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Interpreting the various associations of MiRNA polymorphisms with susceptibilities of cardiovascular diseases

Current evidence based on a systematic review and meta-analysis

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Abstract

Background: To interpret the various associations between miRNA polymorphisms and cardiovascular diseases (CVD).

Methods: Literature search has identified relevant studies up to June 2016. A meta-analysis was performed followed the guidelines from the Cochrane review group and the PRISMA statement. Studies were identified by searching the Cochrane Library, EMBASE, PUBMED and WHO clinical trials registry center. A meta-analysis has been done with a fixed/random-effect model using STATA 14.0, which also has been used to estimate the publication bias and meta-regression.

Results: The results from 11 case-control studies were included. The miR-146a G/C makes a contribution to the causing of CVD as recessive genetic model. And the miR-499 G/A raised the risks of cardiomyopathy, however it could still accelerate the procedure of CVD combined with myocardial infraction. At this point, we consider that it could deepen the adverse of outcomes from coronary artery disease (CAD), but it's hard to draw an association between miR-499 G/A and CAD. At last the miR-196a2 T/C demonstrated a contrary role between development problem and metabolic issues, which protects the development procedure and impairs the metabolism to cause different disease phenotypes.

Conclusion: Despite inter-study variability, the polymorphisms from miR-146a, miR-499 and miR-196a2 have impacts on cardiovascular disease. Each type of miRNA has individual role in either cardiac development or the origins of CVD.

Abbreviations: CAD = coronary artery disease, CHD = congenital heart disease, CI = confidence interval, CVD = cardiovascular disease, HWE = Hardy–Weinberg equilibrium, miR = microRNAs, RR = risk ratio, SNPs = single nucleotide polymorphisms.

Keywords: cardiovascular diseases, meta-analysis, microRNA, polymorphisms

1. Introduction

During the past several decades, major advanced progresses and improvement have been initiated to provide better medical prevention, diagnosis, and treatment.[1,2] However, cardiovascular diseases (CVDs) remain the single leading cause of death in both developing and developed countries. CVD is a concept of a series of diseases, and it contains problems belongs to cardiomyocytes, cardiac conduction system, coronary artery and developmental defect, indicating cardiomyopathy, arrhythmia, coronary artery disease (CAD), and congenital heart disease (CHD).[3,4] CVD is normal considering as resulting from many risk factors, including genetic and environmental factors.[1] However, such cardiovascular disorders are clinically heterogeneous, ranging from affected individuals with clinical presentations of severe symptoms, including heart failure, sudden death, and asymptomatic individuals. So that identification of risk factors that could discriminate CVD patients with high risk of cardiovascular event or die will improved patient outcomes and prolong survival. Some susceptibility genes have been shown to be associated with an increased risk of developing a CVD.[5] Better knowledge of the genetic background and disease susceptibility genes would probably help us in focusing early diagnosis and treatment on right subjects and improving cardiac outcome in the affected patients. Besides, the rapid development of gene editing techniques made it was more necessary to get better understanding of genetic association with particular disease.[6]

MicroRNAs (miRNAs and miRs) are a class of conservative, small, single-strand, noncoding RNAs that regulate gene expression through degradation of target mRNAs or inhibition of translation.[7] A particular miRNA can target multiple of

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target genes. It is estimated that more than half genes expression could be influenced and regulated by specific miRNAs. Moreover, more and more evidences demonstrated that miRNAs had also been involved in critical developmental and pathological procedures, especially for the CVD.[8–10] MiRNAs were proved to regulate several core pathways in guiding coronary artery biogenesis, cardiomyocytes regeneration, hypertrophy, and function maintaining.[9,11–13] Given the important role of miRNAs in CVDs, they are being exploited for diagnosis, prevention, and treatment of CVD.

The impacts on target genes expression of single nucleotide polymorphisms (SNPs) located in the miRNA sequence have been reported.[14,15] Epidemiological studies confirmed that miRNA-associated sequence polymorphism made great contributions in the development and progression of diseases including CVDs, neurodegenerative diseases, and cancers.[16] Such as microRNA-146a (miR-146a) rs2910164 is a G/C polymorphism located in the seed region of mature miR-146a-3p sequence, and the minor C allele of the rs2910164 G/C polymorphism causes a change from a G:U pair to a C:U in the stem structure of miR-146a precursor and produces a less stable secondary structure, which leads to lower levels of mature miR-146a-3p and miR-146a-5p in cells.[17] Besides, miR-196a2 T/C (rs11614913) and miR-499 G/A (rs3746444) have been found similar deficiency in transducing mature miRNA. According to recent data, the miR-146aG, miR-196a2C, and miR-499G alleles are possible genetic predisposing factors, and may associate with CVD. However, even a series of researches have been launched to identify this issue, convinced data are still missing. Currently, several meta-analyses have been published among these 3 polymorphisms on miRNAs, most of them are focusing on the cerebrovascular diseases.[17–19] As heart is a complicated organ, different CVDs might due to different types of cells and tissues. So here, we performed this meta-analysis to evaluate the influences of the polymorphisms in regulating CVD, especially we clarified all the subtypes of CVDs, to investigate the function of each miRNA in particular cell types relating with pathological progresses. These results will facilitate our understanding on the effects of rs2910164, rs11614913, and rs3746444 polymorphisms toward the underlying biological processes and may shed light on the path for more effective disease management and treatment strategies for patients with CVDs.

2. Materials and methods

2.1. Protocol

This analysis was conducted in accordance with a predetermined protocol following the recommendations of Deeks.[20] The data collection and reporting were in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[21] This is a review research, and the approval from institutional ethics committee is not required.

2.2. Search strategy

PubMed, Embase, the Cochrane Central Register of Controlled Trials, and World Health Organization clinical trials registry center were searched with a high-sensitive and high-specific search strategy as "(heart OR cardiology OR cardiac OR cardiology[MeSH Terms] OR heart[MeSH Terms]) AND (miRNA OR microRNA OR miRNA[MeSH Terms] OR miR) AND (single nucleotide polymorphism OR mutation OR variant OR polymorphism OR SNP OR Polymorphism[MeSH Terms])." Search was updated to June 2016.

2.3. Study selection

The inclusion criteria were as followings: case–control design for miRNA polymorphisms with CVD; the association of miR-146a (rs2910164) or miR-499 (rs3746444) or miR-196a2 (rs11614913) and cardiac related diseases' risks should be evaluated; studies should on human beings; the genotype in the control group should be agreed with the Hardy–Weinberg equilibrium (HWE); detailed genotype distribution frequency of cases and controls could be obtained or calculate; and the data in the publication are sufficient for present.

Studies were excluded if any of the following applies.

The exclusion criteria were as followings: repeat publications, abstracts, letters, or reviews; and studies not meeting all of the inclusion criteria.

2.4. Data collection and assessment of study quality

Kaiyu Zhou and Peng Yue assessed eligibility of reports independently at the title and/or at abstract level, with a 3rd reviewer (Yifei Li) determining the divergences together. A 14-item Quality Assessment of genetic-associated studies list has been used to measure the quality of each study's methodology, which was based on the report of NCI-NHGRI Working Group on Replication in Association Studies.

2.5. Evaluation indicators

To evaluate the association between miR-146a (rs2910164) or miR-499 (rs3746444) or miR-196a2 (rs11614913) and specific CVDs (CHD, cardiomyopathy, CAD, and arrhythmia) risk, the relative risk ratio (RR) with 95% confidence interval (CI) was used. The pooled RRs were calculated using genetic model of allelic model, homozygous model, heterozygous model, recessive model, and dominant model, and the statistical significance was determined by the Z-test, and $P < .05$ was considered to be statistically significant. Subgroup analysis was conducted according to the specific types of diseases which were focused on.

2.6. Publication bias

Publication bias was assessed by funnel plots using Egger test by Stata statistical software (STATA), version 14.0. Once an asymmetric distribution of all the data points presented in the funnel plot with a quantified Egger test result of $P < .05$, it indicated the presence of potential publication bias.[22]

2.7. Heterogeneity

Heterogeneity was examined by chi-squared test in pooling results. Heterogeneity was considered to be existed if $P < .05$ in qualitative tests. Also, I^2 test was conducted to quantitatively estimate the proportion of variation among each study with a value >50% indicating significant heterogeneity.

2.8. Meta regression and sensitivity analysis

The meta-regression was carried out to detect where the potential factor for whether results would be various among some significant factors' subgroup analyses. The sensitivity analysis was conducted for every study using STATA 14.0 for meta-analysis fixed-effects estimates to determine if an single study was incurring undue weight in the analysis.

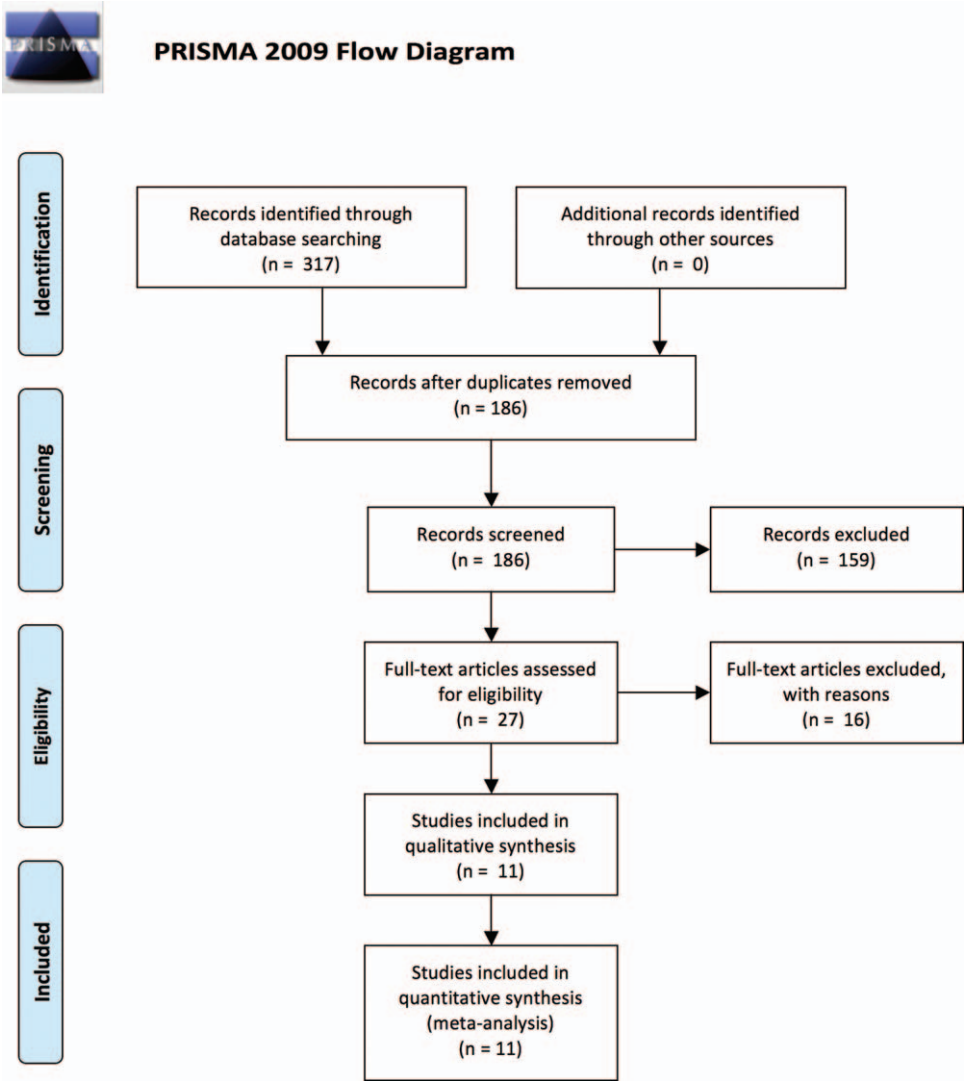

Figure 1. PRISMA flow chart of studies inclusion and exclusion. From Moher et al.[21] For more information, visit www.prisma-statement.org.

2.9. Statistical analysis

Chi-squared test was used to evaluate the HWE of the control group polymorphism. If $P < .05$, it was considered to be deviated from HWE. Data were analyzed using STATA Version 14.0.[23] Besides, publication bias and meta-regression analysis were conducted by STATA version 14.0. If there was an obvious heterogeneity among the studies ($I^2 > 50\%$), the random-effects model (the DerSimonian and Laird method) was used for the meta-analysis. Otherwise, the fixed-effect model using the Mantel–Haenszel method was used.

3. Results

3.1. Study evaluation

One hundred eighty-six citations were retrieved by the search method aforementioned. Then 159 citations were excluded according to the selection criteria, and identified the initially 27 articles after reading titles and abstracts. However, 16 articles were excluded by reviewing their completed articles. Among them, 11 articles were unable to extract useful data for meta-analysis, 3 articles were not focused on CVDs and 2 articles did not provide specific genotyping data. Then, manual retrospective research failed to add missing articles to this meta-analysis. At last 11 articles[7,11,24–32] with case–control studies for miRNA polymorphisms association with CVDs were enrolled into this meta-analysis (Fig. 1). Among them, 3 individual miRNA polymorphisms as miR-146a (rs2910164), mir-499 (rs3746444), and miR-196a2 (rs11614913) were extracted for evaluation. However, there were 4 types of CVDs, which have been hit after literature review as CHD, cardiomyopathy, CAD, and atrial fibrillation. The basic characteristics of included studies were showed in Table 1.

3.2. Study quality

The quality list of questions was used to review the test quality of the included studies following the report of NCI-NHGRI Working Group on Replication in Association Studies.[33] Most of the studies satisfied a majority of the items on the 14-item Quality Assessment of genetic-associated studies' list. All the enrolled studies missed the estimate of sample size. However, no

Table 1

Characteristics of the studies included in this meta-analysis.

Study	Year	Country	Ethnicity	Genotyping method	Source of controls	Total sample size (case/control)	HWE	Reported polymorphism sites	Diseases type
Xu	2009	China	Chinese	PCR–RFLP	Hospital based	miR-146a (501/505) miR-499 (1003/1046) miR-196a2 (1324/1783)	Yes	rs2910164 rs3746444 rs11614913	CHD
Zhou	2010	China	Chinese	PCR–RFLP	Population based	miR-146a (221/321) miR-499 (221/321) miR-196a2 (221/321)	Yes	rs2910164 rs3746444 rs11614913	Dilated cardiomyopathy
Zhi	2012	China	Chinese	PCR–RFLP and PCR–PIRA	Hospital based	miR-499 (916/584) miR-196a2 (916/584)	Yes	rs3746444 rs11614913	CAD
Chen	2014	China	Chinese	PCR–LDR	Population based	miR-146a (919/889) miR-499 (919/889) miR-196a2 (919/889)	Yes	rs2910164 rs3746444 rs11614913	CAD
Hamann	2014	Germany	Caucasian	HRM	Population based	miR-146a (206/200)	Yes	rs2910164	CAD
Ramkaran	2014	India	Indian	PCR–RFLP	Population based	miR-146a (106/100)	Yes	rs2910164	CAD
Xiong	2014	China	Chinese	PCR–RFLP	Hospital based	miR-146a (295/283) miR-499 (295/283) miR-196a2 (295/283)	Yes	rs2910164 rs3746444 rs11614913	CAD
Huang JB	2015	China	Chinese	MALDI-TOF-MS	Population based	miR-146a (173/207) miR-196a2 (173/207)	Yes	rs2910164 rs11614913	CHD
Huang SL	2015	China	Chinese	TaqMan	Population based	miR-146a (717/717) miR-196a2 (718/720)	Yes	rs2910164 rs11614913	CAD
Su	2015	China	Chinese	PCR–RFLP	Hospital based	miR-196a2 (65/58)	Yes	rs11614913	Atrial fibrillation
Bastami	2016	Iran	Iranian	TaqMan	Hospital based	miR-146a (300/300)	Yes	rs2910164	CAD

CAD = coronary artery disease, CHD = congenital heart disease, 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–PIRA = polymerase chain reaction–primer-introduced restriction analysis, PCR–RFLP = polymerase chain reaction–restriction fragment length polymorphism.

study had mentioned the whether there were some blind methods in conducting this project, and no genotyping replication had been done among included researches. Besides, only few studies performed functional studies and made cases adjustment. Overall, all the researches follow the basic line of the genetic analysis with clearly describe the patients and control population baseline and genotypes, providing specific details with how such individual polymorphism identified. The whole questionnaires of quality list had been shown in Table 2.

3.3. Publication bias

Egger test was used to measure the publication bias of included studies. Each study was presented by a dot and the distance between each dot and the vertical line suggests its bias, respectively. The absence of any asymmetric distribution suggested there was no publication bias. The Egger test results of miR-146a were as following: allelic model (C vs G), $P=.593$, $t=0.56$, 95% CI (−2.215, 3.591); homozygous model (CC vs GG), $P=.218$, $t=1.35$, 95% CI (−0.933, 3.428); heterozygous model (CG vs GG), $P=.742$, $t=0.34$, 95% CI (−3.031, 4.060); recessive model (CC vs CG+GG), $P=.385$, $t=0.93$, 95% CI (−0.913, 2.088); and dominant model (CC+CG vs GG), $P=.456$, $t=0.79$, 95% CI (−2.113, 4.231). The Egger test results of miR-499 were as following: allelic model (G vs A), $P=.877$, $t=0.17$, 95% CI (−11.847, 13.166); homozygous model (GG vs AA), $P=.436$, $t=-0.90$, 95% CI (−10.159, 5.694); heterozygous model (GA vs AA), $P=.566$, $t=0.64$, 95% CI (−15.134, 22.798); recessive model (GG vs GA+AA), $P=.387$, $t=-1.01$, 95% CI (−11.225, 5.822); and dominant model (GG+GA vs AA), $P=.674$, $t=0.46$, 95% CI (−13.054, 17.511). The Egger test results of miR-196a2 were as following: allelic model (T vs C), $P=.874$, $t=-0.17$, 95% CI (−6.343, 5.540); homozygous model (TT vs CC), $P=.752$, $t=-0.33$, 95% CI (−6.526, 4.969); heterozygous model (TT vs CC), $P=.707$, $t=-0.39$, 95% CI (−6.059, 4.375); recessive model (TT vs TC+CC), $P=.902$, $t=0.13$, 95% CI (−4.228, 4.697); and dominant model (TT+TC vs CC), $P=.643$, $t=-0.49$, 95% CI (−6.972, 4.656). All the results from Egger tests indicated that there was no potential for publication bias among the studies included in this analysis.

3.4. The association between miR-146a polymorphism with CVDs risk

Overall, 9 studies with 3436 cases and 3520 controls were included in this analysis. And several results revealed significant associations. For the overall analysis, the homozygous model (CC vs GG) (RR = 1.07, 95% CI = 1.01–1.14, $P=.028$) and the recessive model (CC vs CG+GG) (RR = 1.10, 95% CI = 1.01–1.18, $P=.021$) demonstrated dramatically association. No significant associations were found in the overall analysis in allelic (C vs G), heterozygous (CG vs GG), and dominant (CC+CG vs GG) models (Fig. 2). And heterogeneities had been found in allelic ($I^2=50.2\%$, $P=.041$), homozygous ($I^2=52.5\%$, $P=.032$), heterozygous ($I^2=59.6\%$, $P=.011$), and dominant ($I^2=65.1\%$, $P=.003$) models, while there was no significant heterogeneity in recessive ($I^2=0.0\%$, $P=.676$) model.

For the analysis of CHD, no significant association had been identified among all kinds of genetic models as allelic (C vs G), homozygous (CC vs GG), heterozygous (CG vs GG), dominant (CC+CG vs GG), and recessive (CC vs CG+GG) models. And there also was no significant heterogeneity among 5 types of genetic models' analysis (Fig. 2).

For the analysis of cardiomyopathy, also no significant association had been identified among all kinds of genetic

Table 2
The quality of included studies.

Study	Power	Controls characterization	Case characterization	LD exploration	Polymorphism identification	Genotyping error check	Hardy–Weinberg equilibrium	Blinding	Multiple testing	Covariate adjustment	Risks	Population stratification adjustment	Replication	Functional study
1	−	+	+	−	+	−	+	?	+	−	−	−	−	+
2	−	+	+	−	+	−	+	?	+	−	−	+	−	−
3	−	+	+	−	+	−	+	?	+	−	−	−	−	−
4	−	+	+	−	+	+	+	?	+	−	−	−	−	−
5	−	+	+	−	+	−	+	?	+	−	−	−	−	−
6	−	+	+	−	+	−	+	?	+	−	−	+	−	+
7	−	+	+	−	+	−	+	?	+	−	−	−	−	−
8	−	+	+	−	+	−	+	?	+	−	−	+	−	−
9	−	+	+	−	+	−	+	?	+	−	+	+	−	−
10	−	+	+	−	+	−	+	?	+	−	−	−	−	−
11	−	+	+	−	+	−	+	?	+	−	−	+	−	−

+ = yes; − = no; ? = not clear, LD = linkage disequilibrium.

models as allelic (C vs G), homozygous (CC vs GG), heterozygous (CG vs GG), dominant (CC + CG vs GG), and recessive (CC vs CG + GG) models. As this subgroup analysis only enrolled 1 study, so that no heterogeneity analysis was performed (Fig. 2).

For the CAD analysis, the allelic model (C vs G) (RR = 1.09, 95% CI = 1.01–1.18, $P = .000$), homozygous model (CC vs GG) (RR = 1.14, 95% CI = 1.05–1.24, $P = .001$), the dominant model (CC + CG vs GG) (RR = 1.02, 95% CI = 1.02–1.09, $P = .004$), and the recessive model (CC vs CG + GG) (RR = 1.16, 95% CI = 1.05–1.28, $P = .003$) demonstrated dramatically association. While the heterozygous (CG vs GG) showed no significant association. And heterogeneities had been found in heterozygous ($I^2 = 66.3\%$, $P = .011$) and dominant ($I^2 = 68.0\%$, $P = .008$) models, while there was no significant heterogeneity in allelic ($I^2 = 40.7\%$, $P = .134$), homozygous ($I^2 = 47.9\%$, $P = .088$), and recessive ($I^2 = 0.0\%$, $P = .885$) models (Fig. 2).

3.5. The association between miR-499 polymorphism with CVDs risk

Overall, 5 studies with 3354 cases and 3123 controls were included in this analysis. And several results revealed significant associations. For the overall analysis, the allelic (RR = 1.11, 95% CI = 1.03–1.20, $P = .008$), homozygous (RR = 1.53, 95% CI = 1.21–1.93, $P = .000$), and recessive (RR = 1.52, 95% CI = 1.21–1.93, $P = .000$) models demonstrated dramatically association. No significant associations were found in the overall analysis of heterozygous and dominant models (Fig. 3). And heterogeneities had been found in allelic ($I^2 = 74.3\%$, $P = .004$), homozygous ($I^2 = 72.2\%$, $P = .006$), heterozygous ($I^2 = 87.4\%$, $P = .000$), dominant ($I^2 = 82.0\%$, $P = .000$), and recessive ($I^2 = 77.2\%$, $P = .001$) models (Fig. 3).

For the analysis of CHD, no significant association had been identified among all kinds of genetic models as allelic, homozygous, heterozygous, dominant, and recessive models. As this subgroup analysis only enrolled 1 study, so that no heterogeneity analysis was performed (Fig. 3).

For the analysis of cardiomyopathy, the allelic (RR = 1.56, 95% CI = 1.26–1.94, $P = .000$), heterozygous (RR = 1.82, 95% CI = 1.45–2.29, $P = .000$), and dominant (RR = 1.66, 95% CI = 1.36–2.04, $P = .000$) models demonstrated dramatically association. No significant associations were found in the analysis of homozygous and recessive models (Fig. 3). Moreover, as the heterozygous model and dominant model both demonstrated significant associations, but not significance had been identified in homozygous model. As this subgroup analysis only enrolled 1 study, so that no heterogeneity analysis was performed (Fig. 3).

For the CAD analysis, the allelic model (RR = 1.11, 95% CI = 1.03–1.22, $P = .029$), the homozygous model (RR = 1.95, 95% CI = 1.46–2.60, $P = .000$), and the recessive model (RR = 2.06, 95% CI = 1.54–2.75, $P = .000$) demonstrated dramatically association. While the heterozygous and recessive models showed no significant association. And no heterogeneity had been found among allelic ($I^2 = 0.0\%$, $P = .381$), homozygous ($I^2 = 27.4\%$, $P = .252$), heterozygous ($I^2 = 4.3\%$, $P = .352$), dominant ($I^2 = 0.0\%$, $P = .662$), and recessive ($I^2 = 38.9\%$, $P = .195$) models (Fig. 3).

3.6. The association between miR-196a2 polymorphism with CVDs risk

Overall, 5 studies with 4628 cases and 4844 controls were included in this analysis. And several results revealed significant associations. For the overall analysis, the allelic model (T vs C)

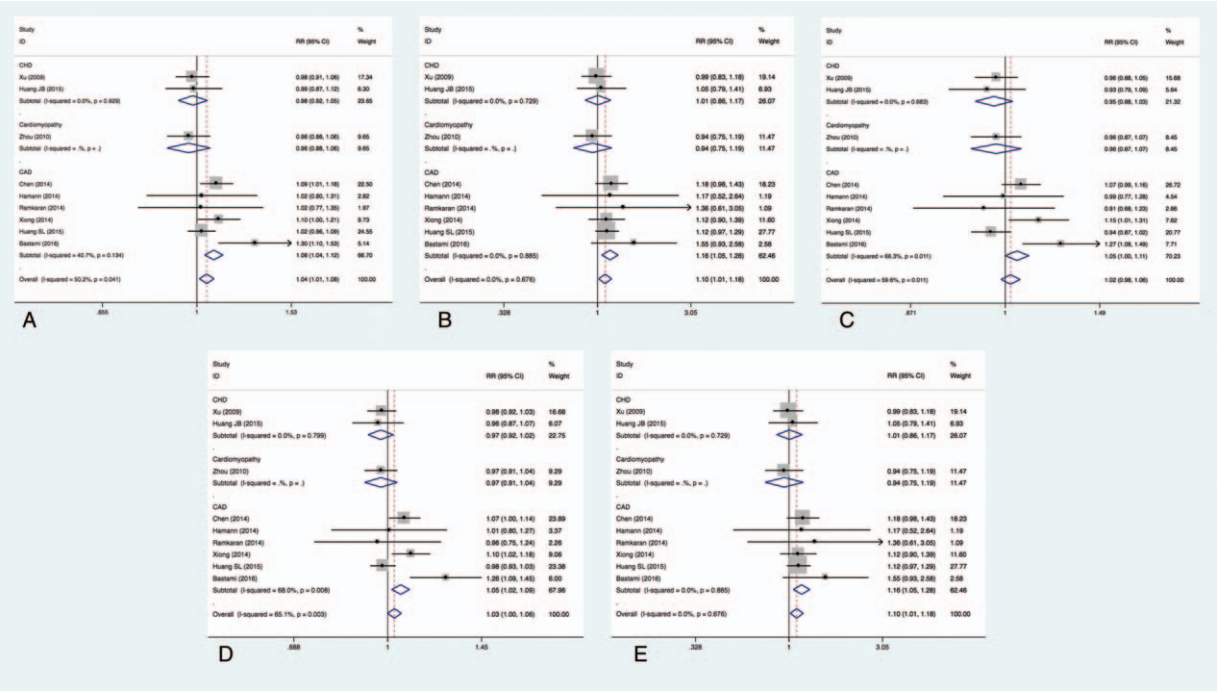

Figure 2. Forest plots of odds ratios for the association between microRNA-146a rs2910164 and the risk of coronary artery disease. (A) C vs G; (B) CC vs GG; (C) CG vs GG; (D) CC + CG vs GG; (E) CC vs CG + GG. CI = confidence interval, df = degrees of freedom.

(RR = 0.96, 95% CI = 0.94–0.99, $P = .005$), homozygous model (TT vs CC) (RR = 0.94, 95% CI = 0.90–0.98, $P = .005$), and dominant model (TT + TC vs CC) (RR = 0.97, 95% CI = 0.96–0.99, $P = .013$) demonstrated dramatically association. No significant associations were found in the overall analysis of heterozygous (TC vs CC) and recessive (TT vs TC + CC) models (Fig. 4). And heterogeneities had been found in allelic ($I^2 = 85.1\%$, $P = .000$), homozygous ($I^2 = 85.8\%$, $P = .000$), heterozygous ($I^2 = 80.4\%$, $P = .000$), dominant ($I^2 = 85.3\%$, $P = .000$), and recessive ($I^2 = 74.4\%$, $P = .000$) models (Fig. 4).

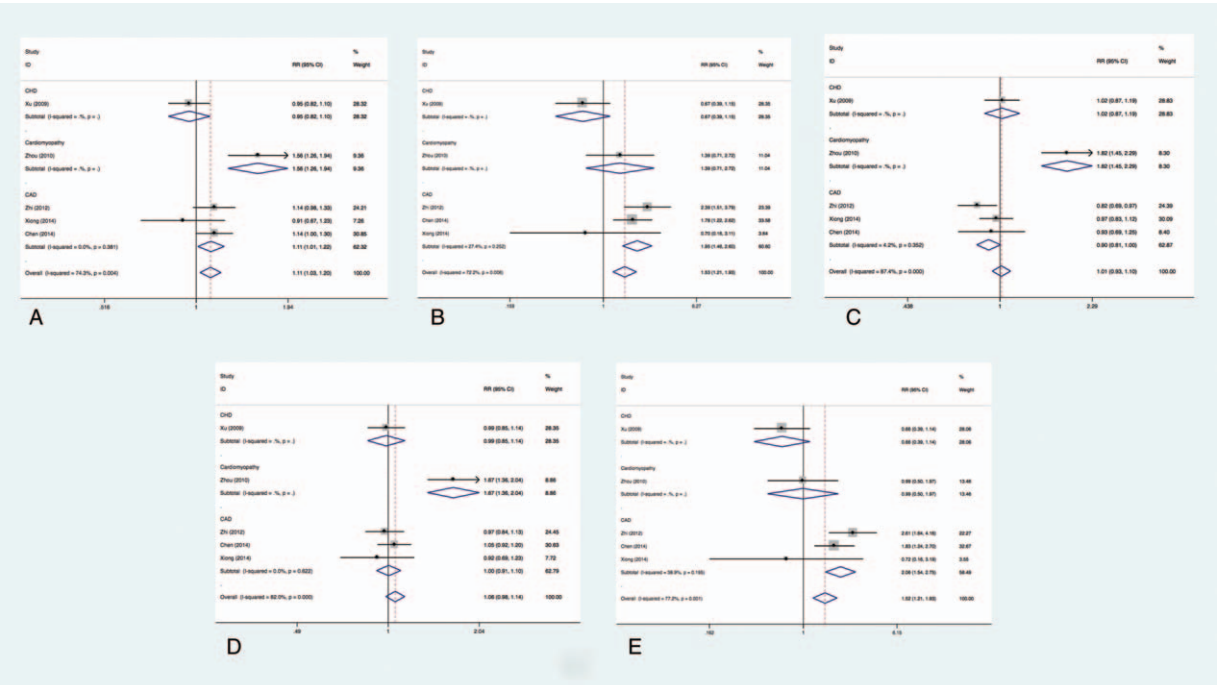

Figure 3. Forest plots of odds ratios for the association between microRNA-499 rs3746444 and the risk of coronary artery disease. (A) G vs A; (B) GG vs AA; (C) GA vs AA; (D) GG + GA vs AA; (E) GG vs GA + AA. CI = confidence interval, df = degrees of freedom.

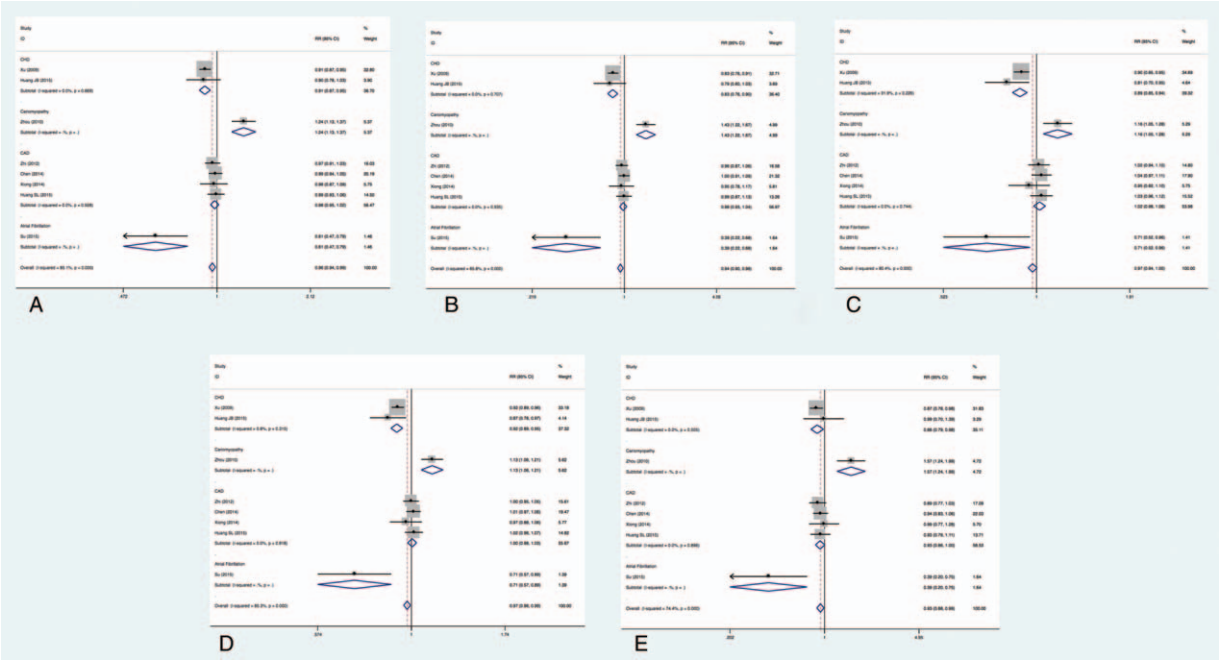

Figure 4. Forest plots of odds ratios for the association between microRNA-196a2 rs11614913 and the risk of coronary artery disease. (A) T vs C; (B) TT vs CC; (C) TC vs CC; (D) TT + TC vs CC; (E) TT vs TC + CC. CI = confidence interval, df = degrees of freedom.

For the analysis of CHD, no significant association had been identified among all kinds of genetic models as allelic, homozygous, heterozygous, dominant, and recessive models. In this subgroup analysis, there was no heterogeneity within an individual group (Fig. 4).

For the analysis of cardiomyopathy, the allelic model (T vs C) (RR = 1.24, 95% CI = 1.13–1.37, P = .000), homozygous model (TT vs CC) (RR = 1.43, 95% CI = 1.22–1.67, P = .000), heterozygous model (TC vs CC) (RR = 1.16, 95% CI = 1.05–1.28, P = .004), dominant model (TT + TC vs CC) (RR = 1.13, 95% CI = 1.06–1.21, P = .000), and recessive model (TT vs TC + CC) (RR = 1.57, 95% CI = 1.24–1.99, P = .000) all demonstrated dramatically association (Fig. 4). As this subgroup analysis only enrolled 1 study, so that no heterogeneity analysis was performed (Fig. 4).

For the CAD analysis, the allelic model (T vs C) (P = .304), homozygous model (TT vs CC) (P = .533), heterozygous model (TC vs CC) (P = .324), dominant model (TT + TC vs CC) (P = .728), and recessive model (TT vs TC + CC) (P = .066) showed no significant association. And no heterogeneity had been found among allelic (I^2 = 0.0%, P = .928), homozygous (I^2 = 0.0%, P = .935), heterozygous (I^2 = 0.0%, P = .744), dominant (I^2 = 0.0%, P = .818), and recessive (I^2 = 0.0%, P = .896) models (Fig. 4).

For the analysis of atrial fibrillation, the allelic model (T vs C) (RR = 0.61, 95% CI = 0.47–0.79, P = .000), homozygous model (TT vs CC) (RR = 0.39, 95% CI = 0.22–0.68, P = .001), heterozygous model (TC vs CC) (RR = 0.71, 95% CI = 0.52–0.96, P = .024), dominant model (TT + TC vs CC) (RR = 0.71, 95% CI = 0.57–0.89, P = .002), and recessive model (TT vs TC + CC) (RR = 0.93, 95% CI = 0.88–0.99, P = .000) all demonstrated dramatically association (Fig. 4). As this subgroup analysis only enrolled 1 study, so that no heterogeneity analysis was performed (Fig. 4).

3.7. Meta-regression and sensitivity analysis

The meta-regression analysis had been carried out to identify whether the ethical race was a potential factor which might cause the heterogeneities. However, as only Chinese populations were enrolled in the meta-analyses of miR-499 and miR-196a2, so that no meta-regression had been carried to measure the potential impacts from ethnicity. According to the results (Suppl Fig. 1, http://links.lww.com/MD/C250), the ethnicities did not make any contribution to the significant heterogeneities among all the groups of miR-146a. For miR-146a, there was no significant result after calculation among allelic model (Coef = 0.063, 95% CI = −0.004 to 0.132, P = .06), homozygous model (CC vs GG) (Coef = 0.172, 95% CI = −0.018 to 0.361, P = .07), heterozygous model (CG vs GG) (Coef = 0.059, 95% CI = −0.021 to 0.139, P = .125), dominant model (CC + CG vs GG) (Coef = 0.058, 95% CI = −0.005 to 0.122, P = .07), and recessive model (CC vs CG + GG) (Coef = 0.080, 95% CI = −0.071 to 0.308, P = .18). Besides, the individual studies for each diagnostic test were not responsible for existed heterogeneities. Moreover, the meta-analysis said that the subjects' number would not shift the heterogeneities. Finally, sensitivity analysis had been done by qualitative measurement among miR-146 (Suppl Fig. 2, http://links.lww.com/MD/C250), miR-196a2 (Suppl Fig. 3, http://links.lww.com/MD/C250), and miR-499 (Suppl Fig. 4, http://links.lww.com/MD/C250), and every analysis confirmed in both direction and magnitude of statistical significance the findings of all the overall analyses.

4. Discussion

Although the fatality of malignant tumor has been rapidly increase recent tears, but the CVD still ranks the first healthy killer since 1990, and this trend is expected to continue in the future.[34,35] Since the attention to miRNAs arising from almost a

decade ago, the researches focusing on miRNA remained the most popular topic across several studying field. So that, a series of publications have claim the emerging role of miRNA in the regulation of heart development, maturation, proliferation, differentiation, as well as the pathological procedure due to kinds of origins. As the theory of "Precision Medicine" had been pointed out, there was emerging desires to get further understandings of the genetic backgrounds of diseases. This meta-analysis was restricted to the characteristics and association between particular miRNA polymorphisms and CVDs. Within this meta-analysis, we systematic reviewed all the related miRNA polymorphisms related with CVD. According to search results, SNP sites of miR-146a, miR-196a2, miR-499, miR-149, miR-423, miR-4513, miR-34b/c, and miR-218 had been identified with association with CVDs.[11,27,36–39] However, among all the listed miRNAs, only few of them had been taken into serious consideration and analysis. For the miR-149, miR-423, miR-4513, miR-34b/c, and miR-218, there were <2 articles focused on them, which could not provide convening ideas on the associations between such miRNA SNPs and CVDs, and it was impossible to initiate qualified meta-analysis based on such limited number of original researches. Given that, we took miR-146a G/C (rs2910164), miR-196a2 T/C (rs11614913), and miR-499 G/A (rs3746444) into extended analysis seriously. To our knowledge, this is the first meta-analysis focused on the systematic review all the related miRNA SNPs and their associations with CVDs. However, He et al[18] had drawn a systematic review of miR-146a polymorphisms on cardio-cerebrovascular diseases. And Bao et al[17] reported the pooled data of miR-146a polymorphism on CAD and stroke. Besides, the work from Xiao et al[19] revealed the associations among miR-146a, miR-499, and miR-196a2 polymorphisms with ischemic stroke. So that, we could see the current meta-based studies were mainly focused on cerebrovascular diseases, which indicated their relationships on vascular deficiency. However, to get better understanding of their roles in cardiac diseases, de novo designed research should be launched to enrolled all the possible data on "heart." So, this meta-analysis was a pioneer 1 to evaluate the potential association between miRNAs' polymorphisms and CVDs on the published protocols, and demonstrated some instruction for current sight on this issue.

In this meta-analysis, we included 11 relevant studies. Only 1 article focused on dilated cardiomyopathy and atrial fibrillation, respectively. While 2 articles measured the impacts of miRNA on CHD, and most of the enroll researches focused on CAD. For the miR-146a G/C (rs2910164), overall evaluation demonstrated that significant differences among allelic model ($P=.009$), homozygous model ($P=.028$), and recessive model ($P=.021$). Moreover, in the analysis of recessive model, there was definitely no heterogeneity ($I^2=0.0\%$). According that the miR-146a G/C SNP played a genetic role as recessive model. Among the subgroup analysis, the polymorphism of miR-146a G/C did not make any contribution to the causes of CHD or cardiomyopathy, while all the results from overall evaluation could be recorded restricted within CAD subgroup. Cowan et al[40] reported that miR-146a could inhibit thrombin-induced NF-kappaB pathway activation and subsequent inflammatory responses in human endothelial cells. Li et al[41] also demonstrated that loss function of microRNA-146a in monocytes and macrophages could activate NF-kappaB and accelerate the progress inflammation and atherosclerosis. So that the miR-146a was mainly involved in the regulation of inflammation responses targeting endothelial cells. That's could explain why the polymorphism of miR-146a G/C should be responsible for the generation of CAD, while negative in CHD a cardiomyopathy analysis which should involved with deregulation of cardiomyocytes.

Taking the results from the measurement of miR-499 G/A (rs3746444), the overall results revealed that such SNP influenced the phenotype among allelic model ($P=.008$), homozygous model ($P=.000$), and recessive model ($P=.000$). However, all the data from the overall analysis were suffered significant heterogeneities. According to our subgroups' measurement, there was no more significant heterogeneity within a single group. The CAD group also revealed dramatic differences with case and control groups, which indicating the right genetic impact model following the overall results. Surprised, miR-499 G/A polymorphism exhibited different genetic mechanism from CAD patients. Significant results have been identified among allelic model ($P=.000$), heterozygous model ($P=.000$), and dominant model ($P=.000$), saying such an SNP within miR-499 shaped the genetic model into 3 independent ones. So that it could work as codominant genetic model in cardiomyopathy origins. Dorn et al[42] proved that miR-499 mutation could impair heart function. Matkovich et al[43] confirmed that miR-499 impairment could lead to cardiomyopathy via Akt and MAPKs' pathways. Besides, a series studies also indicated that miR-499 could be a biomarker for acute myocardial infarction. However, current researches failed to provide a scientific explanation on the increased risk of miR-499 on CAD. Moreover, most studies always enrolled the cases with CAD by hospital appearances after myocardial infarction attacks, which should be a terrible outcome of CAD. So there might be some patients selection bias among them, which could reconclude that the miR-146a G/C might alter patients much more sensitive to ischemic attack with myocardial infarction. It strongly suggested further explorations should be conducted to distinguish the polymorphism of miR-499 contribute to the generation of CAD or extend the adverse outcomes from CAD.

For the analysis focused on miR-196a2, overall results showed that significant differences had been identified among allelic model ($P=.005$), homozygous model ($P=.005$), dominant model ($P=.013$), and recessive model ($P=.029$). However, all the results had significant heterogeneities, so that the overall results should be taken seriously. Following the dissected analysis, the CAD subgroup was negative in detecting the association between miR-196a2 T/C (rs11614913) and risks of CAD. However, the data from CHD patients' analysis revealed great significant differences compared with control ones among allelic model ($P=.000$), homozygous model ($P=.000$), heterozygous model ($P=.000$), dominant model ($P=.000$), and recessive model ($P=.023$). Moreover, the RR value was <1.0, so that the SNP within miR-196a2 performed as a protection role in CHD origins. After interpreting the RR values in each genetic model, it said that the genotype T had advantage role in CHD prevention, while the homozygous genotype TT would provide a stronger protection function with a lower (RR = 0.83, 95% CI = 0.76–0.90) compared with heterozygous (RR = 0.89, 95% CI = 0.85–0.94). Moreover, miR-196a2 T/C (rs11614913) also had administrated protection of atrial fibrillation, which was preferred to treat as a dominant (RR = 0.71, 95% CI = 0.57–0.89) genetic model even all the 5 types of genetic model revealed dramatic differences. Besides, as the analysis of cardiomyopathy showed, the genotype of T would arise more risks of dysfunction of cardiomyocytes, while the homozygous model might extend the risks of cardiomyopathy. Xu et al[11] claimed that the genotype C would increase the expression of mature miR-196a,

and it would bind to HOXB8 mRNA which was involved in development to inhibit its translation. So that the allele T could maintain the expression of HOXB8 to keep the progress of development. Notably, it failed to target a reasonable explanation of lower risk of atrial fibrillation by current research results. However, as the expression of miR-196a had impacts on development, it might be existing developmental malformation of conduction system which could trigger arrhythmias in elder age. Zou et al[44] also supplied evidence that the lower expression of miR-196a2 would impair the metabolism balance and status, so that it could be responsible to the rising risks of cardiomyopathy due to the reduced expression of miR-196a2 with allele T. Interestingly, the miR-196a2 T/C (rs11614913) demonstrated a contrary role between development problem and metabolic issues.

The limitations of this meta-analysis are: Some pooled results showed large heterogeneity. The potential influence factors should be the sample origins. However, some subgroup analyses only involved limited studies, which might also get unconvinced results for few studies, respectively, so that such result should be treated seriously.

5. Conclusion

In conclusion, despite interstudy variability, the polymorphisms from miR-146a, miR-499, and miR-196a2 do have impacts on CVD. The miR-146a G/C makes a contribution to the causing of CVD as recessive genetic model. And the miR-499 G/A raised the risks of cardiomyopathy; however, it could still accelerate the procedure of CVD. At this point, as many of the CVD patients were enrolled by identifying myocardial infarction. So that we consider that it could deepen the adverse of outcomes from CAD, making such patients with more severe clinical manifestation, which leads to an earlier hospital administration. According to that patients' enrollment bias might be existed, and it is hard to draw an association between miR-499 G/A and CAD. At last the miR-196a2 T/C demonstrated a contrary role between development problem and metabolic issues, which protects the development procedure and impairs the metabolism to cause different disease phenotypes.

Author contributions

Kaiyu Zhou, Yimin Hua, and Yifei Li participated in research design. And Kaiyu Zhou, Peng Yue, and Yifei Li collectively contributed to the data collection and analysis. Fan Ma, Hualin Yan, Yi Zhang, Chuan Wang, and Dajian Qiu helped to interpret the results from the analysis. Finally, Yifei Li wrote the manuscript.

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