Systematic Review and Meta-Analysis

Medicine

OPEN

Association between microRNA-146a rs2910164 polymorphism and coronary heart disease

An updated meta-analysis

Qinxue Bao, MD[a,*], Rui Li, BD[a], Chengfeng Wang, BD[a], Shan Wang, BD[a], Minli Cheng, BD[a], Chunhua Pu, MD[a], Lei Zou, BD[a], Chao Liu, BD[a]

Abstract

Background: Coronary heart disease (CHD) is one of the manifestations of atherosclerosis with a high morbidity rate. MicroRNA (miRNA)-146a rs2910164, a single nucleotide polymorphism, is associated with the progression of CHD risk. However, the results are controversial and uncertain. Therefore, an updated meta-analysis was conducted to evaluate the association between rs2910164 and CHD susceptibility.

Methods: PubMed, Cochrane Library, EMBASE, Web of Science, China's National Knowledge Infrastructure, VIP, and Wan fang were searched for the eligible articles until April 30, 2022. The odds ratios (ORs) with 95% confidence interval (CIs) were calculated to assess the correlation. Bonferroni correction was utilized between multiple comparisons. Trial sequential analysis was performed to measure the required information size and assess the reliability of the meta-analysis results.

Results: A total of 18 eligible studies, including 6859 cases and 8469 controls, were analyzed in our meta-analysis. After Bonferroni correction, we found that the G allele at rs2910164 was associated with significantly decreased CHD risk in the allelic model (OR = 0.86), homozygous model (OR = 0.79), and heterozygous model (OR = 0.89) in total population. In the subgroup analysis, the subjects containing the G allele and GG genotype were associated with a lower risk of CHD in the Chinese population, not the GG + CG and CG genotype. In addition, under the allelic, homozygous, heterozygous, and dominant models, miR-146a rs2910164 was at lower CHD risk in the large size population except in the recessive model.

Conclusion: These results show that miR-146a rs2910164 might be associated with lower CHD susceptibility.

Abbreviations: CAD = coronary artery disease, CHD = coronary heart disease, CI = confidence interval, HWE = Hardy-Weinberg equilibrium, IRAK-1 = interleukin-1 receptor-associated kinase 1, miRNA = microRNA, NF-κB = nuclear factor Kappa B, RIS = required information size, SNP = single nucleotide polymorphism, TRAF-6 = tumor necrosis factor receptor-associated factor 6, TSA = trial sequential analysis.

Keywords: coronary heart disease, miR-146a rs2910164, polymorphism

1. Introduction

Coronary heart disease (CHD) is a worldwide chronic complex disease with high morbidity and mortality caused by genetic and environmental factors.[1,2] Although we have many advances to diagnose and predict the prognosis of CHD, some new and feasible ways need to be explored to meet the requirement of clinical work. According to epidemiological studies, many risk factors, including smoking, diabetes, hypertension, and genetic variations, are involved in the pathological progress of CHD.[3–5] With the development of genomics and proteomics, many new candidate biomarkers have emerged to diagnose and predict CHD.

MicroRNA (miRNA) is a set of short non-coding RNA that could negatively regulate mRNA's translation. The length of MiRNA is approximately 18 to 25 nucleotides, and it could bind with several target genes.[6,7] Moreover, one target gene also could bind with several miRNAs to affect the process of mRNA translation. Studies indicate that many pathophysiological procedures for different diseases, such as cancer, hypertension, stroke, diabetes, and CHD, consist of various cellular pathways.[8,9] One of the vital mechanisms is miRNA regulation.[10,11] Studies show that miR-146a is involved in the process of CHD.[12,13] For instance, a previous study showed that miR-146a expression was higher in the coronary artery disease (CAD) group.[14] Furthermore, treatments with angiotensin II receptor blockers and statin could suppress the level of miR-146a and toll-like receptor 4 signal pathway, which might be the molecular mechanism concerning the anti-atherosclerosis

The authors have no funding and conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

[a] Department of Cardiology, Dayi County People's Hospital, Chengdu, Sichuan, China.

**Correspondence: Qinxue Bao, Department of Cardiology, Dayi County People's Hospital, Chengdu 611330, Sichuan, China (e-mail: baoqinxue_1989@163.com).*

How to cite this article: Bao Q, Li R, Wang C, Wang S, Cheng M, Pu C, Zou L, Liu C. Association between microRNA-146a rs2910164 polymorphism and coronary heart disease: An updated meta-analysis. Medicine 2022;101:46(e31860).

Received: 4 July 2022 / Received in final form: 25 October 2022 / Accepted: 26 October 2022

http://dx.doi.org/10.1097/MD.0000000000031860

of angiotensin II receptor blockers and statin in CAD patients. Other studies demonstrated that miR-146a was likely associated with the development of atherosclerosis.[15,16]

Several studies have detected the association between miR-146s rs2910164 polymorphisms and CHD.[17] However, due to the small samples, findings from different groups showed contradictory results that miR-146a rs2910164 might increase the risk of CHD or not be associated with CHD.[18,19] Xu Liu et al have demonstrated that rs2910164 polymorphism of miR-146a was significantly associated with CHD risk.[20] Therefore, we obtained the present meta-analysis referring to 18 case-control studies to assess the relationship between miR146a rs2910164 polymorphisms and CHD risk. Our meta-analysis is an updated meta-analysis with a larger sample size explored to examine further the association (6859 cases and 8469 controls).

2. Materials and methods

2.1. Publication search strategy

We processed a systematic search by Chengfeng Wang, Shan Wang, and Chao Liu independently using the database including PubMed, Cochrane Library, EMBASE, Web of Science, China's National Knowledge Infrastructure, VIP, and Wan Fang until April 30, 2022. The keywords were as follows: ("microRNA-146a" OR "miRNA-146a" OR "miR-146a" OR "rs2910164") AND ("polymorphism" OR "polymorphisms" OR "single nucleotide polymorphism" OR "SNP" OR "variant" OR "variants" OR "variation" OR "genotype" OR "genetic" OR "mutation") AND ("coronary heart disease" OR "CHD" OR "coronary artery disease" OR "CAD" OR "acute myocardial infarction" OR "ACS" OR "myocardial infarction" OR "MI" OR "acute myocardial infarction" OR "AMI" OR "cardiovascular disease" OR "ischemic heart disease" OR "IHD"). Chengfeng Wang, Shan Wang, and Chao Liu also manually examined the reference lists within the eligible studies to figure out additional involved research. The meta-analysis was based on previously published studies; thus, no ethical approval and patient consent were required.

2.2. Inclusion and exclusion criteria

All the eligible studies should match the inclusion criteria: Case-control design; Evaluation concerning miR-146 rs29010164 and CHD; The genotype of the control should accord with the Hardy-Weinberg equilibrium (HWE); The study has sufficient data for present statistics; Languages including Chinese and English. The exclusion criteria were as follows: case report, review, meta-analysis, repeat publication, abstract, letter, animal model, or mechanism research. Moreover, studies that were not conforming to the inclusion criteria should be excluded.

2.3. Data extraction and quality evaluation

Two investigators (Chunhua Pu and Lei Zou) performed the data extraction independently, which consisted of the name of the first author, published year, country of the participants, genotyping methods, tissue, numbers of cases and controls, genotype (GG/CG/CC) frequencies of cases and controls, allele(C/G) frequencies of case and control, and HWE of *P*-value in controls as shown in Table 1. We evaluated the quality of the studies with the software Revman5.4. During data extraction and quality assessment, Qinxue Bao participated in discussions when Chunhua Pu and Lei Zou encountered discrepancies.

2.4. Statistical analysis

To assess the association between miR-146a rs2910164 polymorphism and CHD risk, the review manager (Rveman5.4, Cochrane Collaboration, London, UK) performed the statistical analysis by Qinxue Bao and Minli Cheng. HWE of the control in each study was calculated by X^2-test. When the *P*-value was < 0.05 was considered a disequilibrium of the control group. The odds ratios (ORs) with 95% confidence intervals (CIs) were applied to evaluate the association between miR-146a rs2910164 polymorphism and susceptibility of CHD. There were 5 genetic models, including the allelic model (G vs C), homozygous model (GG vs CC), heterozygous model (CG vs CC), dominant model (GG + CG vs CC), and recessive model (GG vs CG + CC) in the current study. The pooled odds ratios were assessed via *Z*-test, defined as significant with $P < .01(0.05/5)$ after Bonferroni correction. *I*-square statistical test was applied to evaluate the heterogeneity between studies. The random-effect model was employed when $I^2 > 50\%$ using the Mantel-Haenszel method, which indicates evident heterogeneity in our included study.[21] Otherwise, the fixed-effect model was used. Begg's funnel plot was utilized to estimate the publication bias.

2.5. Trial sequential analysis (TSA)

Because of random error or lack of statistical accuracy and power, the results of meta-analysis might acquire false results, defined as type I errors (false-positive errors) and type II errors (false-negative errors). Consequently, Qinxue Bao did the trial sequential analysis (TSA) to evaluate whether cumulative data were sufficiently powerful to draw the conclusion. The meta-analysis results were used to set the incidence in control group and the relative risk reduction. We calculated the required information size (RIS) by TSA 0.9.5.10 Beta (Copenhagen Trial Unit, Centre for Clinical Intervention Research, Denmark.), using $\alpha = 0.05$(2-sided) and $\beta = 0.20$(a power of 80%) to reach a reliable consequence.[22–24]

2.6. Target prediction and enrichment analysis

To determine the possible function of miR-146a, we exploited the target scan human 8.0[25](https://www.targetscan.org/vert_80/) to predict the target gene and then conducted the enrichment analysis via WEB-based GEne SeT AnaLysis Toolkit[26] (Web Geatalt, http://www.webgestalt.org/option.php#). Moreover, we also analyzed the relationship between miR-146a rs2910164 polymorphism and disease by miRNASNPv3[27] (http://bioinfo.life.hust.edu.cn/miRNASNP/#!/).

3. Results

3.1. The features of the eligible articles

Figure 1 shows the entire screening process of our study. A total of 1191 studies were acquired from PubMed, Cochrane Library, EMBASE, Web of Science, China's national knowledge infrastructure, VIP, and Wan Fang databases. Among them, 203 duplicates were precluded from the current study. Another 988 studies were screened according to the titles and abstracts. An amount of 43 alternative articles were evaluated through a full-text review. These 24 articles were excluded because of unavailable data and duplicate data. Ultimately, 18 articles in our study contained 6859 cases and 8469 controls. The features of the original studies are exhibited in Table 1.

3.2. Results of the meta-analysis

The results of the current meta-analysis for the association between miR-146a rs2910164 polymorphism and CHD risk are shown in Table 2 and Figure 2.

We gained 6859 cases and 8469 controls from 18 eligible studies. The G allele at rs2910164 was associated with significantly decreased CHD risk under the allelic model

Table 1

Main characteristics of included studies in our meta-analysis.

Author	Year	Country	Genotyping methods	Tissue	Samples size		GG		CG		CC		G		C		HWE of *P*-value in control
					Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	
Wu Qi	2022	China	RT-PCR	Venous blood	92	100	10	28	37	52	45	20	57	108	127	92	$P > .05$
Agiannitopoulos	2020	Greece	PCR-RFLP, HRM,Sanger Sequencing	Peripheral blood leukocyts	200	200	91	101	95	84	14	15	277	286	123	114	.6657
Mir	2020	Indian	ARMS-PCR	Peripheral blood	100	100	11	5	51	40	38	55	73	50	127	150	$P > .05$
Zhang Linxun	2020	China	RT-PCR	Venous blood	100	100	14	28	64	52	22	20	92	108	108	92	$P > .05$
Qiu	2019	China	SNPscan	Venous blood	505	1109	60	154	246	516	194	436	366	824	634	1368	.946
Manuel	2018	Mexico	TaqMan	Peripheral blood	218	595	116	277	85	267	17	51	317	821	119	369	$P > .05$
SHRESTHA	2018	China	PCR-RFLP	Peripheral blood	295	253	47	52	164	112	84	89	258	216	332	290	$P > .05$
Wang	2017	China	MALDI-TOF MS	Peripheral blood	353	368	62	84	155	179	136	105	279	347	427	389	.645
Bastami	2016	Iran	TaqMan	Peripheral whole blood	300	300	111	150	155	128	34	22	377	428	223	172	.5718
Sung	2016	Republic of Korea	PCR-RFLP	Peripheral blood leukocytes	522	535	77	73	242	260	203	202	227	406	648	664	.46
Huang	2015	China	TaqMan	Peripheral blood	722	721	143	132	308	348	266	237	594	612	840	822	.83
Chen	2014	China	PCR-LDR	Peripheral blood leukocytes	919	889	269	301	463	435	187	153	1001	1037	837	741	$P > .05$
Hamann	2014	Germany	HRM	Whole blood	206	200	120	117	74	73	12	10	314	307	98	93	.748
Xiong	2014	China	PCR-RFLP	Peripheral whole blood	295	283	41	61	141	125	113	97	223	247	367	319	≥.05
Chen Lin	2013	China	TaqMan	Whole blood	658	658	181	194	305	330	172	134	667	718	649	598	$P > .05$
Ramkaran	2013	South Africa	PCR–RFLP	Whole blood	106	100	50	45	43	46	13	9	143	136	69	64	.8501
Yang Ying	2012	China	TaqMan	Peripheral blood leukocytes	853	948	165	189	392	457	272	271	722	835	936	999	$P > .05$
Li Ling	2010	China	TaqMan	Venous blood	415	1010	82	210	184	455	149	345	348	875	482	1145	0.186

ARMS-PCR = Amplification Refractory Mutation System polymerase chain reaction, HRM = high-resolution melting, HWE = Hardy-Weinberg equilibrium, MALDI-TOF MS = matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, PCR-LDR = polymerase chain reaction-ligation detection reaction, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, RT-PCR = reverse transcription-polymerase chain reaction, SBI = silent brain infarction.

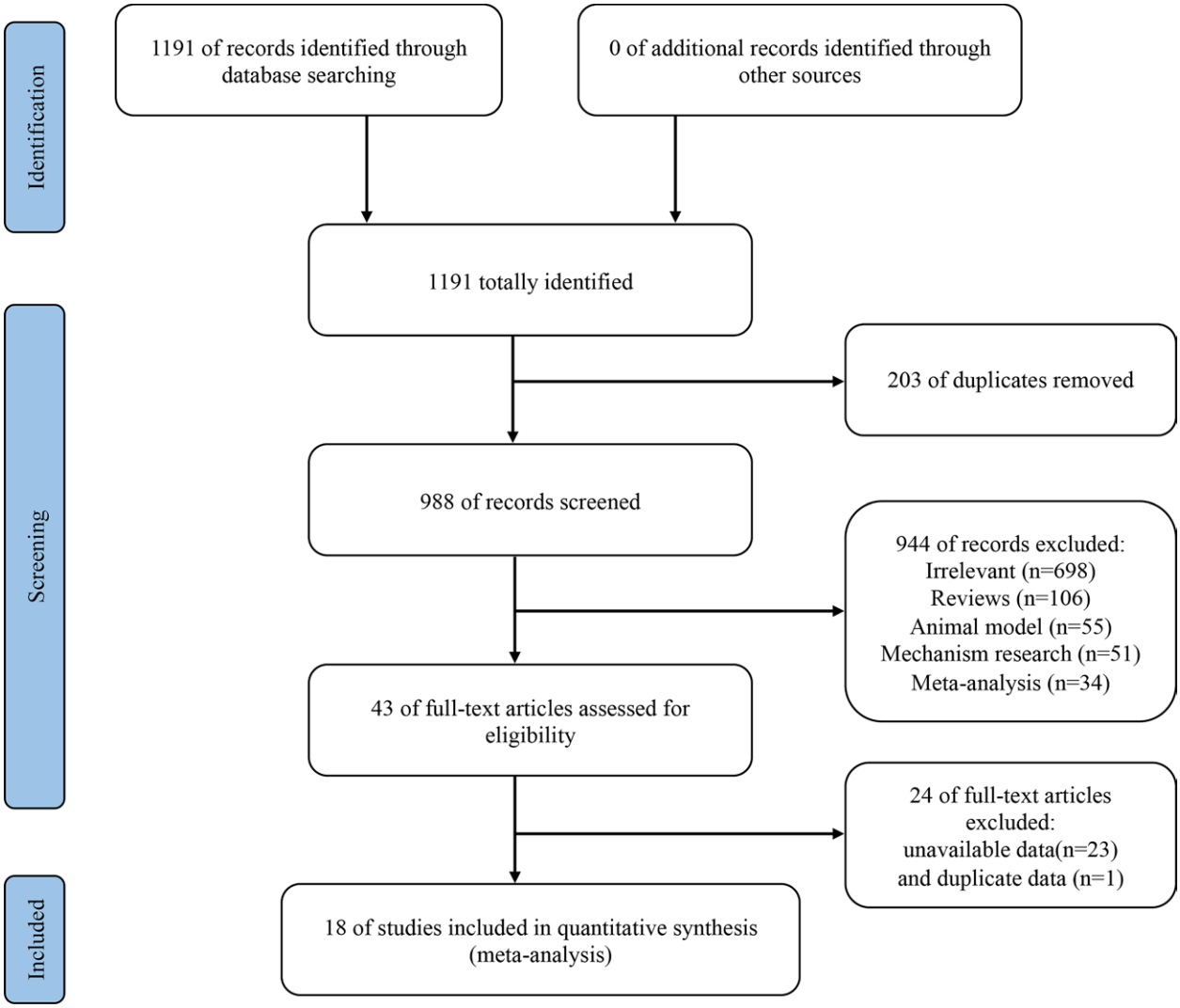

Figure 1. Flow chart of the screening process of our study.

(OR = 0.86, 95% CI = 0.78-0.95, P = .003), homozygous model (OR = 0.79, 95% CI = 0.67-0.92, P = .003), heterozygous model (OR = 0.89, 95% CI = 0.83-0.97, P = .005), dominant model (OR = 0.87, 95% CI = 0.76-0.99, P = .03), and recessive model (OR = 0.86, 95% CI = 0.76-0.97, P = .02) in total population(P < .05). However, under the dominant and recessive model, the association between miR-146a rs2910164 polymorphism and CHD risk did not accomplish statistical significance after Bonferroni correction (P < .01). There were obvious heterogeneities under all genetic models. Then we performed a subgroup analysis by ethnicity and sample size.

We did the subgroup analysis based on ethnicity within 11 studies with about 5207 cases and 6439 controls. The G allele at rs2910164 was related to meaningfully reduce the risk of CHD under the allelic model (OR = 0.86, 95% CI = 0.78-0.94, P = .0009), homozygous model (OR = 0.74, 95% CI = 0.62-0.88, P = .0007), dominant model (OR = 0.83, 95% CI = 0.72-0.96, P = .01) and recessive model (OR = 0.82, 95% CI = 0.71-0.94, P = .004) in Chinese population. Besides, the significance did not remain under the dominant model after the Bonferroni correction. However, we did not observe the association of miR-146a rs2910164 with CHD risk under the heterozygous model (OR = 0.87, 95% CI = 0.75-0.1.01, P = .07).

Significant heterogeneities were also observed among all genetic models in the Chinese population. Therefore, we conducted another subgroup analysis based on sample size. In the large sample size, we did not examine significant heterogeneities in the homozygous model (I^2 = 0%), heterozygous model (I^2 = 4%), dominant model (I^2 = 0%), and recessive model (I^2 = 0%). At the same time, there was obvious heterogeneity in the allelic model (I^2 = 73%). Besides, when the P value was Bonferroni corrected, the pooled data suggested that miR-146a rs2910164 was associated with significantly decreased CHD risk under the allelic model (OR = 0.86, 95% CI = 0.77-0.96, P = .009), homozygous model (OR = 0.85, 95% CI = 0.76-0.96, P = .007), heterozygous model (OR = 0.88, 95% CI = 0.80-0.97, P = .008), and dominant model (OR = 0.88, 95% CI = 0.80-0.96, P = .003). Whereas miR-146a rs2910164 had no remarkable relationship with CHD risk under recessive model (OR = 0.93, 95% CI = 0.84-1.02, P = .12).

3.3. The bias of the publication and sensitivity analysis

In our meta-analysis, Begg's funnel plot was performed to explore the possible bias of the publication. Taking the heterozygous model in the whole population as an example, the Begg's funnel plots showed no marked asymmetry, as shown in Figure 3, meaning there was no significant publication bias risk. After omitting one article at once, there was no prominent effect on the result in the sensitivity analysis, suggesting that our meta-analysis's present finds were reliable.

Table 2

Association between miR-146a rs2910164 polymorphism with coronary heart disease (CHD).

Genotype comparison	Whole/Subgroup	n	OR [95% CI]	Z (*P* value)	Heterogeneity of study design		
					χ^2	df (*P* value)	I^2 (%)
Allelic model (G vs. C)	Whole	18	0.86 [0.78, 0.95]	2.95 (.003*)	64.54	17 (<.00001)	74%
	Chinese	11	0.86 [0.78, 0.94]	3.31(.0009*)	25.70	10 (.004)	61%
	Other	7	0.93 [0.70, 1.22]	0.56(.58)	37.96	6 (<.00001)	84%
	Large size	7	0.86 [0.77, 0.96]	2.63(.009*)	22.24	6 (.001)	73%
	Small size	11	0.86 [0.72, 1.04]	1.58(.11)	42.19	10 (< .00001)	76%
homozygous model (GG *vs.* CC)	Whole	18	0.79 [0.67, 0.92]	2.97 (.003*)	35.68	17 (.005)	52%
	Chinese	11	0.74 [0.62, 0.88]	3.40(.0007*)	22.34	10 (.01)	55%
	Other	7	0.96 [0.68, 1.37]	0.21(.83)	10.93	6 (.09)	45%
	Large size	7	0.85 [0.76, 0.96]	2.68(.007*)	4.35	6 (.63)	0%
	Small size	11	0.70 [0.50, 0.98]	2.07(.04)	27.68	10 (.002)	64%
heterozygous model (CG *vs.* CC)	Whole	18	0.89 [0.83, 0.97]	2.78(.005*)	34.09	17 (.008)	50%
	Chinese	11	0.87 [0.75, 1.01]	1.80(.07)	26.67	10 (.003)	63%
	Other	7	0.99 [0.80, 1.21]	0.14(.89)	6.37	6 (.38)	6%
	Large size	7	0.88 [0.80, 0.97]	2.67(.008*)	6.24	6 (.40)	4%
	Small size	11	0.92 [0.70, 1.22]	0.55(.58)	27.40	10 (.002)	64%
dominant model (GG + CG *vs.* CC)	Whole	18	0.87 [0.76, 0.99]	2.18 (.03)	39.86	17 (.001)	57%
	Chinese	11	0.83 [0.72, 0.96]	2.52(.01)	28.12	10 (.002)	64%
	Other	7	1.00 [0.76, 1.31]	0.00(1.00)	9.54	6 (.15)	37%
	Large size	7	0.88 [0.80, 0.96]	2.99(.003*)	5.44	6 (.49)	0%
	Small size	11	0.85 [0.63, 1.15]	1.06(.29)	34.37	10 (.0002)	71%
recessive model (GG *vs.* CG + CC)	Whole	18	0.86 [0.76, 0.97]	2.36 (.02)	38.04	17 (.002)	55%
	Chinese	11	0.82 [0.71, 0.94]	2.88(.004*)	19.71	10 (.03)	49%
	Other	7	0.99 [0.76, 1.28]	0.10 (.92)	16.48	6 (.01)	64%
	Large size	7	0.93 [0.84, 1.02]	1.55 (.12)	4.86	6 (.56)	0%
	Small size	11	0.77 [0.61, 0.98]	2.10 (.04)	30.05	10 (.0008)	67%

Other (Caucasian, Indian, Korean, Mexico).

95% CI = 95% confidence interval, CHD = coronary heart disease, CI = confidence interval.

* Survived the Bonferroni correction.

3.4. Results of TSA

The results of TSA are shown in Figure 4. For miR-146a rs2910164 polymorphism and susceptibility to CHD in the total population, under the allelic model, homozygous model, and heterozygous model, the cumulative z-curve crossed the TSA boundary and the RIS, which represented that our results were robust and crucial (Fig. 4A, 4B, 4C). We could obtain the same tendency in the Chinese population under the allelic and homozygous models (Fig. 4D, 4E). Furthermore, in Figure 4F, the z-curve crossed the conventional test boundary ($z = 1.96$, $P < .05$) and did not intersect the RIS under the recessive model. Meanwhile, the z-curve was close to the TSA boundary, reflecting that the result might be a false positive. Thus, more studies might be required to certify this consequence further. As shown in Figures 4G, 4H, 4I, and 4J, the results of rs2910164 G versus C group, GG versus CC group, CG versus CC group, and GG + CG versus CC group in subgroup analysis based on sample size revealed that the z-curve did not cross the TSA boundary after reaching the conventional test boundary and the RIS, confirming that the conclusions, even with adequate sample size, were not robust enough.

3.5. Results of enrichment analysis

The results of the enrichment analysis exhibited in Table 3 that miR-146a was involved in many signaling pathways, such as the receptor activator of nuclear factor Kappa B (NF-κB) signaling pathway, which was implicated in cancer, inflammatory and autoimmune diseases, septic shock, and viral infections,[28] and epidermal growth factor receptor (EGFR) signaling pathway which might participate in cell growth, proliferation and differentiation.[29] As indicated in Table 3, several diseases, especially myocardial infarction, were associated with miR-146a. Table 4 exhibited that miR-146a rs2910164 polymorphism was also related to prostate, endometrium, lung, and central nervous system cancers.

4. Discussion

Numerous studies have proved that miR-146a is closely related to cardiovascular diseases. For instance, the expression of miR-146a could affect the proliferation, apoptosis, and migration of vascular smooth muscle cells (VSMC), taking effect on the progress of cardiovascular disease, including atherosclerosis.[30] Besides VSMC, miR-146a was highly expressed in the peripheral blood mononuclear cell (PBMC) of patients who suffered from acute coronary syndrome.[31] Meanwhile, the miR-146a could maintain the stability of atherosclerotic plaques by inhibiting the expression of interleukin-1 receptor-associated kinase 1 (IRAK-1) and tumor necrosis factor receptor-associated factor 6 (TRAF-6) in animal models.[32] Moreover, miR-146a was also meaningfully upgraded in patients' atherosclerotic plaques.[33]

MiR-146a precursor with C allele sequence could downgrade the level of mature miR-146a by influencing the secondary structure compared with the G allele sequence.[34] Then, reducing the miR-146a level would impact its target gene expression and interfere with some biomolecular processes. In consideration of miR-146a relating to cancer, neurological disorders, and cardiovascular diseases, we did this meta-analysis to explore the role of miR-146a on the susceptibility of CHD. In our current meta-analysis, we acquire that miR-146a rs2910164 carrying the G allele has lower CHD risk in the allelic model (OR = 0.86), homozygous model (OR = 0.79), heterozygous model (OR = 0.89) after Bonferroni correction in total population. From the subgroup analysis, the subjects containing the G allele and GG genotype are associated with a lower risk of CHD in the Chinese population, which is not observed in those carrying the GG + CG genotype and CG genotype. In the large sample size, we discover that miR-146a rs2910164 correlates with a lower risk of CHD under allelic, homozygote, heterozygous, and dominant models when the *P* value is Bonferroni corrected. Some previous meta-analyses were completed to explore the correlation of miR-146a

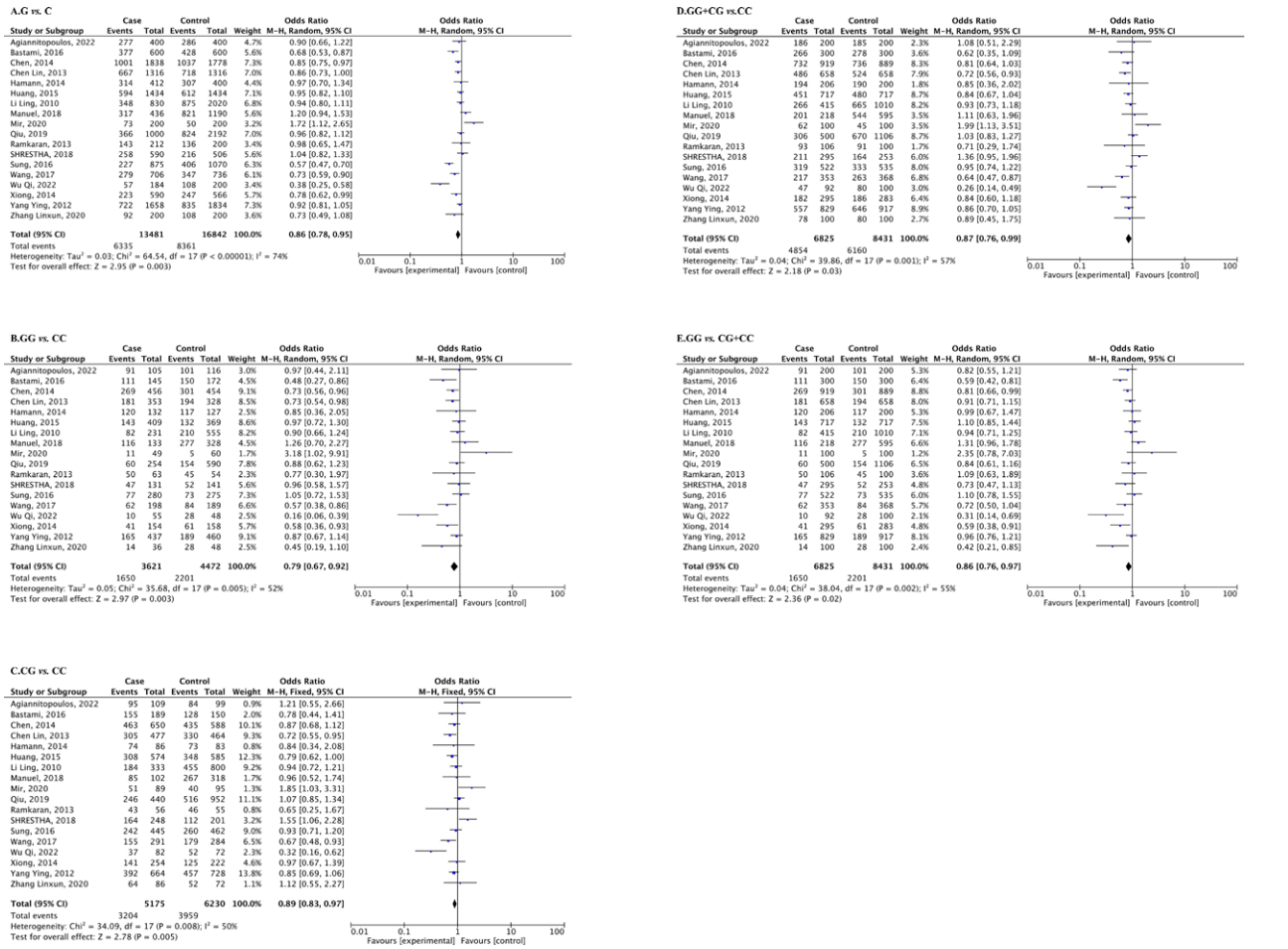

Figure 2. Forest plots of odds ratios for the association between microRNA-146a rs2910164 and coronary heart disease (CHD) risk in the whole population. (A) G *vs.* C; (B) GG *vs.* CC; (C) CG *vs*. CC; (D) GG + CG *vs*. CC; (E) GG *vs.* CG + CC. CHD = coronary heart disease.

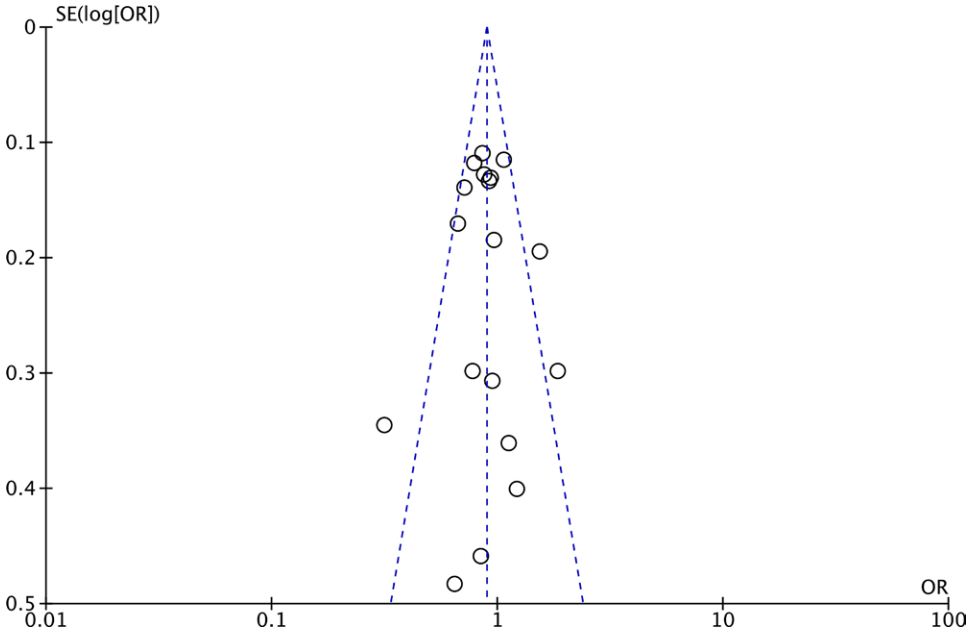

Figure 3. Begg's funnel plot of publication bias for the heterozygous model of miR-146a rs2910164 polymorphism in the whole population.

rs2910164 and susceptibility to CHD. However, the result was mutually contradictory. For example, Zhou et al found an opposite conclusion compared with ours that rs2910164 polymorphism was related to higher CAD risk.[35] The influence of

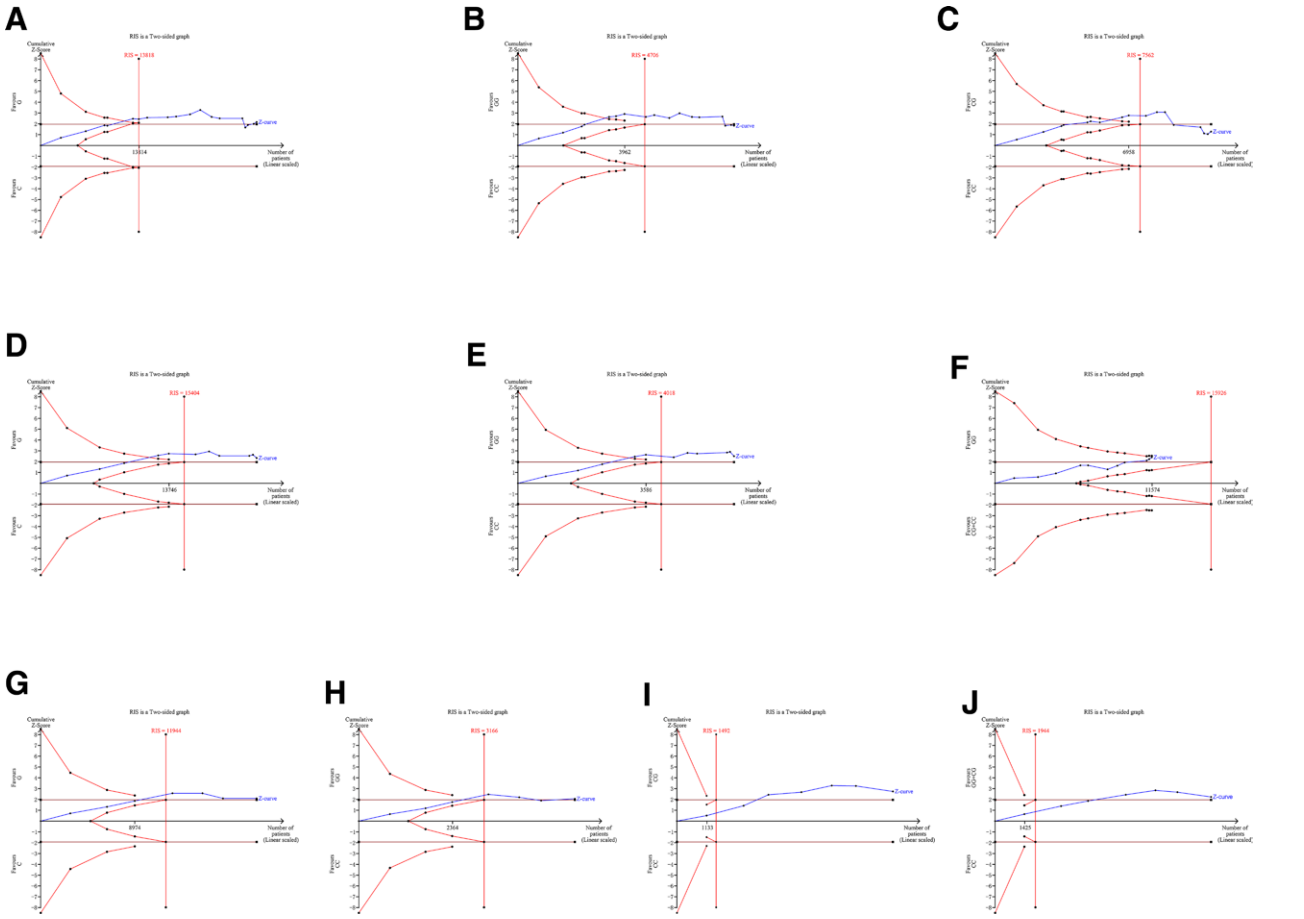

Figure 4. Trial sequential analysis (TSA) analysis for meta-analysis of miR-146a and coronary heart disease (CHD) risk in the total population under G *vs.* C (A), GG *vs.* CC (B), and CG *vs.* CC (C), in the Chinese population under G *vs.* C (D), GG *vs.* CC (E), and GG *vs.* CG + CC (F), as well as in large sample size under G *vs.* C (G), GG *vs*. CC (H), CG *vs.* CC (I), and GG + CG *vs.* CC (J). CHD = coronary heart disease, TSA = trial sequential analysis.

Table 3

Enrichment analysis of miR-146a about pathway and disease.

Pathway		Disease	
Description	***P* value**	**Description**	***P* value**
RANKL/RANK Signaling Pathway	.0016	myocardial infarction, susceptibility to myocardial infarction	2.432E-05
AGE/RAGE pathway	.0013	juvenile myelomonocytic leukemia	.0003387
BDNF signaling pathway	.0005	leukemia, acute myeloid	7.70E-09
TGF-beta Signaling Pathway	7E-05	mitochondrial complex I deficiency	.0003843
ERK Pathway in Huntington's Disease	.0012	lung canceralveolar cell carcinoma	1.083E-05
miR-509-3p alteration of YAP1/ECM axis	.001	tracheoesophageal fistula with or without esophageal atresia	4.254E-05
The effect of progerin on the involved genes in Hutchinson-Gilford Progeria Syndrome	.0003	pulmonary fibrosis, idiopathic	.0003387
EGF/ EGFR Signaling Pathway	.0001	pheochromocytoma	7.272E-05
ErbB Signaling Pathway	.0009	hypogonadotropic hypogonadism 7 with or without anosmia	2.852E-06
Estrogen signaling pathway	.0005	breast cancer	1.50E-09

AGE = advanced glycation end products, BDNF = brain-derived neurotrophic factor, ECM = extracellular matrix, EGF = epidermal growth factor, EGFR/ErbB = epidermal growth factor receptor, ERK = extracellular regulated protein kinases, RANKL = receptor activator of nuclear factor Kappa B ligand, RANK = receptor activator of nuclear factor Kappa B, RAGE = receptor of advanced glycation end products, TGF = transforming growth factor, YAP = yes-associated protein.

miR-146a rs2910164 polymorphism on disease risk was obviously nonuniform, which might be prompted by disease heterogeneity, sample size, and racial difference. For example, the frequency of the rs2910164 G allele in Europeans is 0.76868 and in Asians is 0.411, based on HapMap data (http://hapmap.ncbi.nlm.nih.gov/index.html.en).

Some risk factors, including genetic and environmental elements, inflammation, immunology, and others referring to atherosclerosis, interact to facilitate the formation of CHD.[36–38] Thus, anti-inflammation treatment might be one way to decrease the incidence and mortality of CHD. Recently, miR-146a was reported to be a potential regulator in many mechanisms referring to oxidative stress, metabolism, immunoreaction, inflammation et al, and several diseases like cerebrovascular diseases and cardiovascular diseases.[39,40] MiR-146a has multiple SNP sites involved in several signaling pathways that generate

Table 4

Disease-related variation in miR-146a.

Name	Mutation ID	Position	Ref/Alt	Disease	Region	Exp.	Source
hsa-mir-146a	COSN20075281	chr5:160485353	C/T	Prostate; carcinoma (PMID:26000489)	pre-miRNA	Up	COSMIC
hsa-mir-146a	COSN26984466	chr5:160485356	T/C	Large intestine; carcinoma (PMID:27149842)	pre-miRNA	Up	COSMIC
hsa-mir-146a	COSN20075271	chr5:160485365	T/A	Prostate; carcinoma (PMID:26000489)	pre-miRNA	Up	COSMIC
hsa-mir-146a	COSN20075268	chr5:160485366	C/A	Prostate; carcinoma (PMID:26000489)	pre-miRNA	Down	COSMIC
hsa-mir-146a	COSN1083746	chr5:160485375	G/T	Endometrium; carcinoma	Seed	Down	COSMIC
hsa-mir-146a	COSN8206750	chr5:160485375	G/T	Pancreas; carcinoma	Seed	Down	COSMIC
hsa-mir-146a	COSN24408986	chr5:160485394	G/T	Lung; carcinoma	pre-miRNA	Up	COSMIC
hsa-mir-146a	COSN28692950	chr5:160485411	C/G	Lung; carcinoma	Seed	Up	COSMIC
hsa-mir-146a	COSN1083745	chr5:160485413	G/T	Endometrium; carcinoma	Seed	Down	COSMIC
hsa-mir-146a	COSN8635147	chr5:160485429	G/C	Central nervous system; glioma	Mature	Down	COSMIC

COSMIC (https://cancer.sanger.ac.uk/cosmic).

Down = expression change decreases, miRNA = microRNA, Up = expression increases.

different functions in different diseases, including CHD.[41] Y. Zhu et al reported that one of the SNP sites, rs2910164, was associated with CHD.[42] We conducted the target gene prediction and enrichment analysis to explore the potential mechanism between miR-146a rs2910164 polymorphism and CHD. The results demonstrated that miR-146a might participate in epidermal growth factor receptor (EGFR), NF-κB, and transforming growth factor (TGF)-β signaling pathways, which regulated inflammation and immune response, including innate immune response. Previous studies have demonstrated that miR-146a was involved in regulating innate immune response.[43] Taking the NF-κB pathway as an example, miR-146a participated in the immune response through negative regulation of the target gene of miR-146a, IRAK1, and TRAF6.[44] Moreover, miR-146a coacted with NF-κB to take part in immune cell proliferation.[45] The miR-146a was also relevant to the pathophysiological processes of myocardial infarction and susceptibility to myocardial infarction. The promoter of the miR-146a gene had some NF-κB binding sites, which then induced the expression of interleukin-1b and tumor necrosis factor-alpha.[46] NF-κB participated in the inflammation process via IRAK-1 and tumor TRAF-6.[47] Ramkaran et al investigated that miR-146a was involved in inflammation by downregulating the expression of IRAK-1 and TRAF-6 in CAD patients.[48] They proposed that miR-146a might be a target to decelerate the inflammatory reaction in CHD. Therefore, we could speculate that rs2910164 might contribute to lower susceptibility to CHD by regulating downstream genes, specifically those involved in inflammation via the NF-κB signaling pathway. On the other side, miR-146a might be concerned with cancer development, like lung cancer, prostate cancer, and endometrial cancer.

Due to the following limitations, the results of our present meta-analysis should be discreetly interpreted. Our results of subgroup analysis based on race indicated that the differences in geographical regions and genotypic milieu might affect the consequences, meaning that genetic and environmental factors both participated in the pathophysiological process of CHD. The interactions between gene-gene and gene-environment could impact the role of the miR-146a rs2910164. When we evaluated the association between miR-146a and CHD risk based on sample size, the comparatively small number of patients might influence the conclusions. Therefore, further research with more sample size based on ethnicity, more detailed molecular mechanisms, and more clinical data, such as smoking, lifestyle, age, and sex, is needed to explore the potential function of rs2910164 in CHD patients. Fan et al investigated the correlation between Caveolin-1 polymorphism and the risk of urinary cancer through silico analysis and linkage disequilibrium analysis, which indicated how polymorphisms affected mRNA expression.[49] Some studies utilized the target gene expression between cancer tissue and matched normal tissue from several databases by silico analysis, which could better study the impact of polymorphisms on diseases.[50,51] One of the shortcomings of this study was the lack of proper research on the target gene of miR-146a. We only performed predictive analysis on miR-146a target genes. Thus, more research on the mechanism of the target gene is necessary, which is also our following research direction. Furthermore, this meta-analysis included published studies, which might lead to publication bias. And all included studies were retrospective research prone to information bias.

In conclusion, our meta-analysis indicated that miR-146a rs2910164 carrying the G allele might reduce the CHD risk. Consequently, we predicate that rs2910164 might be a potential factor that plays a protective role in the susceptibility of CHD.

Author contributions

Conceptualization: Qinxue Bao, Rui Li.
Data curation: Qinxue Bao, Chengfeng Wang, Shan Wang, Minli Cheng, Chunhua Pu, Lei Zou, Chao Liu.
Software: Minli Cheng, Chunhua Pu.
Supervision: Rui Li.
Writing – original draft: Qinxue Bao.
Writing – review & editing: Rui Li.

References

[1] Faroux L, Guimaraes L, Wintzer-Wehekind J, et al. Coronary artery disease and transcatheter aortic valve replacement: JACC state-of-the-art review. J Am Coll Cardiol. 2019;74:362–72.
[2] Tong Y, Wang Z, Cai L, et al. NLRP3 inflammasome and its central role in the cardiovascular diseases. Oxid Med Cell Longevity. 2020;2020:4293206.
[3] Chen G, Levy D. Contributions of the Framingham heart study to the epidemiology of coronary heart disease. JAMA Cardiol. 2016;1:825–30.
[4] Peikert A, Kaier K, Merz J, et al. Residual inflammatory risk in coronary heart disease: incidence of elevated high-sensitive CRP in a real-world cohort. Clin Res Cardiol. 2020;109:315–23.
[5] Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. Nat Med. 2000;6:389–95.
[6] Friedman RC, Farh KK, Burge CB, et al. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. 2009;19:92–105.
[7] Bartel DP. Metazoan MicroRNAs. Cell. 2018;173:20–51.
[8] Sharma AR, Sharma G, Bhattacharya M, et al. Circulating miRNA in atherosclerosis: a clinical biomarker and early diagnostic tool. Curr Mol Med. 2022;22:250–62.
[9] Nazarenko MS, Koroleva IA, Zarubin AA, et al. miRNA regulome in different atherosclerosis phenotypes. Mol Biol. 2022;56:166–81.
[10] Srivastava K, Tyagi K. Single nucleotide polymorphisms of microRNA in cardiovascular diseases. Clinica chimica acta; Int J Clin Chem. 2018;478:101–10.
[11] Treiber T, Treiber N, Meister G. Regulation of microRNA biogenesis and its crosstalk with other cellular pathways. Nat Rev Mol Cell Biol. 2019;20:5–20.
[12] Laggerbauer B, Engelhardt S. MicroRNAs as therapeutic targets in cardiovascular disease. J Clin Invest. 2022;132:e159179.

[13] Zhao D, Li Y, Yu X, et al. Associations between miR-146a rs2910164 polymorphisms and risk of ischemic cardio-cerebrovascular diseases. Medicine. 2019;98:e17106.
[14] Takahashi Y, Satoh M, Minami Y, et al. Expression of miR-146a/b is associated with the Toll-like receptor 4 signal in coronary artery disease: effect of renin-angiotensin system blockade and statins on miRNA-146a/b and Toll-like receptor 4 levels. Clin Sci (London, England: 1979). 2010;119:395–405.
[15] Liu H, Wang H, Ma J, et al. MicroRNA-146a-3p/HDAC1/KLF5/IKBα signal axis modulates plaque formation of atherosclerosis mice. Life Sci. 2021;284:119615.
[16] Zha L, Li S, Liu X, et al. Association of miR-146a Gene Polymorphism at loci rs2910164 G/C, rs57095329 A/G, and rs6864584 T/C with susceptibility to kawasaki disease in Chinese Children. Inflam Res. 2019;40:504–12.
[17] Bao MH, Xiao Y, Zhang QS, et al. Meta-analysis of miR-146a polymorphisms association with coronary artery diseases and ischemic stroke. Int J Mol Sci . 2015;16:14305–17.
[18] Li Q, Chen L, Chen D, et al. Influence of microRNA-related polymorphisms on clinical outcomes in coronary artery disease. Am J Trans Res. 2015;7:393–400.
[19] Bastami M, Ghaderian SM, Omrani MD, et al. MiRNA-Related Polymorphisms in miR-146a and TCF21 are associated with increased susceptibility to coronary artery disease in an Iranian population. Genet Test Mol Biomarkers. 2016;20:241–8.
[20] Liu X, You L, Zhou R, et al. Significant association between functional microRNA polymorphisms and coronary heart disease susceptibility: a comprehensive meta-analysis involving 16484 subjects. Oncotarget. 2017;8:5692–702.
[21] Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. Bmj. 2003;327:557–60.
[22] Meng J, Wang S, Zhang M, et al. TP73 G4C14-A4T14 polymorphism and cancer susceptibility: evidence from 36 case-control studies. Biosci Rep. 2018;38:BSR20181452.
[23] Wetterslev J, Thorlund K, Brok J, et al. Estimating required information size by quantifying diversity in random-effects model meta-analyses. BMC Med Res Methodol. 2009;9:86.
[24] Brok J, Thorlund K, Wetterslev J, et al. Apparently conclusive meta-analyses may be inconclusive--trial sequential analysis adjustment of random error risk due to repetitive testing of accumulating data in apparently conclusive neonatal meta-analyses. Int J Epidemiol. 2009;38:287–98.
[25] McGeary SE, Lin KS, Shi CY, et al. The biochemical basis of microRNA targeting efficacy. Science. 2019;366:eaav1741–eaav1741.
[26] Liao Y, Wang J, Jaehnig EJ, et al. Web gestalt 2019: gene set analysis toolkit with revamped UIs and APIs. Nucleic Acids Res. 2019;47:W199–205.
[27] Liu CJ, Fu X, Xia M, et al. miRNASNP-v3: a comprehensive database for SNPs and disease-related variations in miRNAs and miRNA targets. Nucleic Acids Res. 2021;49:D1276–81.
[28] Kumar Rajendran N, George BP, Chandran R, et al. The influence of light on reactive oxygen species and NF-κB in disease progression. Antioxidants (Basel). 2019;8:640.
[29] Burgess AW. Regulation of signaling from the epidermal growth factor family. J Phys Chem B. 2022;126:7475–85.
[30] Cao J, Zhang K, Zheng J, et al. MicroRNA-146a and -21 cooperate to regulate vascular smooth muscle cell proliferation via modulation of the Notch signaling pathway. Mol Med Rep. 2015;11:2889–95.
[31] Yao R, Ma Y, Du Y, et al. The altered expression of inflammation-related microRNAs with microRNA-155 expression correlates with Th17 differentiation in patients with acute coronary syndrome. Cellular Mol Immunol. 2011;8:486–95.
[32] Chu T, Xu X, Ruan Z, et al. miR-146a contributes to atherosclerotic plaque stability by regulating the expression of TRAF6 and IRAK-1. Mol Biol Rep. 2022;49:4205–16.
[33] Raitoharju E, Lyytikäinen LP, Levula M, et al. miR-21, miR-210, miR-34a, and miR-146a/b are up-regulated in human atherosclerotic plaques in the Tampere vascular study. Atherosclerosis. 2011;219:211–7.
[34] Ma XP, Zhang T, Peng B, et al. Association between microRNA polymorphisms and cancer risk based on the findings of 66 case-control studies. PLoS One. 2013;8:e79584.
[35] Zhou HY, Wei Q, Shi XD, et al. miR-146a rs2910164 polymorphism might be associated with coronary artery disease risk in Asians. Cell Mol Biol (Noisy-le-Grand, France). 2017;63:27–9.
[36] Dong Y, Chen H, Gao J, et al. Molecular machinery and interplay of apoptosis and autophagy in coronary heart disease. J Mol Cell Cardiol. 2019;136:27–41.
[37] Hansson GK, Hermansson A. The immune system in atherosclerosis. Nat Immunol. 2011;12:204–12.
[38] Bentzon JF, Otsuka F, Virmani R, et al. Mechanisms of plaque formation and rupture. Circ Res. 2014;114:1852–66.
[39] Xie X, Shi X, Xun X, et al. Association between microRNA polymorphisms and coronary heart disease: a meta-analysis. Herz. 2017;42:593–603.
[40] Roganović J. Downregulation of microRNA-146a in diabetes, obesity and hypertension may contribute to severe COVID-19. Med Hypotheses. 2021;146:110448.
[41] Paterson MR, Kriegel AJ. MiR-146a/b: a family with shared seeds and different roots. Physiol Genomics. 2017;49:243–52.
[42] Zhu Y, Zhu Y, Liu Y, et al. Long noncoding RNA metastasis-associated lung adenocarcinoma transcript 1 correlates with microRNA-125b/microRNA-146a/microRNA-203 and predicts 2-year restenosis risk in coronary heart disease patients. Biomark Med. 2021;15:257–71.
[43] Shukla A, Rastogi M, Singh SK. Zika virus NS1 suppresses the innate immune responses via miR-146a in human microglial cells. Int J Biol Macromol. 2021;193(Pt B):2290–6.
[44] Taganov KD, Boldin MP, Chang KJ, et al. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc Natl Acad Sci USA. 2006;103:12481–6.
[45] King JK, Tran TM, Paing MH, et al. Regulation of T-independent B-cell responses by microRNA-146a. Front Immunol. 2022;13:984302.
[46] Li L, Chen XP, Li YJ. MicroRNA-146a and human disease. Scand J Immunol. 2010;71:227–31.
[47] Mann M, Mehta A, Zhao JL, et al. An NF-κB-microRNA regulatory network tunes macrophage inflammatory responses. Nat Commun. 2017;8:851.
[48] Ramkaran P, Khan S, Phulukdaree A, et al. miR-146a polymorphism influences levels of miR-146a, IRAK-1, and TRAF-6 in young patients with coronary artery disease. Cell Biochem Biophys. 2014;68:259–66.
[49] Fan S, Meng J, Zhang L, et al. CAV1 polymorphisms rs1049334, rs1049337, rs7804372 might be the potential risk in tumorigenicity of urinary cancer: a systematic review and meta-analysis. Pathol Res Pract. 2019;215:151–8.
[50] Meng J, Fan X, Zhang M, et al. Do polymorphisms in protein kinase catalytic subunit alpha-1 gene associated with cancer susceptibility? a meta-analysis and systematic review. BMC Med Genet. 2018;19:189.
[51] Meng J, Wang S, Shen X, et al. Polymorphism of MMP-9 gene is not associated with the risk of urinary cancers: Evidence from an updated meta-analysis. Pathol Res Pract. 2018;214:1966–73.