Lack of association between miR-218 rs11134527 A>G and Kawasaki disease susceptibility

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Research Article

Kawasaki disease (KD) is a type of disease that includes the development of a fever that lasts at least 5 days and involves the clinical manifestation of multicellular vasculitis. KD has become one of the most common pediatric cardiovascular diseases. Previous studies have reported that miR-218 rs11134527 A>G is associated with susceptibility to various cancer risks. However, there is a lack of evidence regarding the relationship between this polymorphism and KD risk. The present study explored the correlation between the miR-218 rs11134527 A>G polymorphism and the risk of KD. We recruited 532 patients with KD and 623 controls to genotype the miR-218 rs11134527 A>G polymorphism with a TaqMan allelic discrimination assay. Our results illustrated that the miR-218 rs11134527 A>G polymorphism was not associated with KD risk. In an analysis stratified by age, sex, and coronary artery lesions, we found only that the risk of KD was significantly decreased for children older than 5 years (GG vs. AA/AG: adjusted OR = 0.26, 95% CI = 0.07–0.94, P = 0.041). The present study demonstrated that the miR-218 rs11134527 A>G polymorphism may have an age-related relationship with KD susceptibility that has not previously been revealed.

Introduction

Kawasaki disease (KD) is an acute, systemic inflammatory vasculitis disease that is also known as lymphatic mucosa syndrome and usually affects infants and young children [1,2]. The clinical features of the disease mainly manifest as a fever lasting 5 days or longer. Patients should be given adequate treatment with intravenous immunoglobulin (IVIG) in the early stage. Coronary artery lesions (CALs) develop in more than 20–25% of untreated patients, some of whom even develop coronary artery aneurysms [3], which are the main cause of death in KD patients. After treatment, the complication rate decreases to 5%. Thus far, the etiology of KD remains unclear. Epidemiological studies have demonstrated that the incidence rates of KD are increasing year by year in the areas of Japan, South Korea, and Taiwan [4]. Most previous studies have focused on the associations of KD susceptibility with single nucleotide polymorphisms (SNPs), such as SNPs of ITPKC, GRIN3A, ITPR3, ADAM17, CASP3, TARC/CCL17 etc. [5–9]. However, the relationships between microRNA polymorphisms and KD susceptibility have not been reported.

MicroRNAs (miRNAs) are a class of endogenous, small RNAs of approximately 18–25 nucleotides. One miRNA can have multiple target genes, and several miRNAs can also regulate the same gene. miRNAs exist in many forms, including pri-miRNAs, pre-miRNAs, and mature miRNAs. SNPs are spread throughout the human genome and play a main role in DNA mutations that are functional or regulate various biological processes and therefore can contribute to the development or prevention of diseases [10,11]. Numerous
studies have revealed the relationship between the miR-218 polymorphism and cancer risk in the Chinese population. Gao et al. [12] reported a meta-analysis about cancer in the Chinese population, and their results revealed that the miR-218 rs11134527 A>G polymorphism is linked to a remarkable reduction in cancer risk in GG genotype people compared with AA and AG genotype people. Other studies have demonstrated that the miR-218 rs11134527 A>G polymorphism is related to the susceptibility to cervical cancer, esophageal squamous cell carcinoma, breast cancer, and neuroblastoma [13-16]. Several studies have demonstrated the relationship between miR-218 and cardiovascular disease. For example, miR-218 has been reported to be associated with pathogenesis of myocardial infarction [17]. miR-218 also regulates heart tube formation in zebrafish by targeting Robo family proteins [18]. Chiavacci et al. [19] demonstrated that miR-218 mediates Tbx5 overexpression during zebrafish heart development. However, the association of the miR-218 genetic polymorphism with KD is unknown. In the present study, we focused on whether the miR-218 rs11134527 A>G polymorphism was related to the risk of KD.

Materials and methods

Study population

The subjects included 532 patients with KD and 623 controls that were collected from January 2012 to January 2017 in the Guangzhou Women and Children Medical Center in China. The KD patients were diagnosed mainly based on the Japanese Kawasaki Disease Research Association’s comprehensive clinical diagnostic criteria [20]. The KD patients attended our hospital as outpatients with follow-ups and inpatients, and the healthy controls were children who came to our hospital for health examinations within the same time period and had no fever or other diseases. The present study was approved by the Guangzhou Women and Children Medical Center Ethics Committee, and the children and their families provided written informed consent.

DNA extraction and genotyping

Samples of 200 μl of anticoagulant-containing blood were collected according to the instructions of the Genomic DNA Extraction Kit. The specific procedures can be found in the literature [21-23]. The centrifuge tube was collected and quantified using a nucleic acid quantifier, which was eventually stored at −80°C. The miR-218 rs11134527 A>G polymorphism was genotyped with TaqMan reagent. Allele-specific probes were purchased from Applied Biosystems. The PCR reaction was performed in 384-well plates that were run on an ABI-Q6 Sequence Detection System machine [24,25]. Moreover, to ensure the quality and accuracy of the genotyping results, we randomly selected 10% of the samples for repeat analysis, and the results were 100% concordant.

Statistical analysis

First, we examined the Hardy–Weinberg equilibrium (HWE) of the samples. Next, the χ² test was employed to assess the significant differences between cases and controls in the frequency distributions and genotypes. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to quantify the association between the miR-218 rs11134527 A>G polymorphism and Kawasaki disease susceptibility with adjustments for age and gender. The association between the miR-218 rs11134527 A>G polymorphism and KD was assessed with analyses that were stratified by age, gender, and coronary artery lesions. The data analyses were performed with SAS software (Version 9.4; SAS Institute, Cary, NC, U.S.A.). A P-value <0.05 indicated a significant difference.

Results

Characteristics of the patients with Kawasaki disease

Table 1 presents the selected demographic characteristics of the cases and controls. There were no differences between the KD patients and controls in the distributions of age (P=0.602) or gender (P=0.143). The mean ages were 28.39 months for the KD patients (±24.68; range 1–166) and 28.48 months for the controls (±25.33; range 0.07–166). Among the cases, 31.39% and 68.61% were female and male, respectively, and the controls were 35.47% and 64.53% female and male, respectively (P 0.143). Among the cases, 31.58% had a CAL, and 61.42% had no coronary artery lesion (NCAL).

Association between the miR-218 rs11134527 A>G polymorphism and the risk of KD

The genotype distributions of the miR-218 rs11134527 A>G polymorphism in the KD patients and controls are displayed in Table 2. The miR-218 rs11134527 A>G genotype distribution analysis for HWE in the control group...
Table 1 Frequency distribution of selected variables for cases and controls

| Variables                      | Cases (N=532) | Controls (N=623) | P*  |
|--------------------------------|---------------|------------------|-----|
| Age range, month               |               |                  |     |
| No.                            | %             | No.              | %   |
| 1.00–166.0                     | 0.07–166      | 0.602            |
| Mean ± SD                      | 28.39 ± 24.68 | 28.48 ± 25.33    |     |
| <12                            | 137           | 165              |     |
| 12–60                          | 351           | 397              |     |
| ≥60                            | 44            | 61               |     |
| Gender                         |               |                  |     |
| Male                           | 365           | 402              |     |
| Female                         | 167           | 221              |     |
| Coronary artery lesion         |               |                  |     |
| CAL                            | 168           | 35.47            |
| NCAL                           | 364           | 64.53            |

Table 2 Genotype distributions of rs11134527 A>G polymorphism and Kawasaki disease susceptibility

| Genotype | Cases (N=523) | Controls (N=623) | P*  | Crude OR (95% CI) | Adjusted OR (95% CI)† | P† |
|----------|---------------|------------------|-----|------------------|-----------------------|----|
|          |               |                  |     |                  |                       |    |
| HWE = 0.779 |             |                  |     |                  |                       |    |
| AA       | 162 (30.98)   | 195 (31.30)      | 1.00|                  | 1.00                  |    |
| AG       | 267 (51.05)   | 328 (52.65)      | 1.00 (0.77–1.30) | 1.00 (0.75–1.27) | 0.863                  |    |
| GG       | 94 (17.97)    | 100 (16.05)      | 1.16 (0.81–1.64) | 0.420            | 1.13 (0.80–1.61) | 0.489                  |    |
| Additive |               |                  | 0.881|                 |                       |    |
| Dominant | 361 (69.02)   | 428 (68.70)      | 0.906| 1.02 (0.79–1.31) | 1.01 (0.79–1.30) | 0.919                  |    |
| Recessive| 429 (82.03)   | 523 (83.95)      | 0.388| 1.15 (0.84–1.56) | 1.15 (0.84–1.57) | 0.380                  |    |

*χ² test for genotype distributions between Kawasaki disease patients and controls.
†Adjusted for age and gender.

revealed an equilibrium (HWE = 0.779). However, there was no significant association between the miR-218 rs11134527 A>G polymorphism and KD susceptibility after adjustments for age and gender (AG vs. AA: adjusted OR = 0.98, 95% CI = 0.75–1.27, P=0.863; GG vs. AA: adjusted OR = 1.13, 95% CI = 0.80–1.61, P=0.489; dominant model: adjusted OR = 1.01, 95% CI = 0.79–1.30, P=0.919; and recessive model: adjusted OR = 1.15, 95% CI = 0.84–1.57, P=0.380).

Stratified analysis

Next, we further assessed the effects of the miR-218 rs11134527 A>G polymorphism in the cases and controls with a stratified analysis (Table 3). The participants were stratified according to age, gender, and the presence of coronary artery lesions. In the analysis stratified by age, we found that there were more controls with the GG genotype among the children who were older than 60 months (adjusted OR = 0.26, 95% CI = 0.07–0.94, P=0.041).

Discussion

In our study, the results did not reveal a significant relationship between the miR-218 rs11134527 A>G polymorphism and KD susceptibility in Southern Chinese children. However, a stratified analysis suggested that there was a significant association between carriers of the AA/AG genotypes and the occurrence of KD compared with carriers of the GG genotype especially among the children older than 5 years.

KD is an acute systemic vasculitis and self-limiting disease that predominantly occurs in children younger than 5 years. The incidence rate of KD varies geographically, and it is more prevalent in Asian populations [26]. Although the etiology of KD remains unknown, extensively studies have suggested that the causes of KD may be affected by viral or bacterial infections, autoimmune factors and genetic factors [27]. With the goal of identifying the genetic factors related to KD, Kuo et al. [28] indicated that the C allele of ITPKC rs28493229 (341 KD patients and 1190 controls)
Table 3 Stratification analysis for the association between rs11134527 A>G polymorphism and Kawasaki disease susceptibility

| Variables                        | AA/AG cases/controls | GG | Crude OR (95% CI) | P   | Adjusted OR* (95% CI) | P*  |
|----------------------------------|----------------------|----|-------------------|-----|-----------------------|-----|
| Gender                           |                      |    |                   |     |                       |     |
| <12                              | 110/145              | 22/20 | 1.45 (0.75–2.79) | 0.266 | 1.51 (0.78–2.94) | 0.221 |
| 12–60                            | 279/331              | 68/66 | 1.22 (0.84–1.78) | 0.293 | 1.22 (0.84–1.78) | 0.297 |
| >60                              | 40/47                | 4/14  | 0.34 (0.10–1.10)  | 0.072 | 0.26 (0.07–0.94) | 0.041 |
| Gender                           |                      |    |                   |     |                       |     |
| Females                          | 130/189              | 35/32 | 1.59 (0.94–2.70)  | 0.086 | 1.56 (0.91–3.67) | 0.104 |
| Males                            | 299/334              | 59/68 | 0.97 (0.66–1.42)  | 0.873 | 0.98 (0.67–1.43) | 0.907 |
| Coronary artery lesion            |                      |    |                   |     |                       |     |
| CAL                              | 140/523              | 26/100 | 0.97 (0.61–1.55)  | 0.904 | 0.97 (0.61–1.56) | 0.912 |
| NCAL                             | 289/523              | 68/100 | 1.23 (0.88–1.73)  | 0.232 | 1.23 (0.87–1.73) | 0.237 |

*Adjusted for age and gender.

is associated with the susceptibility to KD and aneurysm formation in KD patients in a Taiwanese population. A review revealed that many potential susceptibility genes are associated with the risk of KD and CAL, including SNPs of ITPKC, CASP3, T helper type17, TGF-β, BLK, FCGR2A, KCNN2, and other genes [29]. However, Natividad et al. [30] reported that they did not find any statistically significant associations of the HLA-DRB1, TNF-α and ITPKC genes with KD in KD patients relative to controls among a Filipino population. Other studies have reported similar results in that the susceptibilities to KD and CAL were not associated with the HLA-DRB1 gene in a Taiwanese population and a Korean population [31,32]. These findings indicate that the results of such association studies may be affected by race, environment, and family genetics.

miRNAs are endogenous noncoding small RNAs that regulate the expression of genes by the reverse complementation of genes by specific miRNAs, and miRNAs are involved in the regulation of a variety of biological processes, including inflammation, cardiovascular diseases, and cancer. Polymorphisms in miRNAs gene may affect miRNA biogenesis and function; for example, the results from Tian et al. [33] suggested that the miR-146a rs2910164 and miR-196a-2rs11614913 polymorphisms were associated with the hepatitis virus-related hepatocellular cancer risk. Dai et al. [34] recruited 1143 subjects (583 controls; 560 breast cancer patients) and found that the T allele polymorphism of the miR-196a2 rs11614913 gene is associated with a decreased risk of breast cancer and that the miR-499 rs3746444 AG/GG genotypes are associated with an increased risk of breast cancer in Chinese individuals. These authors also found that the miR-196a-2 rs11614913 and miR-27a rs895819 polymorphisms are correlated with reduced breast cancer risk [34,35]. Moreover, several studies have reported that miRNAs also play a critical role in KD. Researchers are focused on the potential of miRNAs as useful diagnostic biomarkers, therapeutic targets, and actors in disease pathogenesis in KD. Rong et al. [36] demonstrated that the serum miR-92a-3p levels were significantly higher in children with KD compared with non-Kawasaki disease subjects, and the miR-92a-3p level had a diagnostic sensitivity of 81.8% for KD. Zhang et al. [37] enrolled 102 patients with KD and 80 healthy controls in a study and found that the serum miR-200c and miR-371-5p levels were significantly higher in the KD patients compared with the controls. These two miRNAs were significantly higher in KD patients who were resistant to IVIG compared with those for whom IVIG was effective; thus, these two miRNAs may serve as diagnostic biomarkers and therapeutic targets in KD. In previous microRNAs target prediction studies, the results indicated that the target genes of serum miR-200c, miR-371-5p, and miR-145 are related to signaling pathways that include the Wnt, MAPK, TGF-β, and mTOR signaling pathways, and these pathways have been reported to be involved in inflammatory responses [38-40]. However, these findings provide no evidence that miR-218 plays an important role in KD.

MiR-218 is a tumor suppressor gene that is expressed at significantly low levels in various tumors. Some targets of miR-218, such as ROBO1, RICTOR, BIRC5 and LAMB3, have been reported to participate in many cancer signaling pathways, such as the ERK/MAPK, Wnt/β-catenin, and Notch pathways [12,41,42]. These findings indicate that the up-regulation of miR-218 may reduce the risk of cancer by down-regulating these targets. SNPs of these miRNAs may improve miRNA binding affinity and alter the mRNA expression levels of the target genes and thus may contribute to the susceptibilities of humans to common diseases [43,44]. Therefore, we examined whether the miR-218 rs11134527 A>G polymorphism is associated with the genetic susceptibility to KD. In our present study, we found
no significant association between the miR-218 rs11134527 A>G polymorphism and KD risk. Interestingly, we observed that the miR-218 rs11134527 A>G carriers of the GG genotype were associated with a significantly decreased risk of KD compared with the AG/AA genotype carriers in the older-age subgroup (>60 months), which may be attributable to the fact that younger patients may have higher KD susceptibility, and the miR-218 rs11134527 A>G variant homozygote GG genotype may affect miR-218 binding and mRNA splicing, which would affect the expression of some targets. Zhou et al. [45] demonstrated that the miR-218 rs11134527 GG genotype is associated with an increased risk of cervical cancer compared with the AA genotype among Chinese women. Furthermore, according to a previous study of cardiovascular disease, Gao et al. [46] and Chen et al. [47] reported no associations of miR-218 rs11134527 A>G with the risks of congenital heart disease or myocardial infarction in a Chinese population. To some extent, these studies may support our results.

Although this is the first investigation of the association between the miR-218 rs11134527 A>G polymorphism and KD risk in Chinese children, our study has potential limitations that should be reviewed. First, due to the retrospective nature of the original study design, we did not have detailed information about other factors, such as medical histories, parental environmental exposures, and dietary intakes. Second, we only performed a case–control study to explore the association between the miR-218 rs11134527 A>G polymorphism and KD susceptibility, and we did not explore the expression level of miR-218 in the peripheral blood or the potential mechanisms of action of the polymorphism. Third, we only examined the rs11134527 A>G polymorphism and other potential SNPs of miR-218 were not included in the present study.

In short, we recruited 1155 children (532 cases and 623 controls) to participate in our research. Compared with previous studies (samples of <120 cases), this project had a relatively larger sample size. Moreover, this is the first report to demonstrate that the miR-218 rs11134527 A>G polymorphism was not associated with the genetic susceptibility to KD; however, the miR-218 rs11134527 A>G polymorphism may have a weak effect on KD risk among those older than 5 years in the Chinese population. Further investigation of the mechanisms by which the miR-218 rs11134527 A>G polymorphism affects KD susceptibility are required.

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Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

Author Contribution
All authors contributed significantly to this work. L.P., L.F., Y.X., D.C., Q.D., X.H., M.L., and L.Z. performed the research study and collected the samples and data. L.P. and D.C. analyzed the data. P.H. and X.G. designed the research study. L.P., L.F., and G.X. wrote the paper, and L.F. prepared all of the tables. All authors have reviewed the manuscript. Additionally, all authors have read and approved the manuscript.

Abbreviations
CAL, coronary artery lesion; CI, confidence interval; HWE, Hardy–Weinberg equilibrium; IVIG, intravenous immunoglobulin; KD, Kawasaki disease; miRNA, microRNA; NCAL, no coronary artery lesion; OR, odds ratio; SNP, single nucleotide polymorphism.

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