs1059703* polymorphisms were not associated with susceptibility to inflammatory arthritis. Further studies are warranted to clarify the role of miRNA genes in the pathogenesis of inflammatory arthritis in different ethnic groups, since miRNA polymorphisms may play different roles in different ethnic populations.

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Compliance with ethical guidelines

Conflict of interest. G.G. Song, S.-C. Bae, Y. H. Seo, J.-H. Kim, S. J. Choi, J. D. Ji and Y.Ho. Lee state that there are no conflicts of interest.

The accompanying manuscript does not include studies on humans or animals.

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