Urinary MicroRNA Expression Analysis of miR-1, miR-215, miR-335, Let-7ain Childhood Nephrotic Syndrome

C.D.Mohana Priya (pricillacharm@gmail.com)
Sri Ramachandra Institute of Higher Education and Research
Vettriselvi Venkatesan
Sri Ramachandra Institute of Higher Education and Research
P.Pricilla charmine
Sri Ramachandra Institute of Higher Education and Research
G.Sangeetha Geminiganesan
Sri Ramachandra Institute of Higher Education and Research
Sudha Ekambaram
Dr Mehta's Hospitals Pvt Ltd

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Abstract

Background

Recently, urinary exosomal miRNAs are gaining increasing attention as their expression profiles are often associated with specific diseases and they exhibit great potential as noninvasive biomarkers for the diagnosis of various diseases. The present study was aimed to evaluate the expression status of selected miRNAs (miR-1, miR-215-5p, miR-335-5p and let-7a-5p) in urine samples from children with NS [steroid sensitive (SSNS)] and [steroid resistant (SRNS)] along with healthy control group.

Methods

MicroRNA isolation was carried out in urine samples collected from SSNS (100 nos), SRNS (100 nos), and healthy controls (50 nos) using MiRNeasy Mini Kit, followed by cDNA conversion for all the four selected miRNAs using Taqman advanced miRNA cDNA synthesis kit and their expression was quantified by Taqman Advanced miRNA assay kits using Real Time PCR Machine and Rotogen-Q in SSNS and SRNS patients and healthy control subjects.

Results

Quantification of all the four miRNAs (miR-1, mir-215, miR-335, let 7a) were found to be upregulated in both SSNS and SRNS as compared to control group. Further, the comparison of microRNAs within the case groups revealed significant downregulation of three microRNAs - miR-1, miR-215, miR- 335 and upregulation of let-7a in SRNS group as compared to SSNS. The t-test performed for all the four miRNAs was found to be statistically significant.

Conclusions

The aberrant expression of all the four microRNAs in both SSNS and SRNS as compared to healthy subjects may serve as novel biomarkers to distinguish between NS and healthy controls. The differential expression of microRNA let-7a is useful to discriminate SSNS and SRNS.

Highlights

- Urinary miRNA are non-invasive biomarkers for the prognosis and disease monitoring of nephrotic syndrome.
- Gene expression status of four selected microRNAs such as mir-1, miR-215-5p, miR-335-5p and let-7a-5p were studied in the urine samples of children with SSNS, SRNS and healthy controls groups.
- All the four miRNAs were found to be upregulated in both SSNS and SRNS as compared to control group.
- Comparison of microRNAs within the case groups revealed significant downregulation of miR-1, miR-215, miR-335 and upregulation of let-7a in SRNS group as compared to SSNS.

Introduction

Nephrotic syndrome (NS) is considered as one of the most common glomerular kidney diseases, often encountered in children with a characteristic triad of massive proteinuria (40 mg/h/m^2 in children), hypoalbuminemia, and dependent edema, due to the disrupted function of glomerular filtration barrier (GFB) (**Bagga, 2008; Bierzynska and Saleem, 2017**) [1, 2]. In children below the age of 18 years, minimal change nephropathy (MCN) and focal segmental glomerulosclerosis (FSGS) represent the cause of NS in over 85% of cases (**Mekahli et al., 2009)** [3]. In children, MCN is the major cause of NS, responds to steroids at conventional doses and hence this disease is termed steroid sensitive NS (SSNS), while in steroid resistant NS (SRNS), FSGS is the most common histopathological lesion, and may progress to end-stage renal disease (ESRD) (**Mekahli et al., 2009; Hjorten et al., 2016**) [3, 4].

Renal biopsy is a standard procedure but an invasive technique with potential complications, to define the histology, and serial monitoring is not normally feasible, especially for children (**Luo et al., 2013**) [5]. In recent studies, urinary miRNAs are regarded as biomarkers as they reflect kidney diseases including NS (**Szeto, 2014; Chen et al., 2018)** [6, 7]. Some of them are involved in the pathogenesis of NS and thus are disease-specific. Recently, **Chen et al.** (2018) have identified elevated levels of urinary miR-194-5p, miR-146b-5p, miR-378a-3p, miR-23b-3p and miR-30a-5p) in children with NS. Similarly, urinary miR-21, miR-216a, and miR-494 which are found to be in NS may predict a high risk of disease progression and loss of renal function, irrespective of the histological diagnosis (**Szeto, 2014**) [6]. In view of its stability and easy quantification, urinary miRNAs could be used as attractive biomarker candidates for disease diagnosis, predicting drug efficacy and for monitoring therapy decisions.

MicroRNAs (miRNA) are endogenous short non-coding RNAs with a length of around 22 bases, that regulate gene expression at the post-transcriptional level, through incomplete binding to the 3' untranslated regions (UTR) of multiple target mRNAs, enhancing their degradation and inhibiting their translation (**Bartel, 2004**) [8]. Dysregulated miRNAs play critical roles during aging, carcinogenesis and cancer progression (**Erturk et al., 2014; Cao et al., 2014; Gao et al., 2014; Sun et al., 2014: Bai et al., 2014**) [9–13].

Among numerous miRNAs, miR-335 has attracted widespread attention as numerous studies have shown dysregulation of miR-335 in many cancers (**Erturk et al., 2014; Cao et al., 2014; Gao et al., 2014; Sun et al., 2014**) [9–12], and a potential biomarker for the prognosis and diagnosis of cancer by acting as an oncogene or tumor suppressor in the development, migration (**Wang et al., 2013**) [14], metastasis and apoptosis (**Yang et al., 2016**) [15].

In kidneys, miRNAs have been implicated in renal development, homeostasis and physiological functions as well as in the pathogenesis of various renal diseases, including nephrotic syndrome (**White et al., 2010; Ribal, 2011**) [16, 17]. miR-335 and rno-miR-7a have been implicated in an aging mechanism related to oxidative stress by inhibiting the expression of the antioxidant genes (**Bai et al., 2014**) [13].

A recent study has shown that the expression of X-linked inhibitor of apoptosis protein (XIAP) was significantly higher and the expression of microRNA-215 (miR-215) was significantly lower in human colonic cancer cell line HCT116. miR-215 overexpression and (or) silencing XIAP expression promote the apoptosis of HCT116 cells by enhancing caspase-9 and caspase-3 activities. These studies indicate inhibition of XIAP expression by MiR-215 (**Lu et al., 2020**) [18]. Zinc finger E-box-binding homeobox 2 (ZEB2), a downstream target of miR-215 plays an essential role in the process of epithelial-mesenchymal transition (EMT), and podocyte depletion and loss (**Fardi et al., 2019**) [19]. Expression of miR-215-5p in the podocyte, attenuates epithelial-mesenchymal transition of podocytes by inhibiting *ZEB2* expression, targeting directly at the 3-UTR, implying that miR-215-5p negatively regulates ZEB2 activity (**Jin et al., 2020**) [20].

miR-1 is abundantly expressed in the myocardium, play a central role in cardiogenesis, heart function and pathology. Human miR-1 has two isomers (miR-1-1 and miR-1-2) that have identical sequences but are encoded by distinct genes. In mice, targeted deletion of miR1-1 or miR1-1 or both leads to abnormalities in heart development (such as ventricular septal defect and myocyte cell cycle aberrations) and cardiac function including heart arrhythmia and disturbances in heart conduction (**Tao and Martin, 2013; Chistiako et al., 2016**) [21, 22]. However, to the best of authors' knowledge, there is hardly any study on the role of miR-1 in kidney physiology or pathology.

Studies on let-7a-5p have shown downregulation of this miRNA in diabetic nephropathy (DN), and serve as a biomarker for DN diagnosis. Renal mesangial cells cultured under high concentrations of glucose *in vitro have* shown significant downregulation of let-7a-5p. Transfection with let-7a-5p mimics significantly inhibited the PI3K/AKT signaling pathway in renal mesangial cells cultured under high-glucose conditions, while transfection with let-7a-5p inhibitors evinced the opposite effects, indicating a pathological role of let-7a-5p in mesangial cell hypertrophy (**Wang et al., 2019**) [23].

The present investigation was undertaken to study the expression status of selected microRNAs such as mir -1, miR-215-5p, miR-335-5p and let-7a-5p in the urine samples of children with SSNS, SRNS and healthy controls groups. These microRNAs were selected based target prediction using the bioinformatics tool based on the database available and the gene expression analysis in children with SSNS, SRNS and compared with the control group and the results are analyzed for the microRNA expression.

Materials And Methods

Selection of study participants

The study was conducted in Sri Ramachandra institute of Higher Education and Research (SRIHER) and the children with NS visiting the out-patient Department of Nephrology, SRIHER and Dr. Mehta's Hospitals(IEC-NI/19/FEB/68/01&001/IRB/MCH/2020) Chennai, India were recruited. The children who have met with the required inclusion and exclusion criteria aged from 1-12 years without the familial

history of NS were enrolled for the study. Age- and sex-matched healthy children with normal renal function who visited OPDs were recruited as the parallel controls.

Urinary miRNA profiles in children with SSNS and SRNS will be compared with those of healthy children.

The sample size taken for the study was 250 in which 200 were cases (SSNS, SRNS) and 50 were controls. Of the 200 NS children included, 100 children who did not respond to prednisilone therapy for about a minimum period of 4 weeks were considered as steroid resistant and the histological studies confirmed the MCN or FSGS. The remaining one hundred samples were SSNS. Exclusion criteria included secondary NS and SRNS with the history other than FSGS or MCN. Informed consent was obtained from all the parents /guardians along with an asset form. Ethical committee approval was obtained from the Institutional Ethics committee of both the institution.

Sample collection

About 5 ml of urine sample was collected in sterile container and processed immediately after collection, centrifuged at 3,000 x g for about 30 min and at 13,000 x g for 5 min 4°C. The supernatant was discarded and the urinary cell pellet was lysed by vortexing with 5 volumes of QIAzol lysis buffer and was stored in -80°C for further use.

RNA isolation

MicroRNA isolation was carried out for SSNS, SRNS and healthy controls using MiRNeasy Mini Kit (Qiagen). About 3.5 µl miRNeasy Serum/Plasma Spike-In Control was added to urine samples, mixed thoroughly with equal volume of chloroform, incubated for 2–3 min at room temperature and centrifuged at 12,000 x g at 4°C for 15 min. The upper aqueous phase was transferred to a new collection tube, and 1.5 volumes of 100% ethanol was added.

About 700 µl of sample was pipetted into an RNeasy Min Elute spin column in a 2 ml collection tube, centrifuged at ≥ 8000 x g for 15 seconds at room temperature. Supernatant was discarded. Around 500 µl of elution buffer was added to the RNeasy Min Elute spin column and centrifuged at the same rpm. About 500 µl of 80% ethanol was added to the RNeasy MinElute spin column and centrifuged for 2 min at ≥ 8000 x g. The flow through was discarded followed by dry spin. Finally, the RNeasy MinElute spin column was placed in a new 1.5 ml collection tube and 14 µl RNase-free water added directly to the center of the spin column followed by centrifugation for one min at full speed to elute the microRNA.

The isolated nucleic acids were quantified to determine the concentrations and purity of the samples. The concentration and quality of extracted RNA were assessed by spectrophotometry on the NanoDrop 1000 (Thermo Scientific, Waltham, MA). The ratio of the absorbance at 260 and 280 nm (A260/280) is used to assess the purity of nucleic acids. About 2 µl of isolated sample was added to the lower pedestal of the nano drop and the purity of nucleic acid was assessed for all the collected urine sample using nano drop

equipment. A ratio of 1.7-2.0 was generally accepted as a good quality. All 250 samples were checked for quality and quantity and found to be good and with adequate proportion for expression study.

cDNA conversion

To the poly A tailing reaction master mix, 1 µl of RNA was added and the reaction was set up for about 55 min in thermal cycler. Then cDNA conversion was done for the selected miRNAs using Taqman advanced miRNA cDNA synthesis kit according to the manufactures protocol. Next ligation reaction was prepared and the cycling reaction was setup at 16°C in the thermal cycler. After ligation reaction, reverse transcription (RT) master mix was prepared according to the manual instructions and kept for cycling reaction at 42°C for about 15 min. Finally, miR- amplification step was carried out by adding 2.5 µl of the RT product and the final volume was made upto 22.5 µl and kept for 45 min in thermal cycler. The concentration of input isolated RNA for c DNA conversion is 10 ng/µl. The converted cDNA templates were further diluted to proceed for microRNA expression analysis using Real Time PCR.

miRNA expression using quantitative Real-time PCR

The expression of the selected miRNAs (miR-1, mir-215, miR-335-5p and let-7a-5p) was quantified by Taqman Advanced miRNA assay kits (Applied Biosystems, USA) using the applied Bio systems Fast 7900 HT Real Time PCR Machine and Rotogen-Q (Qiagen).

MicroRNA target prediction

MicroRNA target predictions tools such as miRTargetLink link human has been used in order to predict and screen the microRNAs involved in the study. This miRTargetLink human is helpful in finding out the microRNAs targeted by a single gene and single microRNA can be targeted by many genes.

Statistical Analysis

The data were analyzed using Student ‘t’ test to calculate the level of significance for the fold change. For all analyses, a difference with $p<0.05$ was considered statistically significant. Pearson r correlation analysis were also performed between cases and control group, a difference with $p<0.05$ was considered to be statistically significant.

Results

3.1 MicroRNA expression analysis

The graph represents the delta Ct (ΔCt) values of the miRNAs among the cases (SSNS, SRNS) and control group. Quantification of all the four miRNAs (miR-1, mir-215, miR-335, let 7a) were found to be upregulated in both SSNS and SRNS in comparison with the control group. Further, the microRNAs are compared within the case groups. miR-1 (Fig.1 A, B; Fig. 3A), miR-215, (Fig.1 C, D; Fig. 3B), miR-335 (Fig.2 A, B; Fig. 3C), were observed to be downregulated in SRNS group in comparison with SSNS, while let 7a registered significant upregulation in SRNS (Fig.2 C, D; Fig. 3D). The t-test was performed for all the four miRNAs and found to be statistically significant with *p* values as mentioned in the Table 1. Pearson r correlation analysis for all the four miRNAs performed among which let-7a was found to be statistically significant with *p* values as mentioned in the Table 2.

3.2 Target prediction of selected microRNAs and functional analysis

The target prediction software miRTargetLink human revealed 18 experimentally validated targets for the microRNAs miR-1, mir-215, miR-335-5p and let-7a-5p, as shown in Table 3. The representative image of molecular network of the selected microRNAs with their enriched targets are depicted in Fig4(a)&4(b). The target genes were further analyzed for the various pathways associated with the disease using gProfiler software in Table 4. The analysis revealed significantly enriched BioGRID interactions including receptor binding for growth factor, cytokines, VEGF, biological process involved in the development of kidney, nephron and renal system, regulation of T cell mediated immunity, AGE-RAGE signaling pathway in diabetic complications, Th17 cell differentiation,abnormal nephron morphology,abnormal renal cortex morphology,abnormal renal glomerulus morphology, abnormal urine protein level, Focal segmental glomerulosclerosis, receptor interaction, cytokine-cytokine nephrotic syndrome, genes controlling nephrogenesis, primary FSGS, proteinuria *etc*. Description of the various processes and pathways with their ID and significance level is represented in Table 5.

Discussion

Physiologically representative and accessible samples such as saliva, blood or urine are referred to as liquid biopsies, harbor circulating cells, protein, DNA, and RNA biomarkers with high potential for characterizing conditions of health and disease (**Perakis et al., 2017**) [24]. Among these, RNA has come to the forefront of readily accessible molecules for the discovery of novel biomarkers (**Weber et al., 2010**) [25]. Urine based biomarkers would be ideal for many studies due to its representation of the physiological state of the organism and accessible nature of urine (**Buschmann et al., 2016**) [26].

Urinary miRNAs represent an attractive, noninvasive tool for the early detection of various human diseases. In the kidneys, miRNAs not only maintain normal regulatory mechanisms but also play indispensable roles in renal dysfunction and structural damage (**Szeto, 2014**) [8]. Urine is an ideal source of biomarkers and provide valuable insight on renal pathophysiology. Podocyte cytoskeleton is regulated by several miRNAs, including miR-30, miR-132, miR-134 and miR-29a (**Tsuji et al., 2020**) [27]. In the

present study, the observed upregulation of all the four miRNAs (miR-1, mir-215, miR-335, let-7a) in both SSNS and SRNS *versus* healthy controls implies of podocyte injury, aberrant expression of these microRNAs and their consequent role in the pathogenesis of NS. As research into microRNAs continues, new microRNAs are being continually discovered, and their functions are being confirmed one by one in different diseases. To the best of author's knowledge, all the four micoRNAs currently studied are identified for the first time in NS subjects, may serve as novel biomarkers to distinguish between NS and healthy controls, meriting further attention to this area.

As a next step, the target genes for all the four microRNAs were identified using microRNA prediction tool such as miR TargetLink link human (Figs. 4(a)&4(b)). The predicted targets of each microRNA are listed in Table 2. The target genes for miR-1 and miR-335 are *ANLN KIRREL*, respectively. The target gene for miR-1 is *ANLN* which encodes anillin, an actin-binding protein, and was identified as a cause of SRNS due to reduced binding to the slit diaphragm-related protein CD2AP (**Gbadegesin et al., 2014**) [28]. **NEPH1**, also known as **Kin of IRRE-like protein 1**, is a protein that in humans is encoded by the *KIRREL* gene. KIRREL1 (also known as NEPH1), is a member of the nephrin-like protein family, which includes KIRREL2 (also known as NEPH2) and KIRREL3 (also known as NEPH3). The cytoplasmic domains of these proteins interact with the C terminus of podocin (NPHS2), and the genes are expressed in kidney podocytes, cells involved in ensuring size- and charge-selective ultrafiltration (**Sellin et al., 2003**) [29]. KIRREL plays a role in maintaining the structure of the filtration barrier that prevents proteins from freely entering the glomerular urinary space (**Donoviel et al., 2001**) [30]. SRNS is a frequent cause of chronic kidney disease almost inevitably progressing to ESRD. More than 58 monogenic causes of SRNS have been discovered and majority of known SRNS causing genes are predominantly expressed in glomerular podocytes, placing them at the center of disease pathogenesis. Mutant KIRREL1 proteins failed to localize to the podocyte cell membrane, indicating defective trafficking and impaired podocytes function (**Solanki et al., 2020**) [31]. Thus, the KIRREL1 gene product has an important role in modulating the integrity of the slit diaphragm and maintaining glomerular filtration function. In the present study, the observed low expression of miR-1 and miR-335 in SRNS as compared to SSNS, surprisingly, the level of miR-335 was found to low in SRNS as compared to SSNS.

MicroRNA prediction tool such as miRTarget link human revealed that microRNA let -7a-5p has been targeted by the gene CD2-associated protein (CD2AP). CD2AP is an adapter molecule, essential for the slit-diaphragm assembly and for maintaining podocyte integrity and reducing proteinuria. CD2AP was a strong candidate gene for NS. Within glomeruli, CD2AP expression is restricted to the podocyte where it has been shown to interact with nephrin and podocin (**Schwarz et al., 2001**) [32]. Mice lacking CD2AP (*Cd2ap* −/− mice), a model for congenital NS develop extensive foot process effacement beginning at 1 wk of age, followed by excessive deposition of extracellular matrix by mesangial cells and blockage of capillaries and die at 6-7 wk of age from proteinuria and renal failure (**Shih et al., 1999**) [33], indicating a pivotal role of CD2AP-nephrin interactions in the glomerular filtration function. Taken together, we assume that the up-regulation of let -7a-5p targets the CD2AP promoter sequences and suppresses gene expression, leading to podocyte injury. A careful screening of let–7a-5p revealed that this microRNA alone remained elevated in SRNS as compared to SSNS. So far, ~10 mutations of CD2AP have been reported in

FSGS or NS patients. Mutations in the CD2AP gene can contribute to FSGS development. Therefore, it is not surprising to find upregulated let–7a-5p microRNA in SRNS subjects. miRTargetLink human is an effective and efficient tool for experimental biologists to comprehensively predict the genes for miRNAs as well as to analyze and interpret their involvement in the biological process, functions and signaling pathways.

To sum up, these urinary miRNAs expressed only in NS could be used as potential non-invasive biomarker candidates for diagnosing and monitoring paediatric NS.

Declarations

Declaration of interest

The authors report no conflicts of interest and they are responsible for the content and writing of this article.

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Tables

Table 1

Expression of miRNAs represented as fold change

miRNA	Fold change	*p* value
miR-215	SSNS = 3.94	SSNS = 0.00001
	SRNS = 1.06	SRNS =0.00001
miR-1	SSNS = 4.04	SSNS = 0.00031
	SRNS = 1.98	SRNS = 0.23442
miR-335	SSNS = 4.20	SSNS = 0.00020
	SRNS = 2.86	SRNS = 0.00388
Let -7a	SSNS = 1.51	SSNS = 0.02164
	SRNS = 4.33	SRNS = 0.00001

Table 2

Pearson r correlation analysis of the microRNAs (miR-1, mir-215, miR-335-5p and let-7a-5p) and mir-484 among cases (SSNS and SRNS) and control group

Control vs SSNS		
miRNA	**r value**	***p* value**
miR-215	215**	0.002
miR-1	1	-
miR-335	.141*	.058
Let -7a	0.276**	<0.01
Control vs SRNS		
miR-215	0.13	0.858
miR-1	1	-
miR-335	.170*	0.16
Let -7a	.023	.744
****Correlation is significant at the level 0.01 level(2-tailed)**		
***Correlation is significant at the level 0.05 level(2-tailed)**		

Table 3

Targets for microRNAs miR-1, miR-215, miR-335 and let-7a predicted using mirTarbase and mirWalk databases

MicroRNA	Target genes	Protein	Location	Target genes location	Associated Disease/ Phenotype
miR-1-5P	*ACTN4,GATA3,C0Q6, CD2AP,IL8*	Anillin	Podocyte cytoskeleton	7p14.2	FSGS
miR-215	*IL1R1, PLCE1*				
miR-335	*ACE, IL8, LEPR, PLCE1, NPHS2, IL4, KIRREL, TFP1* *VEGFA, DDN*	Neph-3 /Filtrin	Slit Diaphragm		
let-7a	*CD2AP*	CD2-associated protein	Slit Diaphragm	6p12.3	FSGS, SRNS
FSGS - focal segmental glomerulosclerosis; SRNS - (cortico)steroid-resistant nephrotic syndrome.					

Table 4

Predicted microRNA target genes from miRTargetLink

MicroRNA	Target genes	Protein	Location	Target genes location	Associated Disease/ Phenotype
miR-1-5p	*ACTN4*	α-Actinin-4	Podocyte cytoskeleton	19q13.2	FSGS, SRNS
	GATA3		Mesangial cells	10p14	Segmental glomerulosclerosis
	C0Q6	Ubiquinone biosynthesis monooxygenase COQ6		14q24.3	NS with sensorineural deafness, CoQ10 deficiency
	CD2AP,	CD2-associated protein	Slit diaphragm	6p12.3	FSGS, SRNS.
	IL8				
miR-215	*IL1R1,*				
	PLCE1 (NPHS3)	Phospho lipase C epsilon 1	Podocyte cytoskeleton	10q23.33	Early onset diffuse mesangial sclerosis and FSGS
miR-335	*ACE, TFP1* *VEGFA, DDN, LEPR*				
	IL8, IL4				
	PLCE1	Phospho lipase C epsilon 1	Podocyte cytoskeleton	10q23.33	Early onset diffuse mesangial sclerosis and FSGS
	NPHS2	Podocin	Podocyte foot process	1q25.2	CNS, FSGS, SRNS, MCN
	KIRREL	Neph-3 /Filtrin	Slit Diaphragm		
let-7a	*CD2AP*	CD2-associated protein	Slit Diaphragm	6p12.3	FSGS, SRNS

FSGS - focal segmental glomerulosclerosis; SRNS - (cortico)steroid-resistant nephrotic syndrome. MCN - Minimal change nephropathy.

TABLE 5

Significantly enriched BioGRID interactions of the predicted target genes indicating the biological process, cellular component, cellular component, molecular function, and biological pathways (KEGG , Reactome,Wiki, HP)

OR

Gene Ontology enrichment analysis by miRTargetLink link human

Description	Term ID	Corrected p-value
Molecular Function		
growth factor receptor binding	GO:0070851	3.836×10-6
cytokine receptor binding	GO:0005126	1.019×10-4
signaling receptor binding	GO:0005102	3128×10-3
vascular endothelial growth factor receptor binding	GO:0005172	7.325×10-3
Biological Process		
kidney development	GO:0001822	2.843×10-2
Nephron development	GO:0072006	1.678×10-3
Renal system development [	GO:0072001	3.200×10-2
Regulation of T cell mediated immunity	GO:0002709	1.850×10-2
Cellular Component		
Extracellular region	GO:0005576	3.226×10-2
Reactome Pathway		
Nephrin family interactions	REAC:R-HSA-373753	2 .879×10-4
KEGG Pathway		
AGE-RAGE signaling pathway in diabetic complications	KEGG:04933	7.917×10-3
Th17 cell differentiation	KEGG:04659	8.896×10-3
Cytokine-cytokine receptor interaction	KEGG:04060	1.008×10-2
WP(WikiPathways)		
Nephrotic syndrome	WP:WP4758	3.139×10-5
Genes controlling nephrogenesis	WP:WP4823	

		2.453×10-3
Primary FSGS	WP:WP2572	2.372×10-4
HP		
Abnormal nephron Morphology	HP:0012575	1.165×10-3
Abnormal renal cortex morphology	HP:0011035	6.359×10-4
Abnormal renal glomerulus morphology	HP:0000095	2.726×10-4
Abnormal urine protein level	HP:0020129	5.863×10-4
Chronic kidney disease	HP:0012622	9.194×10-3
Focal segmental Glomerulosclerosis	HP:0000097	1.206×10-4
Glomerular sclerosis	HP:0000096	1.027×10-3
Proteinuria	HP:0000093	4.058×10-4
Renal insufficiency	HP:0000083	1.257×10-2
Abnormal urine metabolite level	HP:0033354	4.065×10-2
Abnormal renal corpuscle morphology	HP:0031263	2.726×10-4

Figures

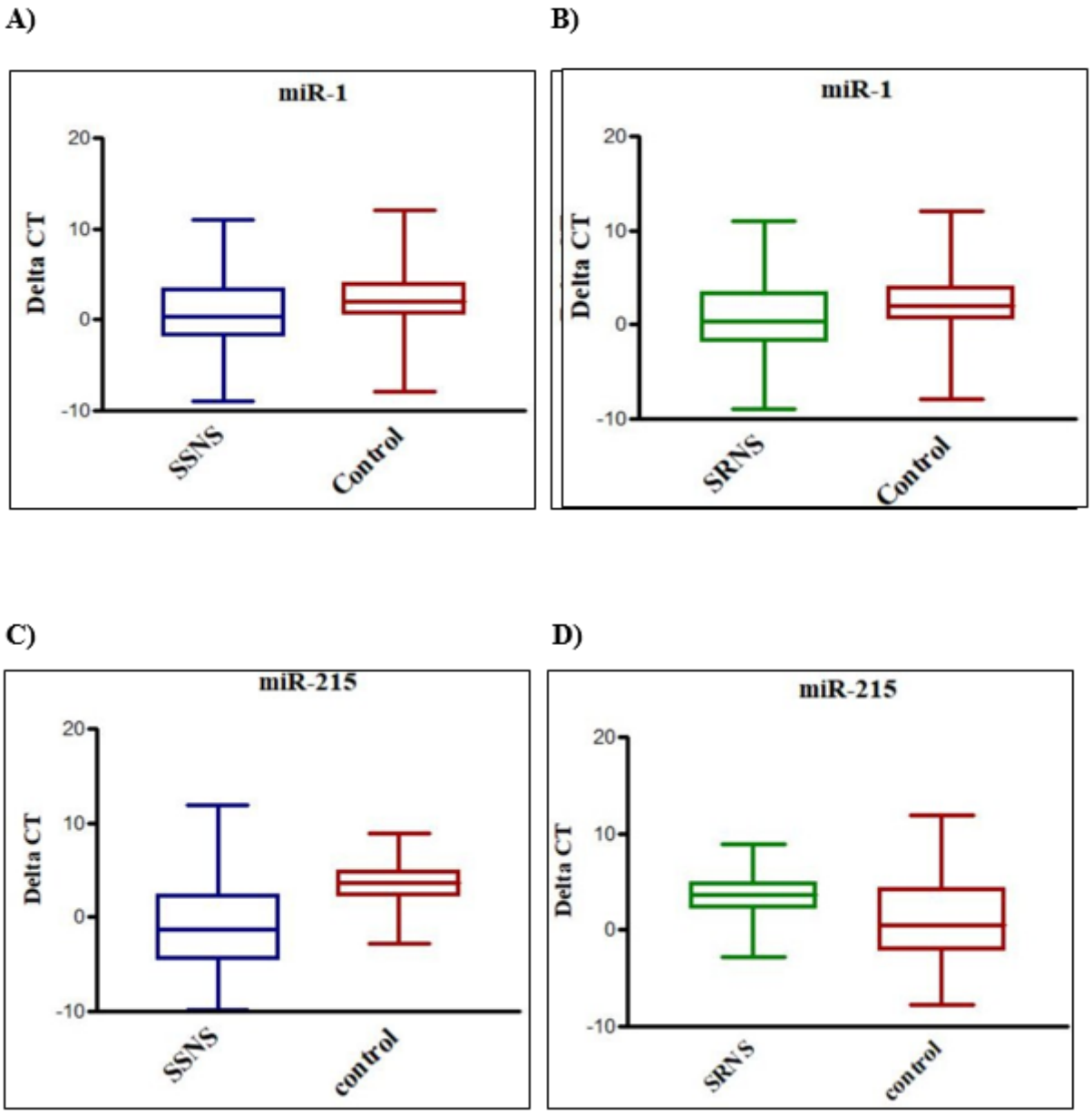

Figure 1

miRNA expression of urinary miR-1 and miR-215 in children with SSNS andSRNS

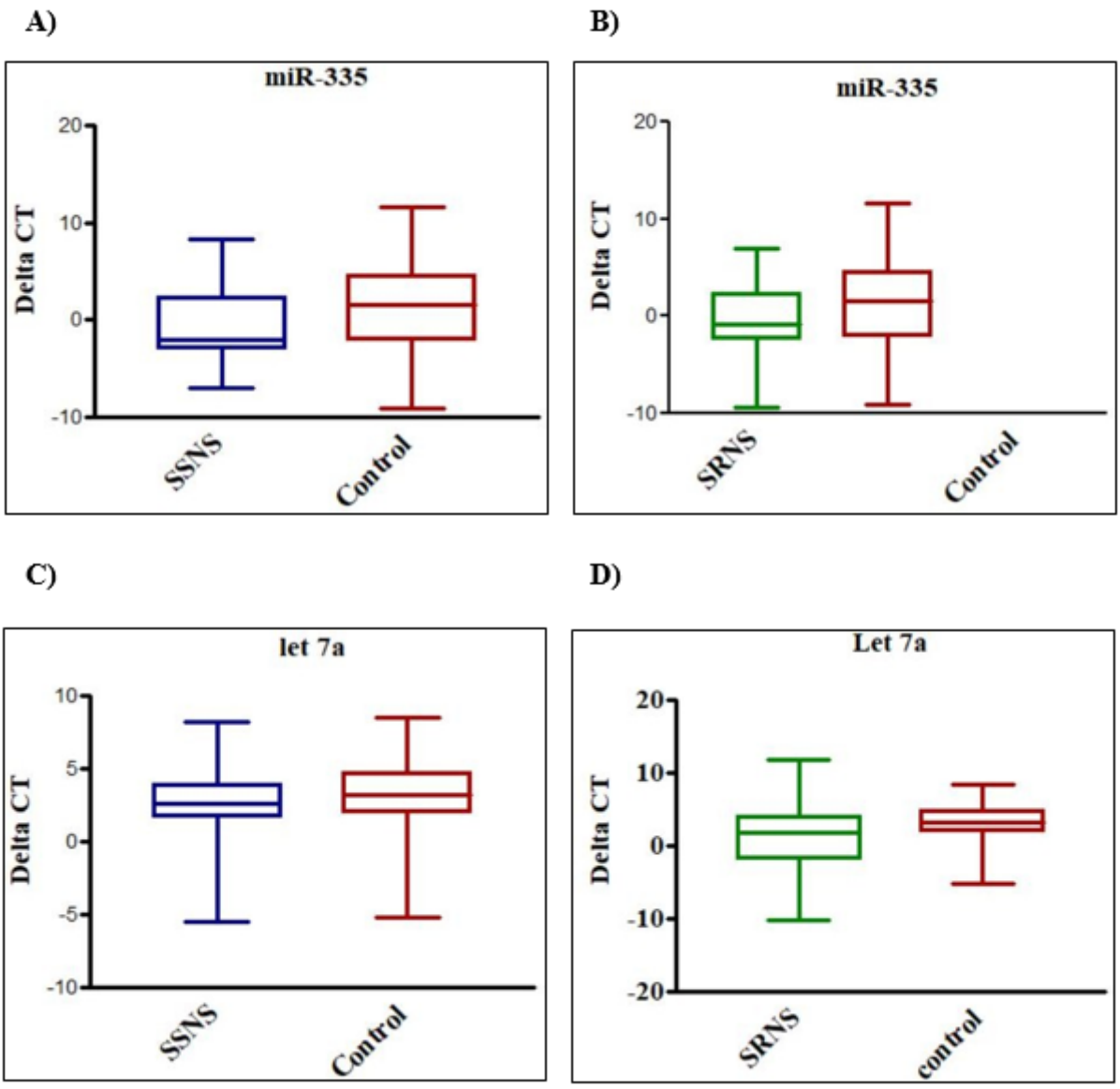

Figure 2

miRNA expression of urinary miR-335 and let-7a in children with SSNS and SRNS

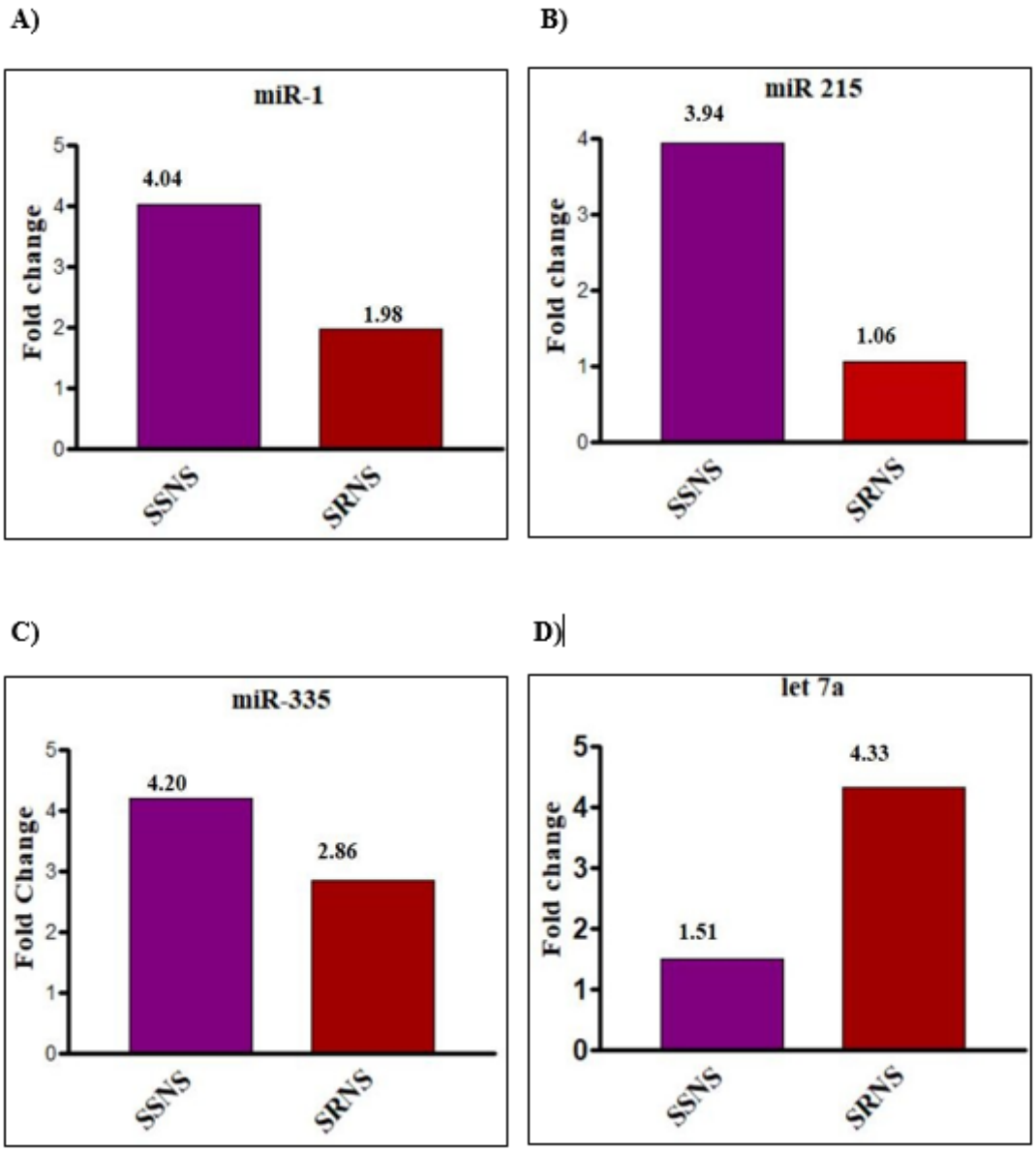

Figure 3

Bar diagrams showing fold changes in the expression levels of miR-1 (Fig. 3a), miRNA 215 (Fig. 3b), miRNA 335 (Fig. 3c) and miRNA let-7a (Fig. 3d)

Figure 4

(a)Molecular interaction network of the candidate miRNAs and targeted genes

(b) Molecular network of hsa-let-7a-5p and hsa- miR-335-5p miRNAs using the miRTargetLink human bioinformatics tool

Supplementary Files

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