Differential expression of long non-coding RNAs SRA, HCG22 and MHRT in children with Kawasaki disease

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Abstract. Kawasaki disease (KD) is an acute, self-limited inflammatory illness during childhood that may lead to thrombosis in the coronary arteries (CA). The major aims of the present study were to estimate the serum levels of long non-coding RNAs (lncRNAs) and the metabolic profiles of patients with KD. A total of 40 specimens were obtained from pediatric patients (40 specimens before and 40 specimens after treatment) who were diagnosed with KD (n=40). The controls comprised healthy children without KD (n=40). The serum levels of lncRNAs steroid receptor RNA activator (SRA), human leukocyte antigen complex group 22 (HCG22) and myosin heavy chain-associated RNA transcript (MHRT) were determined using reverse transcription-quantitative PCR. Subsequently, the correlation between the expression levels of lncRNAs and biochemical parameters of patients was assessed. Receiver operating characteristic curves were constructed to determine the diagnostic value of the lncRNAs. The results indicated that the serum levels of lncRNAs SRA and HCG22 were higher in patients with acute KD compared with those in healthy controls. B-type natriuretic peptide (BNP) and C-reactive protein were positively correlated with HCG22 in patients with acute KD, while total cholesterol and low-density lipoprotein were negatively correlated with HCG22 in patients with acute KD. The lncRNA MHRT was significantly upregulated in convalescent KD compared with acute KD following intravenous immunoglobulin therapy. In patients with convalescent KD, creatine kinase was positively correlated with MHRT, while BNP and adenosine deaminase were negatively correlated with MHRT. In conclusion, to the best of our knowledge, the present study was the first to identify that the serum levels of lncRNAs SRA and HCG22 in patients with acute KD were higher compared with those in control subjects. MHRT levels in patients with convalescent KD were higher than those in the acute phase. LncRNAs SRA and HCG22 may have crucial roles in KD and are potential novel diagnostic biomarkers for KD. LncRNA MHRT may be considered a novel biomarker for predicting the clinical prognosis of patients with KD.

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Introduction

Kawasaki disease (KD), also known as mucocutaneous lymph node syndrome, was first reported by Kawasaki (1) in 1967. KD is an acute febrile disease that may result in vasculitis of the small- and medium-sized arteries, particularly the coronary arteries (CA) (2). KD mainly affects infants and children aged <5 years. Due to the unclear pathogenesis and etiology of the disease, diagnosis relies on the major clinical features of KD and other clinically similar diseases should be excluded with known causes, such as exudative conjunctivitis, exudative pharyngitis, oral ulcerations, splenomegaly and vesiculobullous or petechial rashes (3). However, this frequently results in delayed treatment of pediatric patients with incomplete KD (3). Principal clinical findings of classic KD include prolonged fever (≥5 days), lips and oral cavities (including erythema and cracking of lips, erythema of oral and pharyngeal mucosa), bilateral conjunctival congestion, acute non-purulent cervical lymphadenopathy, polymorphous exanthema and changes in the extremities, such as erythema and edema of the extremities in the acute phase (4). However, the detailed pathogenesis of KD has remained elusive (5). Despite the spontaneous cessation of febrile and other signs of inflammation, up to 25% of untreated patients develop permanent damage to the CA, even resulting in CA aneurysm formation (6). The most common cause of mortality from KD is myocardial infarction due to thrombosis or luminal myofibroblastic proliferation leading to stenosis (7).

Long non-coding RNAs (lncRNAs), which are >200 nucleotides in length and lack any protein-coding capacity, have been discovered to be extensively transcribed

from the genome (8). Multiple lines of evidence indicated that mutations and dysregulations of lncRNAs are related to a variety of human diseases. LncRNAs are readily detectable in numerous human body fluids, including plasma, serum, urine and saliva, making them promising and attractive noninvasive and rapid diagnostic tools for disease diagnosis and prognosis (9).

The past decade has witnessed a rapid increase in the number of studies addressing the roles of lncRNAs in diverse cardiovascular conditions and associated risk factors. For instance, lncRNAs are now associated with different cardiovascular diseases, including vascular disease, atherosclerosis, pathological hypertrophy and development, dyslipidemia and metabolic syndrome (10).

To the best of our knowledge, the expression of cardiac disease-related lncRNAs has not been previously investigated in KD. The present study aimed to explore the levels of cardiac disease-related lncRNAs in blood specimens of pediatric patients with and without KD and between acute and convalescent KD. In addition, their correlation with laboratory parameters was analyzed and the power of candidate lncRNAs in predicting KD was determined in order to identify potential biomarkers for the prediction of the disease.

Materials and methods

Subjects. All specimens were collected at Ningbo Women and Children's Hospital (Ningbo, China) between March 2017 and December 2017. The study cohort comprised 40 pediatric patients with KD (cases) and 40 age-matched healthy subjects (controls; children undergoing physical examination) without any clinical symptoms and signs of inflammation or infection. All patients with KD had fever for at least five days and met at least four of the five clinical criteria for KD (rash, oral mucosal erythema, conjunctival injection and cervical lymphadenopathy and swelling of the hands and feet) or three of the five criteria plus CA abnormalities documented by echocardiogram (3). Table I presents the demographic and clinical data of the study subjects. Serum samples were obtained from healthy children and pediatric patients with KD at both the acute phase [prior to intravenous immunoglobulin (IVIG) administration] and convalescent phase (after IVIG therapy) (11). All patients with KD received 2 g/kg IVIG and aspirin therapy (Table II).

Collection of human blood samples and RNA extraction. Venous blood samples (3 ml/patient) were drawn via direct venous puncture into tubes containing inert separation gel, which was collected from each of the patients with KD (both acute and convalescent phases) and each of the healthy controls. Subsequently, whole blood was centrifuged to obtain serum within 48 h, which was frozen at -80˚C until further use. In the serum of patients, the following laboratory parameters were measured: D-dimer, procalcitonin (PCT), B-type natriuretic peptide (BNP), total protein (TP), albumin, sodium, triglycerides (TG), lactate dehydrogenase (LDH), creatine kinase (CK), CK-myocardial band (CK-MB), adenosine deaminase (ADA), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), total bilirubin (Tbil), total bile acids (TBA) and C-reactive protein (CRP). RNA was isolated using TRIzol-LS (Qiagen GmbH), phenol and chloroform.

Reverse transcription-quantitative PCR (RT-qPCR). Complementary (c)DNA was reverse transcribed from RNA using the HiFi-MMLV cDNA first strand synthesis kit (CoWin Biosciences). RT-qPCR was performed on a 96-well format Roche LightCycler 480 real-time PCR machine for detecting lncRNAs in the case and control groups. The dissociation curve of each sample was then assessed. The relative fold changes were calculated using the comparative threshold cycle (Ct) method (12). Larger ΔCt values indicated lower expression. U6 was used as an internal control. The sequences of the lncRNA primers are listed in Table III.

Statistical analysis. All statistical analyses were performed with SPSS version 24.0 software (IBM Corp.). The mean and standard deviation (SD) were calculated for lncRNA expression levels. Data are presented as the mean ± SD of three independent experiments. Independent-samples Student's t-test was used to compare two different groups, paired Student's t-test was used to compare the same group at two different time-points. Pearson correlation analysis was performed to determine the correlation between levels of different lncRNAs and between lncRNA levels and clinical laboratory parameters assessed using the same serum samples. A receiver operating characteristic (ROC) curve was plotted to evaluate the diagnostic value of lncRNAs. $P<0.05$ was considered to indicate statistical significance.

Results

Demographic data. A total of 40 serum samples from patients with KD (age, 2.84±1.77 years; 24 males) were collected for the present study. Furthermore, 40 healthy controls (age, 3.16±3.63 years; 21 males) were included. The mean duration of fever in patients with KD was 7.97 days (all patients had fever for ≥5 days). Other symptoms included changes in the lips and oral cavity (37 cases; 92.5%), bilateral conjunctival congestion (33 cases; 82.5%), acute non-purulent cervical lymphadenopathy (30 cases, 75%), polymorphous exanthema (29 cases, 72.5%) and changes in the extremities (24 cases, 60.0%). A total of 9 patients (22.5%) were indicated to have CA abnormalities (Table I).

All patients with KD received 2 g/kg IVIG and aspirin therapy. As presented in Table II, between acute KD and convalescent KD, D-dimer ($P<0.001$), PCT ($P=0.012$), BNP ($P<0.001$), TP ($P<0.001$), LDH ($P=0.034$), ADA ($P<0.001$), ALT ($P=0.035$), TC ($P=0.001$), LDL ($P<0.001$), Tbil ($P<0.022$), TBA ($P=0.009$) and CRP ($P<0.001$) differed significantly. However, the laboratory data of the acute group were not significantly different with regards to albumin levels, sodium, TG, CK-MB, CK, AST and HDL. However, when comparing the acute KD and control groups, TP ($P<0.001$), albumin ($P<0.001$), sodium ($P<0.001$), CK ($P<0.001$), LDH ($P<0.001$), ALT ($P=0.005$), TC ($P<0.001$), HDL ($P<0.001$), Tbil ($P=0.03$), TBA ($P=0.01$) and CRP ($P<0.001$) were significantly different. By contrast, TG, CK-MB, ADA, AST and LDL did not exhibit any significant difference between acute KD and controls.

Table I. Demographic and clinical characteristics of the subjects.

Item	Healthy controls (n=40)	Kawasaki disease (n=40)
Age (years)	3.16±3.63	2.84±1.77
Male sex	21 (52.5)	24 (60)
Average duration of fever (days)	0	7.97
Lips and oral cavity changes	-	37 (92.5)
Conjunctival congestion	-	33 (82.5)
Cervical lymphadenopathy	-	30 (75)
Polymorphous exanthema	-	29 (72.5)
Changes in the extremities	-	24 (60)
Coronary artery abnormalities	-	9 (22.5)
IVIG therapy	-	40 (100)

Values are expressed as the mean ± standard deviation or n (%). IVIG, intravenous immunoglobulin.

Table II. Laboratory data of controls and patients with acute KD and convalescent KD.

Parameter	Acute KD (n=40)	Convalescent KD (n=40)	P-value (acute vs. convalescent KD)	Control (n=40)	P-value (acute KD vs. control)	Normal range
D-dimer (μg/l)	1,768.25±1,670.13	721.25±536.72	<0.001	Not assessed	-	<500.00
PCT (ng/ml)	3.70±8.60	0.12±0.26	0.012	Not assessed	-	<0.05
BNP (pg/ml)	1,420.95±1,992.41	280.25±430.55	<0.001	Not assessed	-	<1,000.00
TP (g/l)	62.99±5.07	78.57±6.67	<0.001	67.89±4.03	<0.001	65.00-85.00
Albumin (g/l)	36.86±3.57	35.62±4.13	0.074	44.66±6.97	<0.001	40.00-55.00
Sodium (mmol/l)	134.85±2.02	135.68±2.20	0.070	139.82±1.79	<0.001	137.00-147.00
TG (mmol/l)	1.39±0.73	3.72±11.11	0.192	1.21±0.61	0.24	+<1.70
LDH (U/l)	365.75±99.56	317.17±103.73	0.034	298.61±61.49	<0.001	109.00-245.00
CK (U/l)	59.97±41.82	59.42±42.49	0.952	151.07±70.99	<0.001	38.00-174.00
CK-MB (U/l)	32.93±15.77	35.18±13.29	0.529	32.69±19.41	0.95	<20.00
ADA (U/l)	19.20±4.69	23.70±5.02	<0.001	17.79±5.22	0.20	<25.00
ALT (U/l)	67.80±110.50	28.80±31.01	0.035	15.52±7.24	0.01	9.00-50.00
AST (U/l)	57.98±122.77	49.55±43.55	0.688	36.50±10.06	0.25	15.00-40.00
TC (mmol/l)	3.58±0.62	4.15±0.97	0.001	4.22±0.76	<0.001	<5.20
LDL (mmol/l)	2.06±0.52	2.50±0.58	<0.001	2.17±0.52	0.36	<3.12
HDL (mmol/l)	0.68±0.23	0.83±0.95	0.328	1.17±0.24	<0.001	>1.04
Tbil (μmol/l)	9.28±11.17	5.40±2.47	0.022	5.18±2.59	0.03	5.00-19.00
TBA (μmol/l)	26.61±48.46	6.03±3.96	0.009	6.78±4.27	0.01	<6.70
CRP (mg/l)	77.58±49.86	11.11±2.55	<0.001	4.59±2.96	<0.001	<6.00

Values are expressed as the mean ± standard deviation. KD, Kawasaki disease; PCT, procalcitonin; BNP, B-type natriuretic peptide; TP, total protein; TG, triglycerides; LDH, lactate dehydrogenase; CK-MB, creatine kinase myocardial band; ADA, adenosine deaminase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Tbil, total bilirubin; TBA, total bile acids; CRP, C-reactive protein.

LncRNA serum levels in patients with KD. Initial RT-qPCR analysis included 6 then-known cardiac-related lncRNAs: Steroid receptor RNA activator (SRA), SAF, human leukocyte antigen complex group 22 (HCG22), myosin heavy chain-associated RNA transcript (MHRT), DIO3 opposite strand upstream RNA (DIO3OS) and forkhead box F1 adjacent non-coding developmental regulatory RNA (FENDRR) (9). Fig. 1 presents the levels of these six lncRNAs. The expression levels of SRA were higher in patients with KD (ΔCt=5.40±1.53) compared with those in healthy children (ΔCt=7.00±2.13; P<0.0001). Similarly, serum HCG22 levels were also higher in patients with KD (ΔCt=5.05±1.61) compared with those in the controls (ΔCt=5.69±1.00; P=0.036). Serum SAF, DIO3OS, FENDRR

Table III. PCR primer pairs.

Gene name	Primer sequence	Annealing temperature (˚C)
SRA	Sense: 5'-GGAAGCAGGTATGTGATGAC-3' Anti-sense: 5'-TACCATCCACTGACTGACCT-3'	58
SAF	Sense: 5'-ACATCTCAGCCTCTTGGTG-3' Anti-sense: 5'-ACAGATGGCGAAATGAGG-3'	58
DIO3OS	Sense: 5'-CTTCCTGCTCTTCGTTGTCC-3' Anti-sense: 5'-TGAGGAGGATTGAGTTGGG-3'	58
FENDRR	Sense: 5'-AATTGCTGGGCTGCTTTCTA-3' Anti-sense: 5'-TTCACAATGGCTCAGTGCTC-3'	58
HCG22	Sense: 5'-CGCAGGCACAAATGGATGAG-3' Anti-sense: 5'-CTGGTCTCTTTCCGTGGGAC-3'	58
MHRT	Sense: 5'-CCGACTGCGACTCCTCATAC-3' Anti-sense: 5'-GGCTGAAGAGTGAGCCTTGT-3'	58
U6	Sense: 5'-GCTTCGGCAGCACATATACTAAAAT-3' Anti-sense:5'-CGCTTCACGAATTTGCGTGTCAT-3'	58

SRA, steroid receptor RNA activator; HCG22, human leukocyte antigen complex group 22; MHRT, myosin heavy chain-associated RNA transcript; DIO3OS, DIO3 opposite strand upstream RNA; FENDRR, forkhead box F1 adjacent non-coding developmental regulatory RNA.

Table IV. Single-factor correlation analysis for the association of HCG22 with biochemical parameters between patients with acute KD and controls.

Parameter	Pearson correlation coefficient	P-value
BNP (pg/ml)	-0.453	0.003
TC (mmol/l)	0.337	0.034
LDL (mmol/l)	0.378	0.016
CRP (mg/l)	-0.405	0.010

HCG22, human leukocyte antigen complex group 22; BNP, B-type natriuretic peptide; TC, total cholesterol; LDL, low-density lipoprotein; CRP, C-reactive protein.

Table V. Single-factor correlation analysis for the association of MHRT with biochemical parameters between patients with convalescent Kawasaki disease and controls.

Parameter	Pearson correlation coefficient	P-value
BNP (pg/ml)	0.334	0.003
CK (U/l)	-0.334	0.035
ADA (U/l)	0.337	0.033

MHRT, myosin heavy chain-associated RNA transcript; BNP, B-type natriuretic peptide; CK, creatine kinase; ADA, adenosine deaminase.

and MHRT levels did not significantly change in acute KD and controls.

As presented in Fig. 2, the serum levels of lncRNA MHRT were upregulated in patients with convalescent KD (ΔCt=2.01±1.50) compared with those with acute KD (ΔCt=3.03±1.60; P=0.001) and remained higher compared with those in healthy controls (ΔCt=2.52±1.50; P=0.027). Serum SRA, SAF, DIO3OS, FENDRR and HCG22 levels did not significantly change in patients with KD following IVIG therapy (data not shown).

Evaluation of HCG22 and SRA as novel biomarkers for KD. On single-factor correlation analysis, BNP (P=0.003) and CRP (P<0.010) were positively correlated with HCG22 in patients with acute KD, while TC (P=0.034) and LDL (P=0.016) were negatively correlated with HCG22 in patients with KD (Table IV). However, there was no significant correlation between SRA and any biochemical parameter in patients with KD (data not shown).

Having discovered that the serum levels of HCG22 and SRA were abnormal in patients with KD, the potential use of HCG22 and SRA as diagnostic biomarkers for KD was then tested. ROC analysis was performed to evaluate the predictive power of HCG22 and SRA for KD. To distinguish between acute KD and controls, the area under ROC curve (AUC) was 0.633 (95%CI: 0.509-0.757) for HCG22 (Fig. 3A). The sensitivity and specificity were 55.0 and 72.5%, respectively. The cut-off value was 5.973. For SRA, the AUC was 0.743 (95% CI: 0.627-0.859; Fig. 3B) and the sensitivity and specificity were 70.0 and 82.5%, respectively. The cut-off value was 7.05.

Correlation of MHRT with biochemical parameters. According to the single-factor correlation analysis, CK (P=0.035) was positively correlated with MHRT in patients with convalescent KD, while BNP (P=0.003) and ADA (P=0.033) were negatively correlated with MHRT in patients

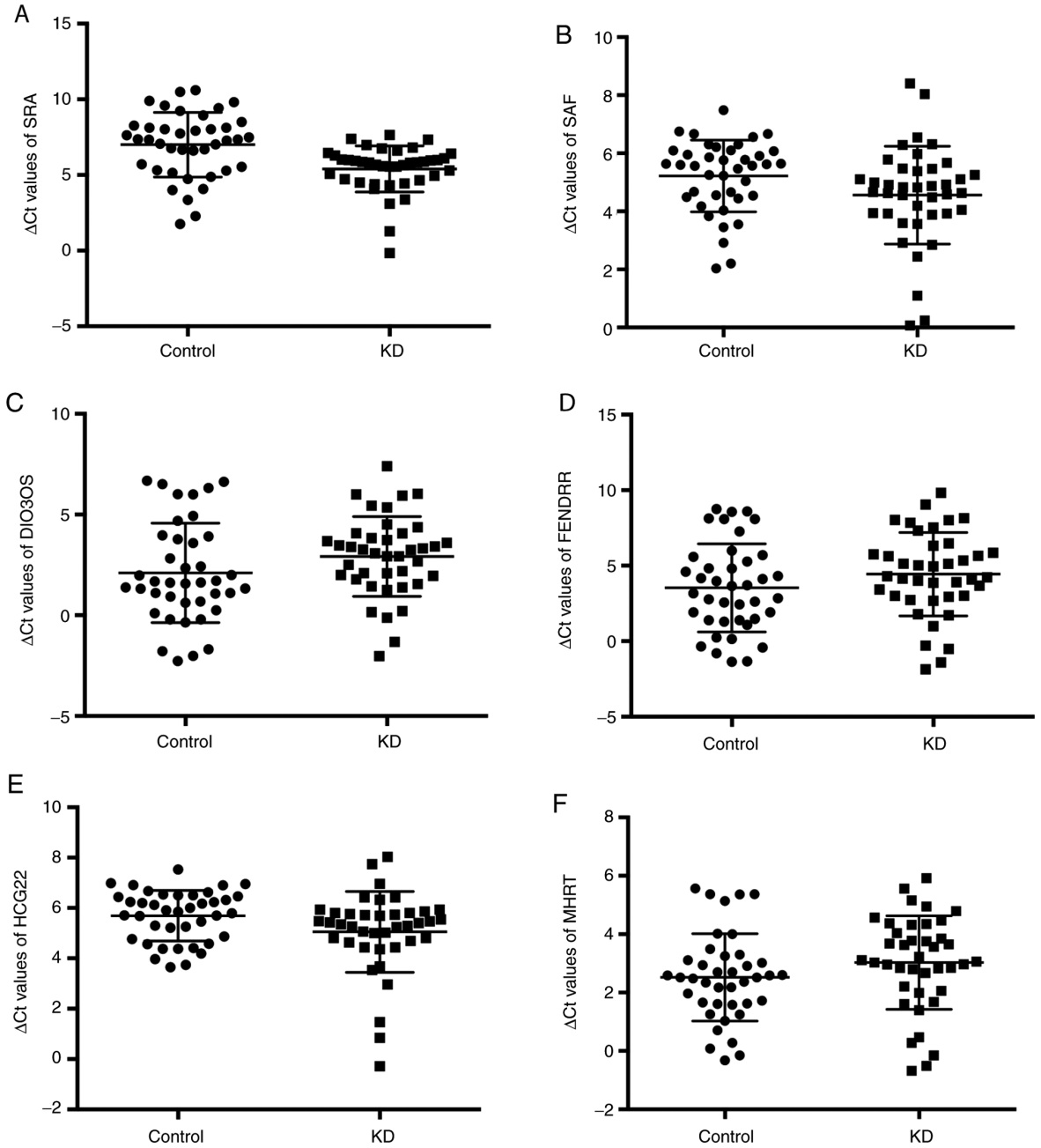

Figure 1. Relative expression of six long non-coding RNAs in patients with acute KD and controls were determined by reverse transcription-quantitative PCR. The RNA expression of (A) SRA and (E) HCG22 was higher in patients with acute KD compared with in healthy controls. The RNA expression of (B) SAF, (C) DIO3OS, (D) FENDRR and (F) MHRT did no significantly change between acute KD and controls. Values are expressed as the mean ± standard deviation. KD, Kawasaki disease; SRA, steroid receptor RNA activator; HCG22, human leukocyte antigen complex group 22; MHRT, myosin heavy chain-associated RNA transcript; DIO3OS, DIO3 opposite strand upstream RNA; FENDRR, forkhead box F1 adjacent non-coding developmental regulatory RNA.

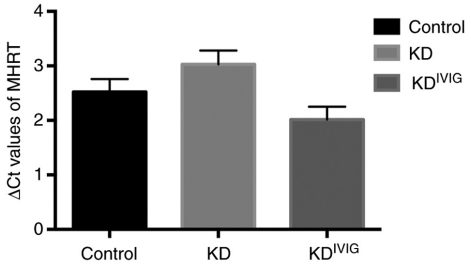

Figure 2. Relative expression of serum lncRNA MHRT levels in patients with acute KD and convalescent KD were determined by reverse transcription-quantitative PCR. Values are expressed as the mean ± standard deviation. KD, Kawasaki disease; MHRT, myosin heavy chain-associated RNA transcript; IVIG, intravenous immunoglobulin.

with convalescent KD (Table V). No significant correlation of MHRT with acute KD was observed (data not shown).

Discussion

Evidence suggests that lncRNAs may be involved in numerous biological processes and lncRNA abnormalities may be associated with human diseases (13). In addition, lncRNAs were proven to be highly stable and readily detectable in a number of body fluids, including saliva, plasma, serum and urine. These characteristics make lncRNAs diagnostic tools for non-invasive and rapid disease diagnosis and prognostication;

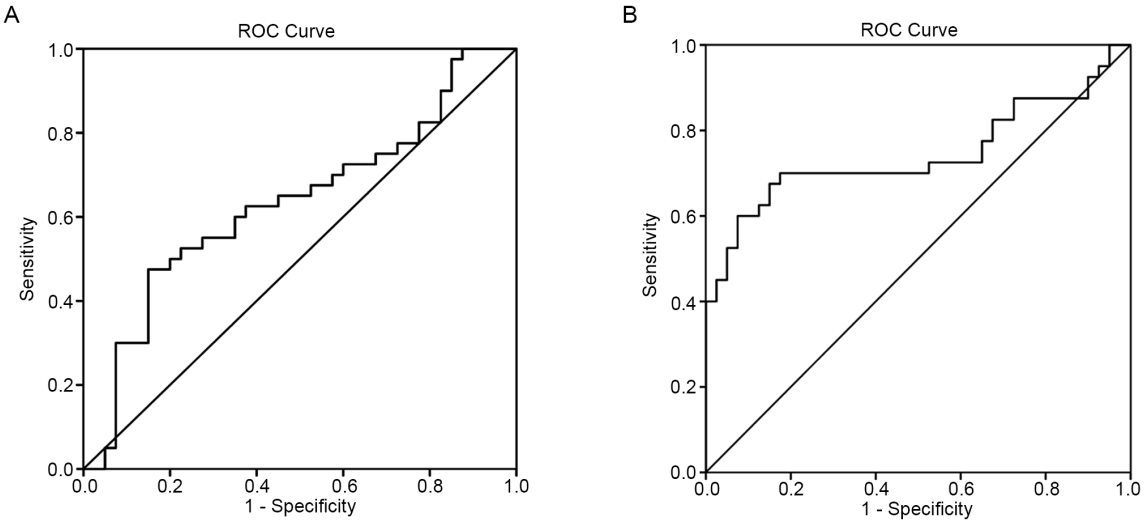

Figure 3. ROC curve analysis for KD. ROC curves were constructed to evaluate the diagnostic value of HCG22 and SRA. (A) For HCG22, the AUC was 0.633 (95% CI: 0.509-0.757). (B) For SRA, the AUC was 0.743 (95% CI: 0.627-0.859). KD, Kawasaki disease; ROC, receiver operating characteristic; AUC, area under the ROC curve; SRA, steroid receptor RNA activator; HCG22, human leukocyte antigen complex group 22.

identification of novel biomarkers in body fluid samples has broad prospects and appeal (14). This strongly suggests the important role of lncRNAs in pathophysiology and their potential in clinical application.

LncRNAs SRA and HCG22 are both associated with cardiovascular disease. SRA is highly expressed in the liver, heart and skeletal muscle and is associated with human dilated cardiomyopathy (DCM) (14). HCG22 is a novel mucin-like gene and is located in a mucin-like gene cluster with DPCR1, mucin 21, cell surface-associated and mucin 22 (15). HCG22 expression is high in lung tissues (16). The pathogenesis of idiopathic DCM is also related to HCG22 expression (17). HCG22 has also been detected in the brain, spleen, thymus, prostate and oral cavity (18). Based on the Ct values of the present RT-qPCR analysis, both SRA and HCG22 were abundantly expressed in serum samples of pediatric patients. KD is characterized by systemic inflammation in all of the medium-sized arteries and in multiple organs and tissues during the acute fever, but inflammation of the CA and heart (valvulitis, pericarditis or myocarditis) results in the most devastating clinical outcomes (19).

Cardiovascular manifestations may be prominent during the acute KD episodes and are the major cause of long-term mortality and morbidity. The results of the present study suggested that SRA and HCG22 levels in the KD group were higher than those in the control group. Abnormal lipid metabolism frequently occurs in pediatric patients with KD and abnormal blood lipid levels are among the most important risk factors for CA injury (20). TG and LDL-C levels are positively correlated with atherosclerosis and coronary heart disease, while HDL-C is a protective factor for coronary heart disease and atherosclerosis and is negatively correlated with its risk. Elevated TG and LDL-C and decreased HDL-C levels are reported in children with KD. They all increase the risk of these children developing cardiovascular disease at the adolescent stage (21). In the present study, HDL-C was significantly lower in patients with KD than in healthy controls; however, TG and LDL-C levels did not significantly differ between the two groups. Based on single-factor correlation analysis, BNP and CRP were positively correlated with HCG22 in patients with acute KD, while TC and LDL were negatively correlated with HCG22 in patients with acute KD.

The ROC is a comprehensive index used to reflect the sensitivity and specificity of continuous variables. In the present study, ROC curves for KD were constructed. The AUC for serum HCG22 to distinguish between KD and normal subjects was 0.633, while the sensitivity and specificity were 55.0 and 72.5%, respectively. For SRA, the AUC was 0.743 and the sensitivity and specificity were 70.0 and 82.5%, respectively. The present results indicated that serum HCG22 and SRA had higher sensitivity and specificity in the screening for KD, which may be more advantageous than the common blood biomarkers of KD. Alterations of lncRNAs in the blood may reflect the underlying mechanisms for a certain disease examined. In the present study, the number of cases of KD was limited and lncRNAs expression levels were analyzed in the serum samples of only 40 patients with KD and 40 healthy controls. More samples should be analyzed in the future.

KD is characterized by endothelial cell damage, which may be due to abnormal production of cytokines and production of cytotoxic antibodies against endothelial cells. Furthermore, IVIG is an effective method to prevent coronary artery abnormalities in patients with KD, possibly by inhibiting the activation of the immune system and reducing endothelial cell damage (22-25). The lncRNA MHRT encodes a spliced lncRNA that may act as a cardioprotective agent of the heart. Based on a study of a similar gene in mice, the encoded transcript may regulate chromatin remodeling by acting as a bait for the SMARCA4 chromatin repressor complex, preventing it from binding to its genomic targets. Blocking the effects of BRG1 may be crucial to protect the heart from pathological hypertrophy (26). In the present study, lncRNA MHRT levels were lower in acute KD, suggesting that lncRNA MHRT is inhibited during acute KD. LncRNA MHRT levels were upregulated in convalescent KD following IVIG treatment and were still higher in the controls. These results suggested

that lncRNA MHRT may have a role during KD following IVIG therapy. In addition, BNP, which is usually secreted by the myocardium, particularly in the case of increased intracardiac pressure and myocardial stress, is one of the important indicators for the diagnosis of human heart failure and left ventricular dysfunction (27). In the present study, BNP was significantly decreased in convalescent KD, which suggested improved cardiac function in pediatric patients with KD during recovery. BNP negatively correlated with lncRNA MHRT in patients with convalescent KD, further suggesting that lncRNA MHRT is a protective factor for KD, indicating that lncRNA MHRT may be a potential therapeutic target.

In conclusion, the present study suggested that the serum levels of lncRNAs SRA and HCG22 were upregulated in pediatric patients with KD compared with those in healthy children. LncRNA MHRT was upregulated in convalescent KD. The present results suggested that lncRNAs SRA and HCG22 may act as novel biomarkers for KD diagnosis and lncRNA MHRT is a novel biomarker for predicting the clinical prognosis of KD.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

QZ contributed to the conception and design of the study. JC collected patient serum samples. QZ and DW performed experimental work and were involved in conceiving the study. HY, FL, YX, FW, JW, HQ and SB were involved in conceiving the study and drafting the manuscript. HQ and SB performed the experimental evaluation. QZ and JC confirmed the authenticity of the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The experimental protocols for the present study were approved by the Ethics Committee of Ningbo Women and Children's Hospital (Ningbo, China). Written informed consent was obtained from the patients' parents prior to study participation.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Kawasaki T: Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes in children. Arerugi 16: 178-222, 1967 (In Japanese).
2. Galeotti C, Kaveri SV, Cimaz R, Koné-Paut I and Bayry J: Predisposing factors, pathogenesis and therapeutic intervention of Kawasaki disease. Drug Discov Today 21: 1850-1857, 2016.
3. Mccrindle BW, Rowley AH, Newburger JW, Burns JC, Bolger AF, Gewitz M, Baker AL, Jackson MA, Takahashi M, Shah P, *et al*: Diagnosis, treatment, and long-term management of kawasaki disease: A scientific statement for health professionals from the American Heart Association. Circulation 135: e927-e999, 2017.
4. Bayers S, Shulman ST and Paller AS: Kawasaki disease: Part I. Diagnosis, clinical features, and pathogenesis. J Am Acad Dermatol 69: 501.e1-e11, 2013.
5. Takahashi K, Oharaseki T and Yokouchi Y: Pathogenesis of Kawasaki disease. Clin Exp Immunol 164 (Suppl 1): S20-S22, 2011.
6. Takahashi K, Oharaseki T and Yokouchi Y: Update on etio and immunopathogenesis of Kawasaki disease. Curr Opin Rheumatol 26: 31-36, 2014.
7. Orenstein JM, Shulman ST, Fox LM, Baker SC, Takahashi M, Bhatti TR, Russo PA, Mierau GW, de Chadarévian JP, Perlman EJ, *et al*: Three linked vasculopathic processes characterize Kawasaki disease: A light and transmission electron microscopic study. PLoS One 7: e38998, 2012.
8. Wapinski O and Chang HY: Long noncoding RNAs and human disease. Trends Cell Biol 21: 354-361, 2011.
9. Schmitz SU, Grote P and Herrmann BG: Mechanisms of long noncoding RNA function in development and disease. Cell Mol Life Sci 73: 2491-2509, 2016.
10. Sallam T, Sandhu J and Tontonoz P: Long noncoding RNA discovery in cardiovascular disease: Decoding form to function. Circ Res 122: 155, 2018.
11. Muta H, Ishii M, Yashiro M, Uehara R and Nakamura Y: Late intravenous immunoglobulin treatment in patients with Kawasaki disease. Pediatrics 129: e291-e297, 2012.
12. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
13. Yang KC, Yamada KA, Patel AY, Topkara VK, George I, Cheema FH, Ewald GA, Mann DL and Nerbonne JM: Deep RNA sequencing reveals dynamic regulation of myocardial noncoding RNAs in failing human heart and remodeling with mechanical circulatory support. Circulation 129: 1009-1021, 2014.
14. Cooper C, Vincett D, Yan Y, Hamedani MK, Myal Y and Leygue E: Steroid receptor RNA activator bi-faceted genetic system: Heads or Tails? Biochimie 93: 1973-1980, 2011.
15. Yatagai Y, Hirota T, Sakamoto T, Yamada H, Masuko H, Kaneko Y, Iijima H, Naito T, Noguchi E, Tamari M, *et al*: Variants near the HLA complex group 22 gene (HCG22) confer increased susceptibility to late-onset asthma in Japanese populations. J Allergy Clin Immunol 138: 281-283.e13, 2016.
16. Taniguchi N, Konno S, Hattori T, Isada A, Shimizu K, Shimizu K, Shijubo N, Huang SK, Hizawa N and Nishimura M: The CC16 A38G polymorphism is associated with asymptomatic airway hyper-responsiveness and development of late-onset asthma. Ann Allergy Asthma Immunol 111: 376-381.e1, 2013.
17. Meder B, Rühle F, Weis T, Homuth G, Keller A, Franke J, Peil B, Lorenzo Bermejo J, Frese K, Huge A, *et al*: A genome-wide association study identifies 6p21 as novel risk locus for dilated cardiomyopathy. Eur Heart J 35: 1069-1077, 2014.
18. Lu F, Houck JR, Lohavanichbutr P and Chen C: Transcriptome analysis reveals differentially expressed lncRNAs between oral squamous cell carcinoma and healthy oral mucosa. Oncotarget 8: 31521-31531, 2017.
19. Amano S, Hazama F, Kubagawa H, Tasaka K, Haebara H and Hamashima Y: General pathology of Kawasaki disease: On the morphological alterations corresponding to the clinical manifestations. Acta Pathol Jpn 30: 681-694, 1980.

20. Cabana VG, Gidding SS, Getz GS, Chapman J and Shulman ST: Serum amyloid A and high density lipoprotein participate in the acute phase response of Kawasaki disease. Pediatr Res 42: 651-655, 1997.
21. Chiang AN, Hwang B, Shaw GC, Lee BC, Lu JH, Meng CC and Chou P: Changes in plasma levels of lipids and lipoprotein composition in patients with Kawasaki disease. Clin Chim Acta 260: 15-26, 1997.
22. Galeotti C, Bayry J, Kone-Paut I and Kaveri SV: Kawasaki disease: Aetiopathogenesis and therapeutic utility of intravenous immunoglobulin. Autoimmun Rev 9: 441-448, 2010.
23. Nonoyama S: Immunological abnormalities and endothelial cell injury in Kawasaki disease. Acta Paediatr Jpn 33: 752-755, 1991.
24. Mostafavi N, Haghjooy-Javanmard S, Presidend N, Manssori NS and Kelishadi R: Persistence of endothelial cell damage late after Kawasaki disease in patients without coronary artery complications. Adv Biomed Res 4: 25, 2015.
25. Leung DY: Clinical and immunologic aspects of Kawasaki disease. Immunodefic Rev 1: 261-271, 1989.
26. Han P, Li W, Lin CH, Yang J, Shang C, Nuernberg ST, Jin KK, Xu W, Lin CY, Lin CJ, *et al*: A long noncoding RNA protects the heart from pathological hypertrophy. Nature 514: 102-106, 2014.
27. De Lemos JA, Mcguire DK and Drazner MH: B-type natriuretic peptide in cardiovascular disease. Lancet 362: 316-322, 2003.