Evaluation of the associations between circulating microRNAs and kidney function in coronary angiography patients

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ABSTRACT

Circulating microRNAs (miRNAs) have been linked to chronic kidney disease. Little is known about the association between circulating miRNAs and kidney function in patients at high cardiovascular risk. We therefore investigated the association between a ~~huge~~ panel of candidate miRNAs and kidney function, based on estimated glomerular filtration rate (eGFR), in two independent cohorts of patients undergoing coronary angiography. The present study totally included 438 coronary angiography patients, who were divided into a discovery cohort (n=120) and a validation cohort (n=318). A candidate miRNA panel comprising 50 renal miRNAs were selected from the literature and expression levels of circulating miRNAs were determined by real-time PCR. Out of initially tested candidate-miRNAs, 38 were sufficiently detectable in plasma. Their association with kidney function was evaluated in the discovery cohort. Associations of seven out of these miRNAs with eGFR were significant after multiple testing correction via false discovery rate (FDR) estimation. To verify obtained results, miRNAs with significant FDR were further analysed in the validation cohort. MiRNAs miR-106b-5p, miR-16-5p, miR-19b-3p, miR-20a-5p, miR-25-3p, and miR-451a proved to be significantly associated with eGFR also in the validation cohort (all p-values <0.001). Association between identified renal miRNAs and kidney function was confirmed by ANCOVA adjusting for age, gender, type 2 diabetes, hypertension, and albumin-to-creatinine ratio. In conclusion, our study showed that miR-16-5p, miR-19b-3p, miR-20a-5p, miR-25-3p, miR-106b-5p, and miR-451a are significantly linked to kidney function in coronary angiography patients.

INTRODUCTION

Chronic kidney disease (CKD) is increasing worldwide and is strongly linked to an elevated risk of cardiovascular and all-cause mortality (31, 36). CKD is characterized by a progressive loss of kidney function over months or years typically showing a long latent period when the disease is clinically silent (18). Therefore, early detection of reduced kidney function is essential to improve risk prediction, particularly in high-risk patients.

Recently, circulating microRNAs (miRNAs) have emerged as novel diagnostic biomarkers in many diseases including kidney disease (20, 21). MiRNAs are small non-coding RNAs of approximately 22 nucleotides in length that usually function as repressors of target genes by either inhibiting translation or promoting degradation of mRNA (5, 30). Currently, approximately 2700 mature miRNAs have been identified in humans according to database miRbase, release 22.1 (15). Many of them are expressed in a tissue and/or cell-specific manner playing important regulatory roles in virtually all cellular processes inclusive of kidney development and function (1, 37). Notably, miRNAs can also be detected outside cells, including circulating cell-free body fluids such as plasma, serum, or urine in a remarkably stable form (41). It is hypothesized that miRNAs are not only passively released by necrotic or injured cells but are actively secreted in membrane-bound vesicles (exosomes, microvesicles) (9, 13), in apoptotic bodies (42), or in vesicle-free but protein-protected protein-miRNA complexes (3). These mechanisms of miRNA packaging may protect circulating miRNAs from degradation. Their highly extracellular stability together with their often tissue-specific expression patterns and their feasible measurability by current techniques makes circulating miRNAs highly attractive as biomarkers in biomedical research.

Several recent studies have investigated circulating miRNAs in patients with severe chronic kidney disease (27, 29), end stage renal disease (10, 39), or acute kidney injury (19, 24, 38). However, little is known about the association between circulating miRNAs and kidney function in patients at high cardiovascular risk, such as coronary patients.

Therefore, we (i) determined the expression of 50 miRNAs, previously associated in the literature with kidney function or kidney disease in a set of plasma samples obtained from coronary angiography patients, (ii) identified those circulating miRNAs putatively related to kidney function and (iii) validated their diagnostic value in a further independent cohort of coronary angiography patients.

METHODS

Study subjects

Patients were selected from a Caucasian patient cohort totally comprising 1048 subjects referred to elective coronary angiography for the evaluation of established or suspected stable coronary artery disease (CAD) at the academic teaching hospital Feldkirch.

The Ethics Committee of the University of Innsbruck approved the present study and written informed consent was given by all participants. Detailed information on the recruitment protocol and the determination of subjects characteristic has been described previously (26, 32). In brief, venous blood samples were collected after an overnight fast of 12 h before angiography was performed. Height and weight were recorded, and body mass index (BMI) was calculated as body weight (kg)/height^2 (m^2). Hypertension was defined according to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and

Treatment of High Blood Pressure (34) and type 2 diabetes mellitus (T2DM) was diagnosed according to American Diabetes Association (ADA) guidelines (2). Coronary angiography was performed with the Judkin's technique and the severity of stenosis was assessed by visual inspection by a team of two investigators, who were blinded to serologic assays as described previously (8). Estimated glomerular filtration rate (eGFR) was assessed by the 'Mayo Clinic Quadratic' (MQ) equation, if not otherwise noted. The MQ equation is based on sex, age, and serum creatinine and has been shown to give an accurate estimate of the glomerular filtration rate in patients with nearly normal renal function (33). Additionally, the 'Chronic Kidney Disease Epidemiology Collaboration' (CKD-EPI) equitation (17) was used to estimate glomerular filtration rate. Renal function was classified as normal kidney function in subjects with eGFR ≥90 mL/min/1.73 m^2, mild impairment of kidney function in subjects with eGFR 60-89 mL/min/1.73 m^2, and chronic kidney disease in subjects with eGFR<60 ml/min/1.73m^2 (28). Urinary albumin excretion was expressed as the albumin/creatinine concentration ratio (ACR) in a random fresh morning urine specimen.

Study design

In a first pre-selecting step, a candidate miRNA panel consisting of 50 miRNAs previously associated in the literature with renal function and/or involved in the development and progression of kidney disease (Supplemental Table S1) was analyzed in 60 plasma samples obtained from patients with angiographically proven CAD to identify those miRNAs, which were sufficiently detectable in plasma. MiRNAs sufficiently detectable in plasma were defined as miRNAs showing raw ct-values <39 cycles in at least 70% of samples. MiRNAs miR-24-3p, miR-92a-3p, and miR-222-3p were selected as endogenous reference miRNAs based on previously performed experiments at our institution and proposed as normalizers in other reports (22, 35, 40).

MiRNAs being detectable below selected cutoff were analyzed in 60 additional coronary patients. In doing so, a final discovery set was generated totally comprising 120 patients, which was used to evaluate the association between selected miRNAs and renal function.

MiRNAs providing significant association with renal function after multiple testing correction in the discovery study cohort were re-tested in further 318 patients randomly selected out of remaining subjects referred to coronary angiography. Finally, the association of selected miRNAs with kidney function was assessed in the two combined patient cohorts totally including 438 coronary angiography patients.

Prospective study

Kidney function was re-assessed based on creatinine values obtained at a follow-up visit after 3.6±1.2 years.

miRNA analysis

RNA was isolated from 0.2 ml plasma using the 'miRNeasy Mini Kit' (Qiagen, Hilden, Germany) according the manufactures protocol for the purification of small RNAs from plasma. Isolated miRNAs were reverse transcribed using 'Universal cDNA Synthesis Kit' (Exiqon, Vedbaeck, Denmark) according to the manufactures instructions for plasma derived miRNAs. Subsequently, quantitative real-time PCR was performed using 'Universal SYBR Green master mix' (Exiqon) and miRNA specific LNA™ PCR primer or 'Pick-&-Mix microRNA PCR Panel' plates (Exiqon) in a 10µl volume on a LightCycler® 480 Real-Time PCR System (Roche Diagnostics, Vienna, Austria). Ct values of each candidate miRNA were recorded and normalized by the global mean of all miRNAs (pre-selecting study) or by the mean expression of the selected reference miRNAs miR-24-3p, miR-92a-3p, and miR-222-3p (discovery and validation study).

Statistical analysis

MiRNA expression levels are given as $2^{-\Delta Ct}$ values, such that increased values reflect increased miRNA concentration. Normal distribution was assessed using Kolmogorov-Smirnov and Shapiro-Wilk test, respectively, showing that miRNA expression levels were not normally distributed. Association between miRNAs and continuous clinical/laboratory parameters were explored using non-parametric Spearman's rank correlation tests. Benjamini and Hochberg false discovery rate correction was used for correcting multiple testing (4). In addition, analysis of covariance models (ANCOVA) were built using a general linear model approach. Statistically significant differences between miRNAs and categorical variables were determined by the Kruskal-Wallis test and the Mann–Whitney U test, respectively. P-values <0.05 were considered significant. Statistical analyses were performed with SPSS 25.0 for Windows (SPSS, Inc., Chicago, IL).

RESULTS

Patients' characteristics

Clinical and biochemical baseline characteristics of patients included in the discovery cohort, the validation cohort as well in the combined patient cohorts are given in table 1. Study cohorts showed a high proportion of patients with male sex, the metabolic syndrome, T2DM, hypertension, and significant coronary artery stenoses. The discovery cohort and the validation cohort showed similar eGFR values (p=0.791). Approximately 10% of patients showed eGFR values below 60 mL/min/1.73 m^2. Consequently, most patients had normal kidney function.

Evaluation of miRNAs levels in plasma samples

A pre-selection study performed in 60 patients of the discovery study cohort showed that 12 miRNAs out of 50 analysed candidate miRNAs showed raw ct-values ≥39 cycles in at least 30% of samples and therefore were excluded from further investigations (Supplemental Table S1). Expression data of miRNAs either normalized by the global mean or by the mean expression of selected reference miRNAs miR-24-3p, miR-92a-3p, and miR-222-3p were highly correlated (mean correlation coefficient = 0.950). Furthermore, selected reference miRNAs were not associated with kidney function in the pre-selection set (all p-values >0.05). Therefore, miR-24-3p, miR-92a-3p, and miR-222-3p were found to be appropriate endogenous reference miRNAs and were used as references in the further study. In a next step, miRNAs with sufficient expression were analysed in additional 60 patients generating a discovery cohort totally comprising 120 patients. Plasma levels of individual miRNAs are shown in Supplemental Fig. S1. MiRNA-451a showed highest expression levels, followed by miR-16-5p.

Associations between miRNAs and eGFR in the discovery and validation cohort

Associations between individual candidate miRNAs and eGFR are given in Supplemental Table S2. Out of 38 miRNAs of the discovery set, 15 miRNAs were significantly associated with eGFR at a nominal level of significance. Associations of seven out of these miRNAs (miR-16-5p, miR-19b-3p, miR-20a-5p, miR-25-3p, miR-106b-5p, miR-320a, and miR-451a) with eGFR remained significant after multiple testing correction via false discovery rate (FDR) estimation. To verify obtained results, miRNAs with significant FDR and additionally miR-320b showing borderline FDR significance with eGFR were further analysed in the validation cohort (n=318). Table 2 shows correlation between these miRNAs and eGFR in the validation study. MiRNAs miR-106b-5p, miR-16-5p, miR-19b-3p, miR-20a-5p, miR-25-3p,

and miR-451a, but not miR-320a and miR-320b, proved to be significantly associated with eGFR also in the validation study cohort.

Associations between identified renal miRNAs and kidney function in the total study cohort

The association of identified renal miRNAs with traits of kidney function was further investigated in the combined study cohorts totally comprising 438 subjects. As to be expected, the associations between identified renal miRNAs and eGFR were significant also in the total study cohort. MiRNAs also remained significantly associated with kidney function alternatively using the CKD-EPI equitation (17) instead of the Mayo Clinic Quadratic equation to estimate glomerular filtration rate (Supplemental Table S3). Identified renal miRNAs were able to significantly discriminate between normal and mildly impaired glomerular filtration rate (figure 1). Furthermore, a significant association between identified renal miRNAs and eGFR was proved by ANCOVA adjusting for age, gender, T2DM, hypertension, and ACR (Supplemental Table S4).

Spearman correlation analysis revealed a high correlation of identified renal miRNAs with each other (Supplemental Table S5). In ANCOVA adjusting for each identified renal miRNA, only associations between the miR-19b-3p, miR-106b-5p and miR-451a with eGFR remained significant (Supplemental Table S6).

To further elucidate the impact of the identified renal miRNAs on kidney function, the association between identified renal miRNAs and parameters, which were associated with kidney function, was assessed. Results of correlation analysis are given in table 3. The identified renal miRNAs were significantly associated with age and established renal markers including ACR, urea, and FGF23 serum levels. However, after adjustment for eGFR in

ANCOVA, the associations between miRNAs and these variables did not remain significant (all p-values >0.05).

Associations between identified renal miRNAs and future kidney function

Creatinine serum concentrations and eGFR assessments were available from 271 subjects out of the 438 initially included patients from a follow-up visit after 3.6±1.2 years. All baseline miRNAs were significantly associated with eGFR also at follow up (Supplemental Table S7).

DISCUSSION

In the present work we report a strong correlation of the six plasma-derived miRNAs miR-16-5p, miR-19b-3p, miR-20a-5p, miR-25-3p, miR-106b-5p, and miR-451a with kidney function in coronary patients. Our findings are based on a multi-step strategy, including a discovery study to identify circulating miRNAs significantly associated with kidney function as well as an independent validation study to verify obtained results. Identified renal miRNAs were even able to significantly discriminate between normal and mildly impaired eGFR.

Our observations are in line with previous studies also demonstrating a significant reduction of circulating miRNAs in patients with CKD. In this regard, Neal et al. (29) showed that in patients with severe chronic kidney disease, circulating levels of total and specific miRNAs, including miR-16-5p, are reduced in comparison to patients with mild renal impairment or normal renal function. Furthermore, Lee et al. (16) reported that circulating miR-20a-5p and miR-106b-5p were significantly lower in CKD patients than in healthy subjects. Notably, the association between plasma-derived miR-19b-3p, miR-25-3p, and miR-451a and kidney function in humans, as described in our study, is new.

That said, it remains unclear whether the identified circulating renal miRNAs are directly involved in kidney function or are a result of impaired kidney function. MiR-20a-5p together with miR-19b-3p and several other miRNAs belongs to the miR-17~92 cluster, which is highly conserved in vertebrates. MiR-106b-5p and miR-25-3p are members of the miR106b~25 cluster, a paralog of the miR-17~92 cluster originated by gene duplication and deletion events during vertebrate evolution (7). Both clusters are essential for development and homeostasis promoting cell division and resistance to apoptosis (7). Apoptosis promotes loss of renal epithelial cells that characterizes acute and chronic kidney disease. Therefore, it may be hypothesized that the observed reduced circulating levels of miRNAs miR-19b-3p, miR-20a-5p, miR-25-3p, and miR-106b-5p as members of the miR-17~92 cluster family reflect an increase in apoptotic processes in the kidneys accelerating renal dysfunction. The supposed impact of the 17~92 cluster family on renal function is supported by animal studies. In this regard, Marrone et al. showed that the miR-17~92 cluster is essential in renal development and that its loss leads to the development of renal disease in mice (23). However, the individual mechanisms by which low plasma levels of members of the miR-17~92 cluster family contribute to renal function remain to be elucidated.

Interestingly, renal miRNAs of the miR-17~92 cluster family were not only highly correlated to each other but also to miR-16-5p and miR-451a, which belong to the miR-15~16 cluster and the miR-144~451 cluster, respectively. The high correlation between circulating miR-16-5p and miR-19b-3p has also been observed by Zhang et al., linking low levels of plasma-derived miR-16-5p and miR-19b-3p to gastric cancer (43). In this regard, the association between miR-16-5p and renal function was no longer significant after adjusting for other identified renal miRNA. Therefore, the significant association between circulating miR-16-5p

and eGFR observed in our study and by others (29) appears to be mediated by other miRNAs, closely correlated to miR-16-5p, such as miR-19b-3p.

However, a direct impact of circulating miR-451-5p on kidney function cannot be excluded. Animal studies showed that miR-451-5p is downregulated in diabetic kidney disease suggesting a protective role of miR-451-5p in kidney tissue (25, 44). It has been shown that overexpression of miR-451-5p inhibits glomerular mesangial cell hypertrophy (44), a key event occurring at a very early stage of diabetic nephropathy. That said, in the present study the association between circulating miR-451-5p and kidney function was independent from the presence of T2DM.

Notably, miR-16-5p together with miR-451-5p showed highest expression levels among the investigated candidate miRNA panel (Supplemental Fig. S1), indicating that miR-16-5p and miR-451-5p account for a significant proportion of total circulating miRNA. Low levels of miR-16-5p or miR-451-5p may therefore reflect reduced levels of total circulating miRNA, which by itself has been associated with reduced kidney function (29). However, the biological background behind the association between reduced miRNA levels and reduced kidney function is still unclear. In this context, it has been shown that subjects with renal dysfunction show enhanced levels of RNases (12, 14) probably leading to increased degradation and, consequently, reduced levels of circulating miRNAs. That said, this hypothesis has been rejected by several authors due to the given protection of circulating miRNAs from degradation by different mechanisms of miRNA packaging such as the incorporation of miRNAs into vesicles or the formation of protein-miRNA complexes (11, 29). Also, the question remains, why some plasma miRNAs are associated with eGFR while others are not.

Evidence suggests that different miRNA transport forms are associated with distinct miRNA signatures (6). Certain miRNAs were mainly detected in microvesicles, whereas others were associated with the RNA binding protein Argonaute 2 (3), which is part of the RNA-induced silencing complex. It may be hypothesized that the kind of extracellular miRNA stabilization contributes to our observation that a specific signature of abundant plasma miRNAs is associated with eGFR, while other common miRNAs (such as miR-223-3p or miR 486-5p) are not. However, sub-classes of miRNA carriers were not determined in our study and, therefore, any conclusions in that regard remain speculative.

Our study has strengths and limitations. One strength of our study is the two-step strategy to identify circulating miRNAs associated with renal function. Significant associations between six miRNAs and eGFR found in a discovery study could be confirmed in a further independent study cohort. However, limited sample size of the discovery study might have reduced the chance of detecting true associations between other candidate miRNAs and kidney function. Another limitation is that GFR was not measured directly, but was estimated based on serum creatinine levels by the Mayo Clinic Quadratic equation. Notably, the Mayo Clinic Quadratic equation has been shown to give an accurate estimate of GFR in patients with nearly normal renal function (33), which was present in the majority our patients. Results could also be reproduced employing the more frequently used CKD-EPI equation. Creatinine values used to estimate GFR were based on a single measurement. Consequently, a non-steady state of kidney function indicated by varying creatinine levels over time cannot be excluded for all of our patients. However, per study design patients included in our study were not acutely ill or hospitalized. Therefore, a steady state of kidney function making eGFR interpretable appears likely at least in most of our patients. Moreover, identified renal miRNAs were significantly linked with eGFR assessments based on creatinine values determined nearly four years after baseline examination confirming their association with

kidney function. Our study participants were a selected group as all of them were referred to coronary angiography for the evaluation of CAD. That said, due to the close correlation between even mild-to-moderate deterioration of kidney function and morbidity or mortality in cardiovascular risk patients, the coronary angiography patients we chose to investigate are of particular clinical interest. The impact of identified renal miRNAs on the incidence of future events has to be investigated in prospective studies.

In conclusion, our study showed that decreased circulating levels of miR-16-5p, miR-19b-3p, miR-20a-5p, miR-25-3p, miR-106b-5p, and miR-451a are significantly linked to reduced kidney function in coronary angiography patients. Their close correlation to each other as well as to kidney function should be considered in future studies. Further studies are needed to clarify the pathophysiological background behind the observed association between reduced levels of identified circulating renal miRNAs and kidney dysfunction.

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534

FIGURE LEGENDS

Figure 1: Associations between identified renal miRNAs and kidney function evaluated in the total study cohort (n=438). Figure 1 shows relative plasma expression levels of miR-16-5p (A), miR-19b-3p (B), miR-20a-5p (C), miR-25-3p (D), miR-106b-5p (E), and miR-451a (F) in patients with normal kidney function (eGFR ≥90 mL/min/1.73 m2), mild impairment of kidney function (eGFR <90-60 mL/min/1.73 m^2), and kidney disease (eGFR <60 mL/min/1.73 m2). Expression levels are given as $2^{-\Delta Ct}$ values (median and interquartile range), such that increased y-axis values reflect increased miRNA concentration; Ct values were normalized by the mean expression of miR-24-3p, miR-92a-3p, and miR-222-3p. Statistically significant differences were determined by the Kruskal-Wallis test and the Mann–Whitney U test, respectively. P-values between stages were given either as n.s. (non significant): $P \geq 0.05$, *: $P < 0.05$, **: $P < 0.01$, or ***: $P < 0.001$.

Table 1: Baseline patients' characteristics

Baseline characteristics	Discovery cohort n=120	Validation cohort n=318	Total cohort n=438
Age (years)	67.5 ± 9.8	67.1 ± 9.8	67.2 ± 9.6
Male gender, n (%)	67 (55.8)	173 (54.4)	240 (54.8)
Body mass index (kg/m^2)	28.7 ± 5.0	28.0 ± 4.6	28.2 ± 4.7
Metabolic syndrome, n (%)	65 (54.2)	135 (42.5)	200 (45.7)
Type 2 diabetes, n (%)	64 (55.3)	131 (42.2)	195 (44.5)
Hypertension, n (%)	92 (67.7)	247 (77.7)	339 (77.4)
History of smoking, n (%)	73 (60.8)	173 (54.4)	246 (56.2)
Significant stenoses, n (%)	81 (67.5)	164 (51.6)	245 (55.9)
Total cholesterol (mg/dl)	196.1 ± 41.5	196.0 ± 47.6	196.1 ± 46.0
HDL-cholesterol (mg/dl)	56.0 ± 15.3	58.6 ± 17.4	57.9 ± 16.9
Triglycerides (mg/dl)	143.5 ± 105.6	135.4 ± 78.7	137.6 ± 86.9
Statin use, n (%)	60 (50.0)	161 (50.6)	221 (50.5)
eGFR (ml/min/1.73m^2)	91.8 ± 22.2	91.1 ± 19.8	91.3 ± 20.5
eGFR 89 – 60 ml/min/1.73m^2, n (%)	42 (35.0)	124 (38.8)	165 (37.8)
eGFR < 60 ml/min/1.73m^2, n (%)	12 (10.0)	28 (8.8)	40 (9.2)
ACR (mg/g)	181.7 ± 429.7	71.9 ± 205.8	100.8 ± 285.8

eGFR, estimated glomerular filtration rate; ACR albumin to creatinine concentration ratio.

Table 2: Associations of renal miRNAs identified in the discovery study with eGFR in the validation study

	rho	p-value
miR-16-5p	0.239	<0.001
miR-19b-3p	0.301	<0.001
miR-20a-5p	0.292	<0.001
miR-25-3p	0.196	<0.001
miR-106b-5p	0.343	<0.001
miR-320a	-0.054	0.338
miR-320b	-0.015	0.788
miR-451a	0.361	<0.001

Statistically significant differences were determined by the Spearman's rho correlation test.

Table 3: Associations between identified renal miRNAs and anthropometrics and laboratory parameters

microRNA	miR-16-5p		miR-19b-3p		miR-20a-5p		miR-25-3p		miR-106b-5p		miR-451a	
	rho	p-value	rho	p-value	rho	p-value	rho	p-value	rho	p-value	rho	p-value
Age	**-0.115**	**0.017**	**-0.115**	**0.017**	**-0.155**	**0.001**	**-0.130**	**0.006**	**-0.175**	**<0.001**	**-0.202**	**<0.001**
BMI	-0.018	0.701	-0.046	0.341	-0.040	0.408	0.050	0.299	-0.051	0.290	0.012	0.797
Glucose	0.069	0.148	0.032	0.507	0.031	0.513	**0.116**	**0.015**	-0.031	0.513	**0.122**	**0.011**
HbA1c	0.044	0.361	-0.016	0.747	0.027	0.575	0.099	0.038	-0.019	0.691	0.086	0.071
SBP (mm Hg)	-0.036	0.452	-0.088	0.069	-0.069	0.151	-0.013	0.787	-0.056	0.244	-0.042	0.379
DBP (mm Hg)	-0.038	0.428	**-0.102**	**0.034**	-0.028	0.561	-0.005	0.918	-0.029	0.551	-0.007	0.881
ACR	**-0.215**	**<0.001**	**-0.153**	**0.008**	**-0.172**	**0.003**	**-0.137**	**0.017**	**-0.195**	**0.001**	**-0.129**	**0.024**
Urinary albumin	-0.071	0.149	-0.061	0.213	**-0.102**	**0.039**	-0.016	0.744	**-0.116**	**0.018**	-0.011	0.826
Serum urea	-0.081	0.089	**-0.147**	**0.002**	**-0.161**	**0.001**	-0.070	0.144	**-0.214**	**<0.001**	**-0.154**	**0.001**
Serum uromodulin	0.009	0.879	-0.012	0.838	0.091	0.114	-0.028	0.627	**0.114**	**0.048**	0.037	0.521
PTH	-0.091	0.067	-0.096	0.055	**-0.130**	**0.009**	-0.076	0.126	-0.096	0.053	-0.092	0.064
FGF23	-0.096	0.071	**-0.180**	**0.001**	**-0.137**	**0.010**	0.008	0.886	**-0.158**	**0.003**	**-0.139**	**0.009**
Klotho	0.014	0.780	0.078	0.125	0.014	0.786	-0.066	0.196	0.041	0.424	0.043	0.395

Associations of identified renal miRNAs with anthropometrics and laboratory parameters were evaluated in the total study cohort (n=438).

Statistically significant differences were determined by the Spearman's rho correlation test. Significant associations are indicated in bold. BMI, body

mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ACR, albumin-to-creatinine ratio; PTH, Parathyroid hormone; FGF23, Fibroblast growth factor 23.