Association study of miR-149, miR-196a2, and miR-499a polymorphisms with coronary artery aneurysm of Kawasaki disease in southern Chinese population

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Abstract

Background: Accumulating evidence suggests that several microRNA (miRNA) polymorphisms are closely associated with disease susceptibility or progression, such as in Kawasaki disease (KD). Our previous studies revealed the association of miR-149 rs2292832 T>C and miR-196a2 rs11614913 C>T polymorphisms with KD susceptibility. The present study further focused on the relationship between three miRNA polymorphisms (miR-149 rs2292832 T>C, miR-196a2 rs11614913 C>T and miR-499a rs3746444 A>G) and the risk of coronary artery aneurysm (CAA) in southern Chinese KD patients.

Methods: We evaluated 318 KD patients with CAAs and 784 patients without CAAs. TaqMan assays were used to estimate genotyping and analyze the relationship between miRNA polymorphisms (miR-149 rs2292832 T>C, miR-196a2 rs11614913 C>T and miR-499a rs3746444 A>G) and risk associations of CAA by odds ratios (ORs) and 95% confidence intervals (CIs).

Results: We found that the miR-149 rs2292832 TC/CC genotype increased the CAA risk (adjusted OR = 1.53, 95% CI = 1.15–2.03, p = 0.003 for TC, adjusted OR = 1.63, 95% CI = 1.08–2.47, p = 0.021 for CC), whereas the miR-499a rs3746444 AG genotype decreased the CAA risk in KD patients (adjusted OR = 0.33, 95% CI = 0.25–0.45 p ≤ 0.001). Moreover, patients carrying two or three of these single nucleotide polymorphism (SNP) genotypes (rs2292832 TC/CC and
showed that miR-34b/c blastoma susceptibility.14 Our team previously found that the dren aged under 5 years.1,2 KD usually causes coronary artery involvement, 3%–5% of patients will develop coronary aneurysms and thrombosis.3 The morbidity and mortality of KD occur in patients with giant aneurysm giant tumors, and KD is the leading cause of acquired pediatric heart disease.4 The incidence of KD continues to increase in East Asian countries.5–7 Recently, KD-like symptoms have been associated with pediatric cases of the novel SARS-CoV-2, which has resulted in an exponential increase in KD cases worldwide.8 Many studies have shown that the etiology of KD may be linked to infection or pathogens and genetic susceptibility.9,10 However, the etiology and risk factors for the disease are still unknown.

MicroRNAs are a class of short (approximately 22 nucleotides) non-coding RNAs that regulate the expression of specific protein-coding genes by multiple target genes.11 miRNAs play a key role in regulating physiological and pathological processes by inhibiting target mRNA translation or promoting degradation.12 An increasing number of studies have evaluated the association between different miRNA polymorphisms and different disease risks. For example, the miR-34b/c rs4938723 C variant allele significantly reduces the risk of neuroblastoma.13 However, a multicenter case–control study showed that miR-34b/c rs4938723 T>C may not confer hepatoblastoma susceptibility.14 Our team previously found that the miR-218 rs11134527 A>G polymorphism was not associated with KD risk, but it may have an age-related relationship with KD susceptibility.15 The miR-608 rs4919510 G>C polymorphism may be a protective factor for coronary artery injury in KD.16 Compared with carriers of the TT genotype, patients aged under 12 months who carried the miRNA-13 rs1625579 TG/GG genotype showed a significantly higher risk of developing KD.17 These results indicate that miRNA polymorphisms may play a key role in the pathogenesis and pathological process of KD.

In recent years, a variety of studies have shown that miR-149 rs2292832 T>C polymorphism increased the risk of CAA in KD patients and that the miR-499a rs3746444 A>G polymorphism decreased the risk of CAA in KD patients. Further studies with larger sample sizes and different centers are needed to confirm the findings of the present study.

**KEYWORDS**
coronary artery aneurysm, Kawasaki disease, microRNA polymorphisms

## 1 | INTRODUCTION

Kawasaki disease (KD) is an acute self-limited systemic vasculitis, with fever as the first clinical feature, and predominantly affects children aged under 5 years.1,2 KD usually causes coronary artery lesions (CAAs), and severe cases lead to coronary artery aneurysms (CAAs) and thrombosis. The latest guide points out that prompt treatment with intravenous immunoglobulin and aspirin remains the mainstay of treatment. However, regarding the incidence of cardiac involvement, 3%–5% of patients will develop coronary aneurysms and thrombosis.3 The incidence of KD continues to increase in East Asian countries.5–7 Recently, KD-like symptoms have been associated with pediatric cases of the novel SARS-CoV-2, which has resulted in an exponential increase in KD cases worldwide.8 Many studies have shown that the etiology of KD may be linked to infection or pathogens and genetic susceptibility.9,10 However, the etiology and risk factors for the disease are still unknown.

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In recent years, a variety of studies have shown that miR-149 rs2292832 T>C, miR-196a2 rs11614913 C>T and miR-499a rs3746444 A>G gene polymorphisms are closely associated with disease susceptibility or disease complication risk, which contributes to understanding the disease mechanism and pathogenesis at the genetic level, and their miRNAs may act as biomarkers or therapeutic agents for autoimmune diseases,18 cardiovascular diseases19 and cancers,20 amongst others. The miR-499a rs3746444 A>G polymorphism has been shown to be related to the risk of rheumatoid arthritis (RA) in an Iranian population.21 This polymorphism and not miR-196a2 rs11614913 C>T has also been observed to be associated with an increased risk of RA, disease activity and methotrexate toxicity in an Egyptian population.22 It has been reported that the miR-149 rs2292832 C allele enhances the cytotoxicity of temozolomide on glioma cells by regulating the miR-149/CDK6 axis.23 The miR-196a2 rs11614913 C>T and miR-149 rs2292832 polymorphisms were not significantly associated with prostate cancer risk, but, compared to the TT genotype, the CC genotype of the miR-499 rs3746444 polymorphism increased the risk of prostate cancer.24 The miR-149 rs2292832 T>C and miR-196a2 rs11614913 C>T polymorphisms significantly increased the coronary artery disease (CAD) prevalence. Furthermore, miR-149 rs2292832 T>C was shown to increase CAD risk in females and patients aged > 63 years.25 Our team has previously studied the association of miR-149 rs2292832 T>C, miR-196a2 rs11614913 C>T and miR-13 rs1625579 T>G polymorphisms with KD susceptibility or the risk of CAAs in KD.17,26,27 However, the results indicated that the miR-149 rs2292832 T>C and miR-196a2 rs11614913 C>T polymorphisms did not affect KD susceptibility, and we did not explore the relationship with CAA risk in KD.

In the present study, we recruited 1102 children with KD (318 children with CAA and 784 without CAA [NCAA]) to participate in this study; 507 of these patients participated in previous studies. We focused on verification of the association between the miR-149 rs2292832 T>C, miR-196a2 rs11614913 C>T and miR-499a rs3746444 A>G polymorphisms and CAA risk in KD by expanding the sample size.
2 | MATERIALS AND METHODS

2.1 | Study design

We selected patients diagnosed with KD who were admitted to Guangzhou Women's and Children's Medical Center as the research subjects and recruited 1102 KD patients from July 2014 to April 2020. All specimens were from the Clinical Biological Resource Bank of Guangzhou Women and Children's Medical Center. The diagnosis of KD before the guidelines published in 2017 was based mainly on the American Heart Association published guidelines. All the patients collected from the patients with KD. In total, 200 Ethylenediaminetetraacetic acid whole blood specimens were collected for each sample according to the instructions for the Genomic DNA Extraction Kit (Tiangen, Beijing, China). A nucleic acid quantifier was used to measure the concentration and quality of genomic DNA (Multiskan GO; Thermo Scientific, Waltham, MA, USA) and then DNA (rs2292832 T>C, miR-196a2 rs11614913 C>T and miR-499a rs3746444 A>G) polymorphisms and the CAA risk in the KD patients. All statistical analyses were performed using SAS, version 9.4 (SAS Institute, Cary, NC, USA). p < 0.05 (two-sided) was considered statistically significant.

3 | RESULTS

3.1 | Clinical characteristics of patients with KD

The clinical characteristics of KD patients are shown in Table 1. We recruited 318 KD patients with CAAs (CAA group) and 784 patients without CAAs (NCAA group) in the present study. The median ages were 15 months for the CAA group (interquartile range = 7.00–34.25) and 20 months for the NCAA group (interquartile range = 11.25–36.00). There were 24.84% females and 75.16% males in the CAA group and 30.23% females and 69.77% males in the NCAA group. There were no significant differences between the CAA and NCAA groups in terms of age (p = 0.349) or gender (p = 0.071).

3.2 | Association between selected SNPs and CAA with KD risk

The genotype frequency distributions of the three selected SNPs of miR-149 rs2292832 T>C, miR-196a2 rs11614913 C>T and miR-499a rs3746444 A>G in KD with or without CAA are shown in Table 2. The genotype distributions were significantly different in both miR-149 rs2292832 T>C (p = 0.005) and miR-499a rs3746444 A>G (p ≤ 0.001) but not in miR-196a2 rs11614913 C>T between the CAA group and the NCAA group. When the miR-149 rs2292832 TT

TABLE 1 Frequency distribution of selected variables for Kawasaki disease patients

| Variables | CAA (n = 318) | NCAA (n = 784) | p* |
|-----------|--------------|----------------|----|
| Age range (months) | 1.00–166.00 | 1.00–131.00 | 0.349 |
| Median | 15 | 20 | 0.071 |
| interquartile range | 7–34.25 | 11.25–36 | |
| ≤ 60 | 294 | 92.45 | 737 | 94.01 |
| > 60 | 24 | 7.55 | 47 | 5.99 |
| Gender | 0.071 |
| Male | 239 | 75.16 | 547 | 69.77 |
| Female | 79 | 24.84 | 237 | 30.23 |

CAA, coronary artery aneurysms; NCAA, no coronary artery aneurysms.

*Two-sided chi-squared test for distributions between cases and controls.
TABLE 2  Genotype distributions of miRNA polymorphisms and susceptibility to coronary artery aneurysms in Kawasaki disease

| Genotype       | CAA  | NCAA | Crude OR (95% CI) | Adjusted OR (95% CI) |
|----------------|------|------|-------------------|----------------------|
|                | (n = 318) | (n = 784) | p<sup>a</sup> | p<sup>b</sup> |
| miR-149 rs2292832 (T>C) |     |       |                  |                      |
| TT             | 150 (47.17) | 455 (58.04) | 0.005 | 1.00 |
| TC             | 125 (39.31) | 248 (31.63) | 1.53 (1.15–2.03) | 1.53 (1.15–2.03) |
| CC             | 43 (13.52) | 81 (10.33) | 1.61 (1.07–2.43) | 1.61 (1.08–2.47) |
| Dominant       | 168 (52.83) | 329 (41.96) | 0.001 | 1.55 (1.19–2.01) |
| Recessive      | 275 (86.48) | 703 (89.67) | 0.135 | 1.36 (0.91–2.02) |
| miR-196a2 rs11614913 (T>C) |     |       |                  |                      |
| TT             | 86 (27.04) | 202 (25.77) | 0.904 | 1.00 |
| TC             | 163 (51.26) | 411 (52.42) | 0.93 (0.68–1.27) | 0.94 (0.69–1.29) |
| CC             | 69 (21.70) | 171 (21.81) | 0.95 (0.65–1.38) | 0.96 (0.66–1.40) |
| Dominant       | 232 (72.96) | 582 (74.23) | 0.662 | 0.94 (0.70–1.26) |
| Recessive      | 249 (78.30) | 613 (78.19) | 0.967 | 0.99 (0.72–1.36) |
| miR-499a rs3746444 (A>G) |     |       |                  |                      |
| AA             | 238 (74.84) | 407 (51.91) | < 0.001 | 1.00 |
| AG             | 73 (22.96) | 370 (47.19) | 0.34 (0.25–0.45) | < 0.001 |
| GG             | 7 (2.02) | 7 (0.89) | 1.71 (0.59–4.94) | 0.321 |
| Dominant       | 80 (25.16) | 377 (48.09) | < 0.001 | 0.36 (0.27–0.49) |
| Recessive      | 311 (97.80) | 777 (99.11) | 0.094 | 2.50 (0.87–7.18) |

Combined effect of risk genotypes<sup>c</sup>

| Trend<sup>d</sup> | 0 | 1 | 2 | 3 | 0–1 | 2–3 |
|-------------------|---|---|---|---|-----|-----|
|                   | 29 (9.12) | 174 (22.19) | < 0.001 | 1.00 | 1.00 |
| 1                 | 123 (38.68) | 336 (42.86) | 2.20 (1.41–3.42) | 0.001 | 2.19 (1.40–3.41) |
| 2                 | 129 (40.57) | 220 (28.06) | 3.52 (2.25–5.51) | < 0.001 | 3.51 (2.24–5.50) |
| 3                 | 37 (11.64) | 54 (6.89) | 4.11 (2.32–7.30) | < 0.001 | 4.12 (2.32–7.33) |
| Trend<sup>d</sup> | 1.62 (1.38–1.89) | < 0.001 | 1.62 (1.38–1.89) |
| 0–1               | 152 (47.80) | 510 (65.05) | < 0.001 | 1.00 | 1.00 |
| 2–3               | 166 (52.20) | 274 (34.95) | 1.60 (1.19–2.16) | 0.002 | 1.61 (1.19–2.17) |

CAA, coronary artery aneurysms; NCAA, no coronary artery aneurysms.
<sup>a</sup>Adjusted for age and gender.
<sup>b</sup>Chi-squared test for genotype distributions between Kawasaki disease patients and controls.
<sup>c</sup>Risk genotypes were miR-149 rs2292832 TC/CC, miR-196a2 rs11614913 TT, miR-499a rs3746444 AA.
<sup>d</sup>“Trend” means that multiple SNP risk genotypes were combined to analyze a high or low risk of coronary artery aneurysm.

genotype was used, the C variant genotypes increased the risk of CAA in KD patients (adjusted OR = 1.53, 95% CI = 1.15–2.03, p = 0.003 for TC, adjusted OR = 1.63, 95% CI = 1.08–2.47, p = 0.021 for CC). Compared to the miR-499a rs3746444 AA genotype, the AG genotypes decreased the risk of CAA in the KD patients (adjusted OR = 0.33, 95% CI = 0.25–0.45, p = 0.001 for AG). In addition, no significant difference was observed for miR-196a2 rs11614913. The analysis of dominant models in the selected SNPs revealed a significant association between the miR-149 rs2292832/miR-499a rs3746444 polymorphism and CAA risk in the KD patients after adjusting for age and gender (for rs2292832, TC + CC vs. TT: adjusted OR = 1.56, 95% CI = 1.20–2.02, p = 0.001; for rs3746444, AG + GG vs. AA: adjusted OR = 0.36, 95% CI = 0.27–0.49, p = 0.001). Under the recessive models, no associations with CAA risk of KD were observed in the selected SNPs. Then, we defined miR-149 rs2292832 TC/CC, miR-196a2 rs11614913 TT and miR-499a rs3746444 AA as risk genotypes. We observed that if the KD patient has those risk genotype (“0” means none of the above genotypes, “1” means one of the above three genotypes, “2” means two of the above three genotypes and “3” means the above three genotypes), KD patients have a significantly increased risk of CAA compared to patients without risk genotypes. To further evaluate the relationship between risk genotypes and the risk of CAA, the combined analysis showed that patients with two or three of these SNP genotypes (rs2292832 TC/CC, rs11614913 TT and rs3746444 AA) had a higher risk of CAA than those who harbored only zero or one of these SNP genotypes (adjusted OR = 1.61, 95% CI = 1.19–2.17, p = 0.002).
3.3 | Stratification analysis

We further evaluated the association between the three selected SNP polymorphisms and CAA risk by stratified analysis based on subgroups of age, gender Table 3. The findings showed the miR-149 rs2292832 TC/CC genotypes were associated with an increased CAA risk in the male KD patients or KD patients aged younger than 60 months (adjusted OR = 1.62, 95% CI = 1.23–2.13, p = 0.001). The miR-499a rs3746444 AG/GG genotypes were associated with a lower risk of CAA in the male KD patients (adjusted OR = 0.35, 95% CI = 0.26–0.48, p ≤ 0.001). Furthermore, compared with those who had only zero or one risk genotypes, combined analysis indicated that carrying two or three risk genotypes was associated with an increased CAA risk in both male and female KD patients who were aged under 5 years.

4 | DISCUSSION

Our previous study revealed that the miR-149 rs2292832 T>C and miR-196a2 rs11614913 C>T polymorphisms were not associated with KD susceptibility in a southern Chinese population. In the present study, we further explored the miR-149 rs2292832 T>C, miR-196a2 rs11614913 C>T and miR-499a rs3746444 A>G polymorphisms and the risk of CAA in KD. We show for the first time that the miR-149 rs2292832 TC/CC genotypes are significantly associated with an increased susceptibility to CAA in male KD patients aged younger than 60 months and that the miR-499a rs3746444 AG genotype, but probably not the miR-196a2 rs11614913 genotype, is associated with a decreased CAA risk in male children aged younger than 60 months. In addition, the patients carrying two or three risk genotypes had a more evident CAA risk at less than 5 years old. These findings provide a theoretical basis for further research on the etiology of KD and may contribute to reducing the incidence of CAA and developing a target for the treatment of CAA.

Emerging evidence has indicated that miRNAs play important roles in various physiological and pathological processes. miRNAs are also involved in the development of cardiovascular diseases, including KD. Recent studies by our team have shown that reduced platelet-derived miR-223 leads to severe coronary pathology, which promotes vascular smooth muscle cell differentiation and reduces the occurrence of KD complicated with CAA and serum exosomal miR-Let-7i-3p as a new potential biomarker for the diagnosis of KD patients with CAA. KD is an acute systemic vasculitis of unknown etiology that usually affects infants and young children aged under 5 years. Children with severe disease will be complicated by CAAs, leading to a series of cardiovascular sequelae. The development of KD causes an inflammatory response and the release of proinflammatory factors, such as tumor necrosis factor (TNF)-α, interleukin-6 and interleukin-1β, which promote vascular endothelial cell damage and the development of CAA. Studies have found that miR-149, miR-196a2 and miR-499a can induce inflammatory reactions and lead to vascular damage. For example, compared with the A allele, the G allele miR-149 rs71428439 precursor decreased
the expression of mature miR-149 and regulated apoptosis in myocardial infarction.33 Carrying the miR-196a2 rs11614913 gene of the T allele can increase the expression of mature miR-196a2 and contribute to an increased risk of CAD.34 The miR-499a rs3746444 CT genotype has been shown to be associated with significantly higher levels of C-reactive protein (CRP) and erythrocyte sedimentation rate than the CC and TT genotypes in a Chinese Han population with RA.35 In addition, our previous study found that the miR-608 rs4919510 polymorphism has a CAL-related relationship with KD susceptibility.29 Therefore, these three miRNA polymorphisms may be closely related to the severity of KD.

Previous evidence suggests that the miR-149 rs2292832 T>C, miR-196a2 rs11614913 C>T and miR-499a rs3746444 A>G gene polymorphisms are closely related to cardiovascular disease but contradictory in different races. For example, studies have shown miR-149 rs2292832 T>C and miR-196a2 rs11614913 C>T polymorphisms were significantly related to the prevalence of CAD, and the miR-499a rs3746444 A>G polymorphism was not associated with CAD risk in a Korean population.33 However, miR-499a rs3746444 A>G was shown to be strongly associated with an increased risk of CAD in a Greek population.36 Furthermore, miR-499a rs3746444 A>G has been shown to be associated with blood pressure and HDL levels in CAD patients and may serve as a potential biomarker for the clinical prognosis of CAD.37 In cardiovascular disease, the polymorphisms of these genes were also associated with other possible pathogenic factors of disease, such as environmental factors, age, lifestyle (smoking, drinking) and other diseases (hypertension, diabetes).19,38 Thus, these three miRNA polymorphisms may be involved in coronary artery injury in KD patients. In the present study, we observed that the miR-149 rs2292832 T>C and miR-499a rs3746444 A>G polymorphisms are associated with the risk of KD with CAA in southern Chinese children. According to previously published studies, the miR-149 rs2292832 T>C polymorphism reduces the expression level of miR-149, and the miR-499a alleles are associated with TNF-α and CRP levels. Based on these results, it is speculated that the miR-149 rs2292832 T>C polymorphism may affect miR-149 expression to regulate disease biogenesis and that miR-499a rs3746444 A>G may reduce TNF-α and CRP levels to avoid vascular damage in patients with KD. Although we did not measure the expression of miRNAs, TNF-α and CRP, this speculation is partially in line with our current results. Our findings may contribute to an improved understanding of KD pathophysiology and the development of new efficient therapeutics to treat KD with CAA.

In summary, this is a relatively large study that provided statistical evidence indicating that the miR-149 rs2292832 T>C and miR-499a rs3746444 A>G polymorphisms did affect the risk of KD with CAA in southern Chinese children. However, we acknowledge that our research has some limitations. First, we only selected three genetic polymorphisms in the present study. Indeed, there are several well-known miRNA genetic polymorphisms related to cardiovascular diseases, such as miR-146a rs2910164 C>G, miR-218 rs1113452 A>G and miR-618 rs2682818 G>C. Second, we recruited KD patients with coronary artery dilation in the NCAA group as a control. However, the question remains as to whether KD patients with coronary artery dilation are a risk factor for CAA. Among NCAA patients, there were 309 patients with coronary artery dilation and 475 patients without CALs in the present study. A comparison analysis between the CAA group and the coronary artery dilation group implied that the polymorphisms of miR-149 rs2292832 and miR-499a rs3746444 were related to CAA occurrence in KD patients (see Supporting information, Table S1). These results do not affect our current conclusions. Third, we only explored the association between these miRNA polymorphisms and KD with CAA risk. Because the original study design was retrospective, we did not have detailed information on other factors, such as children’s disease history, family history and environmental exposures. Fourth, this is a hospital-based study only, it should be confirmed by studying patients from multiple centers, and the results obtained are not representative of all Chinese patients.

5 | CONCLUSIONS

Male carriers of the miR-149 rs2292832 TC/CC genotype who were aged younger than 5 years exhibited a significantly increased risk of CAA, whereas male carriers of the miR-499a rs3746444 AG genotype who were aged younger than 5 years exhibited a reduced risk of CAA. There was no association between the miR-196a2 rs11614913 C>T polymorphism and the risk of CAA. Moreover, children with two or three risk genotypes had a significantly higher risk of CAA than those with zero or one risk genotypes. These findings provide a theoretical basis for further research on the etiology of KD and might contribute to preventing and reducing the development of CAA.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

LYF and GXQ were responsible for study conceptualization. DC and YFX were responsible for study methodology. LYF, TFZ and GXQ were responsible for the formal analysis. JQL and LP were responsible for study investigations. LP, HZZ and LZ were responsible for the resources. LYF and YFX were responsible for preparation of the original draft. LYF, YFX, TFZ and GXQ were responsible for reviewing and
edjing. JQL was responsible for study supervision. DC, LYF and GXQ were responsible for funding acquisition. All authors read and approved the final version of the manuscript submitted for publication.

ETHICAL STATEMENT
The study was approved by the Medical Ethics Committee of Guangzhou Women and Children's Medical Center (2018052105) and was conducted according to the International Ethical Guidelines for Research Involving Human Subjects stated in the Declaration of Helsinki. Informed written consent was obtained from the guardians of the patients and controls.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES
1. Newburger JW, Takahashi M, Gerber MA, et al. Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from the Committee on Rheumatic Fever, Endocarditis and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. Circulation. 2004;110(17): 2747-2771.
2. Sosa T, Brower L, Divanovic A. Diagnosis and Management of Kawasaki Disease. JAMA Pediatr. 2019;173(3):278-279.
3. McCrindle BW, Rowley AH, Newburger JW, et al. Association of pre-miRNA-146a rs2910164 and premiRNA-499 rs3746444 polymorphisms and susceptibility to rheumatoid arthritis. Mol Med Rep. 2013; 7(1):287-291. https://doi.org/10.3892/mmr.2012.1176
4. Hashemi M, Eskandari-Nasab E, Zakeri Z, et al. Association of premiRNA-146a rs2910164 and premiRNA-499 rs3746444 polymorphisms and susceptibility to rheumatoid arthritis. Mol Med Rep. 2013; 7(1):287-291. https://doi.org/10.3892/mmr.2012.1176
5. Wang D, Wang Y, Lin Z, Cai L. Association between miRNA-146a polymorphism and lung cancer susceptibility: A meta-analysis involving 6506 cases and 6576 controls. Gene. 2020;757:144940. https://doi.org/10.1016/j.gene.2020.144940
6. Huang YH, Lin KM, Ho SC, Yan JH, Lo MH, Kuo HC. Increased incidence of Kawasaki disease in Taiwan in recent years: A 15 Years nationwide population-based cohort study. Front Pediatr. 2019;7:1. https://doi.org/10.3389/fped.2019.00121
7. Singh S, Vignesh P, Burgner D. The epidemiology of Kawasaki disease: a global update. Arch Dis Child. 2015;100(11):1084-1088.
8. Verdini L, Mazza A, Gervasoni A, et al. An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. Lancet. 2020;395(10239): 1771-1778.
9. Rowley AH, Shulman ST. The epidemiology and pathogenesis of Kawasaki disease. Front Pediatr. 2018;6:374. https://doi.org/10.3389/fped.2018.00374
10. Kumrah R, Vignesh P, Rawat A, Singh S. Immunogenetics of Kawasaki disease. Clin Rev Allergy Immunol. 2020;59(1):122-139.
11. Saliminejad K, Khorram Khorshid HR, Soleymani Fard S, Ghaffari SH. An overview of miRNAs: biology, functions, therapeutics, and analysis methods. J Cell Physiol. 2019;234(5):5451-5465.
12. Iorio MV, Croce CM. Causes and consequences of microRNA dysregulation. Cancer J. 2012;18(3):215-222.
13. Li Y, Zhuo ZJ, Zhou H, et al. miR-34b/c rs4938723 T>C decreases neuroblastoma risk: a replication study in the Hunan children. Dis Markers. 2019;2019:6514608. https://doi.org/10.1155/2019/6514608
14. Liu P, Zhuo ZJ, Zhu J, et al. Association of TP53 rs1042522 C>G and miR-34b/c rs4938723 T>C polymorphisms with hepatoblastoma susceptibility: A seven-center case-control study. J Gene Med. 2020; 22(7):e3182. https://doi.org/10.1002/jgm.3182
15. Pi L, Fu L, Xu Y, et al. Lack of association between miR-218 rs11134527 A>G and Kawasaki disease susceptibility. Biosci Rep. 2018;38(3). https://doi.org/10.1042/BSR20180367
16. Wang Y, Lu Z, Fu L, et al. The miRNA-608 rs4919510 G>C polymorphism confers reduce coronary injury of Kawasaki disease in a Southern Chinese population. Biosci Rep. 2019;39(5):BSR20181660. https://doi.org/10.1042/BSR20181660
17. Che D, Li J, Fu L, et al. The rs1625579 T>G polymorphism in the miRNA-13 gene confers a risk of early-onset Kawasaki disease in a southern Chinese population. Infect Drug Resist. 2018;11:1055-1060.
18. Latini A, Cicciacci C, Novelli G, Borgiani P. Polymorphisms in miRNA genes and their involvement in autoimmune diseases susceptibility. Immunol Res. 2017;65(4):811-827.
19. Bastami M, Choupani J, Saadatian Z, et al. miRNA polymorphisms and risk of cardio-cerebrovascular diseases: a systematic review and meta-analysis. Int J Mol Sci. 2019;20(2):293. https://doi.org/10.3390/ijms20020293
20. Wang D, Wang Y, Lin Z, Cai L. Association between miRNA-146a polymorphism and lung cancer susceptibility: A meta-analysis involving 6506 cases and 6576 controls. Gene. 2020;757:144940. https://doi.org/10.1016/j.gene.2020.144940
21. Hashemi M, Eskandari-Nasab E, Zakeri Z, et al. Association of premiRNA-146a rs2910164 and premiRNA-499 rs3746444 polymorphisms and susceptibility to rheumatoid arthritis. Mol Med Rep. 2013; 7(1):287-291. https://doi.org/10.3892/mmr.2012.1176
22. Torahi EA, Ismail NM, Torahi AA, Hussein MH, Faywzy MS. Precursor miR-499a variant but not mir-196a2 is associated with rheumatoid arthritis susceptibility in an Egyptian population. Mol Diagn Ther. 2016;20(3):279-295.
23. Xu B, Luo X, Ning X, et al. miR-149 rs1292832 C allele enhances the cytotoxic effect of temozolomide against glioma cells. Neuroreport. 2020;31(6):498-506.
24. Hashemi M, Moradi N, Ziaee SA, et al. Association between single nucleotide polymorphism in miR-499, mir-196a2, mir-146a and mir-149 and prostate cancer risk in a sample of Iranian population. J Adv Res. 2016;7(3):491-498.
25. Sung JH, Kim SH, Yang WI, et al. miRNA polymorphisms (miR146a, miR149, miR196a2 and miR499) are associated with the risk of coronary artery disease. Mol Med Rep. 2016;14(3):2328-2342. https://doi.org/10.3892/mmr.2016.5495
26. Li J, Wang J, Su X, et al. Association between the miRNA-149 rs2292832 T>C polymorphism and Kawasaki disease susceptibility in a southern Chinese population. J Clin Lab Anal. 2020;34(4):e23125. https://doi.org/10.1002/jcla.23125
27. Wang J, Li J, Qiu H, et al. Association between miRNA-196a2 rs11614913 T>C polymorphism and Kawasaki disease susceptibility in southern Chinese children. J Clin Lab Anal. 2019;33(7):e22925. https://doi.org/10.1002/jcla.22925
28. Zhang Y, Wang Y, Zhang L, et al. Reduced platelet miR-223 induction in kawasaki disease leads to severe coronary artery pathology through a miR-223/PDGFRbeta vascular smooth muscle cell axis. Circ Res. 2020;127(7):855-873.
29. Wang YF, Lian XL, Zhong JY, et al. Serum exosomal microRNA let-7i-3p as candidate diagnostic biomarker for Kawasaki disease patients with coronary artery aneurysm. *IUBMB Life*. 2019;71(7):891-900.

30. Noval Rivas M, Arditi M. Kawasaki disease: pathophysiology and insights from mouse models. *Nat Rev Rheumatol*. 2020;16(7):391-405.

31. Yamaji N, da Silva LK, Shoda T, et al. TNF-alpha blockers for the treatment of Kawasaki disease in children. *Cochrane Database Syst Rev*. 2019;8:CD012448. https://doi.org/10.1002/14651858.CD012448.pub2

32. Li Y, Zheng Q, Zou L, et al. Kawasaki disease shock syndrome: clinical characteristics and possible use of IL-6, IL-10 and IFN-gamma as biomarkers for early recognition. *Pediatr Rheumatol Online J*. 2019;17(1):1. https://doi.org/10.1186/s12969-018-0303-4

33. Ding SL, Wang JX, Jiao JQ, et al. A pre-microRNA-149 (miR-149) genetic variation affects miR-149 maturation and its ability to regulate the Puma protein in apoptosis. *J Biol Chem*. 2013;288(37):26865-26877.

34. Qiu H, Chen Z, Lv L, Tang W, Hu R. Associations between microRNA polymorphisms and development of coronary artery disease: a case-control study. *DNA Cell Biol*. 2020;39(1):25-36.

35. Yang B, Chen J, Li Y, et al. Association of polymorphisms in pre-miRNA with inflammatory biomarkers in rheumatoid arthritis in the Chinese Han population. *Hum Immunol*. 2012;73(1):101-106.

SUPPORTING INFORMATION
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