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Original Article

MicroRNA-146a and its target gene IRAK1 polymorphisms confer susceptibility to systemic lupus erythematosus

Zulvikar Syambani Ulhaq[a,*], Evi Harwiati Ningrum[b], Leonel Eslatkin Aguilar Zambrano[c], Alvi Milliana[a], Christian Peinado Garcia[d]

[a] Department of Biomedical Science, Faculty of Medicine and Health Sciences, Maulana Malik Ibrahim State Islamic University of Malang, Malang, Indonesia
[b] School of Nursing, Faculty of Medicine, University of Brawijaya, Malang, Indonesia
[c] Medical School, Technical University of Manabi, Portoviejo, Ecuador
[d] Department of General Surgery, Weston General Hospital, Weston-super Mare, Somerset, United Kingdom

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ABSTRACT

Objective: To clarify the association between *miR-146a*, *miR-499*, and *IRAK1* polymorphism and systemic lupus erythematosus (SLE) predisposition.

Methods: A literature search was conducted until 12 September 2020 in accordance with the PRISMA guidelines. The keywords "*miRNA-146a*", "*miRNA-499*", "*IRAK1*" and "SLE" were used in combination to obtain case-control studies evaluating the abovementioned gene polymorphism and the risk of SLE.

Results: Patients harbouring C allele of *miRNA-146a* rs2431697 exhibited low SLE risk (CC vs. TC + TT, OR = .77, 95% CI = .62–.95, $p = .019$; TC vs. CC + TT, OR = .84, 95% CI = .71–.98, $p = .027$; and TC vs. TT, OR = .73, 95% CI = .61–.86, $p = .000$), whereas patients carrying the A allele and AA genotype of rs3027898 in *IRAK1* had significantly decreased SLE susceptibility (A vs. C, OR = .73, 95% CI = .60–.87, $p = .001$; AA vs. CA + CC, OR = .64, 95% CI = .42–.97, $p = .037$; AA + CA vs. CC, OR = .71, 95% CI = .56–.88, $p = .003$, and AA vs. CC, OR = .49, 95% CI = .31–.77, $p = .002$). No association was observed between *miRNA-146a* rs2910164 and *miRNA-499* rs3746444 with SLE risk.

Conclusion: This study demonstrates associations between *miRNA-146a* and *IRAK1* polymorphisms with SLE risk. Larger studies on these associations are needed in the future to support our results.

* *Corresponding author.*
E-mail address: zulhaq@kedokteran.uin-malang.ac.id (Z.S. Ulhaq).

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El *microARN-146a* y sus polimorfismos del gen diana *IRAK1* confieren susceptibilidad al lupus eritematoso sistémico

Palabras clave:
miRNA-146a
miRNA-499
IRAK1
Lupus eritematoso sistémico
Predisposición
Susceptibilidad

RESUMEN

Objetivo: Aclarar la asociación entre el polimorfismo de *miR-146a, miR-499* e *IRAK1* y la predisposición al lupus eritematoso sistémico (LES).

Métodos: Se realizó una búsqueda bibliográfica hasta el 12 de septiembre del 2020, de acuerdo con las guías PRISMA. Las palabras clave «*miRNA-146*[a]», «*miRNA-499*», «*IRAK1*» y «LES» se utilizaron en combinación para obtener estudios de casos y controles que evaluaran el polimorfismo de los genes antes mencionados y el riesgo de LES.

Resultados: Los pacientes que albergan el alelo C del *miRNA-146a* rs2431697 mostraron un bajo riesgo de LES (CC frente a TC + TT; OR = 0,77; IC 95% = 0,62-0,95; $p = 0,019$; TC frente a CC + TT; OR = 0,84; IC 95% = 0,71-0,98; $p = 0,027$; y TC vs. TT; OR = 0,73; IC 95% = 0,61-0,86; $p = 0,000$), mientras que los pacientes portadores del alelo A y el genotipo AA de rs3027898 en *IRAK1* redujeron significativamente la susceptibilidad al LES (A vs. C; OR = 0,73; IC 95% = 0,60-0,87; $p = 0,001$; AA vs. CA + CC; OR = 0,64; IC 95% = 0,42-0,97; $p = 0,037$; AA + CA vs. CC; OR = 0,71; IC 95% = 0,56-0,88; $p = 0,003$ y AA vs. CC; OR = 0,49; IC 95% = 0,31-0,77; $p = 0,002$). No se observó asociación entre *miRNA-146a* rs2910164 o *miRNA-499* rs3746444 con riesgo de LES.

Conclusión: Este estudio demuestra asociaciones entre los polimorfismos de *miRNA-146a* e *IRAK1* y el riesgo de LES. En el futuro se necesitan estudios más amplios sobre estas asociaciones para garantizar nuestros resultados.

Introduction

Systemic lupus erythematosus (SLE) is a typical chronic autoimmune disease with a wide range of clinical manifestations and is characterized by the production of autoantibodies against nuclear self-antigens.[1,2] Although various autoantibodies production may be observed in SLE individuals, the antinuclear antibody (ANA)[3] is commonly used as a serological marker to diagnose SLE. The etiopathogenesis of SLE is incompletely known. Nonetheless, genetic factors seem to play a pivotal part in disease susceptibility.

MicroRNAs (miRNAs) are highly conserved endogenous non-coding small RNA molecules and regulate the expression of protein-coding genes by either translational repression or messenger RNA degradation.[4] Among these, *miR-146a* and *miR-499*, are two widely reported genes that significantly contribute to autoimmune diseases through their functional roles in modulating both innate and adaptive immune responses.[5–8] Therefore, both miRNAs could potentially responsible to the development and progression of SLE.

Interleukin (IL)-1 receptor-associated kinase (IRAK1) and tumour necrosis factor (TNF) receptor-associated factor 6 (TRAF6) have been reported as targets of *miRNA-146a*,[9] whereas *miRNA-499* targets several factors including IL-17 receptor B (IL-17RB),[10,11] IL-23a,[12] IL-2R,[12] IL-6,[12,13] IL-2,[12] IL-18R,[12] and peptidyl arginine deiminase type 4 (PADI4),[14] in which all of these inflammatory mediators play crucial role in progressing the pathogenesis of autoimmune diseases, including SLE.

Sequence variations observed in miRNAs have been implicated to influence the disease outcome.[3] This is possibly because single nucleotide polymorphisms (SNPs) within miRNAs region may alter their expression and functions,[3,12] which then resulting in differential regulation of its target protein. Functional SNPs in both *miR-146a* and *miR-499* genes have been evaluated in SLE patients from different populations.[12,15,16] However, the results remain inconclusive. Therefore, this study aims to assess the relationship between *miR-146a*, *miR-499* and *IRAK1* polymorphism and the risk of SLE.

Methods

Meta-analysis was performed in accordance to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. Records were identified through electronic databases dated up to September 2020 with search terms such as "*miRNA-146a*", "*miRNA-499*", "*IRAK1*" and "SLE". Studies were included on the basis of the following criteria: (1) aims to evaluate the association between gene polymorphism with predisposition to SLE; (2) conducted with a case-control design.

Meta-analysis for each gene polymorphism was performed for two or more studies. Genotypic frequency was tested for deviation from the Hardy–Weinberg equilibrium (HWE) in the control subjects. The association between gene polymorphism with predisposition to SLE was calculated by pooled odds ratio (OR) and 95% confidence interval (CI). The Z test was used to evaluate the significance of the pooled effect size. Study heterogeneity was evaluated using Q test and I^2 statistic. A significant Q-statistic ($p < 0.10$) indicated heterogeneity across studies, with substantial heterogeneity indicated by

an I^2 value over 50%. The fixed-effect model (FEM) was used in the absence of heterogeneity, while the random-effect model (REM) was implemented if heterogeneity was present. A funnel plot and Begg's test were used to investigate the publication bias if the pooled effect size consisted of 10 or more studies. The value of 0.05 was indicative of the statistical significance. The Newcastle–Ottawa scale (NOS) was used to assess the study quality, in which a score ≥7 is considered a good study.[17–34]

Results

A total of 37 articles were screened, among which 27 were reviewed. Sixteen studies were excluded due to their absent relation to *miRNA-146a*, *miRNA-499*, and *IRAK1* polymorphisms or the inability to extract the data. Eight studies were then included in this meta-analysis[3,5,12,15,16,35–37] (Fig. 1). A total of 5237 (SLE: 2226, control 3011), 2642 (SLE: 1174, control: 1468), 709 (SLE: 263; control: 446), and 1534 (SLE: 747; control: 787) subjects for *miRNA-146a* rs2910164, *miRNA-146a* rs2431697, *miRNA-499* rs3746444, and *IRAK1* rs3027898 polymorphisms, respectively, were further analyzed. All studies complied with the HWE (Table 1). Details of the retrieved studies are depicted in Table 1.

Pooled results on the associations between *miRNA-146a*, *miRNA-499*, and *IRAK1* polymorphisms with SLE are shown in Table 2. We failed to show any significant association between *miRNA-146a* rs2910164 and *miRNA-499* rs3746444 in any inheritance models (Table 2). However, we found that patients harbouring C allele of *miRNA-146a* rs2431697 were significantly lowered SLE predisposition (CC vs. TC + TT, OR = 0.77, 95% CI = 0.62–0.95, $p = 0.019$; TC vs. CC + TT, OR = 0.84, 95% CI = 0.71–0.98, $p = 0.027$; and TC vs. TT, OR = 0.73, 95% CI = 0.61–0.86, $p = 0.000$, Table 2). In addition, *IRAK1*, target of *miRNA-146a*, was also examined. Patients with the A allele and AA genotype of rs3027898 were significantly decreased SLE susceptibility compare to the C allele and CC genotype, respectively (A vs. C, OR = 0.73, 95% CI = 0.60–0.87, $p = 0.001$; AA vs. CA + CC, OR = 0.64, 95% CI = 0.42–0.97, $p = 0.037$; AA + CA vs. CC, OR = 0.71, 95% CI = 0.56–0.88, $p = 0.003$, and AA vs. CC, OR = 0.49, 95% CI = 0.31–0.77, $p = 0.002$, Table 2). Whereas no significant difference between the AC and AA genotype (Table 2). No evidence of publication bias was observed in any models in each gene polymorphisms (Table 2). Because we failed to show any association between *miRNA-499* with SLE risk, genetic polymorphism of miRNA-499 target was not further evaluated.

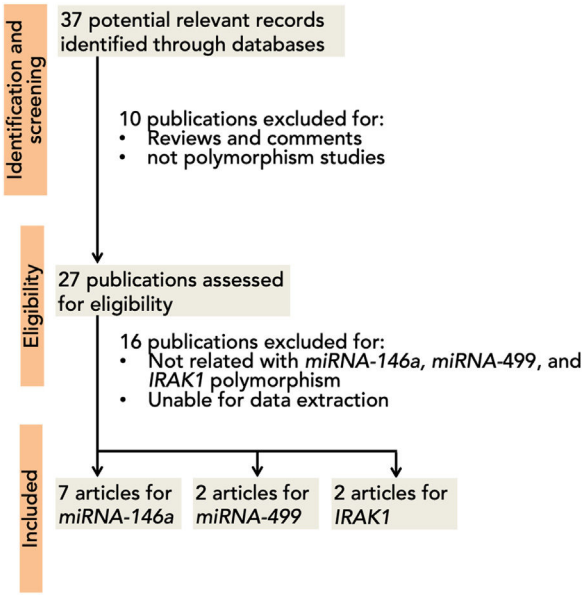

Fig. 1 – PRISMA flow diagram of study selection process for the association between *miR-146a*, *miR-499* and *IRAK1* polymorphism and the risk of SLE.

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

Study	Country	Ethnicity	Numbers		Genotype frequency						NOS	*p*-HWE
			SLE	Control	SLE			Control				
miRNA-146a rs2910164					GG	GC	CC	GG	GC	CC		
Zhang et al.	China	Asian	213	209	33	101	79	23	104	82	9	0.239
Löfgren et al.	Sweden	European	1109	1428	623	422	64	819	531	78	9	0.502
Jimenez-Morales et al.	Mexico	Mexican	367	531	163	167	37	236	229	66	9	0.369
Labib et al.	Egypt	Egyptian	80	120	32	40	8	26	56	38	8	0.530
Aleman-Avilla et al.	Mexico	Mexican	407	486	168	179	60	218	222	46	9	0.327
Ahmadi et al.	Iran	Asian	50	237	28	18	4	113	98	28	8	0.344
miRNA-146a rs2431697					TT	TC	CC	TT	TC	CC		
Löfgren et al.	Sweden	European	1109	1428	423	524	157	434	718	240	9	0.052
Fouda et al.	Egypt	Egyptian	65	40	25	29	11	6	22	12	8	0.428
miRNA-499 rs3746444					TT	TC	CC	TT	TC	CC		
Zhang et al.	China	Asian	213	209	157	50	6	158	45	6	9	0.214
Ahmadi et al.	Iran	Asian	50	237	27	23	0	145	83	9	8	0.495
IRAK1 rs3027898					CC	CA	AA	CC	CA	AA		
Labib et al.	Egypt	Egyptian	80	120	28	24	28	14	32	30	8	0.300
Zhai et al.	China	Asian	667	667	437	167	21	421	202	40	9	0.040

Table 2 – Meta-analysis of the associations between the *miRNA-146a*, *miRNA-499*, *IRAK1* polymorphisms and SLE.

Genetic model	Ethnicity	No. of studies	Test of association				Test of heterogeneity		Publication bias (*p*-value)
			OR	95% CI	*p*-value	Model	*p*-value	I^2 (%)	
miRNA-146a rs2910164									
C vs. G	Overall	6	0.88	0.72–1.09	0.264	REM	0.000	77.31	0.100
	Asian	2	0.83	0.65–1.06	0.141	FEM	0.574	0	NA
	Mexican	2	1.07	0.84–1.37	0.557	REM	0.082	66.93	NA
CC vs. GC + GG	Overall	6	0.85	0.57–1.26	0.431	REM	0.001	74.07	0.188
	Asian	2	0.87	0.60–1.27	0.494	FEM	0.576	0	NA
	Mexican	2	1.14	0.55–2.36	0.712	REM	0.014	83.35	NA
CC + GC vs. GG	Overall	6	0.90	0.72–1.12	0.348	REM	0.031	59.11	0.045
	Asian	2	0.68	0.45–1.04	0.079	FEM	0.918	0	NA
	Mexican	2	1.07	0.89–1.30	0.442	FEM	0.452	0	NA
GC vs. CC + GG	Overall	6	1.01	0.90–1.13	0.821	FEM	0.878	0	0.460
	Asian	2	0.88	0.63–1.22	0.452	FEM	0.754	0	NA
	Mexican	2	1.01	0.83–1.22	0.892	FEM	0.390	0	NA
CC vs. GG	Overall	6	0.76	0.46–1.24	0.277	REM	0.000	78.33	0.113
	Asian	2	0.64	0.37–1.11	0.115	FEM	0.815	0	NA
	Mexican	2	1.17	0.57–2.41	0.660	REM	0.021	81.21	NA
CC vs. GC	Overall	6	0.90	0.63–1.29	0.593	REM	0.015	64.21	0.252
	Asian	2	0.96	0.65–1.42	0.858	FEM	0.699	0	NA
	Mexican	2	1.11	0.53–2.31	0.763	REM	0.019	81.75	NA
GC vs. GG	Overall	6	0.99	0.88–1.12	0.964	FEM	0.348	10.49	0.016
	Asian	2	0.70	0.45–1.09	0.120	FEM	0.840	0	NA
	Mexican	2	1.05	0.86–1.28	0.624	FEM	0.964	0	NA
miRNA-146a rs2431697									
C vs. T	Overall	2	0.67	0.40–1.10	0.115	REM	0.072	68.97	NA
CC vs. TC + TT	Overall	2	0.77	0.62–0.95	**0.019**	FEM	0.293	9.28	NA
CC + TC vs. TT	Overall	2	0.52	0.21–1.26	0.149	REM	0.066	70.21	NA
TC vs. CC + TT	Overall	2	0.84	0.71–0.98	**0.027**	FEM	0.540	0	NA
CC vs. TT	Overall	2	0.45	0.15–1.28	0.137	REM	0.076	68.16	NA
CC vs. TC	Overall	2	0.88	0.70–1.10	0.285	FEM	0.623	0	NA
TC vs. TT	Overall	2	0.73	0.61–0.86	**0.000**	FEM	0.112	60.32	NA
miRNA-499 rs3746444									
C vs. T	Overall	2	1.08	0.79–1.48	0.595	FEM	0.945	0	NA
CC vs. TC + TT	Overall	2	0.80	0.27–2.33	0.691	FEM	0.368	0	NA
CC + TC vs. TT	Overall	2	1.18	0.82–1.68	0.363	FEM	0.613	0	NA
TC vs. CC + TT	Overall	2	1.26	0.87–1.82	0.211	FEM	0.376	0	NA
CC vs. TT	Overall	2	0.84	0.28–2.45	0.752	FEM	0.415	0	NA
CC vs. TC	Overall	2	0.71	0.23–2.16	0.550	FEM	0.323	0	NA
TC vs. TT	Overall	2	1.23	0.85–1.78	0.256	FEM	0.466	0	NA
IRAK1 rs3027898									
A vs. C	Overall	2	0.73	0.60–0.87	**0.001**	FEM	0.602	0	NA
AA vs. CA + CC	Overall	2	0.64	0.42–0.97	**0.037**	FEM	0.328	0	NA
AA + CA vs. CC	Overall	2	0.71	0.56–0.88	**0.003**	FEM	0.143	53.32	NA
CA vs. AA + CC	Overall	2	0.80	0.63–1.00	0.053	FEM	0.336	0	NA
AA vs. CC	Overall	2	0.49	0.31–0.77	**0.002**	FEM	0.873	0	NA
AA vs. CA	Overall	2	0.81	0.51–1.27	0.372	FEM	0.157	50.17	NA
CA vs. CC	Overall	2	0.61	0.30–1 .23	0.170	REM	0.089	65.52	NA

Bold text indicates a statistically significant difference with a *p*-value less than 0.05.

Discussion

Our current study addresses the possible association between the *miR-146a*, *miRNA-499*, and *IRAK1* polymorphism and its susceptibility to SLE. We found that the individuals carrying the C and A allele of rs2431697 and rs3027898, respectively, displayed protection against the occurrence of SLE, thereby implying that both miR-146a and its target protein, IRAK1, are crucial in the pathogenesis of SLE. Similar to previously reported studies[38–41]; our updated meta-analysis strengthens the notion that the *miR-146a* rs2910164 was not responsible for SLE predisposition.

Functional analysis revealed that numerous SNPs within *miR-146a* have been shown to affect the expression level of mature miR-146a.[16] For example, the T allele of rs2431697 decreases matures miR-146a expression.[16] Downregulation of human-miR-146a is associated to the manifestations of SLE by deregulating the activation of the interferon (IFN) pathway.[42,43] This may be due to several reason such as the

decreasing expression of miR-146a and subsequent upregulation of IRAK1 and nuclear factor-κB (NF-κB) that may stimulate transcription of several genes-related inflammatory responses.[44] Hence, it suggests the presence of inverse relationship between miR-146a expression level and SLE. Moreover, a study also identified that the biological axis between miR-146a and TRAF6 is closely linked to the SLE progression.[45] Additionally, mutant or alternative type (T allele) carriers of rs2431697 evidently increased the coronary artery disease risk, but not the severity of the disease.[46] Compiling to our findings, this implies that the rs2431697 is important in determining SLE susceptibility. Further studies investigating the effect of rs2431697 and SLE progression are required.

Although functional analysis of rs3027898 in *IRAK1* is currently unavailable,[47] it is possible to speculate that the C allele may contribute to the upregulation of IRAK1. Indeed, hyperactivation of IRAK1 in $CD4^+$ T cells was observed in SLE individuals.[48] While we failed to show any association between rs2910164 with SLE predisposition, other polymorphism such as rs57095329 in *miR-146a* is correlated with SLE.[40,39] Luo et al.[43] have successfully demonstrated the attenuation of miR-146 expression is partly influenced by low binding capability to Ets-1 due to conformational changes in the promoter region of rs57095329.

Association between *miRNA*-499 polymorphism with SLE was also evaluated, but no association was observed. Interestingly, however, patients harbouring the T allele of rs3746444 had low RA risk.[39] Additionally, Lu et al.[49] demonstrated that rs3746444 polymorphism is closely linked to RA susceptibility in Mediterranean populations. Nonetheless, further studies are still required to determine whether the rs3746444 polymorphisms contribute to SLE susceptibility in different ethnic groups.

The present study has some limitations. Firstly, some of the pooled studies were generated from the limited number of included studies, thus the result may not be precise. Secondly, methodological variation of SNP identification and the opposite findings between studies may influence the results obtained in this particular study. Thirdly, because SLE is a complex disease, other factors, including, genetics, hormonal, environmental, and immune response/status should be closely examined.

In conclusion, our study demonstrates the association between *miRNA-146a* and *IRAK1* polymorphisms with SLE risk. Further investigations evaluating functional analysis of *miRNA-146a* and *IRAK1* in individuals with SLE and controls are necessary to clarify the role of the microRNA and its target gene in the etiology of SLE.

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Conflicts of interest

None to declare.

Appendix A. Supplementary material

The Spanish translation of this article is available as supplementary material at doi:10.1016/j.rcreu.2021.05.007.

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