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Potential candidate maternal serum miRNAs for the diagnosis of fetal congenital heart disease

Yimeng Xi[1, 2, #], Enkang Lu[3, 4, #], Xinyan Ruan[2], Ruoyun Wu[5], Lijun Wu[1], Ting Zhou[1], Haitao Gu[4,*], Yun Wu[1,*]

[1] Department of Ultrasound, Nanjing Maternity and Child Health Care Hospital, Women's Hospital of Nanjing Medical University, Nanjing 210004, China
[2] The First School of Clinical Medicine, Nanjing Medical University, Nanjing 211103, China
[3] Department of Cardiothoracic Surgery, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China
[4] Central Laboratory of Jiangsu Provincial Maternal and Child Health Care Hospital, Maternal and Child Branch of the First Affiliated Hospital of Nanjing Medical University, Nanjing 210036, China
[5] School of Stomatology, Nanjing Medical University, Nanjing 210009, China

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Abstract

Congenital heart disease (CHD) is one of the most significant birth defects leading to infant mortality worldwide. Circulating microRNAs (miRNAs) are emerging as novel biomarkers for the detection of cardiovascular diseases. In this study, we aimed to investigate the role of maternal serum miRNAs expression as biomarkers in the diagnosis and prediction of children with CHD. High-throughput sequencing of peripheral blood from pregnant women with abnormal and normal fetal hearts identified 1939 differentially expressed miRNAs, the first 11 of which were selected as predictive biomarkers of CHD. The expression of miRNAs in more clinical samples was then quantitatively verified by reverse transcriptase polymerase chain reaction and the correlation between abnormal miRNAs and CHD was analyzed. Two miRNAs (hsa-miR-3195 and hsa-miR-122-5p) were found to be significantly down-regulated in pregnant women with fetal CHD. By further bioinformatics analysis, we predicted that hsa-miR-3195 and hsa-miR-122-5p could induce CHD by influencing biometabolic processes. hsa-miR-3195 and hsa-miR-122-5p may serve as novel non-invasive biomarkers for prenatal detection of fetal CHD.

Keywords: Congenital heart disease, Prenatal detection, miRNAs, Biomarker.

1. Introduction

Among congenital structural malformations, congenital heart disease (CHD) is the primary cause of death and a significant public health concern. The enhanced diagnosis accuracy of echocardiography may be the reason for the rising incidence of CHD[1]. Atrial septal abnormalities, valve defects, and lesions affecting the outflow tract are all included in the pathologic spectrum of CHD[2-5]. About 80% of CHD cases are multifactorial, meaning they are caused by both genetic and environmental factors. Approximately 20% of cases have been linked to chromosomal abnormalities, Mendelian syndromes, non-syndromic single-gene disorders, or malformations[6]. If prenatal screening is successful, which includes echocardiography, magnetic resonance imaging, and multilayer spiral CT, the prevalence of CHD may be decreased[7, 8]. However, due to lack of standardization, the rate of prenatal CHD detection remains low[9, 10], and therefore, its use as a screening tool, especially in routine prenatal checkups, remains a challenge. Therefore, more accurate detection techniques are needed to improve detection rates and thereby reduce the potential impact on patient prognosis. The diagnosis and prognosis of individuals with CHD can be greatly improved by having a thorough grasp of the molecular mechanisms underlying the aetiology and progression of the condition.

Small, highly conserved non-coding RNA strands known as microRNAs (miRNAs) control the expression of genes at the post-transcriptional and translational levels[11, 12]. MiRNAs play complex roles in various pathophysiological states. Complex diseases can result from changes in the expression of a single miRNA, which can impact several target genes and impact the whole genetic network[13]. Many miRNAs are expressed as key regulators in the embryonic, postnatal, and adult heart; their aberrant expression or gene deletion is associated with disruption of cardiac development, abnormal cardiac cell growth and differentiation, and cardiac dysfunction[14,

* Corresponding author.
E-mail address: share20240310@163.com (Y. Wu); guhaitao65@163.com (H. Gu).
# These authors contributed equally
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15].In 2008, miRNAs in plasma and serum were found to be surprisingly stable[16]. Afterwards, a number of research teams reported using miRNAs as circulating biomarkers for diabetes mellitus[17, 18], cardiovascular diseases[19, 20], and malignancies [21, 22], suggesting a high degree of specificity of circulating miRNAs for disease pathology. Particularly in congenital malformations, many studies found that mothers carrying foetuses with CHD have highly up- or down-regulated miRNAs[23, 24].

High-throughput sequencing was utilised in this investigation to look at the gene expression profiles of miRNAs in the sera of pregnant women with normal foetal heart development and pregnant women with impaired foetal heart development due to congenital heart disease. Using real-time reverse transcription polymerase chain reaction (qRT-PCR), the differentially expressed miRNAs that satisfied the screening criteria were then verified. Lastly, an analysis and evaluation of these components' possible use within the medical diagnostic of heart disease have been conducted.

2. Materials and Methods

2.1. Processing and collection of clinical specimens

The Ethics Committee of the Women's Hospital at Nanjing Medical University was consulted in order to obtain ethical permission before any work began. The Women's Hospital Ethics Committee of Nanjing Medical University authorised all experiments, that adhere to the Declaration of Helsinki's tenets (approval date: November 26, 2020; protocol code: 2020KY072). Blood samples from 31 pregnant women (N = 31, age = 30.16 ± 5.14) whose foetuses were diagnosed with congenital cardiac disease and 41 pregnant women (N = 41, age = 32.21 ± 4.60) whose foetuses were healthy were acquired with consent.

Table 1. Clinical features for pregnant women with fetal diagnosis of CHD[25].

Sample Number	Age at Diagnosis	Reproductive History	Gestational Age	Exposure to Harmful Substances	Clinical Diagnosis
Samples were used for high-throughput sequencing					
D5	26	G2P1	23W + 3D	NO	Tetralogy of Fallot, Pulmonary atresia
D25	30	G1P0	23W + 2D	NO	Total anomalous pulmonary venous drainage
D26	31	G2P1	23W + 0D	NO	Endocardial cushion defect
D24	35	G1P0	23W + 0D	NO	Tetralogy of Fallot
D27	36	G1P0	24W + 2D	NO	Hypoplastic right heart, Tricuspid Atresia, Pulmonary valve atresia
D40	33	G1P0	24W + 2D	NO	Tricuspid Atresia, Ventricular septal defect
Samples were used for follow-up qRT-PCR					
A01	30	G1P0	22W + 6D	NO	Double outlet of the right ventricle
A02	34	G3P1	24W + 4D	NO	Tetralogy of Fallot
A03	34	G2P1	26W + 3D	NO	Pulmonary artery crossover
A04	36	G2P1	28W + 0D	NO	The right heart is larger than the left heart
A05	23	G1P0	25W + 6D	NO	Ventricular septal defect, Right-sided aortic arch
A06	39	G3P1	33W + 0D	NO	The right heart is larger than the left heart, Aortic arch narrowing
A07	10	G5P4	24W + 3D	NO	Hypoplastic left heart syndrome
A08	34	G2P0	28W + 6D	NO	The right heart is larger than the left heart
A09	30	G2P1	23W + 5D	NO	Transposition of the great arteries
A10	29	G1P0	24W + 6D	NO	Ventricular septal defect, Coarctation of the Aorta, Transposition of the great arteries
A11	31	G1P0	34W + 5D	NO	Hypoplastic right heart
A12	33	G2P1	25W + 3D	NO	The right ventricle is smaller than the left ventricle
A13	29	G1P0	24W + 5D	NO	Aortic arch stenosis
A14	28	G1P0	27W + 4D	NO	Aortic arch stenosis
A15	29	G2P1	28W + 5D	NO	Small left atrium
A16	33	G1P0	25W + 3D	NO	Complete atrial septal defect
A17	33	G2P1	24W + 2D	NO	Ventricular septal defect, Aortic arch stenosis, Possible aortic arch dissection
A18	31	G3P2	27W + 3D	NO	Mid-large tricuspid regurgitation
A19	27	G2P0	22W + 3D	NO	Total anomalous pulmonary venous drainage
A20	27	G1P0	25W + 3D	NO	Ventricular septal defect
A21	24	G1P0	25W + 4D	NO	Transposition of the great arteries, Ventricular septal defect
A22	29	G1P0	24W + 6D	NO	Right-sided aortic arch
A23	31	G1P0	24W + 2D	NO	Ventricular septal defect, Aorta riding across
A24	29	G1P0	28W + 2D	NO	Ventricular septal defect
A25	31	G3P1	24W + 5D	NO	Ventricular septal defect, Aortic valve stenosis

Fetal echocardiography was used to confirm all diagnostic findings. We gathered healthy fetal hearts from expectant mothers who came for prenatal checkups. In compliance with Early Detection Research Network operational protocols, serum was obtained following the acquisition of written permission from both the patient and healthy persons. Tables 1[25] and 2[25] display the study individuals' fundamental clinical features. In an outpatient clinic, whole blood was drawn and put into tubes with yellow caps that contained citrate dextrose. We transferred the serum into fresh RNase-free tubes after centrifuging it at 3000x g for 10 minutes and incubating it at room temperature for 15 minutes. We processed the sample entirely on ice. Lastly, in preparation for additional research, the serum was frozen at -80◦ C [26].

2.2. Circulating microRNA extraction and preservation

Utilizing a predetermined serum volume (200 µL/part), quantitative reverse transcription polymerase chain reaction (qRT-PCR) and microRNAs sequencing assays were performed. Using Trizol LS (Life Technologies, Carlsbad, CA, USA) in line with the manufacturer's guidelines[27], we isolated total RNA from patient serum. Then, all OD 260/280 absorbance ratios of the NanoDrop ND-1000 were confirmed to be between 1.8 ~ 2.0. Lastly, in preparation for later research, we refrigerated the isolated RNA at -80°C.

2.3. Library Preparation and Sequencing

First, preprocessing was done on total RNA samples from six pregnant women who had a fetal diagnosis of CHD (Table 1) and six pregnant women whose fetal hearts were normal (Table 2). To accomplish effective reverse transcription, we eliminated the following RNA alterations that impeded the creation of tiny RNA sequence libraries: In order to ligate the 3′-adaptor, after being eliminated, 3′-cP (2′,3′-cyclic phosphate) was substituted with 3′-OH; similarly, 3′-aminoacyl (charged) diacylation was carried out and replaced with 3′-OH; and last, phosphorylation of the hydroxyl group 5′-OH resulted in 5′-P in order to ligate the 5′-adaptor and demethylate m1A and m3C. Illumina RT primers and amplification primers were used to amplify the cDNA that had been generated. The 135–160 bp PCR-generated fragments, which correspond to a narrow RNA size range of 15–40 nt, were subsequently extracted and purified using a PAGE gel[28]. Ultimately, The generated library was quantified using an Agilent BioAnalyzer 2100, and an Illumina NextSeq 500 was used for sequencing[29, 30].

2.4. Bioinformatic analysis of microRNAs

Using the R package EdgeR [31], the microRNAs' expression patterns and varying expressions were evaluated. MicroRNAs with a fold change of >2 or <-2 and a p-value that is below 0.01 were seen as having a notable variation in expression. We were able to create heat maps and volcano plots for visual identification using GraphPad Prism 9.3.1 (GraphPad Software, California, CA, USA) software (v4.1.2) [32].

2.5. Quantitative Real-Time PCR (qRT-PCR) for microRNA

Utilizing the Bulge-loopTM miRNA qRT-PCR Starter Kit (Ribo Bio, Guangzhou, China), the microRNA expression levels in serum samples from various pregnant women were ascertained following the use of qRT-PCR to validate the sequencing results. The experiment was set out precisely like this: The initial ingredients used were RT Primer (1 µL), 5X Reverse-Transcription Buffer (2 µL), RNA template (5 µL), and RTase Mix (2 µL), per the manufacturer's instructions. To extract cDNA, the mixture was then cultivated for 60 minutes at 42 ◦ C and then for 10 minutes at 70 ◦ C. Then, 1 µL of cDNA, 1.1 µL of RNase-free H2O, 0.2 µL of Bulge-loopTM miRNA forward primer, 0.2 µL of Bulge-loopTM reverse primer, and 2.5 µL of 2X SYBR Green mixture were added to the qPCR. In a Roche Light Cycler 480 Real Time PCR system (Roche Light Cycler® 480 system, Basel, Switzerland), 40 cycles of 95 °C for two seconds, 60 °C for twenty seconds, and 70 °C for twenty seconds were used to carry out the reactions. The external control was the miDETECTTM miRNA external control (cel-miR-39-3p). Using the $2^{-\Delta\Delta Ct}$ method, the microRNA expression levels in each sample were recorded. Table S1 contains a list of the microRNAs that were selected for validation.

2.6. Receiver operating characteristic analysis

Software called GraphPad Prism 9.3.1 (GraphPad Software, California, CA, USA) was used to analyse receiver operating characteristics (ROC). Each chosen microRNA's expression level was rated to indicate the patient's condition (0 for healthy, 1 for CHD). ROC curves were generated by calculating the area under the curve (AUC) using the binomial exact confidence interval[33].

2.7. Functional analysis of microRNAs

First, We employed the following resources to forecast the desired genes of the chosen microRNAs: TargetScan (https://www.targetscan.org/ (access date: August 14, 2023)), miRTarbase (https://miRTarBase.cuhk.edu.cn (access date: August 14, 2023)). Next, Using the DAVID Bioinformatics Resource (https://david.ncifcrf.gov/ (accessed August 14, 2023)), enrichment studies for Kyoto Encyclopaedia of Genes and Genomes (KEGG) and Gene Ontology (GO) were conducted. Finally, we elucidated the interactions between different related genes using the String database (https://cn.string-db.org/ (accessed August 14, 2023)), which is designed to search for interacting genes/proteins.

2.8. Statistical analysis

Mean standard deviation for the qRT-PCR validation data was subjected to employing GraphPad Prism 9.3.1 for administration and analysis (GraphPad Software, California, USA). The unpaired Student's t-test was used to analyze statistical variations. P-values were deemed statistically significant if they were less than 0.05. Every experiment was carried out three times to guarantee the accuracy of the findings.

3. Results

3.1. Selection of candidate miRNA biomarkers

In this analysis, 1939 differently regulated miRNAs were found. Particularly expressed miRNAs represented miRNAs with CPM values less than 20 in one group and greater than 20 in the other, whereas commonly expressed miRNAs represented miRNAs with CPM values greater

Table 2. Clinical features for pregnant women with a healthy fetal heart[25].

Sample Number	Age at Diagnosis	Reproductive History	Gestational Age	Exposure to Harmful Substances	Whether the Fetal Heart Is Healthy
Samples were used for high-throughput sequencing					
C28	34	G2P1	23W + 6D	NO	YES
C29	28	G1P0	24W + 1D	NO	YES
C30	31	G2P0	23W + 6D	NO	YES
C31	28	G1P0	23W + 6D	NO	YES
C32	27	G1P0	24W + 0D	NO	YES
C35	28	G1P0	23W + 6D	NO	YES
Samples were used for follow-up qRT-PCR					
B1	34	-	-	NO	YES
B2	35	-	-	NO	YES
B3	34	-	-	NO	YES
B4	36	-	-	NO	YES
B5	33	-	-	NO	YES
B6	28	-	-	NO	YES
B7	34	-	-	NO	YES
B8	36	-	-	NO	YES
B9	32	-	-	NO	YES
B10	33	-	-	NO	YES
B11	48	-	-	NO	YES
B12	30	-	-	NO	YES
B13	31	-	-	NO	YES
B14	31	-	-	NO	YES
B15	28	-	-	NO	YES
B16	36	-	-	NO	YES
B17	36	-	-	NO	YES
B18	35	-	-	NO	YES
B19	18	-	-	NO	YES
B20	34	-	-	NO	YES
B21	30	-	-	NO	YES
B22	36	-	-	NO	YES
B23	36	-	-	NO	YES
B24	32	-	-	NO	YES
B25	27	-	-	NO	YES
B26	31	-	-	NO	YES
B27	32	-	-	NO	YES
B28	33	-	-	NO	YES
B29	32	-	-	NO	YES
B30	41	-	-	NO	YES
B31	30	-	-	NO	YES
B32	30	-	-	NO	YES
B33	34	-	-	NO	YES
B34	32	-	-	NO	YES
B35	27	-	-	NO	YES

than 20 in both groups. MiRNAs were then analyzed for their expression profiles through heatmap and volcano plotting. Next, we used heatmaps and volcano diagrams to examine the features of miRNA expression. The heatmap (Figure 1a) demonstrated that the two groups of miRNAs had significantly different expression levels. When comparing the disease group to the control group, eleven miRNAs were dysregulated, with six being up-regulated and five being down-regulated, as demonstrated by the volcano plot and meeting the criterion of log2FoldChange >2 and p-value < 0.01, as illustrated in Figure 1b.

Using qRT-PCR, we confirmed the miRNAs' expression levels in the peripheral serum of twelve disease- and twelve control-group pregnant women. Among the 11 miRNAs tested, pregnant women whose fetuses were diagnosed with CHD had significantly lower expression levels

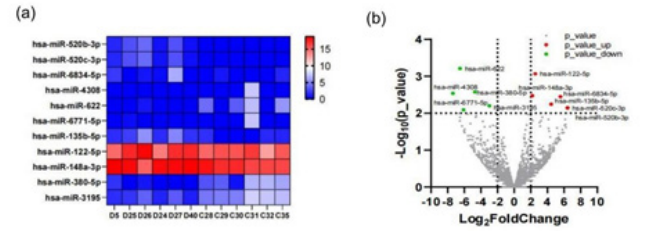

Fig. 1. Distinct expression patterns of miRNAs in foetuses with aberrant versus healthy hearts. (a) Heatmaps showing the abnormal expression of miRNAs in foetuses with normal and defective hearts. (b) Volcanic diagrams showing the aberrant expression of miRNAs in pregnant women with normal foetal hearts and foetal cardiac abnormalities. Eleven miRNAs, six of which were down-regulated and five of which were up-regulated, were found to be dysregulated in the disease group when compared to the control group.

of hsa-miR-3195 and hsa-miR-122-5p than did controls. These two miRNAs (hsa-miR-3195 and hsa-miR-122-5p) were chosen for additional examination.

3.2. Validation of miRNA characterization in a cohort of pregnant women

Using the entire dataset of pregnant women, we looked into the biomarker capacity of the two chosen miRNAs (25 cases, 35 controls). Expectant mothers whose fetuses had a cardiac disease diagnosis had considerably reduced hsa-miR-3195 and hsa-miR-122-5p levels compared to control pregnant women (Figure 2). We further assessed hsa-miR-3195 and hsa-miR-122-5p's capacity to differentiate between expectant mothers whose fetuses had a cardiac disease diagnosis and those whose fetuses were heart healthy using ROC curve analysis[33] (Figure 2). Hsa-miR-3195 and hsa-miR-122-5p had AUCs of 0.6754 (p-value < 0.05, 95% CI = 0.5405-0.8104) and 0.7017 (p-value < 0.01, 95% CI = 0.5705-0.8329), accordingly, according to the results[34]. Thus, it is plausible that hsa-miR-3195 and hsa-miR-122-5p could serve as prospective indicators of coronary heart disease. When combining these two miRNAs, the AUC increased slightly to 0.7291 (p-value < 0.01, 95% CI = 0.6001-0.8581) (Table 3).

3.3. Predicting potential targets for miRNA differential expression and target gene function analysis

The potential targets of hsa-miR-3195 and hsa-miR-122-5p were identified using TargetScan and miRTarBase. Then, to explore the possible functions and mechanisms of hsa-miR-3195 and hsa-miR-122-5p in CHD occurrence, We analysed the target genes using KEGG and GO pathways. We categorized miRNA target genes according to cellular composition, molecular function, and biological process. GO annotation showed that the desired gene of hsa-miR-3195 was enriched in protein binding (Figure 3a). The desired gene of hsa-miR-3195 was shown to be significantly expressed in endocytosis, according to KEGG annotation (Figure 3b). According to GO annotation, the desired gene for hsa-miR-122-5p was substantially expressed in protein binding (Figure 3c). The desired genes of hsa-miR-122-5p were found to be highly expressed in metabolic pathways, according to KEGG annotation (Figure 3d). Their function in The occurrence of congenital heart abnormalities and the fetal heart may be explained by these findings.

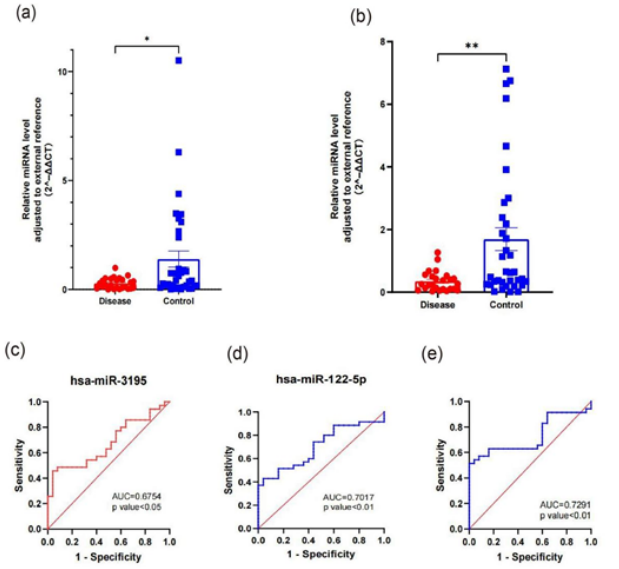

Fig. 2. Verification of the ROC curve for certain miRNAs and two regulatory abnormalities miRNAs in pregnant women with abnormal and normal foetal hearts. (a) hsa-miR-3195 (25 Disease vs. 35 Control) verification. (b) Hsa-miR-122-5p Verification (25 Disease vs. 35 Control). One specificity is the horizontal axis and sensitivity is the vertical axis. The value of (c)hsa-miR-3195, (d)hsa-miR-122-5p, and (e) the combination of the two miRNAs in the diagnosis of foetal CHD are measured using the AUC parameter.

Table 3. ROC curve analysis showing the discriminative power of the selected microRNAs (miRNA) or the combined form in pregnant women.

miRNA	AUC	95% CI	*p*-value
hsa-miR-122-5p	0.7017	0.5705-0.8329	0.0081
hsa-miR-3195	0.6754	0.5405-0.8104	0.0214
two combined	0.7291	0.6001-0.8581	0.0026

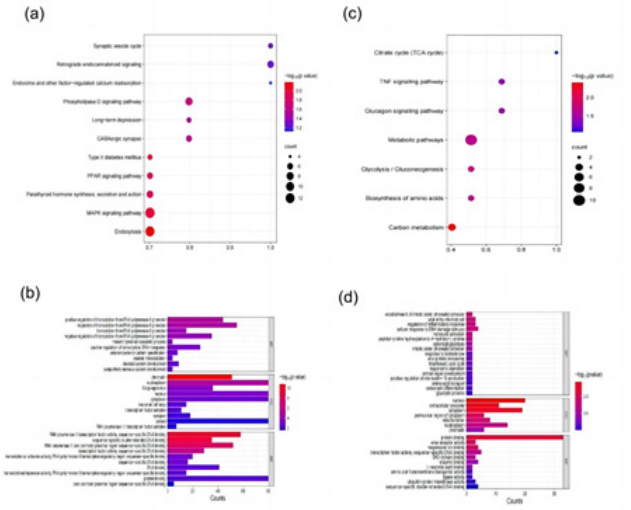

Fig. 3. KEGG pathway and GO analysis of certain miRNAs. (a) Analysis of the hsa-miR-3195 GO pathway. (b) Analysis of the hsa-miR-3195 KEGG pathway. (c) Analysis of the hsa-miR-122-5p GO pathway. (d) Analysis of the hsa-miR-122-5p KEGG pathway.

3.4. Protein–Protein Interaction (PPI) network analysis

We examined the anticipated target gene interactions to obtain a more profound comprehension of the funda-

mental function of miRNAs. Initially, we discovered that H3C13 was the key protein by examining the 423 genes of hsa-miR-3195 (Figure 4a). According to Rashid M. (2021), H3C13 was shown to be up-regulated in malignancies of the stomach, breast, colon, head & neck, brain, and liver[35]. Therefore, we hypothesized that hsa-miR-3195 might influence the pathophysiology of CHD by controlling the H3C13 pathway's activity. Subsequently, we analyzed the proteins encoded by the 41 desired genes of hsa-miR-122-5p and determined the protein PKM that interacts most frequently (Figure 4b). In addition to metabolism, PKM acts as a protein kinase that functions to control a number of biological activities, including transcription, mitotic progression, DNA repair, and cell division[36-43]. Therefore, We postulated that by controlling PKM pathway activation, hsa-miR-122-5p would have an impact on the pathophysiology of CHD.

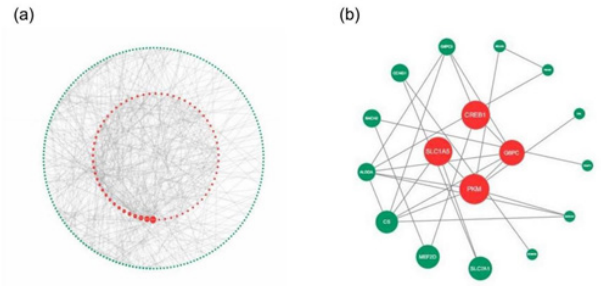

Fig. 4. Networks of interactions between proteins between hsa-miR-3195 and hsa-miR-122-5p. The protein-protein interaction network of hsa-miR-3195 and hsa-miR-122-5p, which correspond to the same target genes, is shown in (a).

4. Discussion

Since CHD is the most frequent kind of CHD in neonates, its pathophysiology is largely unknown [44]. Prenatal diagnostic tools and medical conditions have advanced to the point that most cases of CHD may now be recognised during pregnancy; nonetheless, some cases of CHD may still remain undetectable during pregnancy. Certain straightforward forms of CHD, like atrial septal defect, ventricular septal defect, and arterial conduit failure, can be surgically corrected postnatally and have good prognoses [45]. Even after numerous surgeries, the prognosis for a few intricate types of CHD, such as hypoplastic left heart syndrome, can be dismal if they are not identified and treated promptly [46]. Thus, in addition to routine cardiac ultrasonography, if we have a blood biomarker that allows for more comprehensive screening and multi-angle CHD diagnosis will greatly reduce CHD frequency and death.

There currently exist no reliable biomarkers for CHD in the clinical context, despite numerous attempts to find them. A novel class of ncRNA-miRNAs has emerged in the realm of biomarkers for diagnosis and prognosis as a result of the advancement of sequencing technologies. miRNAs are short, naturally occurring, single-stranded RNA molecules with around 22 nucleotides that do not code for proteins. They have been recognized as significant modulators of the expression of genes because they attach to target mRNAs to either stop translation or start the process of degradation[47-49]. In order to determine whether miRNAs in pregnant women's serum could be useful as potential biomarkers for CHD, we investigated the expression of miRNAs in serum and conducted biological studies.

First, we sequenced pregnant women's peripheral serum who had foetal heart abnormalities and those who had normal foetal hearts using high-throughput sequencing. To confirm the accuracy of the data, we used qRT-PCR to identify the top 11 aberrantly expressed miRNAs based on the sequencing results. Next, in blood samples taken from two cohorts of expectant mothers, hsa-miR-3195 and hsa-miR-122-5p were the most down-regulated and statistically important miRNAs. The illness group had lower levels of hsa-miR-3195 and hsa-miR-122-5p compared to the control group, according to the results of qRT-PCR. Interestingly, the results for hsa-miR-3195 were in agreement with the sequencing findings, whereas the outcomes for has-miR-122-5p were different from the sequencing results. To learn more about the possible role that miRNAs play in the pathophysiology of CHD, GO and KEGG pathway analysis has been performed. The outcomes demonstrated that hsa-miR-3195 enriched target genes of protein binding and endocytosis signaling pathways in cardiomyocytes. As a result, hsa-miR-3195 might have a role in the biochemical mechanisms that cause congestive heart failure. We discovered that the desired genes of hsa-miR-122-5p were primarily enriched in metabolic and protein-binding pathways; as a result, hsa-miR-122-5p is crucial for normal cell development and proliferation. CHD is generally diagnosed in the early stages of pregnancy, and its development is a complicated, protracted process involving numerous genetic and epigenetic changes. There are currently several varieties of congenital cardiac illness. Therefore, in order to more precisely identify and examine various forms of CHD, we must keep growing the sample size and obtaining more comprehensive pathology data. More investigation is needed to ascertain the link of causation between variations in miRNA expression levels and the onset of CHD, the relationship between differences in the amount of miRNA expression and the duration of pregnancy, and the possible biological processes that may be regulated by the downstream targets found in this study.

5. Conclusions

We conclude that hsa-miR-3195 may be crucial for the fetal heart's development based on our thorough analysis of miRNAs in the sera of expectant mothers who have healthy fetuses and those who have defective fetuses. Subsequent bioinformatics analysis led us to the conclusion that hsa-miR-3195 and hsa-miR-122-5p together could act as a molecular tool for determining the propensity for congenital heart disease in the next generation as well as a possible biomarker for the clinical detection of the condition. We think that our research can offer valuable perspectives for additional exploration of the mechanism of miRNA action in coronary heart disease and the use of serum factors for predictive and preventive clinical diagnosis.

Acknowledgements

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Conflicts of interest

The authors declare no conflict of interest.

Data availability statement

High-throughput sequencing data has been uploaded to the GEO database, and the accession number is GSE221349 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE221349 (uploaded on 17 December 2022 and acquired accession number on 19 December 2022)).

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