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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 $p \leq 0.001$). Moreover, patients carrying two or three of these single nucleotide polymorphism (SNP) genotypes (rs2292832 TC/CC and

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rs11614913 TT and rs3746444 AA) had a higher risk for CAA than those who harbored only zero or one of these SNP genotypes.

Conclusions: Our results demonstrated that the *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 (CALs), 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 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, *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 diseases[19]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 CALs 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.[1] All the patients collected after 2017 were diagnosed in accordance with the latest guidelines.[3] All the patients were confirmed by professional cardiologists and included in this study; patients with congenital heart disease, cardiac hypoplasia and incomplete clinical case data were excluded. CAA patients were referred for absolute value of coronary vessel inner diameter and a *z* score: large CAA (≥ 8.0 mm or $z \geq 10$), medium CAA (5.0–8.0 mm or $z \geq 5$ to < 10) and small CAA (< 5.0 mm or $z \geq 2.5$ to < 5).[1,3] KD Patients were divided into two subgroups: patients with CAA as the CAA group, age and gender-matched subjects without CAA (normal coronary artery and coronary artery dilation patients) were randomly selected as the NCAA group. The ages of all the subjects ranged from 0 to 14 years. The parents of all the patients knew the purpose of the study and signed an informed consent form. This study was approved by the Ethics Committee of Guangzhou Women and Children's Medical Center (2015090113).

2.2 | DNA extraction and genotype

Ethylenediaminetetraacetic acid whole blood specimens were collected from the patients with KD. In total, 200 μL of whole blood was 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 the samples were stored in a refrigerator at −80°C for later use. Detection of the genotype of these miRNAs (*miR-149* rs2292832 T>C, *miR-196a2* rs11614913 C>T and *miR-499a* rs3746444 A>G) was determined using TaqMan probes (C_11533078_30 for rs2292832, C_31185852_10 for rs11614913 and C_2142612_40 for rs3746444). A TaqMan® single nucleotide polymorphism (SNP) genotyping kit was used on an ABI Q6 instrument (QuantStudio™ 6 Flex Real-Time PCR System; Applied Biosystems, Foster City, CA, USA). To validate the genotyping results, we randomly selected approximately 5% of the samples for repeat genotyping, and the results were found to be consistent.

2.3 | Statistical analysis

We used chi-squared tests to assess the distributions of demographic variables and genotype frequency distributions in the KD patients. Univariate and multivariate logistic regression models were used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) after adjusting for age and gender to evaluate the association between the *miR-149* 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 ($p \leq 0.001$)) 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

	CAA ($n = 318$)		NCAA ($n = 784$)		
Variables	***n***	**%**	***n***	**%**	***p*[a]**
Age range (months)	1.00–166.00		1.00–131.00		0.349
Median	15		20		
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.
[a]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 ($n = 318$)	NCAA ($n = 784$)	p^a	Crude OR (95% CI)	p	Adjusted OR (95% CI)[a]	p^b
miR-149 rs2292832 (T>C)							
TT	150 (47.17)	455 (58.04)	**0.005**	1.00		1.00	
TC	125 (39.31)	248 (31.63)		1.53 (1.15–2.03)	**0.003**	1.53 (1.15–2.03)	**0.003**
CC	43 (13.52)	81 (10.33)		1.61 (1.07–2.43)	**0.024**	1.63 (1.08–2.47)	**0.021**
Dominant	168 (52.83)	329 (41.96)	**0.001**	1.55 (1.19–2.01)	**0.001**	1.56 (1.20–2.02)	**0.001**
Recessive	275 (86.48)	703 (89.67)	0.135	1.36 (0.91–2.02)	0.130	1.37 (0.92–2.04)	0.117
miR-196a2 rs11614913 (T>C)							
TT	86 (27.04)	202(25.77)	0.904	1.00		1.00	
TC	163(51.26)	411 (52.42)		0.93 (0.68–1.27)	0.655	0.94 (0.69–1.29)	0.713
CC	69 (21.70)	171 (21.81)		0.95 (0.65–1.38)	0.780	0.96 (0.66–1.40)	0.834
Dominant	232(72.96)	582 (74.23)	0.662	0.94 (0.70–1.26)	0.662	0.95 (0.71–1.27)	0.724
Recessive	249 (78.30)	613 (78.19)	0.967	0.99 (0.72–1.36)	0.967	1.00 (0.73–1.37)	0.993
miR-499a rs3746444 (A>G)							
AA	238 (74.84)	407(51.91)	**< 0.001**	1.00		1.00	
AG	73 (22.96)	370 (47.19)		0.34 (0.25–0.45)	**< 0.001**	0.33 (0.25–0.45)	**< 0.001**
GG	7 (2.20)	7 (0.89)		1.71 (0.59–4.94)	0.321	1.61 (0.56–4.66)	0.380
Dominant	80 (25.16)	377 (48.09)	**< 0.001**	0.36 (0.27–0.49)	**< 0.001**	0.36 (0.27–0.48)	**< 0.001**
Recessive	311 (97.80)	777 (99.11)	0.094	2.50 (0.87–7.18)	0.089	2.35 (0.81–6.76)	0.115
Combined effect of risk genotypes[c]							
0	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)	**0.001**
2	129 (40.57)	220 (28.06)		3.52 (2.25–5.51)	**< 0.001**	3.51 (2.24–5.50)	**< 0.001**
3	37 (11.64)	54 (6.89)		4.11 (2.32–7.30)	**< 0.001**	4.12 (2.32–7.33)	**< 0.001**
Trend[d]				1.62 (1.38–1.89)	**< 0.001**	1.62 (1.38–1.89)	**< 0.001**
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)	**0.002**

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

[a]Adjusted for age and gender.

[b]Chi-squared test for genotype distributions between Kawasaki disease patients and controls.

[c]Risk genotypes were *miR-149* rs2292832 TC/CC, *miR-196a2* rs11614913 TT, *miR-499a* rs3746444 AA.

[d]"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, $p \leq 0.001$ 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, $p \leq 0.001$). 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, $p = 0.002$).

TABLE 3 Stratification analysis for the association between miRNA polymorphisms and susceptibility to CAA in Kawasaki disease

Variables	rs2292832 (CAA/NCAA)		Adjusted OR[a] (95% CI)	p^a	rs3746444 (CAA/NCAA)		Adjusted OR[a] (95% CI)	p^a	Combined effect of risk genotypes (CAA/NCAA)		Adjusted OR[a] (95% CI)	p^a
	TT	TC/CC			AA	AG/GG			0–1	2–3		
Age (months)												
≤ 60	136/428	158/309	**1.62 (1.23–2.13)**	**0.001**	71/347	223/390	**0.35 (0.26–0.48)**	**< 0.000**	136/474	158/263	2.11 (1.61–2.78)	**< 0.000**
> 60	14/27	10/20	0.96 (0.35–2.61)	0.934	9/30	15/17	0.32 (0.12–0.91)	0.032	16/36	8/11	1.76 (0.58–5.33)	0.316
Gender												
Males	113/322	126/225	1.60 (1.17–2.16)	**0.003**	60/271	179/276	0.35 (0.25–0.49)	**< 0.000**	113/361	126/186	2.13 (1.56–2.90)	**< 0.000**
Females	37/133	42/104	1.45 (0.87–2.41)	0.159	20/106	59/131	0.39 (0.22–0.70)	0.002	39/149	40/88	1.78 (1.06–3.00)	**0.028**

[a]Adjusted for age and gender.

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, $p \leq 0.001$)). 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[28] and serum exosomal miR-Let-7i-3p as a new potential biomarker for the diagnosis of KD patients with CAA.[29] KD is an acute systemic vasculitis of unknown etiology that usually affects infants and young children aged under 5 years.[3] Children with severe disease will be complicated by CAAs, leading to a series of cardiovascular sequelae.[30] 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.[31,32] Studies have found that miR-149, miR-196a2 and miR-499a can induce inflammatory reactions and lead to vascular damage.[25] 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.[20] 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.[25] 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 carries 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

editing. 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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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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