SMALL NON-CODING RNA PROFILES IN CHRONIC KIDNEY DISEASE IN THE GENERAL POPULATION AND HIGH-RISK SUB-GROUPS (WITH HYPERTENSION AND/OR DIABETES MELLITUS) IN A SOUTH AFRICAN COMMUNITY-BASED COHORT

By

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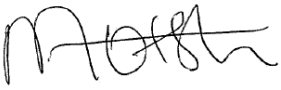
DECLARATION

I, Dipuo Dephney Motshwari, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signature:  **Date: 18 August 2022**

ABSTRACT

Background: Chronic kidney disease (CKD) is a major public health concern and contributor to morbidity and mortality globally. Identification of CKD in its early stages is critical to mitigate its progression to advanced stages or development of cardiovascular-associated complications, effectively improving health outcomes. With the established limitations of currently adopted clinical indicators of CKD, identification of more accurate and reliable biomarkers has been the focus of recent clinical research. MicroRNAs are emerging as promising diagnostic and prognostic markers as well as targets for therapeutic intervention for a multitude of disease including CKD. However, published data on the same miRNA signatures in CKD is contradictory across studies and limited studies have explored the value of these miRNAs in CKD in individuals of African ancestry. Therefore, we aimed to identify all published miRNAs found to be associated with CKD and/or measures of kidney function and kidney damage, as well as their expression patterns, in the general population and in high-risk subgroups [hypertension (HTN), diabetes mellitus (DM) and human immunodeficiency virus (HIV)-infected). Furthermore, we aimed to characterize the expression profile of five known whole blood miRNAs (miR-126-3p, miR-30a-5p, miR-1299, miR-182-5p and miR-30e-3p) in the general population with CKD and six novel whole blood miRNAs (hsa-miR-novel-chr1_36178, hsa-miR-novel-chr2_55842, hsa-miR-novel-chr7_76196, hsa-miR-novel-chr5_67265, hsa-miR-novel-chr15_18383 and hsa-miR-novel-chr13_13519) in high-risk individuals with HTN and DM, for the first time in a South African cohort.

Methods: We conducted a systematic search of Medline via PubMed, Scopus, Web of Science, and EBSCOhost databases to identify relevant studies according to a predefined eligibility and exclusion criteria published in English or French languages on or before 31 October 2021. Using quantitative reverse transcriptase PCR (RT-qPCR), we quantified the expression of known whole blood miRNAs in a general population (n= 1449), whilst the quantification of novel whole blood miRNAs was performed in 911 individuals with HTN and/or DM. We used adjusted and unadjusted regression models to assess the association between the studied miRNAs and prevalent CKD as well as markers of kidney function and kidney damage. The area under the receiver operating characteristics curve was used to determine the discriminatory power of the studied novel blood miRNAs to identify those with prevalent CKD.

Results: Through a systematic search of the literature, we identified a number of frequently dysregulated miRNAs in CKD (miR-126, miR-223, miR-155, miR-21) and in diabetic kidney disease (DKD) (miR-155, miR-126, miR-192, miR-21, miR-15a-5p, miR-29a, miR-29b and miR-29c). Of these, miR-126 and miR-223 were commonly downregulated in CKD whilst in individuals with DKD, miR-21 and miR-29b were consistently upregulated. In our South African population, an

upregulated expression of known whole blood miRNAs in the general population and novel whole blood miRNAs in high-risk individuals, was observed in those with CKD relative to those without CKD. Moreover, upregulated expression of known miRNAs (miR-126-3p, -182-5p and -30e-3p) and all the studied novel miRNAs were independently associated with prevalent CKD. Whilst only the increased expression of hsa-miR-novel-chr2_55842 and hsa-miR-novel-chr7_76196 were independently associated with reduced estimated glomerular filtration rate (eGFR). Furthermore, all the novel whole blood miRNAs were acceptable predictors of CKD, however, only hsa-miR-novel-chr13_13519 added to CKD prediction beyond conventional factors.

Conclusion: This study provides evidence of miRNA dysregulation in CKD in the general population and high-risk individuals for the first time in a South African population. We also highlighted a list of miRNAs with consistent dysregulated patterns commonly studied in the general population with CKD and those with DKD in various geographic locations. The dysregulated miRNA expression pattern suggests that altered miRNA may play a role in the pathogenesis of CKD. These findings form the basis for future research aimed at investigating the clinical value of miRNAs in CKD particularly in Africa.

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And finally: To the Almighty God *"For I know the plans I have for you," declares the LORD, "plans to prosper you and not to harm you, plans to give you hope and a future. Jeremiah 29:11"* I'm overwhelmed with gratitude; you had a plan for me too.

DEDICATION

To my dearly beloved mother (Salphinah N Motshwari), my first friend, my best friend and my forever friend, this work was done in your honour. You left me when I had just started my PhD, and miraculously I made it to the finish line. It's been a roller coaster ride, but I know you were right here with me every step of the way, spiritually. All the sacrifices were worth it mama, so this right here is a payback from way back for you, and to many more paybacks to come. Continue looking down on us with a smile and bless us always, our "biggest" ancestor. Continue to rest peacefully, Love you Always and Forever.

>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>***To God be the Glory***<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<

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ABBREVIATIONS

ACR : Albumin-to-Creatinine Ratio
AER : Albumin Excretion Rate
AGO : Argonaute
Ang : Angiopoietin
APOL1 : Apolipoprotein L1
BACE 1 : β-site Amyloid precursor protein-Cleaving Enzyme 1
BMI : Body Mass Index
cDNA : Complementary DNA
CKD : Chronic Kidney Disease
CKD-EPI : Chronic Kidney Disease Epidemiology Collaboration
CVD : Cardiovascular Disease
DALYs : Disability-Adjusted Life-years
DGCR8 : DiGeorge Syndrome Critical Region 8
DKD : Diabetic Kidney Disease
DM : Diabetes Mellitus
DNA : Deoxyribonucleic Acid
eGFR : Estimated Glomerular Filtration Rate
ESKD : End-Stage Kidney Disease
GBD : Global Burden of Disease
GFR : Glomerular Filtration Rate
HbA1C : Glycated Haemoglobin
HC : Hip Circumference
HICs : High-Income Countries
HIV : Human Immunodeficiency Virus
HIVAN : HIV-Associated Nephropathy
HTN : Hypertension
KDIGO : Kidney Disease Improving Global Outcomes
KRT : Kidney Replacement Therapy
LMICs : Low-And-Middle Income Countries
MDRD : Modification of Diet in Renal Disease
MICs : Middle-Income Countries
miRNAs : MicroRNAs
mRNA : Messenger RNA
ncRNAs : Non-Coding RNAs
NGS : Next Generation Sequencing

piRNAs : Piwi-Interacting RNAs
pmp : Per Million Population
pre-miRNAs : Precursor miRNAs
pri-miRNA : Primary miRNA
RISC : RNA-induced silencing complex
RNAi : RNA interference
RNAse : Ribonuclease
ROC : Receiver Operating Characteristic
rRNA : Ribosomal RNA
RT-qPCR : Quantitative Reverse Transcription Polymerase Chain Reaction
SA : South Africa
SBP : Systolic Blood Pressure
siRNAs : Small Interfering RNAs
SSA : sub-Saharan Africa
TGF-1 : : Transforming Growth Factor 1
TGF-β1 : Transforming Growth Factor Beta 1
tRNA : Transfer RNA
UMOD : Uromodulin
USA : United States of America
UTR : Untranslated Region
WC : Waist Circumference
ZEB : Zinc Finger E-Box Binding Homeobox

CHAPTER 1

1. INTRODUCTION

Chronic kidney disease (CKD), which is a growing global public health concern, affects more than 700 million adults globally and is increasing in prevalence [1]. It is currently ranked the 12th leading cause of death, and it is expected that CKD will rise to be the 5th leading cause of death by the year 2040 [2]. This is alarming because CKD is associated with adverse health outcomes. Indeed, if untreated, CKD can progress to end-stage kidney disease (ESKD), which may require costly and limited kidney replacement therapy (KRT) to sustain life [3]. Chronic kidney disease is also associated with an increased risk of cardiovascular disease (CVD), which is currently the leading cause of mortality and premature death worldwide [3]. Moreover, the risk of people with CKD dying from CVD-associated complications is higher as compared to those who progress to ESKD, with about half of all deaths among CKD individuals resulting from CVD-related complications [4]. Recent evidence suggests that the burden of CKD, which differ substantially across countries and regions, disproportionately affects disadvantaged populations, particularly those residing in low-and middle-income countries (LMICs) [5,6]. In addition, global inequality in access to KRT has been reported, with more than 90% of people receiving KRT residing in high-income countries (HICs) and upper-middle-income countries (MICs) [7,8]. The lack of resources and high level of poverty for the majority of people residing in LMICs, results in poorer health outcomes for people with CKD [9–11].

A number of risk factors contribute to the growing burden of CKD. The high prevalence of hypertension (HTN) and diabetes mellitus (DM) is associated with CKD, partly explaining the increasing burden of CKD globally [12]. This is further compounded by population growth and aging [13] and the increasing adoption of Western habits, which results in unhealthy lifestyles like poor diet quality, physical inactivity and high rates of obesity which further accelerates CKD progression [14]. Infectious diseases such as human immunodeficiency virus (HIV) also play a role in the increasing risk of CKD and this is problematic in developing countries particularly in Africa where the highest rate of HIV infection exists [5,15].

Effective preventive measures for CKD have been described and it is suggested that in order to significantly lower the burden of CKD in LMICs, the primary and secondary preventive measures should be prioritized instead of the tertiary preventive measure whose main objective is investing in KRT [16]. The primary and secondary preventive measures aim to prevent the development of CKD in high-risk individuals and early detection of CKD to enable early initiation of targeted therapy to prevent or delay the adverse effects of CKD [17]. Studies have shown that good management of HTN and DM can reduce the risk and improve health outcomes related to CKD [18].

There are well-established clinical indicators of CKD including glomerular filtration rate (GFR), albuminuria and kidney biopsies [3]. Estimated GFR (eGFR) indicates the level of kidney function

whereas albuminuria informs on the presence of kidney damage. However, these measures present with some limitations, including poor sensitivity for early detection of CKD, which is essential to slow CKD progression, reduce the risk of CVD-associated complications and improve disease outcomes [19]. Therefore, there is a serious need for reliable and easily detectable biomarkers that will allow for early detection of CKD and screening of high-risk individuals and therefore enable early initiation of targeted therapy that will delay or prevent the adverse health outcomes of CKD.

Emerging evidence has suggested the important role of epigenetic factors, particularly microRNAs (miRNAs) in the development of various diseases and their potential value as biomarkers of disease diagnosis and prognosis [20–23]. miRNAs are small non-coding RNAs (ncRNAs) that regulate protein coding genes post-transcriptionally through translation inhibition or degradation of the target messenger RNA (mRNA) [24]. Although considered to be intracellular regulators of gene expression, increasing evidence has shown that miRNAs are also detectable in body fluids in a highly stable manner [25]. This may be due to the fact that miRNAs are released into circulation bound to proteins or encapsulated in micro-vesicles protecting them from degradation by ribonucleases (RNAses) [26]. miRNAs play an important role in many cellular functions such as differentiation, proliferation, development and apoptosis [24]. Consequently, dysregulation in the expression of miRNAs might result in the development or progression of many diseases [27]. Several studies have evaluated the potential role of miRNAs in the development and/or progression of CKD in the general adult population and high-risk groups [23,28–30]. However, most of these studies were performed in populations of European, Asian or American decent, with minimal studies conducted in individuals of African ancestry.

1.1. Aims and objectives

This study consisted of three major aims:

Aim 1:

To identify all published miRNAs found to be associated with CKD and/or measures of kidney function and kidney damage, as well as their expression patterns in the general population and high-risk subgroups (HTN, DM and HIV-infected)

Objectives:

Through a systematic review of published studies on miRNAs associated with prevalent CKD and/or measures of kidney function and kidney damage, the following questions were addressed:

- Which miRNAs are associated with prevalent CKD in (a) the general population and high-risk individuals with (b) HTN, (c) DM and (d) HIV infection?
- Which miRNAs are associated with measures of kidney function and kidney damage?
- What are the expression patterns of the identified miRNAs in CKD?
- Do the expression patterns of the identified miRNAs differ depending on the human sample type used?

- Does the expression profile of the identified miRNAs differ depending on the stage of CKD?

Aim 2:

To characterize the expression profile of five known whole blood miRNAs previously shown to be associated with kidney function and/or kidney disease pathophysiology (miR-126-3p, -30a-5p, -1299, -182-5p and -30e-3p) and to investigate the association between these five miRNAs and prevalent CKD in a community-based sample of adult South Africans with and without CKD

Objectives:

2.1 Previously described miRNAs (miR-126-3p, -30a-5p, -1299, -182-5p and -30e-3p) were extracted from whole blood using RNA isolation kits and quantified by quantitative reverse transcription polymerase chain reaction (RT-qPCR) and the expression profiles of these miRNAs compared between individuals with and without CKD

2.2 Multivariable logistic regression models were used to investigate the association between the studied miRNAs and prevalent CKD as well as the markers of kidney function (eGFR) and damage (albumin creatinine ratio (ACR)).

Aim 3:

To characterize the expression profile of six novel whole blood miRNAs (hsa-miR-novel-chr1_36178, hsa-miR-novel-chr15_18383, hsa-miRnovel-chr2_55842, hsa-miR-novel-chr7_76196, hsa-miR-novel-chr5_67265, and hsa-miR-novel-chr13_13519), previously shown to be associated with DM and HTN in a high-risk group of adult South Africans with and without CKD as well as to determine the diagnostic ability of these six novel miRNAs to discriminate between individuals with and without CKD

Objectives:

3.2 Novel miRNAs (hsa-miR-novel-chr1_36178, hsa-miR-novel-chr15_18383, hsa-miRnovel-chr2_55842, hsa-miR-novel-chr7_76196, hsa-miR-novel-chr5_67265, and hsa-miR-novel-chr13_13519) were extracted from whole blood using RNA isolation kits and quantified by RT-qPCR and the expression profiles of these miRNAs compared between high-risk individuals (with HPT and/or DM) with and without CKD

3.2 Receiver operating characteristic (ROC) curve was used to investigate the predictive ability of the six novel whole blood miRNAs alone or combined, and to determine whether these miRNAs added to the diagnostic predictive ability beyond that of conventional risk factors in high-risk groups of South African adults

CHAPTER 2

2. LITERATURE REVIEW

2.1. Global burden of chronic kidney disease

Chronic kidney disease has been neglected as a global health priority, yet it is associated with high rates of morbidity and mortality and presents a large socio-economic burden. The Global Burden of Disease (GBD) study recently reported that almost 700 million (9.1%) people worldwide were diagnosed with CKD in 2017, which is a 29.3% increase from the year 1990 [31]. According to the same report, the age-standardized prevalence of CKD was higher in women (9.5%) as compared to men (7.3%) [31]. The prevalence of CKD globally is way above other non-communicable diseases such as DM (537 million) [32], CVDs (523 million) [33] and cancers (85.8 million) [34], which are being prioritized by most governments.

Chronic kidney disease accounted for 2.6 million deaths in 2017 globally, of which 1.2 million resulted directly from CKD and 1.4 million were indirectly from CVDs caused by reduced GFR [31]. Deaths attributable to CKD have increased by 41.5% between 1990 and 2017, shifting CKD from the 17th to the 12th ranked leading global cause of death [31]. Furthermore, it has been reported that CKD is currently the third fastest-growing cause of death worldwide, and current projections estimate that it will become the 5th leading cause of death globally by the year 2040 [2]. According to the 2019 GBD statistics, the cases of disability-adjusted life-years (DALYs) caused by CKD has nearly doubled over the past three decades, rising from the 29th rank in 1990 to the 18th rank leading causes of DALYs [34]. The largest increase in DALYs attributable to CKD between 1990 and 2017 was seen in countries with a low sociodemographic index (23.6 %) as compared to those with middle (5.5%) and high sociodemographic index (4%) [31].

Another contributing factor is the limited awareness of CKD, with only 6% and 10% of the general population and high-risk populations, being aware of their disease status [35]. Due to the asymptomatic nature of the disease, individuals are usually diagnosed when the disease has progressed to more advanced stages, when the risk of developing ESKD or cardiovascular complications is high [3]. Regardless, little attention is paid to the early detection of CKD to prevent or delay disease progression by many societies [36].

Taken together, these findings highlight that there has been a dramatic increase in CKD incidence, prevalence, mortality and DALYs due to CKD globally over the past three decades. Furthermore, most of the growth in the burden of CKD has been observed in LMICs as compared to HICs. This is partly attributable to the significant rise in population growth, longevity, westernization and high incidences of HTN, DM and HIV [9].

2.2. The burden of chronic kidney disease in low-to-middle-income countries

The burden of CKD in LMICs remains poorly characterized due to lack of community-based studies, inconsistent assessment of kidney function and lack of CKD registries. However, available data indicate that CKD disproportionately affects disadvantaged populations, with a rapid rise in CKD incidences reported in LMICs [34]. The prevalence of CKD ranges between 10% [37] and 16% [38], in Southeast Asia, some Latin American countries and in sub-Saharan Africa. Moreover, although CKD should be recognized as a health priority, it is not seen as such by most governments and as a result the roll-out of CKD screening programs is still very low (6%) in these countries [39]. There is an underrepresentation of LMICs in large global CKD databases used to study and understand the development and burden of CKD, as these databases predominantly include studies done in HICs [40]. Even within HICs, the burden of CKD is higher in individuals with lower socioeconomic status [41]. Therefore, this indicates that CKD is a global burden that is influenced by disparities in care, treatment and inequalities, hence affecting mainly the most vulnerable of each society [42].

2.1.1. CKD burden in Africa

CKD remains a low priority in governments within Africa not only due to the competing health agenda related to communicable diseases such as tuberculosis, HIV/AIDS and malaria but also factors such as poverty, limited resources, political disputes and corruption [43]. This is further compounded by the lack of continent-wide or regional studies, regularly updated databases on CKD outcomes, in addition to the global problem of inconsistent assessment of kidney function [6]. The two most recent systematic reviews on CKD prevalence (stages 1 to 5) in the general population have reported a high prevalence of CKD in Africa, with Abd Elhafeez et al. reporting a pooled prevalence of 10.1% [44] and Kaze et al. reporting CKD prevalence at 15.8% [38]. Moreover, CKD affects individuals at a younger age in Africa compared to other regions of the world. Studies have reported an earlier onset of ESKD in African individuals at the age of 45 years as compared to the age of 63 years of individuals of other ethnic groups in Western countries [45]. These studies indicate that there is a pronounced burden of CKD in Africa, and this is in light of limited accessibility to treatment. The high burden of CKD in Africa may be attributable to factors such as high incidences of DM and HTN as well as communicable diseases like HIV/AIDS [5]. This double burden of communicable and non-communicable diseases is causing additional strain on the already overwhelmed healthcare systems in African countries, and this may partly result from the adoption of westernized lifestyles associated with unhealthy habits.

2.1.2. CKD burden in South Africa

Even though South Africa (SA) was recently reclassified as an upper-middle-income country by the World Bank [46], it is still confronted by the double burden of communicable and non-communicable

diseases, which has caused a strain on an already overwhelmed health system. Although there is a dearth of data on the burden of CKD due to a lack of large epidemiological studies in SA, a few studies have reported on the prevalence of CKD, with varying results. In 2013, Matsha and colleagues found that the prevalence of CKD (stages 3 to 5), using the CKD-EPI equation without the black race correction factor to estimate GFR, was 17.3% in a sample of 1202 participants of mixed-ancestry [47]. A study by Peer et al (2020) evaluated the prevalence of CKD in a study sample of 1092 black South Africans and they found a lower prevalence of 3.4%, based on GFR estimated using the CKD-EPI equation without the ethnicity correction factor, t[48]. The study by Matsha and colleagues [47] had a slightly older population with a mean age of 53 years as well as a higher prevalence of DM (26.4 %) compared to that by Peer et al (2020) [48] with individuals aged 21 years and older, and a DM prevalence of 14.7%, which may partly explain the differences observed. South Africa is one of the few African countries with national renal registries with data on KRT, with the first one being published in 2012. The latest report, the Eighth Annual Report of the South African Renal Registry, indicated that access to KRT is still very limited in SA, as in 2019, only 9937 individuals received KRT from a pool of 169 per million population (pmp) [49]. Moreover, the majority of these individuals were being treated in the private sector (778 pmp) whereas 57 pmp received their treatment within the public sector [49]. This is alarming because the majority of SAs (>80%) depend on the public sector for treatment access due to the inability to afford medical insurance [50].

2.3. Risk factors of chronic kidney disease

Chronic kidney disease is a complex multifactorial disease arising from various modifiable and non-modifiable risk factors. Identification of these factors is of importance as these may allow early interventions which may prevent or delay the progression of CKD to ESKD, development of CVD-related complications and premature mortality. Diabetes mellitus and HTN are the leading modifiable risk factors of CKD and ESKD globally [51]. High incidences of DM and HTN have been reported globally.

2.3.1. Diabetes mellitus

The most recent edition of the International Diabetes Federation Diabetes Atlas reported that 10.5% (536.6 million) of the global adult population had DM in 2021, with this number expected to rise to 12.2% (783.2 million) by the year 2045 [32]. The highest increase in the prevalence of DM has been projected for the African region (134%) in 2045 [32]. Moreover, the highest prevalence of DM was seen in LMICs, with about 90% of undiagnosed people (44%) found to reside in LMICs. South Africa had the highest prevalence of DM (11.3%) in Africa, with 45.4% of those living with DM being undiagnosed [32]. These statistics are alarming because studies have reported that approximately 30 to 40% of individuals with DM have CKD [52]. Diabetic kidney disease (DKD) is a microvascular complication of DM and a leading cause of ESKD, accounting for approximately 48% of ESKD

cases in the US [12]. The risk of CKD in individuals with DM may be attenuated by metabolic changes associated with DM, such as hyperfiltration, hyperfusion and hyperglycaemia, which will result in altered kidney haemodynamics and cause glomerular hypertrophy, inflammation, interstitial fibrosis and glomerulosclerosis [53].

2.3.2. Hypertension

The prevalence of HTN has also increased substantially over the years, from 594 million in 1975 to an estimated prevalence of 1.39 billion (31.1% of the adult population) in 2010, with the majority of affected individuals residing in LMICs (1.04 billion) as compared to HICs (359 million) [54]. Africa has the highest prevalence of HTN, with a recent meta-analysis estimated a prevalence of 57% in older adults aged 50 years and older [55]. Moreover, the prevalence of HTN was estimating to be 30% in younger adults residing in sub-Saharan Africa, and of these only 27% were aware of their diagnosis [56]. Hypertension is both a risk factor and a consequence of CKD, associated with CKD progression to ESKD, cardiovascular events and mortality [57]. More than 75% of individuals with CKD are at risk of developing HTN [58] whereas 60 to 90% of individuals with HTN develop CKD [59]. Studies have shown that systolic blood pressure (SBP) ≥130mmHg is associated with a higher risk of CKD progression to ESKD [60]. Hypertension accounts for approximately 29% of cases of ESKD in the US [12]. The mechanisms driving CKD and HTN include, but not limited to, glomerulosclerosis, volume overload, sodium retention, endothelial dysfunction and sympathetic overactivity [59,61].

2.3.3. HIV-infection

HIV is one of the major causes of glomerulonephritis, which is a type of kidney disease that is very prevalent in Africa. The complication of CKD in HIV is commonly referred to as HIV-associated nephropathy (HIVAN) [15]. This is very common in Africa, where almost two-thirds (25.7 million) of the global HIV-positive population reside [62]. Additionally, more than 20% of individuals diagnosed with HIV globally reside in SA [63]. As a result, the highest prevalence of HIVAN (ranging from 6.0 – 48.5%) has been reported in Africa, and of these, 24–83% of the cases were reported in SA [15]. Individuals with HIVAN are at increased risk of developing ESKD, with a likelihood of 2- to 20-fold greater risk than those with only CKD [64]. Further, HIVAN was found to be more common in young adults of African ancestry with advanced HIV infection and this was associated with apolipoprotein L1 (APOL1) high-risk variants, a risk factor for several glomerular disorders, including HIVAN [65]. Other factors such as poor healthcare and late start with antiretroviral therapy, may contribute to the increased risk of CKD in HIV-infected individuals, although antiretroviral therapy can also mediate kidney toxicity [64]. HIV causes CKD through a number of direct and indirect mechanisms affecting all structures of the nephron, such as the cytopathic effect of the virus within the kidney parenchymal cells, the pseudopathological response of the immune system to HIV infections and the use of nephrotoxic drugs [64].

2.3.4. Demographic factors

Demographic factors such as age, sex and race have been associated with the risk of developing CKD. Chronic kidney disease is an age-related condition, reported to be eight times more common in individuals aged 70 years and older [66]. However, this was found to not be the case in Africa, where CKD more often affects young adults, with progression to ESKD occurring 20 years earlier as compared to Western countries [45]. This is partly due to poor health care, limited KRT and poor awareness of CKD in the African region [45]. Furthermore, although the prevalence of CKD is higher in women as compared to men, it was found that men are more likely to progress to advanced stages of CKD such as ESKD at a faster rate as compared to women [52]. These suggest that loss of kidney function or kidney damage progresses at a slower rate in women compared to men. Studies have reported that endogenous oestrogen might offer protective effects against severe loss of kidney function or kidney damage in women contributing to these disparities [67]. Racial and ethnic minorities are disproportionately affected by CKD and its consequent outcomes. Although similar prevalence of early-stage CKD has been reported, ethnic minorities such as African Americans, Hispanics and Native Americans are 1.5 to 4 times more likely to progress to ESKD [42]. Moreover, the high incidences of CKD and ESKD in younger individuals of African descent have been reported and it has been suggested that this could be partly explained by APOL1 mutations observed mainly in native Africans [68] as well as their inappropriate use of traditional medications [69].

2.3.5. Lifestyle

Negative health behaviors such as physical inactivity, alcohol consumption, smoking and poor diet are associated with an increased risk of CKD and its progression [14], accounting for 20% of CKD cases globally [70]. Studies have shown that obesity increases the risk of CKD by its contribution to pathological mechanisms such as inflammation, endothelial dysfunction, oxidative stress and hypervolaemia [71]. In a large epidemiologic study, including 167548 participants, it was found that obese women and men body mass index (BMI) $\geq 30 kg/m^2$ were at a three-to-four-fold increased risk of developing CKD as compared to those with normal weight BMI$< 25 kg/m^2$ [72]. Moreover, individuals with CKD and a BMI of $<25 km/m^2$ were found to be at a reduced risk of progression to ESKD [14]. Socio-economic status is also an important risk factor of CKD; although CKD was once considered a disease of "affluence", recent evidence suggests that the burden of CKD and ESKD is more prevalent in populations with low socio-economic status [41]. This may be attributable to factors such as poorly resourced health facilities, high incidences of HTN, DM and HIV, as well as the adoption of westernized lifestyles [5]. A meta-analysis study showed that the incidence, prevalence and progression of CKD were significantly associated with low socio-economic status particularly in the US as compared to Europe and Asia [41]. Moreover, high rates of untreated ESKD have been reported in Africa and Asia due to the inaccessibility or unaffordability of KRT [8].

2.3.6. Genetic components

Individuals with relatives who have been diagnosed with CKD or ESKD are at an increased risk of developing the disease. A study performed in individuals with a family history of ESKD found increased prevalence of reduced kidney function and increased kidney damage, suggesting an increased risk of developing ESKD in those with family history as compared to those with no family history [73]. A recent large population-based family study investigated familial aggregation of CKD and revealed that first-degree relatives of the affected individual are at risk of CKD, with heritability of kidney-related markers and serum electrolytes ranging between 20% and 50% [74]. These findings highlight the importance of genetic factors in modulating susceptibility to CKD in general populations. Advances in genetic sequencing have led to the identification of a number of genetic variants associated with the risk of CKD by genome-wide association studies [75]. Individuals of African ancestry have been found to have mutations in the APOL1 gene which predisposes them to a higher risk of CKD and ESKD as compared to individuals of European descent [68]. Indeed, individuals with one copy of either the G1 or G2 variant are immune to African trypanosomiasis (sleeping sickness), however individuals who have two copies of either variant are at an increased risk of developing a non-diabetic kidney disease. Inheritance of the high-risk APOL1 gene variants (G1 and G2) has been associated with an increased risk of CKD [76]. Additionally, individuals of African ancestry were also found to be at increased risk of progression to ESKD because of haemoglobin variants such as sickle cell traits associated with increased risk of progression to ESKD [77]. Other genetic variants such as Uromodulin (UMOD) gene variant were found to be associated with gradual loss of kidney function and the development of CKD [75]. The mutations in the UMOD gene were also found to be associated with rare autosomal dominant tubulointerstitial kidney disease with progression to ESKD [76]. However, the identified genetic variants are not enough to explain the genetic variation of CKD markers; for example, only 1.4% of the variation in kidney function as measured by eGFR could be accounted for by 16 variants identified in a meta-analysis of genome-wide association study [78]. This suggests that there might be other factors contributing to the risk of CKD. Recent evidence suggests that there is a strong environmental influence on genetic susceptibility to CKD which cannot be explained by traditional genetics, also known as epigenetics, which can provide insights into the pathogenesis of CKD [79].

2.4. Cardiovascular disease-associated complications

Chronic kidney disease is an independent risk factor of CVD, and hence been associated with an increased risk for CVD mortality. Cardiovascular diseases are the leading cause of global disease burden, affecting approximately 523 million people and accounting for 18.6 million deaths and 34.4 million DALYs globally in 2019 [33]. Furthermore, it has been observed that individuals with CKD are more likely to die from CVD-related complications than progressing to ESKD [80]. Indeed, the risk of CVD-related complications is high in individuals with early-stage CKD and increases as the disease

progresses, with approximately 50% of individuals at stage 4 and 5 CKD developing CVD [81]. Cardiovascular disease-associated mortality accounts for approximately 40 to 50 % of death in advanced CKD and ESKD as compared to those with normal kidney function, for which the rate of mortality is 26% [82]. The GBD study reported that 4.6% of all-cause mortality in 2017 resulted from deaths due to CKD or to CKD-attributable CVD and 25.3 million DALYs from CVD-related complications were attributable to reduced GFR [31]. The high risk of CVD in people with CKD may be attributed to the traditional risk factors associated with CVD such as DM, HTN, and dyslipidaemia. For instance, studies have shown that individuals with both CKD and type 2 DM or individuals with CKD, type 2 DM, HTN and hyperlipidaemia had a higher risk of major adverse cardiovascular events, heart failure and all-cause mortality as compared to those with just type 2 DM [83]. Another study found that individuals with both CKD and type 2 DM had higher risk of all-cause and CVD-related mortality as opposed to individuals with just type 2 DM [84].

2.5. End-stage kidney disease

End-stage kidney disease requires KRT to sustain life; hence in the absence of KRT, it remains uniformly fatal [85]. KRT can be in the form of kidney transplant or dialysis, the former is limited by the scarce supply of kidney donors and the latter is limited by the availability of resources. With the growth in the global economy, the costs for KRT are a major issue, with the annual medical cost for a patient on haemodialysis approximately USD 5000 in HICs. Globally, approximately 30% of individuals in need of KRT received treatment in 2010, with the majority of these individuals (80%) from HICs such as Japan, the USA and Europe. On the contrary, countries in Africa had the lowest access to KRT (9-16%) yet with the second highest number of people (432 000) needing KRT [8]. Although the number of individuals on KRT is expected to rise from five to 10 million by the year 2030, the number of those without access to KRT is expected to remain high in Africa and Asia [8]. It was estimated that approximately 2.3 to 7.1 million individuals had died prematurely due to limited access or lack of KRT, particularly in LMICs in Asia and Africa [8]. Therefore, the best risk mitigation implementation is early detection of CKD which will allow early initiation of cost-effective treatment to slow CKD progression to ESKD and prevent the development of CVD-associated complications [6].

2.6. Definition and classification of chronic kidney disease

Chronic kidney disease is defined as a condition characterized by a gradual loss of kidney function and/or the presence of kidney damage that persists for a period of 3 months or longer, with implications for health [86]. The loss of kidney function is defined as GFR < 60 mL/min/1.73 m^2 and evidence of kidney damage referring to either albuminuria (measured as albumin-to-creatinine ratio [ACR] of >30mg/g or urinary albumin excretion rate [AER] ≥30 mg/24h), history of kidney transplant, electrolyte and other abnormalities due to tubular dysfunction and urinary sediment abnormalities [86]. The classification of CKD has evolved over the years. Currently, the 2012 Clinical

Practice Guidelines for the Evaluation and Management of CKD, according to the Kidney Disease Improving Global Outcomes (KDIGO), states that CKD be classified based on the cause, the GFR category and albuminuria category [86]. The GFR category classifies CKD into six stages (G1, G2, G3a, G3b, G4 and G5) with increasing risk from G1 to G5 as summarised in Table 2.1. The National Institute for Health Excellence further suggested that stage G3 should be divided into two stages, G3a and G3b to reflect an increased risk of CVDs [87]. G1 and G2 indicate the early onset of CKD with normal to mildly decreased levels of GFR, G3a and G3b stages have mild to moderately decreased levels of eGFR, G4 and G5 are the advanced stages of CKD with severely decreased levels of eGFR [3]. Moreover, individuals with a GFR below 15 mL/min per 1.73 m² are classified as having ESKD, also referred to as kidney failure [79]. CKD can also be classified based on the level of urinary albumin (albuminuria) into three stages, namely A1, A2 and A3, with the severity of the disease increasing with in an increase in the level of albuminuria as summarised in Table 2.1. CKD is frequently referred to as a "silent condition" because during the early stages of the disease (stage G1- G3), the affected individuals are usually asymptomatic. As a result, CKD is commonly diagnosed when the disease has progressed to advanced stages, where there is an increased risk of CVD-associated complications, mortality and kidney failure. Some of the common clinical symptoms of CKD include dyspnea, fatigue, joint pain, itching skin, muscle cramps, peripheral oedema, change in output, and weight loss [79].

Table 2.1: *Classification of CKD, according to the current CKD international guidelines*

GFR descriptors and range			Albumin to creatinine ratio (ACR) (mg/mmol)		
		ml/min per 1.73 m2	A1 (Normal to mildly increased (<3))	A2 (Moderately increased (3–30))	A3 (Severely increased (>30))
G1	Normal or high	90+			
G2	Mildly decreased	60 to 89			
G3a	Mildly to moderately decreased	45 to 59			
G3b	Moderately to severely decreased	30 to 44			
G4	Severely decreased	15 to 29			
G5	Kidney failure	<15			

The colours indicate risk of progression. Green: low risk (In the absence of other markers of kidney disease, CKD is not diagnosed); Yellow: Moderately increased risk; Orange: High risk; Red: Very high risk. Deep red:

Highest risk. Abbreviations: CKD (chronic kidney disease); GFR (glomerular filtration rate) ACR (albumin to creatinine ratio). Adapted from the Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group [3].

2.7. Current diagnostic markers of CKD

Currently, the diagnosis of CKD lies heavily on laboratory parameters such GFR, proteinuria and/or albuminuria, with kidney biopsies done to assess histological features for diagnosis confirmation in rare cases [86].

2.7.1. Markers of kidney function

The kidney is an intricate organ consisting of millions of nephrons also known as the functional units of the kidney. The nephrons have various functions, including filtering waste products and toxins from the blood, as well as regulating blood pressure, extracellular fluid volume and producing vitamin D, renin and hormones, such as erythropoietin [88]. GFR, which is the gold standard measure of kidney function, measures the total fluid that is filtered by all functioning nephrons per unit of time. GFR can be measured by determining the rate by which an endogenous or exogenous substance clears from the kidney. Inulin is the most common exogenous filtration marker used for measured GFR [79]. Inulin, which is a polysaccharide produced by many types of plants, cannot be digested and absorbed in the gastrointestinal tract, however it is uniquely treated by the nephrons, it is completely filtered by the glomerulus and rapidly excreted into the urine by the kidneys after ingestion [89]. Although it has been considered the ideal filtration marker used to determine GFR, the use of inulin is not convenient as its measurement procedures are complex, lengthy, expensive, cumbersome, invasive, and not ideal to perform in routine practice or for screening purposes. Moreover, the protocol for measuring inulin requires continuous intravenous infusion and multiple blood and urine sample collections [89]. Other markers such as iothalamate, iohexol, ethylenediaminetetraacetic acid or diethylenetriaminepentaacetic acid have been explored as alternative exogenous filtration markers for GFR measurement, however similar to inulin, they are complex, they require continuous infusion or bolus administration thereby limiting their utility in the clinic or for research purposes [90]. To circumvent these limitations, several prediction equations have been developed to estimate GFR (eGFR) based on endogenous filtration markers.

2.7.1.1. Predictive equations of eGFR

Estimated GFR remains the most widely used method for the determination of kidney function in clinical practice and epidemiologic research. The first equation to estimate GFR was developed in 1957 by Effersoe, and to date, more than 20 equations have been developed [91]. The three most commonly used equations for the estimation of GFR are the Cockcroft–Gault, Modification of Diet in Renal Disease (MDRD) and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations. The Cockcroft-Gault equation was developed in a population of 249 Caucasian males with

CKD [92]. This equation has some major drawbacks as it does not take into consideration the effect of sex, ethnicity, and muscle mass on GFR and it was never revised to include the standardized creatinine measurements. The MDRD equation was developed and validated in a population of 1628 individuals, mostly Caucasians, with a small percentage of African Americans aged between 18 and 70 years with CKD (GFR <60 mL/min/1.73 m^2) [93]. The equation was originally based on six variables, including demographic variables (age, sex, and ethnicity), serum creatinine, urea, and albumin; however, a year later the equation was simplified to a shorter four-variable version (4v-MDRD) consisting of only demographic variables and serum creatinine. The inclusion of demographic variables in the GFR estimation equation was done to adjust for non-GFR determinants of serum creatinine and provide more accurate estimates of GFR. In 2006, the 4v-MDRD equation was modified to include standardized serum creatinine which is measured using specific assays with calibration traceable to the isotope-dilution mass spectrometry [94]. However, the 4v-MDRD equation is limited in that it was not validated in individuals with normal kidney function and has been reported to be imprecise during higher levels of GFR where it tends to underestimate GFR and misdiagnose individuals with normal kidney function as having CKD. Therefore, the CKD-EPI was developed in the year 2009 in order to overcome some of the limitations of the 4v-MDRD equation [95]. The CKD-EPI equation included the same variables as the 4v-MDRD equation however, it also included individuals with normal kidney function (GFR values > 60 mL/min/1.73 m^2). Hence it performs better in individuals with higher eGFR. The dataset used to develop the CKD-EPI equation was diverse; it was pooled from 10 studies with a total population of 8250 participants from various ethnic groups such as Caucasians, Black Americans, Hispanics and Indian Americans, and it was validated in a similar cohort with a total population of 3900 individuals pooled from 16 studies [95]. Although the current international guidelines of CKD recommend the use of the CKD-EPI equation to estimate GFR, this equation was found to perform poorly in individuals with lower eGFR [3]. Therefore, it is clear that neither the 4v-MDRD nor the CKD-EPI predictive equation can be used across all GFR ranges [96].

Another important limitation is that both the 4v-MDRD and CKD-EPI predictive equations were derived and validated in HICs such as North America, Europe and Australia, with predominately Caucasian populations and, as a result, were found to perform poorly in populations outside these countries, particularly in relation to the adjustments for the African American ethnicity [96]. Although both equations included the African American cohorts to adjust for black ethnicity, recent studies report that the inclusion is not applicable in other geographic regions. A study in individuals of African and Afro-Caribbean ancestry in the United Kingdom found that disregarding the African American race factor significantly improved the bias and accuracy of the eGFR predictive equations, particularly in black individuals with higher eGFR (≥60 mL/min/1.73m^2) [97]. Similar findings from populations in sub-Saharan African showed that the inclusion of the black race coefficient factor did

not improve the performance of either the 4v-MDRD and CKD-EPI in estimating GFR, and in actual fact, its exclusion improved their performance in individuals with higher eGFR (≥60 mL/min/1.73m^2) [98]. Another study performed in a mixed ancestry population of South Africa found that both equations showed less bias when the black race factor was excluded [99]. This may partly be due to the difference between African Americans and native Africans in terms of body structure and muscle mass [100]. The use of the black race correction factor may result in overestimation of GFR and subsequently CKD underdiagnosis particularly in black Africans. Therefore, these equations are biased and needs to be validated in African populations for correct estimates of CKD burden. These limitations further stimulate the interest in novel diagnostic markers with improved diagnostic/prognostic accuracy for CKD particularly in Africa.

2.7.1.2. Endogenous filtration markers

The 4v-MDRD and CKD-EPI predictive equations are limited by the inherent use of endogenous filtration markers, including serum creatinine [94] and cystatin C [101]. Serum creatinine is limited in that its concentration can be affected by factors independent of glomerular filtration. Creatinine is a waste product of skeletal muscle tissue breakdown and the digestion of dietary protein that is filtered and secreted by the proximal tubules but not reabsorbed or metabolized by the kidney [90]. The concentration of serum creatinine increases with a reduction in GFR, however, it can also be affected by factors independent of creatinine glomerular filtration such as muscle mass, diet, in particular, meat intake or use of protein supplements, physical activity, certain drugs and tubular secretion or excretion [102].

Another alternative endogenous filtration marker that has come to light in the past decade is cystatin C. Cystatin C is a small molecular weight protein produced by the cells in the body at a constant rate, and it is freely filtered and reabsorbed by the kidneys (tubular cells) [90]. Cystatin C has been reported to be more advantageous and a better marker for eGFR as compared to serum creatinine. It has less intra-variability, stable production and it is not affected by factors such as diet and muscle mass [103]. However, like serum creatinine, it is also affected by non-glomerular filtration factors such as age, sex, ethnicity, diabetes, inflammation, smoking, use of corticosteroids and adipose tissue and these factors should be taken into consideration when interpreting its concentration in clinical practice [101]. In a cross-sectional analysis including a diverse cohort of 5352 individuals and an independent validation cohort of 1119 individuals, an estimation equation for GFR was developed based on cystatin C alone as well as in combination with serum creatinine [103]. It was found that the equation that used both markers performed better than either the serum creatinine or cystatin C based eGFR equation [103]. Recently, Inker and colleagues modified the eGFR equations in an even larger cohort, with the equation incorporating both serum creatinine and cystatin C and the individual serum creatine and cystatin C based equations, however, they eliminated a black race correction factor in

these equations. It was found that the equation incorporating both serum creatinine and cystatin C was more accurate in black and non-black individuals as compared to the equations with either creatinine or cystatin C alone [104].

Conflicting findings have been reported in SSA concerning the performance of cystatin C in eGFR. In a cross-sectional study that was performed in individuals residing in the Democratic Republic of Congo (n=210) and Ivory Coast (n=284), it was found that the use of cystatin C alone or in combination with serum creatinine in the absence of the race factor did not improve the performance of eGFR equations whereas creatinine-based equations performed reasonably well [98]. Another study performed in Malawi in a population of 363 adults of which 32% were HIV-positive, found that creatinine-based relative to cystatin-based CKD-EPI equation in the absence of the race factor performed better in HIV-negative individuals but not in HIV-positive individuals where it was associated with overestimation of GFR. However, they still recommended that creatinine-based equation should be used because of the high costs and lack of standardization in SSA [105]. However, both studies [98,105] had significantly smaller study populations compared to the studies that reported on the advantage of cystatin C which might have influenced their results. Recently, a large multicentre study, including 2578 participants, using data from South Africa, Malawi and Uganda, evaluated 10 different eGFR equations based on serum creatinine and cystatin C compared to measured GFR using iohexol to identify a reliable method for eGFR in SSA [106]. Their findings indicated that the use of creatinine-based GFR equations substantially overestimated kidney function when compared to iohexol and cystatin C measures, particularly at lower GFR levels, and this was exacerbated by the inclusion of the race coefficient factor in Africans [106]. The use of serum creatinine for estimation of GFR remains the preferred option in resource limited settings particularly in Africa because of its affordability and accessibility, however, it is a suboptimal biomarker of eGFR and larger nationally representative studies are needed to explore the performance of cystatin C-based eGFR equations which may be superior in SSA.

2.7.2. Kidney damage markers

Markers of kidney damage are of importance for the classification of CKD in stages where kidney function alteration is not apparent [107]. The current international guidelines of CKD uses the level of albuminuria, which is a well-established marker of kidney damage, to define stage 1 and 2 of CKD where the level of GFR is above 60 mL/min/1.73m^2 [3]. The persistent loss of albumin or protein in urine above the normal ranges (30 mg or 150 mg per day, respectively) may be indicative of kidney damage.

The reference method for measuring albuminuria is by determining albumin excretion rate (AER) in a urine sample collected in a timed 24-hour period [3]. However, this method is time-consuming and susceptible to errors due to incomplete urine collection and timing errors and is impractical for large-

scale studies [108]. The current international guidelines recommended that measurement of ACR in random (preferably first-morning void) spot urine samples to determine the level of albuminuria is accurate enough to substitute AER and should therefore be used as a standard [86]. However, there are limitations that need to be taken into consideration when using ACR, as it can be influenced by factors independent of albumin filtration, such as menstruation, physical activity, hyperglycaemia, HTN, fever and urinary tract infection [108]. The levels of ACR also differ between males and females partly because of differences in muscular mass. Therefore, it is recommended that the diagnosis of albuminuria should be made based on two abnormal ACR out of three measured within a period of three to six months [108]. Moreover, albuminuria is limited during the early stages of CKD as there is generally minimal damage to the kidneys and elevated urinary albumin is not detectable at this stage [109]. Dipstick tests, which are predominantly used in poor resource settings to measure urine protein loss, particularly in Africa, have poor sensitivity and may not detect albuminuria at levels lower than 30-300 mg [102].

Other potential biomarkers for diagnosis and prognosis of CKD have also been explored as reviewed by Rysz et al. (2017), namely asymmetric dimethylarginine, symmetric dimethylarginine, uromodulin, kidney injury molecule-1, neutrophil gelatinase-associated lipocalin, and proteomic and metabolomic biomarkers, however, these markers have not been validated in clinical practice [102]. With all these being said, there is a need for biomarkers that will allow for early detection of CKD and monitoring of treatment response.

2.7.3. Kidney biopsy

Kidney biopsies, on rare occasions, are conducted as confirmation of diagnosis, or to determine the cause or stratification of CKD into various stages [18]. Moreover, they can also inform on the underlying pathological mechanisms, prognosis and help make an informed decision on the intensity and magnitude of treatment prescribed [18]. However, kidney biopsy is an invasive diagnostic procedure, expensive and complex to perform as it requires special facilities, resources and expertise and is not recommended for repeated and population-based testing [3].

2.8. Epigenetics

The term epigenetics was first described in 1942 by Conrad Waddington, literally meaning above or in addition to genetics [110]. Waddington described the term as “the branch of biology which studies the causal interactions between genes and their products which bring the phenotype into being” [111]. With the rapid growth in genetic studies over the years, the definition has evolved and is currently defined as functional alterations of gene expression that are heritable and do not affect the sequence of the DNA [112]. These epigenetic changes result in the silencing or activation of genes, thereby determining which proteins are produced [113]. Some epigenetic changes have been reported to be reversible by

interactions with nutritional and environmental factors. There are three main epigenetic mechanisms; DNA methylation, histone modification and non-coding RNAs (ncRNAs) [112]. DNA methylation, which is the first described and most researched mechanism in epigenetic inheritance, involves addition of a methyl group on the fifth cytosine of the cytosine phosphate guanine dinucleotide thus repressing gene expression through inhibition of transcription. As such, DNA methylation is a key regulator of gene expression and plays a critical role in mammalian development and physiology [114]. Histone modification is a post-translational modification that regulates chromatin structure and gene transcription by histone-acetylation and -methylation, and phosphorylation mechanisms, thereby impacting various important cellular phenotypes [115].

2.8.1. Non-coding RNAs

Another major epigenetic mechanism that has gained prominence in the field of biology over the years has non-protein coding RNAs as key role-players. Although enhanced interest in ncRNA research came about as a result of the discovery of miRNAs, the discovery of ncRNAs started with the identification of ribosomal RNA (rRNA) and transfer RNA (tRNA) in the 1950s [116]. For years, RNA research focused on rRNA and tRNA (also known as protein translation machinery), as well as mRNA, as they all play a major role in protein synthesis [117]. However, with advancements in sequencing technologies and computational analysis, more ncRNAs have been discovered [118]. Moreover, the completion of the human genome sequencing two decades ago, which is one of the biggest landmark achievements in biomedical sciences, led to the functional characterization of human genes which attracted more interest in the field of ncRNAs [119,120]. It was initially believed that the majority of the human genome comprised of protein-coding genes and only these sequences of the genome were transcribed. However, the completion of the human genome sequencing revealed that only 1.5% of the genome comprises of protein-coding genes [121]. Moreover, approximately 80% of the genome that was previously deemed “junk” is ultimately transcribed at some level into functional ncRNAs that regulate gene expression and mediate cellular processes involved in cell physiology and pathology [122].

Non-coding RNAs are a class of functional, highly abundant and diverse group of RNAs that do not encode proteins, but regulate gene expression at transcriptional and post-transcriptional levels [123]. Their classification, as summarized in Figure 2.1, is based on size, with a cut-off of 200 nucleotides used to separate small ncRNAs (<200 nucleotides) such as miRNAs, small interfering RNAs (siRNAs) and piwi-interacting RNAs (piRNAs) from long ncRNAs (>200 nucleotides) such as lncRNAs and circRNAs [123]. Non-coding RNAs play an important role in normal development, physiology and the development of disease, as the majority of genetic variations associated with disease development are located within the ncRNAs [124,125]. The functional relevance of ncRNAs

became more apparent with the discovery of small ncRNAs called miRNAs, which are the main focus of our study.

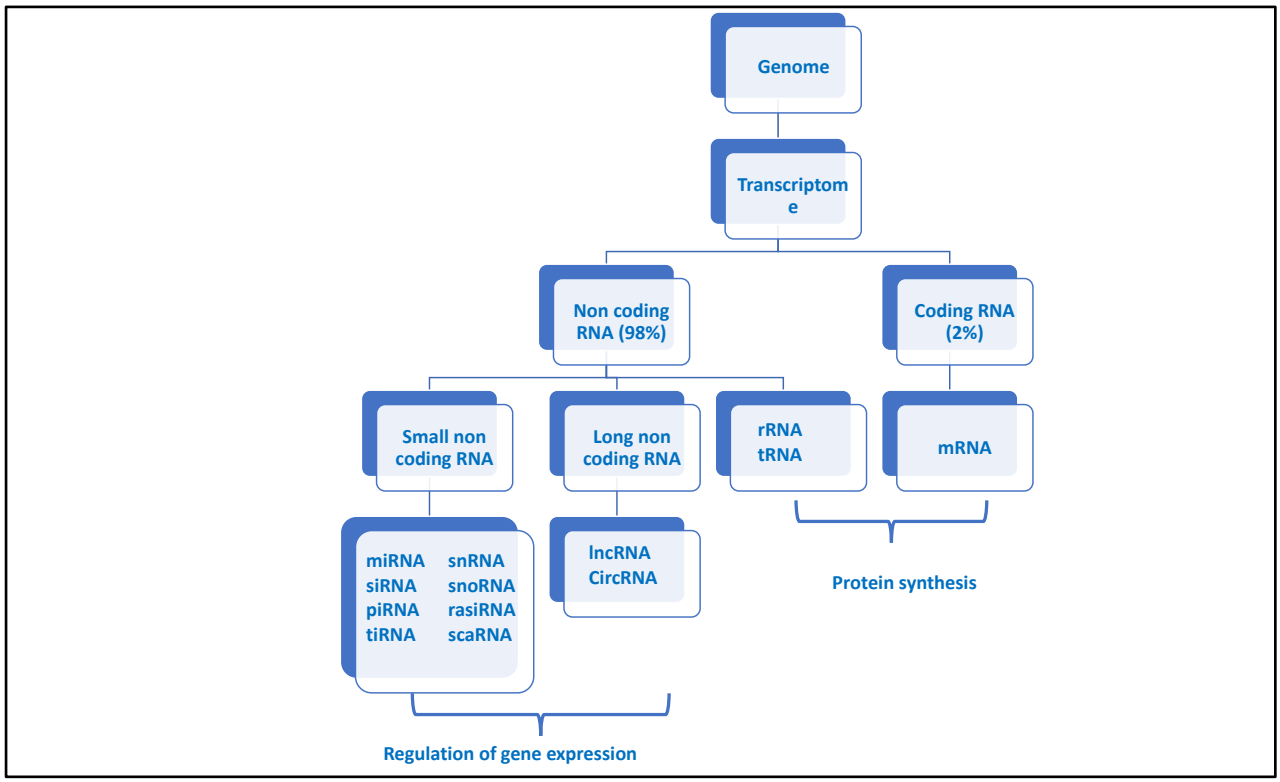

Figure 2.1: *The definition and classification of non-coding RNAs (ncRNAs).* The genome is transcribed into coding and ncRNAs, the latter constituting the majority of the transcriptome. The ncRNA is classified into small and long ncRNAs, which do not code for proteins but regulate gene expression and rRNA and tRNA, which play a major role in protein synthesis. The figure was adapted from Brandenburger (2018) [123].

2.8.2. Discovery of microRNAs

In the early 1990s, two ground-breaking independent studies led to the discovery of the first miRNA. Lee et al. (1993) from Harvard University and Wightman et al. (1993) from the Massachusetts General Hospital, both discovered that the *lin 4* gene in nematode *Caenorhabditis (C.) elegans* produces small (22 nucleotides long) RNAs, now called miRNAs. *Lin-4* does not encode a protein, but regulates mRNA *lin-14* expression post-transcriptionally, by antisense complementary binding to its 3' untranslated region (UTR) and as a result negatively regulating Lin-14 protein levels, which are essential for the development of *C.elegans* [126,127]. This discovery led to the identification of a cellular regulatory mechanism for gene expression. However, it was only seven years later that the role of miRNAs as post-transcriptional regulators of gene expression gained momentum. This was when *let-7,* a second miRNA with similar characteristics, was identified in *C.elegans* [128]. Soon after, *let-7* was identified in other organisms, including humans [129,130], indicating that these miRNAs were not unique to nematodes. These findings led to a new era of post-transcriptional gene regulatory mechanisms, and in 2001, this new class of small ncRNAs were then named miRNAs [131]. Since then, an increasing

number of miRNAs have been detected in all animal models and other species through random cloning, other molecular biology techniques and also by the help of computational and bioinformatic tools [132]. In 2018, approximately 11000 papers were published on the potential role of miRNAs in the diagnostic fields [133].

2.8.3. Description of microRNAs

MicroRNAs are a class of small (18 to 25 nucleotide bases in length), endogenous single-stranded ncRNAs encoded by plant, animal, and viral genomes, which function as post-transcriptional regulators of gene expression [117]. They represent a major class of small ncRNAs and regulate the expression of more than 60% of human protein-coding genes [134]. The regulatory process of miRNAs was first described in 1998 as RNA interference (RNAi) by Andrew Fire and Craig Mello [135], who were later awarded a Nobel Prize in Medicine and Physiology in 2006 for this discovery [136]. The turn of the millennium was accompanied by a rapid increase in the discovery of new miRNAs, and as such, miRBase, a comprehensive and up-to-date central registry and repository was established in 2002, with the aim of assigning unique names to different miRNAs to provide a searchable database for all published miRNAs [137]. At its release, the database contained only 218 miRNAs from five different species [138]. Two decades later and the latest miRBase version 22.1, which was released in 2018, holds approximately 2700 mature miRNAs identified in *Homo sapiens*, 1978 miRNAs in mice, 1095 miRNAs in *C. elegans*, and 469 in Drosophila melanogaster [139].

2.8.4. Biogenesis of microRNAs

The biogenesis of miRNAs is a complex, multistep process that is initiated in the nucleus, undergoing a lot of processing by various enzymes and proteins, and ending in the cytoplasm as illustrated in Figure 2.2. The initial clues to the synthesis of miRNAs came in 2001, when it was shown in *C.elegans* that long double-stranded transcripts are processed into smaller RNAs, approximately 21-25 nucleotides long [140]. Initially, the miRNA genes located within the intergenic and a few in the introgenic regions of the genome [131], are transcribed by RNA polymerase II into a long hairpin-like structure called a primary miRNA transcript (pri-miRNA) in the nucleus [141]. Following this, pri-miRNA is recognized and cleaved by a ribonuclease (RNAse) III endonuclease called Drosha with the help of DiGeorge syndrome critical region 8 (DGCR8) protein, to produce one or more shorter hairpin-like structures called precursor miRNAs (pre-miRNAs) approximately 60 -70 nucleotides long [142]. The pre-miRNA is then transported out of the nucleus via exportin 5 into the cytoplasm, where it is further processed by RNAse III enzyme Dicer, resulting in short (20-23 nucleotides long) double-stranded mature miRNA molecules [143]. The Argonaute (AGO) family of proteins (AGO 1-4) interacts with the mature miRNA duplex, leading to the selection of the strand, the 5p (arising from the 5’ end of pre-miRNA) or the 3p (arising from the 3’ end of pre-miRNA) based on the thermodynamic stability at the 5’ ends of the miRNA duplex [144]. The least stable 5’ end strand is

selected as the guide strand and loaded into AGO, and then incorporated into the RNA-induced silencing complex (RISC), and this marks the completion of miRNA biogenesis [24]. The miRNA will then guide the RISC complex to the target mRNA by partial complementarity, resulting in inhibition of mRNA translation to protein or degradation of mRNA or both [24],[145]. On the other hand, the passenger strand is released from the AGO, and is presumably degraded, although there are few cases where it is incorporated with the RISC, and remains functional [146].

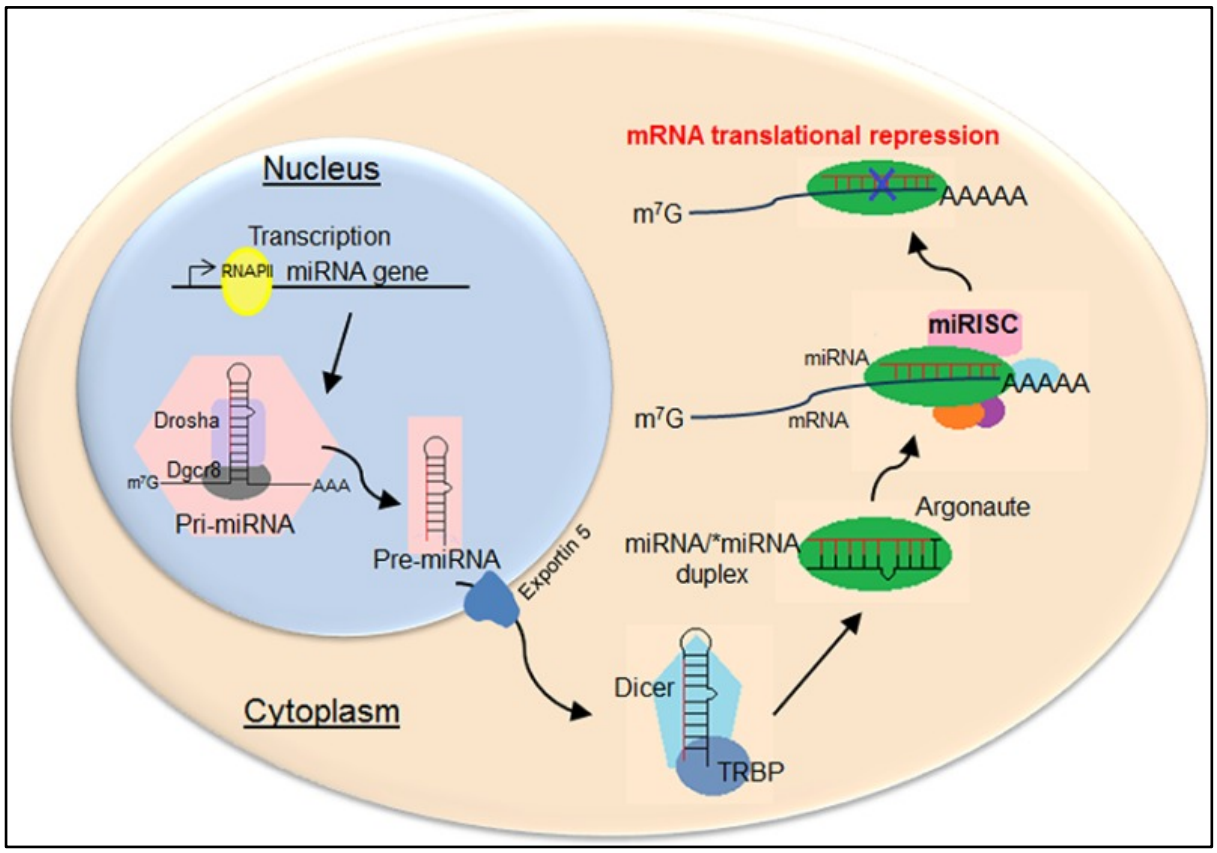

Figure 2.2: *The biogenesis of miRNAs*

The figure illustrates how miRNAs are transcribed in the nucleus by RNA polymerase II into primary miRNA (pri-miRNA), which is then cleaved by Drosha, RNAse III endonuclease with the help of proteins into smaller stem-looped structures called precursor miRNAs (pre-miRNAs). Pre-miRNAs are then exported into the cytoplasm via exportin 5 where they are further processed by Dicer, RNAse III enzyme into mature miRNA which is loaded into argonaute protein and then incorporated into the mRNA-induced silencing complex (miRISC). The miRISC will bind to the target miRNA, thereby inhibiting the translation of mRNA or leading to the degradation of miRNA. Adapted from Hajarnis et al. (2015) [147], See license for permission of reuse under Appendix A.

2.8.5. Function of microRNAs

The primary function of miRNAs is to regulate gene expression at the post-transcriptional level, thereby impacting the synthesis of certain proteins [117]. This is predominantly achieved by mediating destabilization of their target mRNA, although a minor component of translation inhibition has been

detected [148]. At a molecular level, miRNAs bind to the target site generally located in the 3'UTR of the target mRNA by canonical and non-canonical mechanisms [117]. In the canonical mechanism, the guide miRNA incorporated in the RISC complex binds to the 3'UTR region of the target mRNA based on the seed sequence, the 3'UTR complementary sequence, located between the second and eighth nucleotides on the 5' end of the miRNA, resulting in deadenylation, translation inhibition and then degradation of the target mRNA [24]. However, 60% of the time, these sequences are not completely complementary, and therefore, the miRNA will bind to the target mRNA via non-canonical mechanisms containing mismatched nucleotides, resulting in the degradation of the mRNA [117]. Due to the nature of their binding, a single miRNA can regulate the expression of a multitude of mRNAs, and on the other hand, one mRNA possesses multiple binding sites for different miRNAs, and therefore may be regulated by multiple miRNAs [149]. This highlights how miRNAs are involved in various biological pathways, as well as the development of diseases. However, it is important to note that only miRNAs expressed at the highest levels can exert transcriptional regulation on their target mRNA. The use of prediction tools such as in silico target prediction algorithms are being employed to identify miRNA potential targets based on the complementarity between the miRNAs and 3'UTR on the mRNA, as well as their degree of conservation across species [150].

MicroRNAs also play a role in intercellular signaling, and although they are mostly found in the cytoplasm of cells, they are also secreted into body fluids, whereas some are exogenous miRNAs from food, bacteria and fungi [151]. They are carried by extracellular vesicles of different cells to to be released together with other genetic material into their targeted cells, thereby facilitating cell communication. Because of the regulatory role of miRNAs, they are implicated in a variety of biological processes such as cell proliferation, differentiation, apoptosis, development and metabolism [24]. Considerably high levels of miRNAs were expressed in organs of human fetuses relative to matched adult organs [152]. Similarly, mutations in *lin-4* and *let-7* (the first two identified miRNAs) were associated with defects in the development of larvae, and it was suggested that these miRNAs are involved in the early and late larval developmental transition in *C. elegans* [127],[128]. Furthermore, miRNAs also play an important role in homeostasis maintenance and development. Knockout of genes encoding Dicer and Drosher enzymes involved in the biogenesis of miRNAs, was found to be associated with underdeveloped organs and/or death [153].

2.8.6. Location of microRNAs

Understanding the distribution and expression of miRNAs across different human biological samples is important in understanding their physiological and pathological roles. The expression of miRNAs usually correlates with the expression of its host genes. At a molecular level, assessment of the chromosomal location and genomic distributions demonstrated that approximately 52%, 40% and 8% of the human miRNA transcription sites are located within the intergenic, intronic and exonic regions

of the genome, respectively [154]. Moreover, it was shown that some miRNAs are expressed in clusters and this is because their genes are co-expressed as they are in close proximity to each other [155]. Studies have highlighted that miRNAs are ubiquitously expressed across human biological samples, whilst a small percentage is expressed in a tissue-specific manner [156]. This may be because certain cell types are found in many organs, therefore, their specific miRNAs may be misinterpreted as ubiquitously expressed [157]. Landgraf and colleagues carried out a sequencing-based study and reported the expression of 340 miRNAs in 26 organs, providing initial evidence of differing miRNA expression patterns across various tissues [158]. A year later, it was demonstrated that placental miRNAs are detectable in maternal plasma in a protected fashion and in stable forms [159]. In the same year, another study reported the extracellular pattern of miRNAs in individuals with large B-cell lymphoma [160]. Expression patterns of miRNAs previously identified in specific tissues were reported in 12 different extracellular body fluids from normal including, plasma, urine, saliva, breast milk, tears, cerebrospinal fluid, colostrum, seminal fluid, amniotic fluid, bronchial lavage, pleural fluid and peritoneal fluid by Weber and co-workers [151]. Approximately one third of tissue-specific miRNAs are also expressed in circulation. The stability of these miRNAs in bodily fluids is remarkable, and it has been suggested that this stability is due to their ability to bind proteins such as Argonaute2 or lipoprotein complexes or be encapsulated within the cell in multivesicular compartments, which fuse with the cell membrane to release exosomes into circulation , thereby protecting them from degradation by RNAses found in bodily fluids [161]. The mechanisms in which miRNAs are released into circulation are still not fully understood to date. However, others suggest that following tissue injury, cell death and necrosis or through active secretion, protein-bound miRNAs are secreted into the circulation to facilitate intercellular communication [162].

2.8.7. Techniques for microRNA detection

There are four main techniques for the quantification of miRNAs, namely quantitative reverse transcriptase northern blot hybridization, RT-qPCR, microarray and next-generation sequencing (NGS) [163]. Initially, northern blot hybridization, RT-qPCR and microarrays were the only techniques used to quantify miRNAs. However, these techniques are of low yields and could only quantify a limited number of miRNAs [163]. With the advancement in technology, NGS with greater yield is now employed to quantify and discover novel miRNAs [164]. Northern blot hybridization is a widely used technique for quantifying miRNAs [163]. It was the first technique utilized to study miRNAs, by using polyacrylamide gel electrophoresis to visualize the miRNA. Whilst this technique allows the identification of new miRNAs, it requires large quantities of RNA, is time-consuming and has low sensitivity and throughput [165]. RT-qPCR is the gold-standard technique for miRNA quantification. This technique uses total RNA as a starting material and is converted to complementary DNA (cDNA) by reverse transcription followed by amplification of miRNA with specific primers [166]. RT-qPCR is highly sensitive and specific, quite affordable, easy to use and fast [166]. However, it can be

affected by the purity and quality of RNA, and also requires the inclusion of normalization miRNAs which are not yet standardized [167]. Microarrays utilise fluorescence to hybridize target miRNAs to the complementary fixed probes. Although it is easy to perform and allows parallel analysis of hundreds of miRNAs in a single sample with short turnaround times, it is expensive, biased by cross-hybridization and it does not allow identification of new miRNAs [168]. NGS allows parallel sequencing of millions of miRNAs in a single sample and can be used to identify novel miRNAs. However, this technique is costly, time-consuming, and requires complex data analysis [165].

2.8.8. MicroRNAs and disease association

Understanding the molecular, physiological, and pathological mechanisms underlying the development of diseases in humans is critical in biomedical research. A growing body of evidence demonstrates that miRNAs play an extensive role in gene expression through their involvement in a variety of biological processes such as cell development, proliferation, differentiation and apoptosis, determination of cell fate, adaptation to stress, immune reaction, host-viral interactions, signal transduction, hematopoietic lineage differentiation and tumorigenesis [117],[126]. Therefore, dysregulation of their expression profiles may be associated with the development of various diseases. Dysregulated miRNA patterns may result from various factors, including those that interfere with miRNA biogenesis and sequestration, genomic alterations that may result from deletion or amplification of genes that encode miRNAs or aberrant expression of transcription factors or enzymatic differences [169]. The initial study to provide evidence on altered miRNA expression in diseases was published in 2002 [170]. In this study, the authors searched for tumor suppressors at chromosome 13q14 region which contains genes encoding miR-15 and -16, in B-cell chronic lymphocytic leukemia cells and found that this region is downregulated or frequently deleted in individuals with B-cell chronic lymphocytic leukemia disease. Following this study, several other independent laboratories reported dysregulated miRNA profiles in various cancers. Increasing evidence has shown that miRNAs can act as oncogenes or tumor suppressors in the development, progression and metastases of cancers of the breast [171], ovary [172], prostate [173], colon [174] and lung [175].

MicroRNAs are involved in the functioning of the nervous system, and dysregulation in their expression pattern may result in the development of diseases such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, epilepsy and glioblastoma [176]. For example, the expression patterns of miR-20b-5p, miR-30a-5p, and miR-146a-5p were altered in immunological and neurologic pathways underlying the development of multiple sclerosis [177]. MiR-107 has been reported to be downregulated in Alzheimer's disease and may be associated with disease progression through its regulation of mRNA β-site amyloid precursor protein-cleaving enzyme 1 (BACE1) [178]. MiR-103a, -30b and -29a were upregulated in treated individuals with Parkinson's disease [176]. In addition, aberrant miRNA expressions have been associated with CVDs. Several studies have shown that miRNAs, including

miR-1, miR-133a, miR-208a/b, and miR-499, are usually upregulated following myocardial infarction [179].

2.8.9. MicroRNAs in chronic kidney disease

Studies have shown that some miRNAs are specifically expressed in the human kidney and may be involved in the development, homeostasis, and physiology of the kidney. MiRNAs including miR-192, miR-194, miR-204, miR-215, miR-216, miR-449c-5p and miR-449b-5p, were found in greater abundance in the kidney as compared to other organs and as a result, were targeted for investigation by most studies to determine their role in kidney dysfunction [156,180]. On the other hand, other miRNAs such as miR-21, miR-200a, and let-7a–g were found to be highly expressed in the kidney as well as in other organs [158]. Conversely, there are a number of other miRNAs that are expressed in lower levels or absent in the kidneys as compared to other organs, thereby permitting the expression of certain proteins essential for the normal functioning of the kidneys [181]. When all this is considered, it is apparent that miRNAs may play an important role in kidney development, maintenance and functioning, as well as the development of kidney disease. Differential expression of miRNAs has been observed between the inner and outer regions of the kidney. For example, a 20-fold increase in expression of miR-192 was reported in the cortex where it regulates sodium transport, relative to the medulla of the kidney [181]. Animal model studies were the first to reveal evidence of the involvement of miRNAs in the development of kidney disease. The deletion of an RNA nuclease involved in miRNA biogenesis in mice was associated with the development of kidney injury, proteinuria and kidney failure [182].

One of the first studies to evaluate extracellular miRNAs in individuals with CKD was by Neal and colleagues. They found that plasma miR-16, miR-21, miR-155, miR-210, and miR-638 were significantly downregulated in individuals on dialysis [183]. Several other studies reported on the dysregulation of circulating miRNAs in individuals with CKD. Fourdnier et al. reported that reduced expression of serum miR-126 and miR-223 was associated with lower eGFR in individuals with CKD stages 1 to 5 in a longitudinal cohort study [29]. A cross-sectional analysis in elderly Japanese individuals demonstrated serum miR-126, miR-197 and miR -223 downregulation in individuals with CKD [184].

2.8.9.1. MicroRNAs in diabetic kidney disease

Dysregulation of miRNA expression has been associated with diabetic kidney disease (DKD), a leading cause of ESKD. Kato and colleagues performed microarray profiling and discovered that the expression of miR-192 was upregulated by transforming growth factor beta 1 (TGF-β1) in diabetic mice and subsequently activated the expression of type 1 and 2 collagen gene in mesangial cells by targeting the E-box repressors Zeb1 and Zeb2 which control the expression of TGF-1-induced collagen [185]. In a further study, they showed that miR-192 induced the upregulation of TGF-β1 and

amplification of TGF-β1 signalling, accelerating the progression of chronic fibrotic kidney diseases, including DKD [186]. Others have demonstrated that miR-21 plays a role in the development of DKD through its involvement in fibrosis, apoptosis, hypertrophy and protein kinase B activation by targeting genes such as Phosphatase and TENsin homolog [187]. A mouse model study showed that miR-21 was associated with fibrogenesis and epithelial injury in the kidneys and showed potential as a therapeutic target [188]. Due to their effect on pathways such TGF-β1 signalling pathway, and their impact on epithelial-mesenchymal transformation, miRNAs are also involved in the development of renal fibrosis, a common cause of DKD and a major pathological feature of ESKD. Inflammation plays an important role in the progression of DKD, and anti-inflammatory miRNAs like miR-146a are reportedly involved in the pathogenesis of DKD. An animal model study showed that in mice where miR-146a was knocked-out, there was increased activation and suppression of macrophages M1 and M2, respectively, as well as increased expression of pro-inflammatory cytokines as opposed to mice with miR-146a, thereby highlighting the anti-inflammatory role of this miRNA in the development of DKD [189]. Increased expression of miR-21-5p has been observed in serum and kidney samples of humans or rodents with DKD [190],[191]. Knockdown of miR-21-5p in mice with induced DM was associated with ameliorated inflammation, reduced interstitial fibrosis, reduced albuminuria and prevented podocyte loss [190].

2.8.9.2. MicroRNAs in hypertensive nephropathy

Altered miRNA expression patterns have been reported in hypertensive nephropathy. Global miRNA profiling on kidney biopsies demonstrated that a number of miRNAs, including miR-200a, miR-200b, miR-141, miR-429 and miR-192 were highly expressed in individuals with hypertensive nephrosclerosis and the degree of upregulation was associated with disease progression [192]. A study performed in Dahl salt-sensitive rat models showed that the upregulation of miR-29b suppressed the expression of collagen and extracellular matrix-related genes, thereby causing hypertensive kidney injury [193]. In a study by Lu and colleagues, they demonstrated miR-103a-3p upregulation in individuals with hypertensive nephropathy when compared to normotensive individuals. Furthermore, they showed that mice which overexpressed miR-103a-3p and were induced with angiotensin II presented with kidney inflammation, kidney fibrosis and albuminuria, as compared to mice with silenced miR-130a-3p, thereby highlighting the role of this miRNA in the development of hypertensive nephropathy [194].

2.8.10. MicroRNAs as potential biomarkers of disease

Biomarkers have evolved over the years with human advancement in technology and research. Nowadays, a biomarker is defined as “a molecule that can be objectively measured and evaluated in human body fluid to reflect the presence or absence of disease as well as response to treatment” [195]. Therefore, an ideal biomarker has to meet certain qualities, including 1) being measurable in a sample

that was obtained in a minimally invasive nature, such as in urine, blood or saliva, 2) being disease-specific, sensitive with production in the early stages of the disease before the presentation of clinical symptoms, 3) being inexpensive to quantify, 4) changing in quantity with disease progression or in response to treatment, and 5) being translatable from research to clinic [195]. Most of the biomarkers currently used for disease diagnosis are protein-based. Due to the complexity of the structure of proteins and their diverse nature, protein biomarkers have proven to be costly, time-consuming and difficult to analyse as they require high-affinity specific analysers [196]. Although it was believed that RNA molecules could not be used as biomarkers, particularly in blood, because of high levels of ribonucleases (RNAses), this notion was dismissed when miRNAs were detected in the circulation in highly stable forms.

In 2007, it was discovered that miRNAs were among the RNA molecules that were exported into the extracellular space in vesicles, thereby protecting them from degradation by RNAses [197]. A year later, it was demonstrated that placental miRNAs are detectable in maternal plasma in a protected fashion and in highly stable forms [159]. MicroRNAs have become more desirable as potential diagnostic and prognostic markers of disease due to a number of advantageous qualities, including that they are expressed in a tissue specific manner, detectable in human biofluids in highly stable manner due to their short length, and can be easily and reliably quantified using fast and robust techniques [117]. Moreover, due to their altered expression in biofluids or tissues of unhealthy compared to healthy individuals, miRNAs can therefore discriminate disease conditions or correlate with mechanisms of disease development or progression [20–23]. Taken together, these qualities suggest that miRNAs may present a new class of potential biomarkers that can be detected in a minimally invasive, highly specific and sensitive manner, and are capable of early diagnosis or prediction of disease, allowing early initiation of treatment and therefore improving patient outcomes as compared to traditional diagnostic methods [198].

The field of cancer was the first to establish miRNAs as disease biomarkers. They observed that dysregulated expression of serum miR-21, miR-155 and miR-210 was associated with a diagnosis of large B cell lymphoma [160]. Thereafter, the potential role of circulatory miRNAs as disease biomarkers was explored by independent laboratories in various cancers, including prostate [25] and lung cancer [199]. Moreover, miRNAs have allowed for the identification of the tissue of origin for metastatic cancer, thereby reducing time-consuming procedures and overall costs of the procedure [200]. Since then, the research into specific biofluid miRNA profiles for a wide range of diseases has increased substantially, and miRNAs have emerged as promising diagnostic and prognostic biomarkers for a wide range of diseases [133]. In 2011, Keller and colleagues conducted a comprehensive study to evaluate whole blood specific miRNA profiles in the following 14 diseases: lung, prostate, pancreatic and ovarian cancer; gastric, Wilms and pancreatic tumours, as well as multiple sclerosis, acute

myocardial infarction, pancreatitis, periodontitis and sarcoidosis [26]. They used microarrays to analyse miRNA profiles in 454 blood samples and identified two miRNAs capable of discriminating between healthy controls and each profiled disease. In a study by Mohan and colleagues, they demonstrated that urinary miRNAs secreted following nephron injury may serve as potential biomarkers for the prediction or early detection of DKD [201].

2.8.11. MicroRNAs as therapeutic targets

Similarly, a number of studies are currently underway evaluating the potential use of miRNAs as therapeutic targets in the form of miRNA mimics and antagomiRs. In cases where the expression of a specific miRNA is downregulated, the expression can be re-established by using a miRNA mimic [202], whereas when a specific miRNA is overly expressed an antagomiR is used to suppress the expression of that miRNA [203]. miRNA mimics are synthetic RNA molecules with the same nucleotide sequence as their specific endogenous miRNA, whereas antagomiRs are synthetic RNA molecules that bind and sequester their specific endogenous miRNA, thereby inhibiting its functions [204]. The unique ability of miRNAs to bind to a multitude of mRNAs and regulate their expression creates a broad and intricate regulatory network, making them attractive as potential therapeutic candidates [205]. Although none of the miRNA-based therapies has been formally approved, the possibility of utilising miRNAs as potential therapeutic targets has gained interest after various therapies have reached phase I and II of their respective clinical trials. An example is the successful treatment of individuals with hepatitis C virus infection using miravirsen, which is a sequester and inhibitor of miR-122. This miRNA-based therapy is currently in phase II of clinical trials and is moving towards the market [206]. Also, miR-29 has been shown to have antifibrotic qualities, and miR-29 mimic (MRG-201) is currently in phase II of the clinical trial for the treatment of individuals with skin fibrosis, although it might be applicable to kidney fibrosis, a common cause of ESKD [207].

2.9. References

1. Global Collaboration of CKD. Global, regional, and national burden of chronic kidney disease, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017 - PubMed [Internet]. 2020 [cited 2022 May 31]. Available from: https://pubmed.ncbi.nlm.nih.gov/32061315/

2. Foreman KJ, Marquez N, Dolgert A, Fukutaki K, Fullman N, McGaughey M, et al. Forecasting life expectancy, years of life lost, and all-cause and cause-specific mortality for 250 causes of death: reference and alternative scenarios for 2016–40 for 195 countries and territories. Lancet. 2018;392(10159):2052–90.

3. Levin A, Stevens PE, Bilous RW, Coresh J, De Francisco ALM, De Jong PE, et al. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical

practice guideline for the evaluation and management of chronic kidney disease. Kidney Int Suppl. 2013;3(1):1–150.

4. Oguntola SO, Hassan MO, Duarte R, Vachiat A, Manga P, Naicker S. Atherosclerotic vascular disease is more prevalent among black ESKD patients on long-term CAPD in South Africa. BMC Nephrol. 2019;20(1):1–10.

5. George C, Mogueo A, Okpechi I, Echouffo-Tcheugui JB, Kengne AP. Chronic kidney disease in low-income to middle-income countries: The case f increased screening. BMJ Glob Heal. 2017;2(2):1–10.

6. Stanifer JW, Von Isenburg M, Chertow GM, Anand S. Chronic kidney disease care models in low-and middle-income countries: a systematic review. BMJ Glob Heal. 2018;3(2):e000728.

7. Thurlow JS, Joshi M, Yan G, Norris KC, Agodoa LY, Yuan CM, et al. Global epidemiology of end-stage kidney disease and disparities in kidney replacement therapy. Am J Nephrol. 2021;52(2):98–107.

8. Liyanage T, Ninomiya T, Jha V, Neal B, Patrice HM, Okpechi I, et al. Worldwide access to treatment for end-stage kidney disease: A systematic review. Lancet [Internet]. 2015;385(9981):1975–82. Available from: http://dx.doi.org/10.1016/S0140-6736(14)61601-9

9. Xie Y, Bowe B, Mokdad AH, Xian H, Yan Y, Li T, et al. Analysis of the Global Burden of Disease study highlights the global, regional, and national trends of chronic kidney disease epidemiology from 1990 to 2016. Kidney Int. 2018;94(3):567–81.

10. Norton JM, Eggers P. Poverty and Chronic Kidney Disease. Chronic Ren Dis. 2020;181–96.

11. Davids MR, Chothia MY. Chronic kidney disease for the primary care clinician. South African Fam Pract. 2019;61(5):19–24.

12. Saran R, Robinson B, Abbott KC, Agodoa LYC, Bhave N, Bragg-Gresham J, et al. US renal data system 2017 annual data report: epidemiology of kidney disease in the United States. Am J Kidney Dis. 2018;71(3):A7.

13. Tonelli M, Riella M. Chronic kidney disease and the aging population. Brazilian J Nephrol. 2014;36:1–5.

14. Ricardo AC, Anderson CA, Yang W, Zhang X, Fischer MJ, Dember LM, et al. CRIC Study Investigators: Healthy lifestyle and risk of kidney disease progression, atherosclerotic events, and death in CKD: Findings from the Chronic Renal Insufficiency Cohort (CRIC) Study. Am J Kidney Dis. 2015;65(3):412–24.

15. Rosenberg AZ, Naicker S, Winkler CA, Kopp JB. HIV-associated nephropathies:

epidemiology, pathology, mechanisms and treatment. Nat Rev Nephrol. 2015;11(3):150–60.

16. Levey AS, Schoolwerth AC, Burrows NR, Williams DE, Stith KR, McClellan W. Comprehensive public health strategies for preventing the development, progression, and complications of CKD: report of an expert panel convened by the Centers for Disease Control and Prevention. Am J Kidney Dis. 2009;53(3):522–35.

17. Li P, Garcia-Garcia G, Lui S-F, Andreoli S, Fung W, Hradsky A, et al. Kidney health for everyone everywhere–from prevention to detection and equitable access to care. Brazilian J Med Biol Res. 2020;53.

18. Levin A, Tonelli M, Bonventre J, Coresh J, Donner JA, Fogo AB, et al. Global kidney health 2017 and beyond: a roadmap for closing gaps in care, research, and policy. Lancet. 2017;390(10105):1888–917.

19. Cañadas-Garre M, Anderson K, McGoldrick J, Maxwell AP, McKnight AJ. Genomic approaches in the search for molecular biomarkers in chronic kidney disease 11 Medical and Health Sciences 1103 Clinical Sciences [Internet]. Vol. 16, Journal of Translational Medicine. BioMed Central; 2018. Available from: https://doi.org/10.1186/s12967-018-1664-7

20. Chen X, Xie D, Zhao Q, You ZH. MicroRNAs and complex diseases: From experimental results to computational models. Brief Bioinform. 2019;20(2):515–39.

21. Zhu H, Wang G, Zhou X, Song X, Gao H, Ma C, et al. miR-1299 suppresses cell proliferation of hepatocellular carcinoma (HCC) by targeting CDK6. Biomed Pharmacother [Internet]. 2016;83:792–7. Available from: http://dx.doi.org/10.1016/j.biopha.2016.07.037

22. Amiri A, Tehran MM, Asemi Z, Shafiee A, Hajighadimi S, Moradizarmehri S, et al. Role of resveratrol in modulating microRNAs in human diseases: From cancer to inflammatory disorder. Curr Med Chem. 2019;26(March 2020).

23. Wang J, Wang G, Liang Y, Zhou X. Expression profiling and clinical significance of plasma microRNAs in diabetic nephropathy. J Diabetes Res. 2019;2019.

24. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009;136(2):215–33.

25. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci. 2008;105(30):10513–8.

26. Keller A, Leidinger P, Bauer A, ElSharawy A, Haas J, Backes C, et al. Toward the blood-borne miRNome of human diseases. Nat Methods. 2011;8(10):841–3.

27. Abdellatif M. Differential expression of microRNAs in different disease states. Circ Res. 2012;110(4):638–50.

28. Liu Y, Usa K, Wang F, Liu P, Geurts AM, Li J, et al. MicroRNA-214-3p in the kidney contributes to the development of hypertension. J Am Soc Nephrol. 2018;29(10):2518–28.

29. Fourdinier O, Schepers E, Metzinger-Le Meuth V, Glorieux G, Liabeuf S, Verbeke F, et al. Serum levels of miR-126 and miR-223 and outcomes in chronic kidney disease patients. Sci Rep. 2019;9(1):1–12.

30. Fujii R, Yamada H, Munetsuna E, Yamazaki M, Ohashi K, Ishikawa H, et al. Associations of Circulating MicroRNAs (miR-17, miR-21, and miR-150) and Chronic Kidney Disease in a Japanese Population. J Epidemiol. 2019 Apr;30(4):177–82.

31. Bikbov B, Purcell CA, Levey AS, Smith M, Abdoli A, Abebe M, et al. Global, regional, and national burden of chronic kidney disease, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2020;395(10225):709–33.

32. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract. 2022;183:109119.

33. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, et al. Global burden of cardiovascular diseases and risk factors, 1990–2019: update from the GBD 2019 study. J Am Coll Cardiol. 2020;76(25):2982–3021.

34. Vos T, Lim SS, Abbafati C, Abbas KM, Abbasi M, Abbasifard M, et al. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2020;396(10258):1204–22.

35. Ene-Iordache B, Perico N, Bikbov B, Carminati S, Remuzzi A, Perna A, et al. Chronic kidney disease and cardiovascular risk in six regions of the world (ISN-KDDC): a cross-sectional study. LANCET Glob Heal. 2016;4(5):E307–19.

36. Honeycutt AA, Segel JE, Zhuo X, Hoerger TJ, Imai K, Williams D. Medical costs of CKD in the Medicare population. J Am Soc Nephrol. 2013;24(9):1478–83.

37. Stanifer JW, Jing B, Tolan S, Helmke N, Mukerjee R, Naicker S, et al. The epidemiology of chronic kidney disease in sub-Saharan Africa: A systematic review and meta-analysis. Lancet Glob Heal [Internet]. 2014;2(3):e174–81. Available from: http://dx.doi.org/10.1016/S2214-109X(14)70002-6

38. Kaze AD, Ilori T, Jaar BG, Echouffo-Tcheugui JB. Burden of chronic kidney disease on the African continent: A systematic review and meta-analysis. BMC Nephrol. 2018;19(1):1–11.

39. Bello AK, Levin A, Tonelli M, Okpechi IG, Feehally J, Harris D, et al. Assessment of global kidney health care status. JAMA - J Am Med Assoc. 2017;317(18):1864–81.

40. Evans M, Grams ME, Sang Y, Astor BC, Blankestijn PJ, Brunskill NJ, et al. Risk factors for prognosis in patients with severely decreased GFR. Kidney Int reports. 2018;3(3):625–37.

41. Zeng X, Liu J, Tao S, Hong HG, Li Y, Fu P. Associations between socioeconomic status and chronic kidney disease: a meta-analysis. J Epidemiol Community Heal. 2018;72(4):270–9.

42. Garcia-Garcia G, Jha V. Chronic kidney disease in disadvantaged populations. Brazilian J Med Biol Res. 2015;48:377–81.

43. Okpechi IG. ESKD in sub-Saharan Africa: will governments now listen? Lancet Glob Heal. 2017;5(4):e373–4.

44. Abd Elhafeez S, Bolignano D, D'Arrigo G, Dounousi E, Tripepi G, Zoccali C. Prevalence and burden of chronic kidney disease among the general population and high-risk groups in Africa: A systematic review. BMJ Open. 2018;8(1).

45. Arogundade FA, Barsoum RS. CKD Prevention in Sub-Saharan Africa: A Call for Governmental, Nongovernmental, and Community Support. Am J Kidney Dis. 2008;51(3):515–23.

46. Bank W. Global economic prospects, June 2020. The World Bank; 2020.

47. Matsha TE, Yako YY, Rensburg MA, Hassan MS, Kengne AP, Erasmus RT. Chronic kidney diseases in mixed ancestry south African populations: Prevalence, determinants and concordance between kidney function estimators. BMC Nephrol. 2013;14(1):1–10.

48. Peer N, George J, Lombard C, Steyn K, Levitt N, Kengne A-P. Prevalence, concordance and associations of chronic kidney disease by five estimators in South Africa. BMC Nephrol. 2020;21(1):1–11.

49. Davids MR, Jardine T, Marais N, Sebastian S, Davids T, Jacobs JC. South African Renal Registry Annual Report 2019. African J Nephrol. 2021;24(1):95–106.

50. Ngobeni V, Breitenbach MC, Aye GC. Technical efficiency of provincial public healthcare in South Africa. Cost Eff Resour Alloc. 2020;18(1):1–19.

51. Bikbov B, Purcell CA, Levey AS, Smith M, Abdoli A, Abebe M, et al. Global, regional, and national burden of chronic kidney disease, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet (London, England) [Internet]. 2020 Feb 29 [cited 2022 May 31];395(10225):709–33. Available from: https://pubmed.ncbi.nlm.nih.gov/32061315/

52. Hill NR, Fatoba ST, Oke JL, Hirst JA, O'Callaghan CA, Lasserson DS, et al. Global

prevalence of chronic kidney disease–a systematic review and meta-analysis. PLoS One. 2016;11(7):e0158765.

53. Alicic RZ, Rooney MT, Tuttle KR. Diabetic kidney disease: challenges, progress, and possibilities. Clin J Am Soc Nephrol. 2017;12(12):2032–45.

54. Mills KT, Stefanescu A, He J. The global epidemiology of hypertension. Nat Rev Nephrol. 2020;16(4):223–37.

55. Bosu WK, Reilly ST, Aheto JMK, Zucchelli E. Hypertension in older adults in Africa: a systematic review and meta-analysis. PLoS One. 2019;14(4):e0214934.

56. Ataklte F, Erqou S, Kaptoge S, Taye B, Echouffo-Tcheugui JB, Kengne AP. Burden of undiagnosed hypertension in sub-saharan Africa: a systematic review and meta-analysis. Hypertension. 2015;65(2):291–8.

57. Mallamaci F, Pisano A, Tripepi G. Hypertension management in chronic kidney disease. Nephrol Dial Transplant. 2020;(1):1–2.

58. Culleton B. Introduction to the Canadian clinical practice guidelines. J Am Soc Nephrol. 2006;17:S1-3.

59. Ku E, Lee BJ, Wei J, Weir MR. Hypertension in CKD: core curriculum 2019. Am J Kidney Dis. 2019;74(1):120–31.

60. Anderson AH, Yang W, Townsend RR, Pan Q, Chertow GM, Kusek JW, et al. Time-updated systolic blood pressure and the progression of chronic kidney disease: a cohort study. Ann Intern Med. 2015;162(4):258–65.

61. Lea JP, Nicholas SB. Diabetes mellitus and hypertension: key risk factors for kidney disease. J Natl Med Assoc. 2002;94(8 Suppl):7S.

62. WHO. HIV/AIDS | WHO | Regional Office for Africa [Internet]. 2018 [cited 2022 Jun 5]. Available from: https://www.afro.who.int/health-topics/hivaids

63. Hodes R. Hiv/Aids in South Africa. In: Oxford Research Encyclopedia of African History. 2018.

64. Alfano G, Cappelli G, Fontana F, Di Lullo L, Di Iorio B, Bellasi A, et al. Kidney disease in HIV infection. J Clin Med. 2019;8(8):1254.

65. Naicker S, Rahmania S, Kopp JB. HIV and chronic kidney disease. Clin Nephrol. 2015;83(Suppl 1):S32.

66. Bash LD, Coresh J, Köttgen A, Parekh RS, Fulop T, Wang Y, et al. Defining incident chronic

kidney disease in the research setting: The ARIC Study. Am J Epidemiol. 2009;170(4):414–24.

67. Dubey RK, Jackson EK. Estrogen-induced cardiorenal protection: potential cellular, biochemical, and molecular mechanisms. Am J Physiol Physiol. 2001;280(3):F365–88.

68. Parsa A, Kao WHL, Xie D, Astor BC, Li M, Hsu C, et al. APOL1 risk variants, race, and progression of chronic kidney disease. N Engl J Med. 2013;369(23):2183–96.

69. Stanifer JW, Kilonzo K, Wang D, Su G, Mao W, Zhang L, et al. Traditional medicines and kidney disease in low-and middle-income countries: opportunities and challenges. In: Seminars in Nephrology. Elsevier; 2017. p. 245–59.

70. Vart P, Gansevoort RT, Crews DC, Reijneveld SA, Bültmann U. Mediators of the association between low socioeconomic status and chronic kidney disease in the United States. Am J Epidemiol. 2015;181(6):385–96.

71. Mirrakhimov AE. Obstructive sleep apnea and kidney disease: is there any direct link? Sleep Breath. 2012;16(4):1009–16.

72. Ejerblad E, Fored CM, Lindblad P, Fryzek J, McLaughlin JK, Nyrén O. Obesity and risk for chronic renal failure. J Am Soc Nephrol. 2006;17(6):1695–702.

73. McClellan WM, Warnock DG, Judd S, Muntner P, Patzer RE, Bradbury BD, et al. Association of family history of ESRD, prevalent albuminuria, and reduced GFR with incident ESRD. Am J kidney Dis. 2012;59(1):25–31.

74. Zhang J, Thio CHL, Gansevoort RT, Snieder H. Familial aggregation of CKD and heritability of kidney biomarkers in the general population: the lifelines cohort study. Am J Kidney Dis. 2021;77(6):869–78.

75. Cañadas-Garre M, Anderson K, Cappa R, Skelly R, Smyth LJ, McKnight AJ, et al. Genetic susceptibility to chronic kidney disease–some more pieces for the heritability puzzle. Front Genet. 2019;10:453.

76. Pollak MR, Friedman DJ. The genetic architecture of kidney disease. Clin J Am Soc Nephrol. 2020;15(2):268–75.

77. Naik RP, Irvin MR, Judd S, Gutiérrez OM, Zakai NA, Derebail VK, et al. Sickle cell trait and the risk of ESRD in blacks. J Am Soc Nephrol. 2017;28(7):2180–7.

78. Köttgen A, Pattaro C, Böger CA, Fuchsberger C, Olden M, Glazer NL, et al. New loci associated with kidney function and chronic kidney disease. Nat Genet. 2010;42(5):376–84.

79. Webster AC, Nagler E V., Morton RL, Masson P. Chronic Kidney Disease. Lancet [Internet].

2017;389(10075):1238–52. Available from: http://dx.doi.org/10.1016/S0140-6736(16)32064-5

80. Tuegel C, Bansal N. Heart failure in patients with kidney disease. Heart. 2017;103(23):1848–53.

81. Gansevoort RT, Correa-Rotter R, Hemmelgarn BR, Jafar TH, Heerspink HJL, Mann JF, et al. Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention. Lancet. 2013;382(9889):339–52.

82. Thompson S, James M, Wiebe N, Hemmelgarn B, Manns B, Klarenbach S, et al. Cause of death in patients with reduced kidney function. J Am Soc Nephrol. 2015;26(10):2504–11.

83. Cherney DZI, Repetto E, Wheeler DC, Arnold S V, MacLachlan S, Hunt PR, et al. Impact of cardio-renal-metabolic comorbidities on cardiovascular outcomes and mortality in type 2 diabetes mellitus. Am J Nephrol. 2020;51(1):74–82.

84. Afkarian M, Sachs MC, Kestenbaum B, Hirsch IB, Tuttle KR, Himmelfarb J, et al. Kidney disease and increased mortality risk in type 2 diabetes. J Am Soc Nephrol. 2013;24(2):302–8.

85. Swanepoel CR, McCulloch MI, Abraham G, Donner JA, Alrukhaimi MN, Blake PG, et al. Challenges for sustainable end-stage kidney disease care in low-middle-income countries: the problem of the workforce. Kidney Int Suppl [Internet]. 2020;10(1):e49–54. Available from: https://doi.org/10.1016/j.kisu.2019.11.007

86. Outcomes KDIG, Group CKDW. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int. 2013;3(1):1–150.

87. Carville S, Wonderling D, Stevens P. Early identification and management of chronic kidney disease in adults: summary of updated NICE guidance. BMJ. 2014;349.

88. Kato M, Park JT, Natarajan R. MicroRNAs and the glomerulus. Exp Cell Res. 2012;318(9):993–1000.

89. Hsu C, Bansal N. Measured GFR as “gold standard”—all that glitters is not gold? Vol. 6, Clinical Journal of the American Society of Nephrology. Am Soc Nephrol; 2011. p. 1813–4.

90. Lopez-Giacoman S, Madero M. Biomarkers in chronic kidney disease, from kidney function to kidney damage. World J Nephrol. 2015 Feb;4(1):57–73.

91. Effersoe P. Relationship between endogenous 24-hour creatinine clearance and serum creatinine concentration in patients with chronic renal disease. Acta Med Scand. 1957;156(6):429–34.

92. Cockcroft DW, Gault H. Prediction of creatinine clearance from serum creatinine. Nephron. 1976;16(1):31–41.

93. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D, et al. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Ann Intern Med. 1999;130(6):461–70.

94. Levey AS, Coresh J, Greene T, Marsh J, Stevens LA, Kusek JW, et al. Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. Clin Chem. 2007;53(4):766–72.

95. Levey AS, Stevens LA, Schmid CH, Zhang Y, Castro III AF, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604–12.

96. Earley A, Miskulin D, Lamb EJ, Levey AS, Uhlig K. Estimating equations for glomerular filtration rate in the era of creatinine standardization: a systematic review. Ann Intern Med. 2012;156(11):785–95.

97. Gama RM, Clery A, Griffiths K, Heraghty N, Peters AM, Palmer K, et al. Estimated glomerular filtration rate equations in people of self-reported black ethnicity in the United Kingdom: Inappropriate adjustment for ethnicity may lead to reduced access to care. PLoS One [Internet]. 2021 Aug 1 [cited 2022 Jun 5];16(8). Available from: /pmc/articles/PMC8360513/

98. Bukabau JB, Yayo E, Gnionsahé A, Monnet D, Pottel H, Cavalier E, et al. Performance of creatinine-or cystatin C–based equations to estimate glomerular filtration rate in sub-Saharan African populations. Kidney Int. 2019;95(5):1181–9.

99. Holness JL, Bezuidenhout K, Davids MR, Warwick JM. Validation of equations to estimate glomerular filtration rate in South Africans of mixed ancestry. S Afr Med J. 2020;110(3):229–34.

100. Inker LA, Shafi T, Okparavero A, Tighiouart H, Eckfeldt JH, Katz R, et al. Effects of race and sex on measured GFR: the multi-ethnic study of atherosclerosis. Am J Kidney Dis. 2016;68(5):743–51.

101. Stevens LA, Schmid CH, Greene T, Li L, Beck GJ, Joffe MM, et al. Factors other than glomerular filtration rate affect serum cystatin C levels. Kidney Int. 2009;75(6):652–60.

102. Rysz J, Gluba-Brzózka A, Franczyk B, Jabłonowski Z, Ciałkowska-Rysz A. Novel biomarkers in the diagnosis of chronic kidney disease and the prediction of its outcome. Int J Mol Sci. 2017;18(8):1702.

103. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med. 2012;367(1):20–9.

104. Inker LA, Eneanya ND, Coresh J, Tighiouart H, Wang D, Sang Y, et al. New creatinine-and cystatin C–based equations to estimate GFR without race. N Engl J Med. 2021;385(19):1737–49.

105. Glaser N, Deckert A, Phiri S, Rothenbacher D, Neuhann F. Comparison of various equations for estimating GFR in Malawi: how to determine renal function in resource limited settings? PLoS One. 2015;10(6):e0130453.

106. Fabian J, Kalyesubula R, Mkandawire J, Hansen CH, Nitsch D, Musenge E, et al. Measurement of kidney function in Malawi, South Africa, and Uganda: a multicentre cohort study. Lancet Glob Heal. 2022;10(8):e1159–69.

107. Hallan SI, Ritz E, Lydersen S, Romundstad S, Kvenild K, Orth SR. Combining GFR and albuminuria to classify CKD improves prediction of ESRD. J Am Soc Nephrol. 2009;20(5):1069–77.

108. Lin C-H, Chang Y-C, Chuang L-M. Early detection of diabetic kidney disease: Present limitations and future perspectives. World J Diabetes. 2016;7(14):290.

109. Al-Rubeaan K, Siddiqui K, Al-Ghonaim MA, Youssef AM, Al-Sharqawi AH, AlNaqeb D. Assessment of the diagnostic value of different biomarkers in relation to various stages of diabetic nephropathy in type 2 diabetic patients. Sci Rep. 2017;7(1):1–9.

110. Waddington CH. The epigenotype. Endeavour. 1942;1:18–20.

111. Waddington CH. Towards a Theoretical Biology: Symposia Organized by the International Union of Biological Sciences: Prolegomena. Bellagio, 28 August to 3 September 1966. Edinburgh University Press; 1968.

112. Wing MR, Ramezani A, Gill HS, Devaney JM, Raj DS. Epigenetics of Progression of Chronic Kidney Disease: Fact or Fantasy? Semin Nephrol [Internet]. 2013;33(4):363–74. Available from: http://dx.doi.org/10.1016/j.semnephrol.2013.05.008

113. Smyth LJ, Duffy S, Maxwell AP, McKnight AJ. Genetic and epigenetic factors influencing chronic kidney disease. Am J Physiol - Ren Physiol. 2014;307(7):757–76.

114. Wang C, Xu G, Wen Q, Peng X, Chen H, Zhang J, et al. CBS promoter hypermethylation increases the risk of hypertension and stroke. Clinics. 2019;74(16).

115. Magro VM. A New Approach in the Study of Hypertension in the Elderly Subject: The Role of Epigenetics. Arch Neurol Neurosci. 2019;2(4):0–2.

116. Palazzo AF, Lee ES. Non-coding RNA: What is functional and what is junk? Front Genet. 2015;5(JAN).

117. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Front Endocrinol (Lausanne). 2018;9:402.

118. Micheel J, Safrastyan A, Wollny D. Advances in Non-Coding RNA Sequencing. Non-coding RNA. 2021;7(4):70.

119. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, et al. Initial sequencing and analysis of the human genome. 2001;

120. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, et al. The sequence of the human genome. Science (80-). 2001;291(5507):1304–51.

121. Kumar V, Abbas AK, Aster JC. Robbins basic pathology e-book. Elsevier Health Sciences; 2017.

122. Consortium EP. An integrated encyclopedia of DNA elements in the human genome. Nature. 2012;489(7414):57.

123. Brandenburger T, Salgado Somoza A, Devaux Y, Lorenzen JM. Noncoding RNAs in acute kidney injury. Kidney Int [Internet]. 2018;94(5):870–81. Available from: https://doi.org/10.1016/j.kint.2018.06.033

124. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. Nat Rev Genet. 2009;10(3):155–9.

125. de Pontual L, Yao E, Callier P, Faivre L, Drouin V, Cariou S, et al. Germline deletion of the miR-17∼ 92 cluster causes skeletal and growth defects in humans. Nat Genet. 2011;43(10):1026–30.

126. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993;75(5):843–54.

127. Wightman B, Ha I, Ruvkun G. Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. Cell. 1993;75(5):855–62.

128. Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, et al. The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature. 2000;403(6772):901–6.

129. Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, et al. Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature. 2000;408(6808):86–9.

130. Slack FJ, Basson M, Liu Z, Ambros V, Horvitz HR, Ruvkun G. The lin-41 RBCC gene acts in the C. elegans heterochronic pathway between the let-7 regulatory RNA and the LIN-29

transcription factor. Mol Cell. 2000;5(4):659–69.

131. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. Science (80-). 2001;294(5543):853–8.

132. Krek A, Grün D, Poy MN, Wolf R, Rosenberg L, Epstein EJ, et al. Combinatorial microRNA target predictions. Nat Genet. 2005;37(5):495–500.

133. Bonneau E, Neveu B, Kostantin E, Tsongalis GJ, De Guire V. How close are miRNAs from clinical practice? A perspective on the diagnostic and therapeutic market. Electron J Int Fed Clin Chem Lab Med. 2019;30(2):114–27.

134. Gomes CPDC, Cho J-H, Hood LE, Franco OL, Pereira RWD, Wang K. A review of computational tools in microRNA discovery. Front Genet. 2013;4:81.

135. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature. 1998;391(6669):806–11.

136. Fire AZ, Mello CC. The nobel prize in physiology or medicine 2006. Nobel Media AB 2014. 2006;

137. Griffiths-Jones S. The microRNA registry. Nucleic Acids Res. 2004;32(suppl_1):D109–11.

138. Kozomara A, Griffiths-Jones S. miRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res. 2014;42(D1):D68–73.

139. Kozomara A, Birgaoanu M, Griffiths-Jones S. MiRBase: From microRNA sequences to function. Nucleic Acids Res. 2019;47(D1):D155–62.

140. Tuschl T. RNA interference and small interfering RNAs. Chembiochem. 2001;2(4):239–45.

141. Lee Y, Kim M, Han J, Yeom K, Lee S, Baek SH, et al. MicroRNA genes are transcribed by RNA polymerase II. EMBO J. 2004;23(20):4051–60.

142. Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, et al. The nuclear RNase III Drosha initiates microRNA processing. Nature. 2003;425(6956):415–9.

143. Ambros V, Bartel B, Bartel DP, Burge CB, Carrington JC, Chen X, et al. A uniform system for microRNA annotation. Rna. 2003;9(3):277–9.

144. Schwarz DS, Hutvágner G, Du T, Xu Z, Aronin N, Zamore PD. Asymmetry in the assembly of the RNAi enzyme complex. Cell. 2003;115(2):199–208.

145. Bagga S, Bracht J, Hunter S, Massirer K, Holtz J, Eachus R, et al. Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. Cell. 2005;122(4):553–63.

146. Matranga C, Tomari Y, Shin C, Bartel DP, Zamore PD. Passenger-strand cleavage facilitates assembly of siRNA into Ago2-containing RNAi enzyme complexes. Cell. 2005;123(4):607–20.

147. Hajarnis S, Lakhia R, Patel V. MicroRNAs and polycystic kidney disease. Exon Publ. 2015;313–34.

148. Guo H, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature. 2010;466(7308):835–40.

149. Lorenzen JM, Haller H, Thum T. MicroRNAs as mediators and therapeutic targets in chronic kidney disease. Nat Rev Nephrol [Internet]. 2011;7(5):286–94. Available from: http://dx.doi.org/10.1038/nrneph.2011.26

150. Yousef M, Showe L, Showe M. A study of microRNAs in silico and in vivo: bioinformatics approaches to microRNA discovery and target identification. FEBS J. 2009;276(8):2150–6.

151. Weber JA, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, et al. The microRNA spectrum in 12 body fluids. Clin Chem. 2010;56(11):1733–41.

152. Tang Y, Liu D, Zhang L, Ingvarsson S, Chen H. Quantitative analysis of miRNA expression in seven human foetal and adult organs. PLoS One. 2011;6(12):e28730.

153. Hippen KL, Loschi M, Nicholls J, MacDonald K, Blazar BR. Effects of microRNA on regulatory T cells and implications for adoptive cellular therapy to ameliorate graft-versus-host disease. Front Immunol. 2018;9:57.

154. Hsu PWC, Huang H-D, Hsu S-D, Lin L-Z, Tsou A-P, Tseng C-P, et al. miRNAMap: genomic maps of microRNA genes and their target genes in mammalian genomes. Nucleic Acids Res. 2006;34(suppl_1):D135–9.

155. Baskerville S, Bartel DP. Microarray profiling of microRNAs reveals frequent coexpression with neighboring miRNAs and host genes. Rna. 2005;11(3):241–7.

156. Ludwig N, Leidinger P, Becker K, Backes C, Fehlmann T, Pallasch C, et al. Distribution of miRNA expression across human tissues. Nucleic Acids Res. 2016;44(8):3865–77.

157. Kent OA, McCall MN, Cornish TC, Halushka MK. Lessons from miR-143/145: the importance of cell-type localization of miRNAs. Nucleic Acids Res. 2014;42(12):7528–38.

158. Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, et al. A mammalian microRNA expression atlas based on small RNA library sequencing. Cell. 2007;129(7):1401–14.

159. Chim SSC, Shing TKF, Hung ECW, Leung T, Lau T, Chiu RWK, et al. Detection and

characterization of placental microRNAs in maternal plasma. Clin Chem. 2008;54(3):482–90.

160. Lawrie CH, Gal S, Dunlop HM, Pushkaran B, Liggins AP, Pulford K, et al. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br J Haematol. 2008;141(5):672–5.

161. Londina E, Lohera P, Telonis AG, Quann K, Clark P, Jinga Y, et al. Analysis of 13 cell types reveals evidence for the expression of numerous novel primate- And tissue-specific microRNAs. Proc Natl Acad Sci U S A. 2015;112(10):E1106–15.

162. Chen X, Liang H, Zhang J, Zen K, Zhang C-Y. Secreted microRNAs: a new form of intercellular communication. Trends Cell Biol. 2012;22(3):125–32.

163. Condrat CE, Thompson DC, Barbu MG, Bugnar OL, Boboc A, Cretoiu D, et al. miRNAs as Biomarkers in Disease: Latest Findings Regarding Their Role in Diagnosis and Prognosis. Cells. 2020;9(2):276.

164. Burgos KL, Javaherian A, Bomprezzi R, Ghaffari L, Rhodes S, Courtright A, et al. Identification of extracellular miRNA in human cerebrospinal fluid by next-generation sequencing. Rna. 2013;19(5):712–22.

165. Smoczynska A, Sega P, Stepien A, Knop K, Jarmolowski A, Pacak A, et al. miRNA detection by stem-loop RT-qPCR in studying microRNA biogenesis and microRNA responsiveness to abiotic stresses. In: Plant MicroRNAs. Springer; 2019. p. 131–50.

166. Chen C, Ridzon DA, Broomer AJ, Zhou Z, Lee DH, Nguyen JT, et al. Real-time quantification of microRNAs by stem–loop RT–PCR. Nucleic Acids Res. 2005;33(20):e179–e179.

167. Fiedler SD, Carletti MZ, Christenson LK. Quantitative RT-PCR methods for mature microRNA expression analysis. In: RT-PCR Protocols. Springer; 2010. p. 49–64.

168. Li W, Ruan K. MicroRNA detection by microarray. Anal Bioanal Chem. 2009;394(4):1117–24.

169. Ravegnini G, Cargnin S, Sammarini G, Zanotti F, Bermejo JL, Hrelia P, et al. Prognostic role of miR-221 and miR-222 expression in cancer patients: a systematic review and meta-analysis. Cancers (Basel). 2019;11(7):970.

170. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci. 2002;99(24):15524–9.

171. Iorio M V, Ferracin M, Liu C-G, Veronese A, Spizzo R, Sabbioni S, et al. MicroRNA gene expression deregulation in human breast cancer. Cancer Res. 2005;65(16):7065–70.

172. Zheng H, Liu J-Y, Song F-J, Chen K-X. Advances in circulating microRNAs as diagnostic and prognostic markers for ovarian cancer. Cancer Biol Med. 2013;10(3):123.

173. Porkka KP, Pfeiffer MJ, Waltering KK, Vessella RL, Tammela TLJ, Visakorpi T. MicroRNA expression profiling in prostate cancer. Cancer Res. 2007;67(13):6130–5.

174. Hollis M, Nair K, Vyas A, Chaturvedi LS, Gambhir S, Vyas D. MicroRNAs potential utility in colon cancer: Early detection, prognosis, and chemosensitivity. World J Gastroenterol WJG. 2015;21(27):8284.

175. Yu H, Guan Z, Cuk K, Brenner H, Zhang Y. Circulating microRNA biomarkers for lung cancer detection in Western populations. Cancer Med. 2018;7(10):4849–62.

176. Serafin A, Foco L, Zanigni S, Blankenburg H, Picard A, Zanon A, et al. Overexpression of blood microRNAs 103a, 30b, and 29a in l-dopa–treated patients with PD. Neurology. 2015;84(7):645–53.

177. Freiesleben S, Hecker M, Zettl UK, Fuellen G, Taher L. Analysis of microRNA and gene expression profiles in multiple sclerosis: integrating interaction data to uncover regulatory mechanisms. Sci Rep. 2016;6(1):1–14.

178. Wang W-X, Rajeev BW, Stromberg AJ, Ren N, Tang G, Huang Q, et al. The expression of microRNA miR-107 decreases early in Alzheimer's disease and may accelerate disease progression through regulation of β-site amyloid precursor protein-cleaving enzyme 1. J Neurosci. 2008;28(5):1213–23.

179. Cheng C, Wang Q, You W, Chen M, Xia J. MiRNAs as biomarkers of myocardial infarction: a meta-analysis. PLoS One. 2014;9(2):e88566.

180. Sun Y, Koo S, White N, Peralta E, Esau C, Dean NM, et al. Development of a micro-array to detect human and mouse microRNAs and characterization of expression in human organs. Nucleic Acids Res. 2004;32(22):e188–e188.

181. Liang M, Liu Y, Mladinov D, Cowley AW, Trivedi H, Fang Y, et al. MicroRNA: A new frontier in kidney and blood pressure research. Am J Physiol - Ren Physiol. 2009;297(3).

182. Harvey SJ, Jarad G, Cunningham J, Goldberg S, Schermer B, Harfe BD, et al. Podocyte-specific deletion of dicer alters cytoskeletal dynamics and causes glomerular disease. J Am Soc Nephrol [Internet]. 2008 Nov;19(11):2150–8. Available from: https://search.ebscohost.com/login.aspx?direct=true&db=cmedm&AN=18776121&site=ehost-live

183. Neal CS, Michael MZ, Pimlott LK, Yong TY, Li JYZ, Gleadle JM. Circulating microRNA expression is reduced in chronic kidney disease. Nephrol Dial Transplant. 2011;26(11):3794–

802.

184. Fujii R, Yamada H, Yamazaki M, Munetsuna E, Ando Y, Ohashi K, et al. Circulating microRNAs (miR-126, miR-197, and miR-223) are associated with chronic kidney disease among elderly survivors of the Great East Japan Earthquake. BMC Nephrol. 2019;20(1):1–7.

185. Kato M, Zhang J, Wang M, Lanting L, Yuan H, Rossi JJ, et al. MicroRNA-192 in diabetic kidney glomeruli and its function in TGF-β-induced collagen expression via inhibition of E-box repressors. Proc Natl Acad Sci. 2007;104(9):3432–7.

186. Kato M, Arce L, Wang M, Putta S, Lanting L, Natarajan R. A microRNA circuit mediates transforming growth factor-β1 autoregulation in renal glomerular mesangial cells. Kidney Int. 2011;80(4):358–68.

187. Bhatt K, Kato M, Natarajan R. Mini-review: Emerging roles of microRNAs in the pathophysiology of renal diseases. Am J Physiol - Ren Physiol. 2016;310(2):F109–18.

188. Chau BN, Xin C, Hartner J, Ren S, Castano AP, Linn G, et al. MicroRNA-21 promotes fibrosis of the kidney by silencing metabolic pathways. Sci Transl Med. 2012;4(121):121ra18-121ra18.

189. Bhatt K, Lanting LL, Jia Y, Yadav S, Reddy MA, Magilnick N, et al. Anti-Inflammatory Role of MicroRNA-146a in the Pathogenesis of Diabetic Nephropathy. J Am Soc Nephrol [Internet]. 2016 Aug;27(8):2277–88. Available from: https://search.ebscohost.com/login.aspx?direct=true&db=c8h&AN=131893492&site=ehost-live

190. Zang J, Maxwell AP, Simpson DA, McKay GJ. Differential Expression of Urinary Exosomal MicroRNAs miR-21-5p and miR-30b-5p in Individuals with Diabetic Kidney Disease. Sci Rep [Internet]. 2019;9(1):1–10. Available from: http://dx.doi.org/10.1038/s41598-019-47504-x

191. Kölling M, Kaucsar T, Schauerte C, Hübner A, Dettling A, Park J-K, et al. Therapeutic miR-21 Silencing Ameliorates Diabetic Kidney Disease in Mice. Mol Ther [Internet]. 2017 Jan 4;25(1):165–80. Available from: https://search.ebscohost.com/login.aspx?direct=true&db=cmedm&AN=28129112&site=ehost-live

192. Wang G, Kwan BCH, Lai FMM, Choi PCL, Chow KM, Li PKT, et al. Intrarenal expression of miRNAs in patients with hypertensive nephrosclerosis. Am J Hypertens [Internet]. 2010;23(1):78–84. Available from: http://dx.doi.org/10.1038/ajh.2009.208

193. Liu Y, Taylor NE, Lu L, Usa K, Cowley Jr AW, Ferreri NR, et al. Renal medullary microRNAs in Dahl salt-sensitive rats: miR-29b regulates several collagens and related genes.

Hypertension. 2010;55(4):974–82.

194. Lu Q, Ma Z, Ding Y, Bedarida T, Chen L, Xie Z, et al. Circulating miR-103a-3p contributes to angiotensin II-induced renal inflammation and fibrosis via a SNRK/NF-κB/p65 regulatory axis. Nat Commun. 2019;10(1).

195. Taylor CR. Introduction to predictive biomarkers: definitions and characteristics. In: Predictive biomarkers in oncology. Springer; 2019. p. 3–18.

196. Chandramouli K, Qian P-Y. Proteomics: challenges, techniques and possibilities to overcome biological sample complexity. Hum genomics proteomics HGP. 2009;2009.

197. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007;9(6):654–9.

198. Krauskopf J, Verheijen M, Kleinjans JC, de Kok TM, Caiment F. Development and regulatory application of microRNA biomarkers. Biomark Med. 2015;9(11):1137–51.

199. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res. 2008;18(10):997–1006.

200. Rosenfeld N, Aharonov R, Meiri E, Rosenwald S, Spector Y, Zepeniuk M, et al. MicroRNAs accurately identify cancer tissue origin. Nat Biotechnol. 2008;26(4):462–9.

201. Mohan A, Singh RS, Kumari M, Garg D, Upadhyay A, Ecelbarger CM, et al. Urinary Exosomal microRNA-451-5p Is a Potential Early Biomarker of Diabetic Nephropathy in Rats. PLoS One [Internet]. 2016 Apr 21;11(4):1–18. Available from: http://10.0.5.91/journal.pone.0154055

202. Metias SM, Lianidou E, Yousef GM. MicroRNAs in clinical oncology: at the crossroads between promises and problems. J Clin Pathol. 2009;62(9):771–6.

203. Farooqi AA, Fayyaz S, Shatynska-Mytsyk I, Javed Z, Jabeen S, Yaylim I, et al. Is miR-34a a Well-equipped Swordsman to Conquer Temple of Molecular Oncology? Chem Biol Drug Des. 2016;87(3):321–34.

204. Bajan S, Hutvagner G. RNA-Based Therapeutics: From Antisense Oligonucleotides to miRNAs. Cells. 2020;9(1):137.

205. Li Z, Rana TM. Therapeutic targeting of microRNAs: current status and future challenges. Nat Rev Drug Discov. 2014;13(8):622–38.

206. Janssen HLA, Reesink HW, Lawitz EJ, Zeuzem S, Rodriguez-Torres M, Patel K, et al.

Treatment of HCV infection by targeting microRNA. N Engl J Med. 2013;368(18):1685–94.

207. Harmanci D, Erkan EP, Kocak A, Akdogan GG. Role of the microRNA-29 family in fibrotic skin diseases. Biomed reports. 2017;6(6):599–604.

CHAPTER 3

Protocol: Published by BMJ Open Journal

3. SYSTEMATIC REVIEW PROTOCOL

MicroRNAs associated with chronic kidney disease in the general population and high-risk subgroups: protocol for a systematic review and meta-analysis

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Keywords: chronic kidney disease; microRNAs; kidney function; kidney damage

ABSTRACT

Introduction: Chronic kidney disease (CKD) is a significant health and economic burden, owing to its ever-increasing global prevalence. Due to the limitations in the current diagnostic methods, CKD is frequently diagnosed at advanced stages, where there is an increased risk of cardiovascular complications and end-stage kidney disease. As such, there has been considerable interest in microRNAs (miRNAs) as potential markers for CKD detection. This review seeks to identify all miRNAs associated with CKD and/or markers of kidney function or kidney damage in the general population and high-risk subgroups and explore their expression profiles in these populations.

Methods and analysis: A systematic search of published literature will be conducted for observational studies that report on miRNAs associated with CKD or kidney function or kidney damage markers (serum creatinine and cystatin C, eGFR and urinary albumin excretion) in adult humans. The electronic database search will be restricted to English and French publications up to 31 October 2021. Two investigators will independently screen and identify studies for inclusion, as well as extract data from eligible studies. Risk of bias and methodological quality will be assessed by the Newcastle-Ottawa Quality Assessment Scale for observational studies and Grading of Recommendations Assessment, Development and Evaluation tools. Appropriate meta-analytic techniques will be used to pool estimates from studies with similar miRNAs, overall and by major characteristics, including by country or region, sample size, gender, and risk of bias score. Heterogeneity of the estimates across studies will be quantified and publication bias investigated. This protocol is reported according to Preferred Reporting Items for Systematic reviews and Meta-Analysis protocols (PRISMA-P) 2015 guidelines.

Ethics and dissemination: This study design does not require formal ethical clearance and findings will be published in a peer-reviewed journal.

Conclusion: This review will provide the expression pattern of miRNAs associated with CKD. This will allow for further research into the identified miRNAs, which could later be used as biomarkers for prediction and early detection of CKD, monitoring of disease progression to advanced stages and as potential therapeutic targets.

Registration: PROSPERO (Reference no: CRD42021270028).

STRENGTHS AND LIMITATIONS OF THIS STUDY

- The systematic review and meta-analysis will follow the PRISMA guidelines
- The review will include studies that analysed miRNAs associated with CKD in the general population and high-risk subgroups
- Study eligibility and data extraction will be conducted by two reviewers independently, with a third reviewer to resolve any inconsistencies or disagreement.
- The quality of individual studies will be assessed using a risk of bias tool
- Significant inter-study methodological variations in analysing miRNA expression

3.1. Introduction

Chronic kidney disease (CKD) is a major public health concern globally and continues to be a significant socio-economic and healthcare burden worldwide[1]. This indirectly results from the association of CKD with adverse health outcomes, including cardiovascular disease (CVD), which is a major cause of morbidity and mortality[1]. Studies have shown that CVDs are the leading cause of death in people with CKD, and the risk of death is even higher in individuals with advanced CKD[2,3]. Another major health outcome of CKD is end-stage kidney disease (ESKD), for which individuals require costly kidney replacement therapy for survival, and whose availability is limited in developing countries[4]. According to the 2020 Global Burden of Disease (GBD) report, the period between 1990 and 2017 saw a 29.1% increase in the prevalence of CKD, with the current estimated prevalence being 9.1%[5]. The rise in CKD cases is partly attributable to the high burden of diabetes mellitus (DM) and hypertension (HTN), the major causes of CKD, and other causes include human immunodeficiency virus (HIV) and advanced age[5]. As highlighted in the latest GBD report, greater promise in effectively dealing with the burden of CKD will be seen through implementing the National Strategic Action Plans for kidney disease in all countries, which includes early detection and prevention of CKD in individuals at high risk[6]. However, due to the absence of symptoms in the early stages of CKD, diagnosis is usually made when the disease has progressed to advanced stages that are associated with higher risk of CVD mortality and ESKD[7]. Therefore, early identification of kidney dysfunction will allow for early treatment initiation, thereby preventing or delaying CKD advancement.

Currently, the diagnosis of CKD lies heavily on the estimation of GFR (eGFR) from serum creatinine or cystatin C and/or markers of kidney damage, such as the level of albuminuria, with kidney biopsies done only in rare instances to confirm a diagnosis[7]. However, these markers have several well-established limitations. For example, serum creatinine used for the estimation of GFR is not specific to kidney disease, as creatinine is a waste product of skeletal muscle metabolism and therefore may be affected by other factors such as age, gender, race, body mass index (BMI) and diet[8]. The use of albuminuria is limited during the early stages of CKD as there is minimal damage to the kidneys and

albuminuria is seldom detectable at this stage[9]. Although kidney biopsies can be utilized to confirm a diagnosis, they are highly invasive, expensive, and not recommended for repeated and population-based testing[7]. Moreover, other potential biomarkers for diagnosis and prognosis of CKD, such as asymmetric dimethylarginine, kidney injury molecule 1 and proteomic and metabolic biomarkers, have been explored, as reviewed by Rysz et al (2017)[8]. However, these markers have not been validated in clinical practice. Consequently, the need for new biomarkers that will facilitate early detection of CKD, prediction of CKD progression and monitoring responses to treatment cannot be overstated.

A biomarker is a molecule that can be objectively measured and evaluated in human body fluid to reflect the presence or absence of disease as well as response to treatment. Therefore, an ideal biomarker must meet certain qualities such as, it must be easily accessible, disease-specific, sensitive, and translatable from research to clinic[10]. Ever since the initial detection of microRNA (miRNA) in the blood of individuals with cancer in a highly stable manner[11], miRNAs have emerged as promising diagnostic and prognostic biomarkers for a wide range of diseases. As of 2018, approximately 11000 papers had been published on the potential role of miRNAs in various diagnostic fields[12]. miRNAs are small non-coding transcripts that regulate gene expression post-transcriptionally, through their binding of target messenger ribonucleic acid RNA (mRNA) on the 3' untranslated region (UTR), degrading the mRNA or inhibiting its translation into proteins[13]. Various properties of miRNAs make them attractive propositions as biomarkers of disease and these include their tissue and disease-specific expression[14], detectability in body fluids that can be accessed in a minimally invasive manner[13], relative stability in biofluids as they are released into circulation enclosed in microvesicles and/or bound to proteins and lipids, thereby protecting them from degradation by RNAses[11]. Additionally, miRNAs can be easily quantified by sensitive molecular techniques such as quantitative reverse transcription polymerase chain reaction (RT-qPCR)[15].

Studies have shown that the expression of some miRNAs are specific to the human kidneys and may be involved in the development, homeostasis, and physiology of the kidneys[16]. Therefore, dysregulation in the expression of these miRNAs may interfere with the normal kidney function, resulting in the development of kidney pathology. A significant number of studies have described the potential role of miRNAs in the pathogenesis of CKD[17,18], diabetic kidney disease (DKD)[19,20], hypertensive nephropathy[21,22] and HIV associated nephropathy[23,24]. However, findings from the majority of these studies are contradictory and inconclusive, with further research warranted. We intend to conduct a systematic review and meta-analysis of observational studies to establish which miRNAs are associated with CKD (any stage of CKD) and/or measures of kidney function and/or damage (serum creatinine, serum cystatin C, eGFR and urinary albumin excretion (UAE) in the general population, as well as in high-risk subgroups (HTN, DM and HIV-infected). In addition, the

review aims to report on the expression profiles of these miRNAs in CKD (general population and HTN, DM and HIV-associated CKD). Furthermore, if data allows, the expression patterns of the identified miRNAs will be compared in different human sample types (whole blood, plasma, serum, platelet-poor plasma, exosomes, urine, and kidney tissue) as well as evaluating the expression profile of the identified miRNAs at various stages of CKD (early [stage 1-3], advanced [stage 4] and ESKD [stage 5]).

3.1.1. Review questions

The purpose of this review is to address the following questions:

1. Which miRNAs are associated with CKD in, (a) the general population and high-risk individuals with (b) HTN, (c) DM and (d) HIV infection?
2. Which miRNAs are associated with measures of kidney function and kidney damage, including serum creatinine and cystatin C, as well as eGFR and UAE?
3. What are the expression patterns of the identified miRNAs in CKD? In other words, are they up-regulated or down-regulated?
4. Do the expression patterns of the identified miRNAs differ depending on the human sample type used? The different sample types include whole blood, plasma, serum, platelet-poor plasma, exosomes, urine, and kidney tissue.
5. Does the expression profile of the identified miRNAs differ depending on the stage of CKD? The stages will include early (CKD stages 1-3), advanced (CKD stage 4) and ESRD (stage 5).

3.2. Methods and analysis

Reporting of this review

This proposed systematic review and meta-analysis will be conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[25]. The methods of the analysis and inclusion criteria have been specified in advance and documented in a protocol in the PROSPERO database (Reference no: CRD42021270028).

3.2.1. Eligibility criteria

Inclusion criteria

Published peer-reviewed studies (cross-sectional, case-control and cohort) reporting on miRNAs associated with CKD and/or kidney function or kidney damage markers (serum creatinine, serum cystatin C, eGFR and UAE) in the general adult population and/or high-risk subgroups (HTN, DM, HIV-infection). Chronic kidney disease will be defined as an eGFR <60mL/min/1.73 m^2 and/or albuminuria (UAE, ≥3 mg/mmol). All studies reported in the English and French languages and conducted on human participants will be considered. All studies need to describe the characteristics of

the study population, include the methods used to classify CKD, have determined miRNA expression by using microarrays, RT-qPCR, next-generation sequencing technology and/or northern blot hybridization, in addition to the use of an endogenous/exogenous control for normalization of miRNA expression data in kidney tissue, whole blood, serum, plasma, platelet-poor plasma, exosomes or urine.

Exclusion criteria

Studies will be excluded if they do not contain primary data obtained from adult human samples. Thus reviews, case reports, newspaper articles, letters to the editor, editorials, commentaries, book chapters and studies that are unpublished or deposited in preprint servers will be excluded. Studies reporting miRNA expression in animals, animal disease models and cell lines, acute kidney disease, causes of CKD other than HTN, DM or HIV infection, pregnant women and participants under the age of 18 years will also be excluded. Moreover, studies will be excluded if a control group or an endogenous/exogenous control for normalization of miRNA expression data was not included when conducting the investigation or published in languages other than English or French.

3.2.2. Search strategy

We will search for potentially eligible studies in Medline (via PubMed), Scopus, Web of Science and EBSCOhost databases. The search will be conducted using a predefined comprehensive and sensitive search strategy combining relevant terms to obtain the maximum possible number of studies, which will be restricted to studies up to 31 October 2021. The following Medical Subject Headings (MeSH) terms and/or phrases, “microRNAs, miRNA, miRNAs, chronic kidney disease, CKD, chronic kidney injury, chronic renal disease, chronic renal injury, renal failure, end-stage renal disease, diabetic kidney disease, diabetic nephropathy, hypertensive nephrosclerosis, chronic kidney failure, chronic renal failure, end-stage renal failure, HIV-associated nephropathy, HIVAN, HIV-associated renal disease, HIV-associated kidney disease” together with Boolean operators (AND/OR/NOT) will be applied to identify relevant studies. Table 2.1 depicts the main search strategy to be employed. A manual search of publication reference lists from eligible studies for additional relevant literature will also be conducted.

Table 1: *Search strategy to be employed*

Search	Query	Number of hits
#1	(Chronic kidney disease) OR (chronic kidney failure) OR (chronic renal disease) OR (chronic renal failure) OR (end-stage renal disease) OR (end-stage renal failure) OR (diabetic kidney disease) OR (diabetic nephropathy) OR (hypertensive nephrosclerosis) OR albuminuria OR proteinuria OR (HIV associated nephropathy) OR HIVAN OR (HIV-associated kidney disease)	
#2	(Serum creatinine) OR (serum cystatin C) OR (estimated glomerular filtration rate) OR (urinary albumin excretion rate) OR (albumin-to-creatinine ratio) OR (urinary albumin)	
#3	microRNAs, miRNA, miRNAs	
#4	animal OR rat OR mouse OR (cell-line)	
#5	cancer OR (acute kidney injury)	
#6	#1 OR #2	
#7	#6 AND #3	
#8	#7 NOT #4	
#9	#8 NOT #5	

Identified studies will be uploaded into the citation management database Mendeley (London, UK), and the duplicate check function used to identify citations retrieved from multiple sources. Unique citations will be uploaded into the systematic review software, Covidence (Covidence, Melbourne, Australia), and independently screened, in a sequential manner (title, abstract, full text) by two authors (DDM and DMM). The latest citation version of the selected studies will be searched to exclude retracted papers or outdated versions of corrected papers. In instances of disagreements, a third author (CG) will arbitrate for eligibility. Reasons for exclusion of non-eligible studies will be documented and summarized in a flow chart.

3.2.3. Data extraction

Two authors (DDM and DMM) will independently perform the data extraction, by using a predetermined data extraction sheet. Any inconsistencies or disagreement will be resolved by consensus or consultation with a third author (CG).

The data to be extracted will include the following: publication details (name of the first author, year of publication, country); study details (design, sample size, demographics [ethnicity, age, sex, nationality], disease outcome [CKD (of unspecified cause), or DKD/HTN-associated CKD/HIVAN or

ESKD] and population [general population or high risk subgroups]); participant clinical characteristics (body mass index [BMI], C-reactive protein [CRP], smoking status, alcohol consumption, lipid profile (low density lipoprotein [LDL], high density lipoprotein [HDL], triglycerides and total cholesterol) and comorbidities [HTN, DM and HIV]); clinical outcomes (diagnostic criteria, classification/staging, duration of disease [if applicable], medication status); CKD diagnostic criteria (eGFR or proteinuria/albuminuria and eGFR equation used); miRNA analysis (sample type, molecular techniques [RNA extraction, assessment of RNA quality, cDNA synthesis, miRNA expression quantification], inclusion of screening and validation cohorts for miRNA analysis, expression pattern (up- or downregulated) of differentially expressed miRNAs and normalization control used; statistical analysis (tests, adjustments made for confounding variables [if any]).

3.2.4. Assessment of methodological quality and risk of bias

Two authors (DDM and DMM) will independently score the quality of included studies. The Grading of Recommendations Assessment, Development and Evaluation (GRADE)[26] will be used to grade the quality of evidence of each paper, as "very low" to "high" and the strength of recommendations as "strong" or "weak". The risk of bias will be assessed using the Newcastle-Ottawa Quality Assessment Scale for observational studies (NOS) tools[27]. The NOS tool assesses the risk of bias based on the critical appraisal of three domains, namely: - i) participant group selection; ii) how comparable the groups are; and iii) determination of the exposure of interest as shown in (Table 2.1). The domains consist of 9 quality items that will be judged using a scoring system. An asterisk (*) will be assigned to each quality item, serving as a visual assessment of that item. The manner in which an asterisk is assigned is such that studies of the highest quality will have the highest number of asterisks assigned to them. As illustrated in Table 2.2, studies will be judged as having a low, moderate and high risk of bias if their total number of assigned asterisks is seven and greater, five or six, and less than five respectively. Disagreements on final study assessments will be resolved with the aid of discussions involving the third reviewer (CG).

Table 2.1: *Domain risk of bias and applicability assessment using the NOS tool*

RISK OF BIAS		Stars awarded
Selection		**Total (4)**
Is the case definition adequate? a) Yes, with independent validation * b) Yes, for example - record linkage or based on self-reports c) No description		
Representativeness of the cases a) Consecutive or obviously representative series of cases * b) Potential for selection biases or not stated		
Selection of Controls a) Community controls * b) Hospital controls c) No description		
Definition of Controls a) No history of disease (end-point) * b) No description of source		
Comparability		**Total (2)**
Comparability of cohorts on the basis of the design or analysis a) Study controls for.....(select the most important factor) * b) Study controls for any additional factor *		
Exposure		**Total (3)**
Ascertainment of exposure a) Secure record * b) Structured interview where blind to case/control status * c) Interview not blinded to case/control status d) Written self-report or medical record only e) No description		
Same method of ascertainment for cases and controls a) Yes * b) No		
Non-Response rate a) Same rate for both groups * b) Non-respondents described c) Rate different and no designation		

Table 2.2: *Overall risk of bias assessment and study quality*

	Selection				**Comparability**		**Exposure**				
Study	Case definition	Representativeness of cases	Selection of Controls	Definition of Controls	Adjust for the most important risk factors	Adjust for other risk factors	Ascertainment of exposure	Same method of ascertainment for cases and controls	Non-Response rate	**Overall RoB score**	**Quality (high, moderate or low risk)**
Study A											
Study B											

3.2.5. Data synthesis and analysis

In instances of sufficient data, meta-analysis using random effects models (DerSimonian–Laird method) will be conducted. The odds ratio (OR) and linear regression coefficients, with the corresponding 95% confidence intervals, extracted from the studies, will be combined using the "*metan*" command in STATA version 17 (College Station, Texas, USA). Heterogeneity will be assessed using Cochran's Q test and I^2 statistic. I^2 values of 25%, 50% and 75% will respectively be deemed to represent low, medium, and high heterogeneity. Sources of heterogeneity will be explored by conducting subgroup analyses according to major study-level characteristics, such as by country or region (Western Europe, Central and Eastern Europe, Asia, Africa, Mediterranean and Middle East and the Americas), sample size (below vs. at or above median sample size across included studies), gender, platform technology used (microarrays, RT-qPCR, next-generation sequencing technology and northern blot hybridization) and risk of bias score. Data will be presented as forest plots. Sensitivity analysis will be conducted to estimate the influence of each individual study on the summary results by repeating the random effects meta-analysis, omitting one study at a time. Egger test with funnel plots will be used to assess publication bias.

In the instance where a meta-analysis of the included miRNAs is not possible, a narrative synthesis of evidence will be conducted, with tables and figures used to summarize the findings.

Patient and public involvement: No patients involved

Ethics and dissemination: This study design does not require formal ethical clearance, and findings will be published in a peer-reviewed journal.

Potential amendments

We do not foresee nor intend to make any amendments to the protocol, in an attempt to avoid outcome reporting bias. However, any amendments that do prove necessary will be documented and reflected online on the PROSPERO website where the protocol has been registered [Reference no: CRD42021270028].

3.3. Discussion

The complications associated with CKD including CVD, progression to ESKD and premature death can be prevented or delayed by early detection of CKD. Aberrant expression patterns of miRNAs have been reported in various diseases including CKD[17–22]. Findings from various studies suggest that these dysregulated miRNA patterns may be useful as potential diagnostic and/or prognostic tools in disease, as well as therapeutic targets[11,28]. However, evidence that kidney function affects miRNA expression levels or vice versa is sparse, coupled with contradictions in reported findings. This may partly be due to varying populations from different geographical locations, and the use of different

human sample types and molecular quantification techniques. Studies have shown that racial disparities[29], differences in environmental factors[30] and pre-analytical factors[31] (analyzed sample and its processing, and qPCR data normalizer used) may influence the observed miRNA expression patterns. Therefore, this systematic review and meta-analysis will seek to report the expression patterns of dysregulated miRNAs associated with CKD, with potential to be used as biomarkers/tools for prediction and early detection of CKD, monitoring of disease progression to advanced stages as well as potential therapeutic targets. Possible limitations of this study could include: limited studies in this area of research in African population, a predominance of poor-quality studies and significant heterogeneity, precluding further analysis.

AUTHORS' CONTRIBUTIONS

Study conceptualization was done by DDM, DMM and CG. The search strategy was developed by DDM, DMM and CG. Preparation of the protocol manuscript was done by DDM and DMM. Critical evaluation of the protocol manuscript was done by CG, APK, TEM, RTE. The search and selection of studies will be done by DDM and DMM. Data extraction and quality assessment will be done by DDM and DMM, and CG as the third author in cases of disagreement. Data analysis will be done by CG. All authors have read and agreed to this version of the protocol manuscript.

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COMPETING INTEREST STATEMENT

None declared.

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3.4. References

1. Bansal N, Katz R, Robinson-Cohen C, *et al*. Absolute rates of heart failure, coronary heart disease, and stroke in chronic kidney disease: an analysis of 3 community-based cohort studies. *JAMA Cardiol*. 2017;2(3):314–8.
2. Fox CS, Matsushita K, Woodward M, *et al*. Associations of kidney disease measures with mortality and end-stage renal disease in individuals with and without diabetes: a meta-analysis. *Lancet*. 2012;380(9854):1662–73.
3. Thompson S, James M, Wiebe N, *et al*. Cause of death in patients with reduced kidney function. *J Am Soc Nephrol*. 2015;26(10):2504–11.
4. Wetmore JB, Collins AJ. Global challenges posed by the growth of end-stage renal disease.

Ren Replace Ther. 2016;2(1):1–7.

5. Bikbov B, Purcell CA, Levey AS, *et al*. Global, regional, and national burden of chronic kidney disease, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet*. 2020;395(10225):709–33.
6. Ellen F C. The impact of chronic kidney. *Nat Rev Nephrol*. 2020;16(May):2020.
7. Levin A, Stevens PE, Bilous RW, *et al*. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int Suppl*. 2013;3(1):1–150.
8. Rysz J, Gluba-Brzózka A, Franczyk B, *et al.* Novel biomarkers in the diagnosis of chronic kidney disease and the prediction of its outcome. *Int J Mol Sci*. 2017;18(8):1702.
9. Al-Rubeaan K, Siddiqui K, Al-Ghonaim MA, *et al.* Assessment of the diagnostic value of different biomarkers in relation to various stages of diabetic nephropathy in type 2 diabetic patients. *Sci Rep*. 2017;7(1):1–9.
10. Taylor CR. Introduction to predictive biomarkers: definitions and characteristics. In: Predictive biomarkers in Oncology. Springer; 2019. p. 3–18. Available from: https://doi.org/10.1007/978-3-319-95228-4_1.
11. Mitchell PS, Parkin RK, Kroh EM, *et al*. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci*. 2008;105(30):10513–8.
12. Bonneau E, Neveu B, Kostantin E, *et al*. How close are miRNAs from clinical practice? A perspective on the diagnostic and therapeutic market. *Electron J Int Fed Clin Chem Lab Med*. 2019;30(2):114–27.
13. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell*. 2009;136(2):215–33.
14. Londina E, Lohera P, Telonis AG, *et al*. Analysis of 13 cell types reveals evidence for the expression of numerous novel primate- And tissue-specific microRNAs. *Proc Natl Acad Sci USA*. 2015;112(10):E1106–15.
15. Wonnacott A, Bowen T, Fraser DJ. MicroRNAs as biomarkers in chronic kidney disease. *Curr Opin Nephrol Hypertens*. 2017;26(6):460–6.
16. Sun Y, Koo S, White N, *et al*. Development of a micro-array to detect human and mouse microRNAs and characterization of expression in human organs. *Nucleic Acids Res*. 2004;32(22):e188–e188.
17. Fourdinier O, Schepers E, Metzinger-Le Meuth V, *et al*. Serum levels of miR-126 and miR-223 and outcomes in chronic kidney disease patients. *Sci Rep*. 2019;9(1):1–12.
18. Fujii R, Yamada H, Yamazaki M, *et al*. Circulating microRNAs (miR-126, miR-197, and miR-223) are associated with chronic kidney disease among elderly survivors of the Great East Japan Earthquake. *BMC Nephrol*. 2019;20(1):1–7.
19. Wang LP, Gao YZ, Song B, *et al*. MicroRNAs in the Progress of Diabetic Nephropathy: A

Systematic Review and Meta-Analysis. Evidence-based Complement. *Altern Med*. 2019;2019.

20. Assmann TS, Recamonde-Mendoza M, Costa AR, *et al*. Circulating miRNAs in diabetic kidney disease: case–control study and in silico analyses. *Acta Diabetol*. 2019;56(1):55–65. Available from: http://dx.doi.org/10.1007/s00592-018-1216-x
21. Lu Q, Ma Z, Ding Y, *et al*. Circulating miR-103a-3p contributes to angiotensin II-induced renal inflammation and fibrosis via a SNRK/NF-κB/p65 regulatory axis. *Nat Commun*. 2019;10(1).
22. Berillo O, Huo K-G, Fraulob-Aquino JC, *et al*. Circulating let-7g-5p and miR-191-5p are independent predictors of chronic kidney disease in hypertensive patients. *Am J Hypertens*. 2020;33(6):505–13.
23. Cheng K, Rai P, Plagov A, *et al*. MicroRNAs in HIV-associated nephropathy (HIVAN). *Exp Mol Pathol*. 2013;94(1):65–72.
24. Wang X, Liu R, Zhang W, *et al*. Role of SIRT1 in HIV-associated kidney disease. *Am J Physiol*. 2020;319(2):F335–44.
25. Moher D, Liberati A, Tetzlaff J, *et al*. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med*. 2009;6(7):e1000097.
26. Guyatt G, Oxman AD, Akl EA, *et al*. GRADE guidelines: 1. Introduction—GRADE evidence profiles and summary of findings tables. *J Clin Epidemiol*. 2011;64(4):383–94.
27. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol*. 2010;25(9):603–5.
28. Chandrasekaran K, Karolina DS, Sepramaniam S, *et al*. Role of microRNAs in kidney homeostasis and disease. *Kidney Int*. 2012;81(7):617–27. Available from: http://dx.doi.org/10.1038/ki.2011.448
29. Gong Z, Wang J, Wang D, *et al*. Differences in microRNA expression in breast cancer between women of African and European ancestry. *Carcinogenesis*. 2019;40(1):61–9.
30. Vrijens K, Bollati V, Nawrot TS. MicroRNAs as potential signatures of environmental exposure or effect: a systematic review. *Environ Health Perspect*. 2015;123(5):399–411.
31. Witwer KW. Circulating microRNA biomarker studies: pitfalls and potential solutions. *Clin Chem*. 2015;61(1):56–63.

CHAPTER 4

Systematic review: To be published

4. SYSTEMATIC REVIEW

MicroRNAs associated with chronic kidney disease in the general population and high-risk subgroups - A systematic review

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ABSTRACT

MicroRNAs (miRNAs) are small non-protein coding RNAs that regulate gene expression post transcriptionally. Emerging evidence supports that miRNAs are dysregulated in chronic kidney disease (CKD). Because miRNAs are easily detectable in biological fluids, their potential utility as diagnostic or prognostic biomarkers, as well as therapeutic targets, for CKD have been advocated. However, studies on dysregulated miRNA expressions in CKD have been inconsistent. Therefore, we performed this systematic review to characterize miRNAs associated with CKD and/or measures of kidney function and kidney damage in the general population, and in high-risk subgroups including people with hypertension (HTN), diabetes mellitus (DM) and human immunodeficiency virus (HIV) infection. Medline via PubMed, Scopus, Web of Science, and EBSCOhost databases were searched to identify relevant studies published in English or French languages on or before 30 September 2022. Furthermore, for the studies to meet the inclusion criteria, they had to be original research studies containing primary data obtained from adult human samples. The Newcastle-Ottawa Quality Assessment Scale for observational studies tool was used to assess the risk of bias in the included studies. A total of 75 studies fulfilled the eligibility criteria and were retained for the systematic review. These studies were classified according to disease outcome, as follows: CKD (n=18), diabetic kidney disease (DKD) (n=51) and HTN-associated CKD (n=6), with no study reporting on miRNA profiles in people with HIV-associated nephropathy. We identified 53, 155 and 13 dysregulated miRNAs in individuals with CKD, DKD and HTN-associated CKD relative to controls, respectively,

with various miRNA expression profiles consistent across subgroups of kidney disease. In individuals with CKD, miR-126 and miR-223 were consistently downregulated, whilst in DKD, miR-21, and miR-29b were consistently upregulated and miR-30e and let-7a were consistently downregulated in at least three studies. The consistent alteration of miRNAs with kidney disease suggests that these miRNAs may be involved in the pathogenesis of kidney disease and therefore invites further research to explore their clinical utility for CKD prevention and control. However, this review was limited by the inability to perform a pooled meta-analysis of the studies due to variabilities such as technical and methodological variabilities between studies.

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Keywords: Chronic kidney disease, Diabetic kidney disease, Hypertension-associated CKD, MicroRNAs

4.1. Introduction

The incidence of chronic kidney disease (CKD) is on the rise globally, and it is expected that CKD will be the fifth leading cause of death by 2045 [1]. This is partly attributable to the high burden of diabetes mellitus (DM) and hypertension (HTN), which are the leading causes of CKD, as well as other causes, including human immunodeficiency virus (HIV) infection and advanced age [2]. Chronic kidney disease is described as a silent condition due to a lack of obvious clinical symptoms, particularly in the early stages of the disease. As a result, most affected individuals are unaware of their disease status, and only detected at the advanced stage of the disease [3]. Furthermore, CKD is an independent risk factor for cardiovascular disease (CVD), and individuals with CKD are more likely to die of CVDs than progress to End-stage kidney disease (ESKD) [4]. Early diagnosis of CKD and effective screening of high-risk individuals is critical to mitigate disease progression and substantially reduce related poor health outcomes [5].

The indirect measurement of glomerular filtration rate (GFR) by clearance of exogenous filtration markers remains the reference standard method for determining kidney function. However, this method is complex, lengthy, expensive, invasive, and as such, not ideal for routine practice or research purposes[6]. As a result, endogenous filtration markers such as serum creatinine and cystatin C are used to estimate GFR (eGFR), and kidney function in clinical practice. However, serum creatinine can be affected by factors independent of glomerular filtration such as muscle mass, age and gender, whereas the measurement of cystatin C is complex, expensive and has not been standardized [7]. Furthermore, the predictive equations for eGFR are biased and imprecise, translating into overestimation of GFR and underdiagnosis of CKD, particularly in black Africans [8,9]. Although,

albuminuria is a well-established marker of kidney damage used to define stages 1 and 2 of CKD where the level of GFR is above 60 mL/min/1.73 m^2, it has limited predictive ability and specificity for early detection of CKD [10]. Kidney biopsies can be used to confirm a diagnosis, but this option comes with significant risk and possibility for complications, and therefore is not ideal for routine practice or research purposes [11]. Put together, these diagnostic challenges highlight the need for more accurate, minimally invasive, highly sensitive and specific, and readily available biomarkers that will improve the diagnosis/prognosis of CKD.

Research into microRNAs (miRNAs) as potential biomarkers of disease diagnosis and prognosis, as well as therapeutic targets, has gained traction over the last 10 years [12]. MiRNAs are a class of small non-coding RNAs, whose main function is regulating gene expression by degrading messenger RNA (mRNA) or inhibiting mRNA translation into functional proteins [13]. They play an important role in various cellular regulatory processes such as differentiation, proliferation, development and apoptosis [13], and are also involved in the development and normal functioning of the kidneys [14]. Although they were initially considered to be intracellular gene regulators, emerging evidence suggests that a number of miRNAs are also detectable in biological fluids such as urine, plasma, serum, and saliva in highly stable forms [15]. Previous studies found that these extracellular miRNAs presented unique patterns in pathological conditions and suggested that they may be utilized as potential diagnostic and prognostic biomarkers [16–19]. There has been a growing interest in exploring the role of extracellular miRNAs in the development and progression of CKD [19–22]. However, most findings describing miRNA expression in various biological fluids from CKD patients are inconsistent.

As such, the main purposes of this review were 1) to identify all reported miRNAs associated with CKD and/or measures of kidney function and kidney damage in the general population, as well as in high-risk subgroups (HTN, DM and HIV-infected), and 2) to explore the specific expression patterns of the identified miRNAs in prevalent CKD. We also aimed to explore 3) whether the expression patterns of the identified miRNAs differed depending on the human sample type used and/or 4) whether the expression profile of the identified miRNAs differed depending on the stage of CKD.

4.2. Materials and methods

4.2.1. Protocol and registration

This review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The protocol for this systematic review was registered on the PROSPERO database (Registration No: CRD42021270028), and detailed methods outlining the steps followed in conducting the systematic review have been previously published [23].

4.2.2. Search strategy

A comprehensive and systematic search of Medline via PubMed, Scopus, Web of Science, and EBSCOhost databases was conducted to identify eligible studies, published in English or French languages on or before 30 September 2022, without a starting date. The search strategy made use of keywords and phrases such as "microRNAs, miRNA, miRNAs, chronic kidney disease, CKD, chronic kidney injury, chronic renal disease, chronic renal injury, renal failure, end-stage renal disease, diabetic kidney disease, diabetic nephropathy, hypertensive nephrosclerosis, chronic kidney failure, chronic renal failure, end-stage renal failure, HIV-associated nephropathy, HIVAN, HIV-associated renal disease, HIV-associated kidney disease, serum creatinine, serum cystatin C, estimated glomerular filtration rate, urinary albumin excretion rate (UAER), urinary albumin-to-creatinine ratio (UACR), urinary albumin" in combination with Boolean operators (AND/OR/NOT) (refer to Additional files: Tables S1–S4). Furthermore, we manually scanned reference lists of the included studies for additional studies.

4.2.3. Data collection

Two authors (DDM and DMM) independently conducted the database searches and screened studies by title, abstract and full-text to identify those meeting the inclusion criteria, as shown in Figure 1. Disagreements encountered were resolved through discussions or consultation with a third author (CG). Studies were included if they: (i) were original articles reporting on miRNAs associated with prevalent CKD and/or measures of kidney function (serum creatinine, serum cystatin C, eGFR) or kidney damage (urinary albumin excretion rate, albumin-to-creatinine ratio, urinary protein) in the general adult population and/or high-risk subgroups (HTN, DM, HIV-infection), ii) written in English and French languages, (iii) clearly described the type of sample in which miRNA analysis was done, methods used for miRNA detection and quantification, as well as the normalization control used, (iv) with clearly defined cases and controls. Studies were excluded if they were: (i) conducted in animal or cell models, (ii) qualitative in nature (reviews, case reports, newspaper articles, editorials, commentaries, book chapters), or (iii) pre-prints or unpublished research.

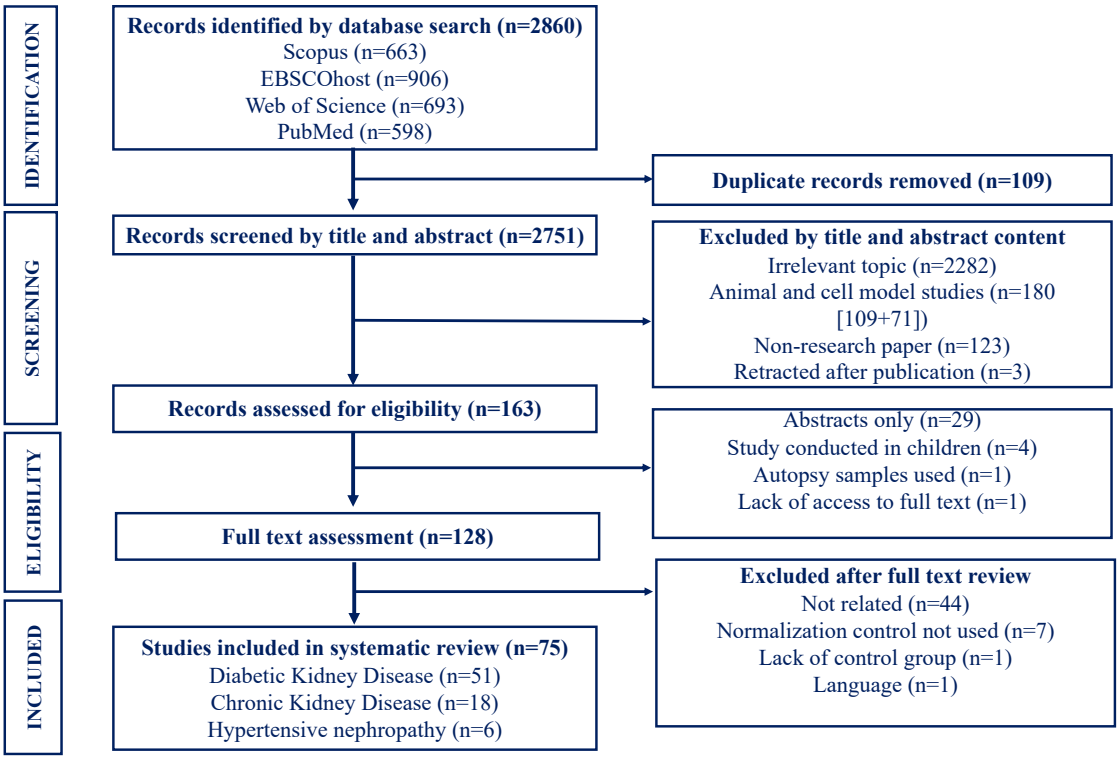

Figure 1: *Selection process for studies included*

4.2.4. Data extraction, assessment, and synthesis

The following data were independently extracted by two reviewers (DDM and DMM) from the eligible studies: publication details (first author, year of publication, country); study details [design, sample size, demographics (age, sex)]; disease outcome [CKD (of unspecified cause), diabetic kidney disease (DKD) / HTN-associated CKD / HIV-associated nephropathy (HIVAN) or ESKD]; population [general or high-risk subgroups (HTN, DM and HIV)]; participant clinical characteristics [body mass index (BMI), C-reactive protein (CRP), smoking status, alcohol consumption, lipid profile (low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides and total cholesterol)]; clinical outcomes (diagnostic criteria, classification/staging, medication status); CKD diagnostic criteria (eGFR or proteinuria/albuminuria and eGFR equation used); miRNA analysis [sample type, molecular techniques, inclusion of screening and validation cohorts, expression pattern (upregulated or downregulated) and normalization control used]. Any inconsistencies or disagreements were resolved by discussions or consultation with a third author (CG). Furthermore, we assessed the quality of studies using the Newcastle-Ottawa Quality Assessment Scale for observational studies (NOS) tool [24]. The assessment was done based on a critical appraisal of three domains, namely: (i) participant group selection, (ii) how comparable the groups are, and (iii) determination of the exposure of interest. The quality of evidence was then assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework [25]. Given that very few studies investigated the association of the same miRNA with CKD risk or markers of kidney function or

damage in the same sample type, making use of different normalization controls and miRNA quantification techniques as well as variabilities in disease outcome measures, and attempting to pool studies was meaningless. We, therefore, instead opted for a narrative synthesis of evidence.

4.3. Results

4.3.1. Search results

We obtained a total of 2860 related citations (663 from Scopus, 906 from EBSCOhost, 693 from Web of Science and 598 from PubMed) from database searches. Of these, 2732 citations were excluded for various reasons (Figure 1). The remaining 128 articles were further assessed for eligibility and 53 studies were subsequently excluded from the review because they were irrelevant to our review (n=44); they did not report on the quantitative reverse transcription polymerase chain reaction (RT-qPCR) normalization controls used (n=7), did not include a control group (n=1) and did not meet our reporting language restrictions (n=1). Ultimately, 75 studies fulfilled the eligibility criteria and were retained for the systematic review (Figure 1). The eligible studies were classified according to disease outcome, as follows: CKD (n=18), DKD (n=51) and HTN-associated CKD (6). Database searches did not return studies reporting on miRNA profiles in humans with HIVAN.

4.3.2. Characteristics of included studies

Tables 1, 2 and 3 detail the main characteristics of studies that were included in the systematic review according to disease outcome. Table 1 summarizes the 18 studies that quantified miRNA expression patterns in CKD compared to controls, whilst Table 2 is a summary of the 51 studies that quantified miRNA expression patterns in DKD relative to controls. A summary of the six studies that quantified miRNA expression patterns in individuals with HTN-associated CKD compared to controls is shown in Table 3. All 75 studies were published between 2013 and 2022, and from diverse geographical locations, including China (n=26), the United States of America (n=6), Spain (n=4), Egypt (n=6), South Africa (n=1), Germany (n=3), Italy (n=2), Austria (n=2), Japan (n=3), Iran (n=3), Belgium (n=1), Sweden (n=1), Turkey (n=1), Poland (n=1), Bahrain (n=2), Brazil (n=2), United Kingdom (n=1), India (n=2), Romania (n=2), France (n=1), Canada (n=1), Ireland (n=1), Republic of Korea (n=1), Netherlands (n=1) and Malaysia (n=1). The design of most studies was either case-control or cross-sectional, with study participant numbers ranging between 28 to 1385 in CKD, 11 to 1018 in DKD and 30 to 150 in HTN-associated CKD. The included studies used varying diagnostic methods to classify kidney disease, with 20% of the studies using only eGFR to classify CKD, 42% defined CKD by the level of albuminuria alone, whilst 34% of the studies used both albuminuria and eGFR to classify CKD. The remaining 4% of studies were not clear on the methods used for CKD classification. For estimation of GFR, most studies used the Modification of Diet in Renal Disease Study (MDRD) equation[26] (44% of the studies) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [27] (38% of the studies). Of those included, 26% of the studies

further validated their findings in a separate cohort [CKD (n=3), DKD (n=14) and HTN with CKD (n=1)].

Of the included studies, 29% performed initial miRNA expression discovery using next-generation sequencing (NGS) techniques [CKD (n=2); DKD (n=2), HTN with CKD (n=4)] and microarrays [CKD (n=1); DKD (n=15)], followed by a validation step using a PCR-based technique. PCR-based techniques were used for the quantification of miRNAs in instances where the miRNA was already identified by NGS and microarrays in previous studies. A wide range of normalization techniques was employed by the studies included in this review, with 31% using an exogenous spike-in control containing non-mammalian synthetic miRNAs such as *Caenorhabditis elegans*-miR-39 (cel-miR-39) (n=22) and 52% of the studies used endogenous controls such as small non-human ubiquitous miRNA (U6) (n=28) or miR-16 (n=8). Of the included studies, 15% of the studies used more than one normalization control [CKD (n=4), DKD (n=5) and in HTN-associated CKD (n=2)]. The sample types in which miRNA expression levels were determined varied widely across studies, with 36% conducted in serum [CKD (n=13) and DKD (n=17)], 21% in plasma [CKD (n=2), DKD (n=10) and HTN-associated CKD (n=4)], 1% in plasma endothelial vesicle [DKD (n=1)], 8% in whole blood [CKD (n=1), DKD (n=4), and HTN-associated CKD (n=1)], 19% in urine [CKD (n=2), and DKD (n=12)], 19% in urinary exosomes [CKD (n=2), DKD (n=8) and HTN-associated CKD (n=2)], 7% in kidney tissue biopsy [CKD (n=1) and DKD (n=4)] and 1% peripheral blood mononuclear cells [DKD (n=1)]. Only 17% of the studies quantified miRNA expression in two or more sample types, and this included two studies in CKD, 10 in DKD and one in HTN-associated CKD.

The expression patterns of 288 miRNAs were investigated across the 75 studies included in this review. Of the 288 miRNAs, 67 miRNAs were evaluated in populations with prevalent CKD, with 53 miRNAs found to be dysregulated (25 downregulated and 28 upregulated). Of the 193 miRNAs evaluated in populations with DKD, 155 miRNAs were found to be dysregulated (67 downregulated and 88 upregulated), whilst 13 (10 downregulated and 3 upregulated) of the 28 miRNAs evaluated in populations with HTN-associated CKD were dysregulated. The dysregulation discussed below refers only to miRNAs evaluated in three or more studies, of which HTN-associated CKD had none.

Table 1: *Characteristics of studies evaluating microRNA expression patterns in chronic kidney disease*

Study	Country	Study population [Cases]	Study population [Control]	Quantification method	Sample type	microRNAs	Upregulated	Downregulated
Carmona, 2020 [28]	Spain	45	10	RT-qPCR	Serum	miR-126-3p, miR-191-5p, miR-223-3p, miR-363-3p, miR-495-3p	-	miR-126-3p, miR-191-5p, miR-223-3p
Chen, 2013 [29]	United States of America	110	8	RT-qPCR	Serum	miR-125b, miR-145, miR-155	-	miR-125b, miR-145, miR-155
Donderski, 2021 [30]	Poland	45	17	RT-qPCR	urine, serum	miR-155-5p, miR-214-3p, miR-200a-5p, miR-29a-5p, miR-21-5p, miR-93-5p, miR-196a-5p	**Urine** - miR-29-5p, miR-21-5p, miR-196a-5p. **Serum** - miR-155-5p, miR-214-3p and miR-200a-5p	**Urine** - miR-155-5p, miR-214-5p, miR-200a-5p, miR-93-5p
Eckersten, 2017 [31]	Sweden	30	18	RT-qPCR	Serum	miR-155	miR-155	-
Fourdinier, 2019 [21]	Belgium	601	31	RT-qPCR	Serum	miR-223, miR-126	-	miR-223, miR-126
Fujii, 2019 [22]	Japan	395	118	RT-qPCR	Serum	miR-17, miR-21, miR-150	-	-
Fujii, 2019 [32]	Japan	229	1156	RT-qPCR	Serum	miR-126, miR-197, miR-223	-	miR-126, miR-197, miR-223
Fujii, 2021 [33]	Japan	29	140	RT-qPCR	Serum	miR-126, miR-197, miR-21, miR-150, miR-17	-	-
Lange, 2019 [34]	Germany	41	5	RT-qPCR	urine exosomes	miR-21-5p, miR-30a-5p, miR-92a-3p	miR-21	-
Li, 2020 [35]	China	116	127	RT-qPCR	Serum	miR-155	miR-155	-
Liu, 2020 [36]	China	110	35	NGS, RT-qPCR	serum	miR-483-5p, miR-363-3p	miR-483-5p	miR-363-3p
Motshwari, 2021 [37]	South Africa	171	740	NGS, RT-qPCR	whole blood	miR-novel- chr1_36178, miR-novel-chr2_55842, miR-novel-chr7_76196, miR-novel-chr5_67265, miR-novel-chr13_13519, and miR-novel-chr15_18383	All novel miRNAs	-

Muralidharan, 2017 [38]	United States of America	19	9	Microarray, RT-qPCR	plasma and urine exosomes	**Urine** - miR-1281, miR-1825, miR-130a-3p, let-7a-5p **Plasma** - miR-1825p miR-1281, miR-423	**Urine** - miR-1825, miR-1281. **Plasma** - miR-1825, miR-1281, miR-144-5p, miR-548ap-5p	**Urine** - miR-4525. **Plasma** - miR-423-5p, miR-3648
Rudnicki, 2016 [39]	Austria	20	52	RT-qPCR	Kidney biopsy	miR-30d, miR-140-3p, miR-532-3p, miR-194, miR-190, miR-204, miR-206	miR-206, miR-532-3	-
Sayilar, 2016 [40]	Turkey	30	15	RT-qPCR	plasma, urine	miR-21, miR-124, miR-192, miR-195, miR-451	**Urine** - miR-124 **Plasma** - miR-195, miR-451	**Urine** - miR-195, miR-451
Shang, 2017 [41]	China	208	37	RT-qPCR	serum	miR-92a, miR-126, miR-155, miR-483	miR-92a	-
Trojanowicz, 2019 [42]	Germany	48	23	RT-qPCR	serum	miR-421	miR-421	-
Ulbing, 2017 [43]	Austria	137	36	RT-qPCR	serum	miR-223-3p, miR-93-5p, miR-142-3p, miR-146a-5p	-	miR-223-3p, miR-93-5p, miR-142-3p

Table 2: *Characteristics of studies evaluating microRNA expression patterns in diabetic kidney disease*

Study	Country	Study population (n)			Quantification method	microRNAs	Sample type	Upregulated	Downregulated
		Healthy	Normoalbuminuria	Diabetic Kidney Disease					
Abdelsalam, 2020 [44]	Egypt	30	30	60	RT-qPCR	miR-451	plasma	miR-451	-
							urine	-	miR-451
Abdou, 2022[45]	Egypt	20	20	40	RT-qPCR	miR-152-3p	serum	miR-152-3p	-
Akhbari, 2018 [46]	Iran	22	21	40	Real time PCR	miR-93	serum	-	miR-93
Akhbari, 2019 [47]	Iran	22	-	61	Real time PCR	miR-155	cell-free serum	-	miR-155
Al-kafaji, 2016 [48]	Bahrain	50	52	50	RT-qPCR	miR-126	peripheral whole blood	-	miR-126
Al-kafaji, 2018 [49]	Bahrain	30	30	25	RT-qPCR	miR-377, miR-192	whole blood	miR-377	miR-192
Argyropoulos, 2013 [50]	United States of America	-	10	30	RT-qPCR	27 microRNAs	urine	miR-214-3p, miR-92b-5p, miR-765, miR-429, miR-373-5p, miR-1913, miR-638	miR-323b-5p, miR-221-3p, miR-524-5p, miR-188-3p
Assmann, 2019 [51]	Brazil	20	33	54	RT-qPCR	miR-16-5p, miR-21-3p, miR-29a-3p, miR-378a-5p, miR-503-5p	plasma	miR-21-3p, miR-378a-5p	miR-16-5p, miR-29a-3p
Barutta, 2013 [52]	Italy	10	12	12	RT-qPCR	miR-130a, miR-424, miR-155, miR-145	urine exosomes	miR-145, miR-130a	miR-424, miR-155
Beltrami, 2018 [53]	United Kingdom	61	62	109	MicroRNA array, RT-qPCR	miR-126-3p, miR-155-5p, miR-29b-3p	urine	miR-126-3p, miR-155-5p, miR-29b-3p	-

Cardenas-Gonzalez, 2017 [54]	United States of America	93	71	132	RT-qPCR, miRNA in situ hybridization	miR-1915-3p, miR-2861, miR-4532, miR-4536-3p, miR-6747-3p	urine	miR-4536-3p, miR-6747-3p	miR-1915-3p, miR-2861, miR-4532
Conserva, 2019 [55]	Italy	20	-	37	Microarray, RT-qPCR	miR-27b-3p, miR-1228-3p	kidney biopsy, cell-free urine	-	miR-27b-3p, miR-1228-3p
Delić, 2016 [56]	Germany	14	14	13	Microarray, RT-qPCR	miR-320c, miR-6068	urine exosomes	miR-320c, miR-6068	-
Dieter, 2019 [57]	Brazil	-	17	23	RT-qPCR	miR-15a-5p, miR-30e-5p	plasma	-	miR-30e-5p
							urine	-	miR-30e-5p
Eissa, 2016 [58]	Egypt	56	60	116	MicroRNA array, RT-qPCR	miR-15b, miR-34a, miR-636	urine pellets, exosomes	miR-15b, miR-34a, miR-636	-
Eissa, 2016b [59]	Egypt	54	56	110	RT-qPCR	miR-133b, miR-342, miR-30a	urine exosomes	miR-133b, miR-342, miR-30a	-
Florijn, 2019 [60]	Netherlands	12	-	33	RT-qPCR	miR-1, miR-21, miR-29a, miR-126, miR-132, miR-145, miR-152, miR-212, miR-223, miR-574, miR-660	plasma endothelial vesicles	miR-21, miR-126	-
							Plasma	miR-126	
							high density lipoprotein fraction	-	miR-132
							Apo-2	miR-126, miR-145, miR-660	-
Fouad, 2020 [61]	Egypt	100	120	120	RT-qPCR	miR-21	plasma	miR-21	-
Guo, 2017 [62]	China	45	33	42	Microarray, RT-qPCR	miR-29c	plasma	miR-29c	-
							urine	-	miR-29c
							kidney tissue	-	miR-29c

Han, 2021 [63]	China	-	5	6	Microarray, RT-qPCR	miR-95-3p, miR-185-5p, miR-1246, miR-631	urine sediment	miR-95-3p, miR-185-5p, miR-1246, miR-631	-
He, 2014 [64]	China	6	-	6	Microarray hybridisation, RT-qPCR	miR-15a, miR-17, miR-21, miR-30b, miR-126, miR-135a, miR-192, miR-377, miR-34a, miR-194-1, miR-205, miR-215	serum	miR-15a, miR-17, miR-21, miR-30b, miR-126, miR-135a, miR-192, miR-377	miR-34a, miR-194-1, miR-205, miR-215
							kidney tissue	miR-135a	-
Hong, 2021 [65]	China	36	36	51	Microarray, RT-qPCR	miR-193a-3p, miR-320c, miR-27a-3p	plasma	miR-193a-3p, miR-320c	-
Jia, 2016 [66]	China	10	30	50	RT-qPCR	miR-192, miR-194, miR-215	urine extracellular vesicles	miR-192, miR-194, miR-215	-
Khokhar, 2021 [67]	India	36	38	35	RT-qPCR	miR-21-5p	whole blood	miR-21-5p	-
Lin, 2021[68]	China	30	36	32	RT-qPCR	miR-638	serum		miR-638
Liu, 2021[69]	China	180	64	116	RT-qPCR	miR-29a	serum	miR-29a	
Ma, 2016 [70]	China	127	157	307	RT-qPCR	miR-192	serum	-	miR-192
Milas, 2018 [71]	Romania	11	26	42	RT-qPCR	miR-21, miR-124, miR-192	urine	miR-21, miR-124	miR-192
Monjezi, 2022[72]	Iran		30	31	RT-qPCR	miR-124-3p	peripheral blood mononuclear cells		miR-124-3p
Motawi, 2018 [73]	Egypt	25	25	25	RT-qPCR	miR-130b	serum	-	miR-130b
Park, 2022[74]	Republic of Korea	7	-	12	NGS	miR-320b, miR-30d-5p, miR-30e-3p, miR-30c-5p, miR-190a-5p, miR-29c-5p, miR-98-3p, miR-331-3p, let-7a-3p, miR-106b-3p, miR-30b-5p, miR-99b-5p, let-7f-1-3p	plasma and urine extracellular vesicles	miR-320b	miR-30d-5p, miR-30e-3p, miR-30c-5p, miR-190a-5p, miR-29c-5p, miR-98-3p, miR-331-3p, let-7a-3p, miR-106b-3p,

									miR-30b-5p, miR-99b-5p, let-7f-1-3p
Peng, 2013 [75]	China	-	41	42	RT-qPCR	miR-29a, miR-29b, miR-29c	urine supernatant	miR-29a	-
Petrica, 2019 [76]	Romania	11	36	81	RT-qPCR	miR-125a, miR-126, miR-146a, miR-21p, miR-124, miR-192	serum	miR-192, miR-21p	miR-124, miR-125a, miR-126, miR-146
							urine	miR-21p, miR-124, miR-125a, miR-126	miR-192, miR-146a
Pezzolesi, 2015 [77]	United States of America	-	40	76	RT-qPCR	let-7b-5p, let-7c-5p, miR-21-5p, miR-29a-3p, miR-29c-3p	plasma	let-7b-5p, miR-21-5p	let-7c-5p, miR-29a-3p
Prabu, 2019 [78]	India	40	40	80	RT-qPCR	let-7i-5p, miR-135b-5p, miR-15b-3p, miR-197-3p, miR-24-3p, miR-27b-3p	urine exosomes	let-7i-5p, miR-24-3p, miR-27b-3p, miR-30a-5p	miR-15b-3p
Regmi, 2019 [79]	China	25	50	42	RT-qPCR	miR-20a, miR-99b, miR-122-5p, miR-486-5p	serum	miR-99b, miR-122	miR-20a, miR-486
Ren, 2019 [80]	China	280	273	465	RT-qPCR	miR-154-5p	serum	miR-154-5p	-
Ren, 2020 [81]	China	-	136	254	RT-qPCR	miR-154-5p	serum	miR-154-5p	-
Roux, 2018 [82]	France	-	73	73	NGS, RT-qPCR	miR-362-5p, miR-152-3p, miR-196b-5p, miR-140-3p	plasma	miR-152-3p	-
Rovira-Llopis, 2018 [83]	Spain	24	13	13	RT-qPCR	miR-31	serum	-	miR-31
Shao, 2017 [84]	China	195	186	309	RT-qPCR	miR-217	serum	miR-217	-
Sham, 2022[85]	Malaysia	-	15	26	miS-cript miRNA qPCR array, RT-qPCR	miR-874-3p, miR-101-3p, miR-145-5p	serum	miR-874-3p, miR-101-3p	
Su, 2020 [86]	China	20	-	20	MicroRNA array, RT-qPCR	miR-140-5p	peripheral blood, kidney tissue	-	miR-140-5p

Wang, 2019 [19]	China	40	40	66	MicroRNA array, qPCR	miR-27a-3p, miR-30e, miR-33b, miR-50, miR-125b-5p, miR-150-5p, miR-155-5p, miR-296, miR-320e, miR-328, miR-484, miR-487, miR-550a-5p, miR-590-5p, miR-744, miR-885-5p, miR-933. miR-3196, let-7a-5p, let-7c-5p	plasma	miR-125b-5p, miR- 484, miR-550	miR-30e, miR-155-5p, miR-320, let-7a-5p, miR-150-5p, miR-3196
Xiao, 2017 [87]	China	35	-	140	Real time PCR	miR-9	serum	miR-9	-
Xie, 2017 [88]	China	-	35	5	MicroRNA array, qPCR	miR-362-3p, miR-877-3p, miR-15a-5p, miR-150-5p	urine exosomes	miR-362-3p, miR-877-3p, miR-150-5p	miR-15a-5p
Zang, 2019 [89]	Ireland	18	30	36	MicroRNA arrays, RT-PCR	miR-21-5p, let-7e-5p, miR-23b-3p, miR-30b-5p, miR-125b-5p	urine sediment exosome	miR-21-5p	miR-30b-5p
Zhang, 2017 [90]	China	28	30	27	Microarray, qPCR	miR-223-3p, miR-106b-5p, miR-103a-3p, miR-126-3p, miR-27a-3p, miR-29a-3p, miR-29c-3p, miR-425-5p, miR-93-5p, miR-1249-5p, miR-2276-3p, miR-1225-5p, miR-345-3p, miR-3679-5p, miR-4281, miR-4442	plasma	-	miR-223-3p
Zhang, 2020 [91]	China	-	30	30	RT-qPCR	miR-135a-5p	serum	miR-135a-5p	-
Zhao, 2020 [92]	China	-	17	17	MicroRNA arrays, qRT-PCR	miR-4491, miR-2117, miR-4507, miR-5088-5p, miR-1587, miR-219a-3p, miR-5091, miR-498, miR-4687-3p, miR-516b-5p, mir-4534, miR-1275, miR-5007-3p, miR-4516	urine exosomes	miR-4687-3p, miR-4534, miR-5007-3p	-

Zhou, 2013 [93]	China	62	104	108	MicroRNA microarrays, real time RT-PCR	let-7a, let-7d, let-7f, miR-4429, miR-363	whole blood	-	let-7a

Table 3: *Characteristics of studies evaluating microRNA expression patterns in hypertension-associated chronic kidney disease*

Study	Country	Study population			Quantification method	Sample type	microRNAs	Upregulated	Downregulated
		Healthy	Hypertensive	Hypertensive CKD					
Berillo, 2020 [94]	Canada	15	31	16	Hi-seq, RT-qPCR	platelet-poor plasma	let-7g-5p, miR-191-5p	-	let-7g-5p, miR-191-5p
Huang, 2018 [95]	China	0	50	100	RT-qPCR	plasma	miR-29a	miR-29a	-
Huang, 2020 [96]	China	0	50	100	RT-qPCR	plasma	miR-29b	miR-29b	-
Nandakumar, 2017 [97]	United States of America	-	15	15	NGS	whole blood	miR-17-5p, miR-130a-3p, miR-15b-5p, miR-106b-3p, miR-106a-5p, miR-16-5p, miR-181a-5p, miR-1285-3p, miR-15a-5p, miR-29c-5p, miR-345-5p, miR-142-3p, miR-339-3p, miR-210-3p	-	miR-17-5p, miR-15a-5p, miR-15b-5p, miR-16-5
Perez-Hernandez, 2018 [98]	Spain	20	28	24	NGS, RT-qPCR	Urinary exosomes	miR-146a and miR-335	-	miR-146a
Perez-Hernandez, 2021 [99]	Spain	15	56	61	NGS, RT-qPCR	plasma and urine exosomes	miR-143-3p, miR-126-3p, miR-26a-5p, miR-144-3p, miR-191-5p, miR-220a-3p, miR-222-3p, miR-423-5p	**Plasma exosome** - miR-191-5p	**Plasma exosome** - miR-222-3p, miR-26a-5p, miR-126-3p

4.3.3. Dysregulated miRNAs in CKD

Of the 53 differentially expressed miRNAs in individuals with CKD, four miRNAs (miR-126, miR-223, miR-155 and miR-21) were reported in at least three studies (Table 4). Of these, serum miR-126 [21,28,32] and serum miR-223 [21,28,32,43] were consistently downregulated in individuals with CKD across studies. Inconsistent expression patterns were observed for miR-155, two studies found the miRNA to be downregulated in serum and urine, [29,30] and two studies found it upregulated in serum [30,31] in CKD, with one study reporting no difference [41] in the expression of miR-155 in both urine and serum, in individuals with and without CKD. Of the studies reporting on the expression pattern of miR-21 in CKD, one study showed a downregulation in this miRNA in serum sample, [30] whilst two studies evaluated miR-21 in urine and urine exosomes and found it upregulated [30,34], and one study found no difference in urine and plasma samples between the case and control groups [40].

4.3.4. Dysregulated miRNAs in DKD

One hundred and ninety-three miRNAs were differentially expressed in DKD, and of these, 12 miRNAs (miR-155, miR-126, miR-192, miR-21, miR-29b, miR-15a-5p, miR-29a, miR-29c, miR-124, let-7a, miR-30e and miR-30b) were reported in at least three studies. miR-21[51,60,61,64,67,71,76,77&89] and miR-29b [50,53], were consistently upregulated whereas let-7a [19,74,93] and miR-30e [19,57,74] were consistently downregulated in individuals with DKD across studies (Table 5). Although discordant results were observed for miR-155, miR-126 and miR-192, they were commonly studied in at least four different studies. miR-155 was downregulated in DKD in three different studies [19,47,52], with one study reporting upregulation [53]. In the five studies where miR-126 expression was investigated in serum, three different studies showed consistent upregulation of the miRNA [53,60,64], whilst two studies reported downregulation [48,76], in individuals with DKD and controls. Three studies reported downregulation of miR-192 [49,70,71] , whilst three other studies [64,66,76] observed upregulation of this miRNA in individuals with DKD compared to controls.

4.3.5. MicroRNAs associated with kidney disease subgroups

Studies reporting on the independent associations of various miRNAs with kidney disease outcomes and/or markers of kidney function and kidney damage are summarized in Tables 5 and 6, as well as in Figure 2. In the general population, miR-17 [22], miR-21[22], miR-150 [22], miR-197 [32] and miR-223 [32] were inversely associated with prevalent CKD, whilst miR-novel-chr2_55842, miR-novel-chr7_76196, miR-novel-chr5_67265, miR-novel-chr13_13519, miR-novel-chr1_36178 and miR-novel-chr15_18383 [37] were positively associated with prevalent CKD. In individuals with DM, miR-377 [49] was inversely associated with DKD, whereas miR-4536-3p [54] was positively associated with DKD. MicroRNAs, let-7b-5p, miR-21-5p were positively associated with progression to ESKD, whereas let-7c-5p, and miR-29a-3p were inversely associated with progression to ESKD in those with DM [77]. In the general population, miR-126 was inversely associated with the risk of CKD [32,33], whereas in

individuals with DM it was inversely associated with DKD, microalbuminuria and macroalbuminuria [48]. In individuals with HTN-associated CKD, miR-29a [95] and miR-29b [96] were positively associated with albuminuria.

4.3.6. MicroRNAs associated with markers of kidney function and kidney damage

Independent associations between kidney function (as measured by eGFR) and certain miRNAs were reported. MiR-125b [29], miR-145 [29], miR-223 [21], miR-17 [22] and miR-150 [22] were positively associated with eGFR, whilst miR-novel-chr2_55842 [37], miR-novel-chr7_76196 [37] and miR-92a [41] were inversely associated with eGFR in the general population with CKD. MiR-155 showed positive [29] and negative [30] associations with eGFR in the general population with CKD. MiR-133b [59] was inversely associated with eGFR in individuals with DKD, whereas let-7g-5p and miR-191-5p [94] were positively associated with eGFR in people with HTN-associated CKD. On the other hand, miR-29a [95] and miR-29b [96] were positively associated with kidney damage (as measured by albuminuria) in individuals with HTN-associated CKD, whilst miR-15b [58], miR-34a[58], miR-636 [58], miR-133b [59], miR-9 [87] were positively associated with kidney damage in individuals with DKD. miR-21 and eGFR showed a positive association in the general population [22] and inverse association in those with DM [71], whilst miR-21 and albuminuria were positively associated in individuals with DM [71]. miR-126 was positively associated with kidney function in the general population [21] and those with DM [33,48], whilst miR-192 [70,71] was positively associated with kidney function and inversely associated with kidney damage in individuals with DM. miR-124 [71] was inversely associated with eGFR and positively associated with albuminuria levels in individuals with DM.

4.3.7. Quality assessment

Based on the NOS tool, most studies (87%) were rated as being of good quality. The remaining studies (13%) received a weak rating mostly due to a lack of adjustments for confounding variables that may have influenced the expression pattern of the miRNAs. Moreover, certain studies did not clearly describe how their controls were selected or how they were defined. Lastly, details on the ascertainment of exposure (how the records were stored and the structure of the interview) as well as the response rate were not clear in most studies.

Table 4: *MicroRNAs reported in at least three studies in chronic kidney disease subtypes*

MicroRNA	Study	Sample type	Expression pattern
CHRONIC KIDNEY DISEASE			
miR-126	Carmona, 2020 [28]	Serum	Downregulated
	Fourdinier, 2019 [21]	Serum	Downregulated
	Fujii, 2019b [32]	Serum	Downregulated
	Shang, 2017 [41]	Serum, urine	No difference
miR-223	Carmona, 2020 [28]	Serum	Downregulated
	Fourdinier, 2019 [21]	Serum	Downregulated
	Fujii, 2019b [32]	Serum	Downregulated
	Ulbing, 2017 [43]	Serum	Downregulated
miR-155	Chen, 2013 [29]	Serum	Downregulated
	Donderski, 2021 [30]	Urine	Downregulated
		Serum	Upregulated
	Eckersten, 2017 [31]	Serum	Upregulated
	Shang, 2017 [41]	Serum, urine	No difference
miR-21	Donderski, 2021 [30]	Urine	Upregulated
		Serum	Downregulated
	Lange, 2019 [34]	Urine exosomes	Upregulated
	Sayilar, 2016 [40]	Urine, plasma	No difference
DIABETIC KIDNEY DISEASE			
miR-155	Akhbari, 2019 [47]	Cell-free serum	Downregulated
	Barutta, 2013 [52]	Urinary exosomes	Downregulated in microalbuminuria
	Beltrami, 2018 [53]	Urine	Upregulated
	Wang, 2019 [19]	Plasma	Downregulated
miR-126	Al-kafaji, 2016 [48]	Whole blood	Downregulated
	Beltrami, 2018 [53]	Urine	Upregulated
	Florijn, 2019 [60]	Plasma exosomal vesicles	Upregulated
		Plasma	Upregulated
		Plasma Ago	Upregulated
	Petrica, 2019 [76]	Urine	Upregulated
		Serum	Downregulated
	He, 2014 [64]	Serum	Upregulated
miR-192	Al-kafaji, 2018 [49]	Whole blood	Downregulated
	Jia, 2016 [66]	Urine extracellular vesicles	Upregulated in microalbuminuria and

			downregulated in macro albuminuria
	Ma, 2016 [70]	Serum	Downregulated
	Milas, 2018 [71]	Urine	Downregulated
	Petrica, 2019 [76]	Urine	Upregulated
		Serum	Upregulated
	He, 2014 [64]	Serum	Upregulated
miR-21	Assmann, 2019 [51]	Plasma	Upregulated in macroalbuminuria
	Florijn, 2019 [60]	Plasma exosomal vesicles	Upregulated
		Plasma	No difference
	Fouad, 2020 [61]	Plasma	Upregulated
	Khokhar, 2021 [67]	Whole blood	Upregulated
	Milas, 2018 [71]	Urine	Upregulated
	Petrica, 2019 [76]	Serum	Upregulated
		Urine	Upregulated
	Pezzolesi, 2015 [77]	Plasma	Upregulated in rapid progressors to ESKD
	Zang, 2019 [89]	Urinary exosomes	Upregulated
	He, 2014 [64]	Serum	Upregulated
miR-29b	Beltrami, 2018 [53]	Urine	Upregulated
	Peng, 2013 [75]	Urine supernatant	No difference
	Argyropoulos, 2013 [50]	Urine	Upregulated
miR-15a-5p	He, 2014 [64]	Serum	Upregulated
	Xie, 2017 [88]	Urinary exosomes	No difference
	Dieter, 2019 [57]	Urine and plasma	No difference
miR-29a	Assmann, 2019 [51]	Plasma	Downregulated in macroalbuminuria
	Peng, 2013 [75]	Urine supernatant	Upregulated
	Pezzolesi, 2015 [77]	Plasma	Downregulated in fast progressors to ESKD
	Liu, 2021 [69]	Serum	Upregulated
miR-29c	Guo, 2017 [62]	Plasma	Upregulated
		Urine sediments	Downregulated
		Kidney tissue	Downregulated
	Pezzolesi, 2015 [77]	Plasma	No difference
	Peng, 2013 [75]	Urine supernatant	No difference
miR-124	Milas, 2018 [71]	Urine	Upregulated

	Monjezi, 2022 [72]	Peripheral blood mononuclear cells	downregulated
	Petrica, 2019 [76]	Serum	Downregulated
		Urine	Upregulated
Let-7a	Park, 2022 [74]	Plasma	Downregulated
		Urinary extracellular vesicles	Downregulated
	Wang, 2019 [19]	Plasma	Downregulated
	Zhou, 2013 [93]	Whole blood	Downregulated
miR-30e	Dieter, 2019 [57]	Plasma	Downregulated
		Urine	Downregulated
	Park, 2022 [74]	Plasma	Downregulated
		Urinary extracellular vesicles	Downregulated
	Wang, 2019 [19]	Plasma	Downregulated
miR-30b	He, 2014 [64]	Serum	Upregulated
	Park, 2022 [74]	Plasma	Downregulated
		Urinary extracellular vesicles	Downregulated
	Zang, 2019 [89]	Urine sediment exosome	Downregulated

Table 5: *Association of miRNAs with kidney disease outcome*

Study	**microRNA**	**Adjustment**	**Effect estimate [OR (95%CI)]**	**Outcome**
Fujii, 2019 [22]	miR-17	sex, age, proteinuria, body mass index, systolic blood pressure, triglyceride, blood glucose, smoking status, alcohol consumption, exercise habit, and medication for non-communicable diseases	0.42 (0.24 to 0.75); p=0.004	CKD
	miR-21		0.47 (0.26 to 0.85); p=0.01	
	miR-150		0.49 (0.27 to 0.88); p=0.02	
Fujii, 2019b [32]	miR-126	age, sex, blood glucose, body mass index, systolic blood pressure, smoking status, alcohol consumption, relocation frequency, degree of	0.67 (0.45 to 0.98); p=0.04	CKD
	miR-197		0.67 (0.46 to 0.99); p=0.05	
	miR-223		0.53 (0.35 to 0.79); p=0.002	

		housing damage, current housing environment, and psychological condition		
Fujii, 2021 [33]	miR-126	Sex, age, body mass index, blood glucose levels, systolic blood pressure, smoking status, alcohol intake, habitual exercise, proteinuria and baseline eGFR or blood urea nitrogen	3.85 (1.01 to 16.8); p=0.05	CKD
Huang, 2018 [95]	miR-29a	age, sex, SBP, fasting blood-glucose, body mass index, glomerular filtration rate, triglyceride, C-reactive protein, and TGF-β1	1.11 (1.08 to 1.37); p=0.002	Proteinuria
Huang, 2020 [96]	miR-29b	age, gender, SBP, fasting blood-glucose, body mass index, glomerular filtration rate, low density lipoprotein cholesterol, C-reactive protein and TGF-β1	0.55 (0.35 to 0.79); p<0.001	Albuminuria
Motshwari, 2021 [37]	miR-novel-chr2_55842	age, gender, smoking status, drinking status, HTN, and DM status	1.65 (1.33 to 2.05); p<0.0001	CKD
	miR-novel-chr7_76196		4.89 (2.48 to 9.64); p<0.0001	
	miR-novel-chr5_67265		1.37 (1.17 to 1.60); p<0.0001	
	miR-novel-chr13_13519		1.79 (1.40 to 2.28); p<0.0001	
	miR-novel-chr1_36178		1.22 (1.10 to 1.37); p<0.0001	
	miR-novel-chr15_18383		1.44 (1.09 to 1.89); p=0.009	
Al-kafaji, 2016 [48]	miR-126	age, gender, BMI and blood pressure, fasting	0.51 (0.37 to 0.71); p=0.002	DKD

		glucose, HbA1c, triglyceride, and LDL	0.78 (0.70 to 0.95); p=0.04	Microalbuminuria
			0.43 (0.30 to 0.70); p=0.03	Macroalbuminuria
Al-kafaji, 2018 [49]	miR-377	age, sex, BMI, HbA1c, mean blood pressure, LDL, triglyceride and total cholesterol	1.12 (0.98 to 1.22); p=0.018	DKD
Cardenas-Gonzalez, 2017 [54]	miR-4536-3p	Not reported	3.03 (1.95 to 4.72)	DKD
Pezzolesi, 2015 [77]	let-7b-5p	Sex, age, HbA1c, duration of type 1 diabetes	2.51 (1.42 to 4.43); p=0.002	ESKD
	miR-21-5p		6.33 (1.75 to 22.92); p=0.005	
	let-7c-5p		0.23 (0.10 to 0.52); p=0.0004	
	miR-29a-3p		0.38 (0.20 – 0.74); p=0.004	

Table 6: *Association of miRNAs with kidney function and damage*

Study	**microRNA**	**Adjustment**	**unstandardized/standardized β-coefficient (95%CI)**	**Outcome**
Chen, 2013 [29]	miR-125b	Not reported	Not reported	eGFR
	miR-145			
	miR-155			
Donderski, 2021 [30]	miR-155-5p	Not reported	0.32; p=0.042	eGFR
Fourdinier, 2019 [21]	miR-223	age, body mass index, diabetes, urea, calcium, phosphate, parathyroid hormone, platelet count, cholesterol, and low density lipoprotein	0.02 (0.01 to 0.03); p<0.0001	eGFR
	miR-126	hypertension, body mass index, diabetes, urea, phosphate, parathyroid hormone, proteinuria,	0.00 (0.000 to 0.001); p=0.002	eGFR

		cholesterol, and low density lipoprotein		
Fujii, 2019 [22]	miR-17	sex, age, proteinuria, body mass index, systolic blood pressure, triglyceride, blood glucose, smoking status, alcohol consumption, exercise habit, and medication for non-communicable diseases	0.121; p=0.004	eGFR
	miR-21		0.134; p=0.002	
	miR-150		0.123; p=0.004	
Fujii, 2021 [33]	miR-126	age, sex, smoking habits, alcohol intake, habitual exercise, BMI, SBP, glucose levels, proteinuria, and baseline eGFR	−3.18; p=0.04	eGFR
Motshwari, 2021 [37]	miR-novel-chr2_55842	age, gender, smoking status, drinking status, hypertension, and diabetes mellitus status	−2.70 (−4.82 to −0.57); p=0.013	eGFR
	miR-novel-chr7_76196		−7.39 (−14.05 to −0.72); p=0.030	
Shang, 2017 [41]	miR-92a	age, sex, smoking, diabetes mellitus, coronary artery disease, and hyperlipidaemia	-0.684; p<0.001 -0.548; p<0.001	eGFR
Berillo, 2020 [94]	let-7g-5p	age, urinary albumin creatinine ratio, carotid distensibility, neutrophil and lymphocyte fractions, neutrophil number and neutrophil-to-lymphocyte ratio	0.41; p<0.001	eGFR
	miR-191-5p		0.30; p<0.014	
Eissa, 2016 [58]	miR-15b	Not reported	0.452 (0.000 to 0.000); p<0.001	UACR
	miR-34a		-0.914 (0.000 to 0.000); p<0.03	
	miR-636		0.889 (0.000 to 0.000); p<0.02	
Eissa, 2016b [59]	miR-133b	Not reported	0.4 (0.395 to 1.855); p<0.01	eGFR
Ma, 2016 [70]	miR-192	Age, duration, body mass index, systolic and diastolic blood pressure, fasting blood glucose, postprandial blood glucose, HbA1C, fasting insulin, postprandial insulin, fasting C peptides, prandial C peptides, blood urea nitrogen, creatinine, low- and high-density lipoprotein	Not reported	UACR

		cholesterol, triglycerides, cholesterol, TGF-β1, and fibronectin		
Milas, 2018 [71]	miR-21	lipid profile, HbA1c, and high-sensitive C-reactive protein	−0.007 (−0.011 to −0.003); p=0.0001	eGFR
	miR-124		−0.007 (−0.011 to −0.003); p=0.0001	
	miR-192		0.005 (0.002 to 0.008); p=0.0001	
	miR-21		−0.0005 (−0.0007 to −0.0002); p=0.0001	UACR
	miR-124		−0.0005 (−0.0007 to −0.0002); p=0.0001	
Xiao, 2017 [87]	miR-9	pigment epithelium-derived factor, vascular endothelial growth factor, low-density lipoprotein cholesterol, total cholesterol, fibrinogen, HbA1c, insulin resistance	0.431; p=0.023	UAER

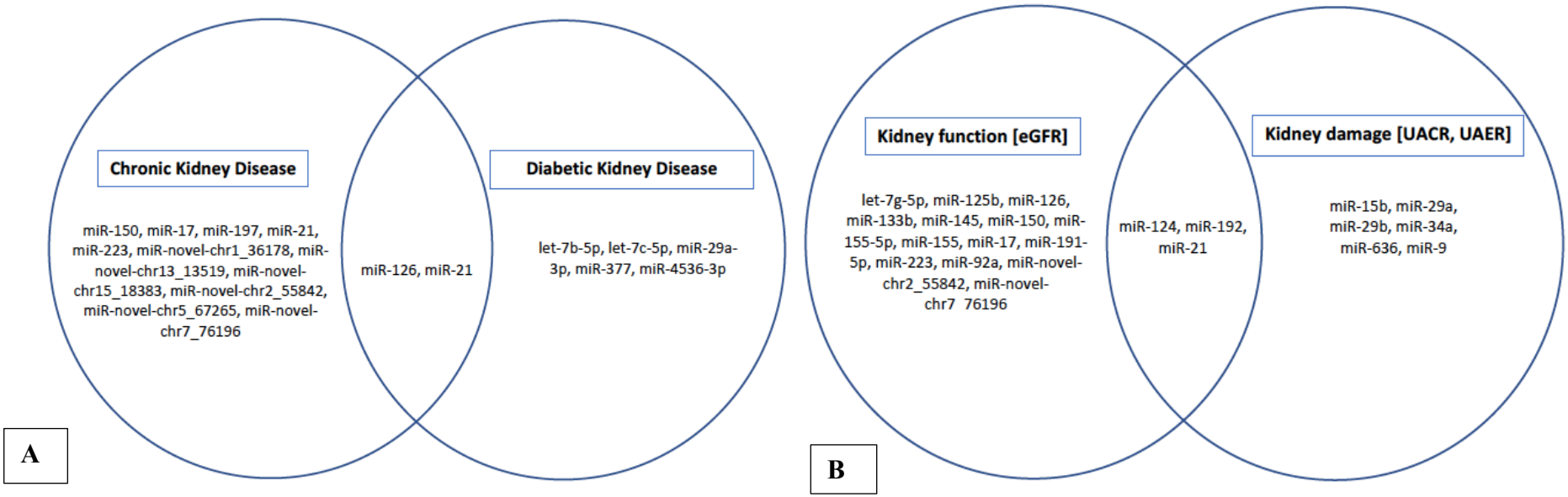

Figure 2: *Associations between miRNAs and CKD subgroups and markers of kidney function and damage.* **a)** Shows miRNAs independently associated with CKD in general population and in high-risk individuals with HTN and DM. **b)** miRNAs associated with markers of kidney function and or kidney damage in general population and in high-risk individuals with HTN and DM.

4.4. Discussion

Recent studies have demonstrated that miRNAs are key mediators in the pathophysiology of CKD, suggesting that circulating miRNAs have potential utility as alternative markers for early detection and progression of CKD, as well as monitoring treatment responses. Circulating and urinary miRNAs are ideal minimally- or non-invasive biomarkers because they are stable in body fluids and exosomes and can be detected using validated techniques for quantification. However, miRNA profile studies in humans have shown contradictory results, with few miRNAs being consistently dysregulated across studies. We performed a systematic review of studies that evaluated miRNA expressions in CKD in the general population, and high-risk subgroups (individuals with HTN and DM). We found that two miRNAs (miR-126 and miR-223) were consistently downregulated in the general population with CKD, whilst miR-21 and miR-29b were consistently upregulated and let-7a-3p and miR-30e were consistently downregulated in individuals with DKD, in whole blood, plasma, serum, urine, or urinary exosomes. Although showing inconsistent data, miR-155, miR-192, miR-15a-5p, miR-29a, miR-29c were also commonly quantified in the studies included in this review. Of note, only a few studies quantified miRNA expression in individuals with HTN-associated CKD, and reported inconsistent findings and none in HIVAN.

MiR-126 is endothelial cell-specific and promotes vascular integrity and angiogenesis via regulation of vascular endothelial growth factor (VEGF) signalling and, as a result, inhibits vascular inflammation [100]. MiR-126, which was downregulated in CKD in the general population [21,28,32] and individuals with DM [48,76] and HTN [99] when quantified in serum and whole blood samples, was inversely associated with prevalent CKD [32,33] and positively associated with eGFR [21,33]. A prospective study showed that lower levels of miR-126 were associated with an increased risk of developing CKD and rapid decline in kidney function over a period of five years [33]. Zhou et al also demonstrated that miR-126 has an atheroprotective role, as it increases vascular smooth muscle cells (VSMCs) turnover, thereby regulating the contractile phenotype of VSMC [101]. Taken together, the downregulation of miR-126 may result in vascular dysfunction, which is very common in early-stage CKD. MiR-126 may therefore be a potential biomarker for the early identification of CKD and a potential target for the prevention or treatment of CKD-related vascular complications. Contrary to these findings, a few studies reported upregulated expression of miR-126 individuals with DKD [53,60,64,76] when quantified in urine, plasma as well as serum. It is plausible that this may be a compensatory mechanism resulting in increased release of miR-126 when microvascular endothelial cells are exposed to stressful conditions [102]. Indeed, Beltrami and colleagues used *in vitro* analyses and showed that miR-126 is released from glomerular endothelial cells in response to DKD-related cytokines [53].

Similarly to miR-126, miR-223 also plays a role in the regulation of VSMC proliferation [103]. This miRNA was consistently downregulated in the general population with CKD in serum samples

[21,28,32,43]. Moreover, lower levels of this miRNA were associated with lower levels of eGFR [32], and prevalent CKD [21]. Although commonly considered to be involved in inflammatory pathways, evidence also suggests a protective role of miR-223 in VSMCs, through the inhibition of calcification by the regulation of interleukin-6 (IL-6)/signal transducer and activator of transcription 3 (STAT3) [63]. These findings imply that increased levels of miR-223 may improve kidney function, and thus may serve as a therapeutic strategy to improve CKD outcomes in the general population.

The involvement of miR-21 in kidney fibrosis is well established, although the mechanisms involved have not been completely clarified. miR-21 acts as a pro-fibrotic factor, and its upregulation induces kidney fibrosis through TGF-β signalling pathway regulation [104]. Consistent upregulation of miR-21 in individuals with DKD [51,61,64,67,71,76,77,89], as well as its inverse association with eGFR [71] and positive association with albuminuria [71] have been reported. Moreover, increased levels of miR-21 were associated with rapid progression to ESKD over a 10-year follow-up period [77]. Correspondingly, *in vitro* and *in vivo* knockdown of miR-21 ameliorated DKD by reducing albuminuria, kidney inflammation, podocyte loss and interstitial fibrosis, suggesting its value as a potential therapeutic target against DKD progression [105]. Although the observed findings imply that increased expression of miR-21 may be associated with the development and progression of DKD, the data was inconclusive in the case of CKD. In the general population with CKD, contrasting results were reported, with miR-21 upregulated in urine [30] and urine exosomes [34] but downregulated in serum samples [30]. Moreover, Fujii and colleagues found that increased levels of miR-21 were positively associated with eGFR and inversely associated with the risk of CKD in the general population [22]. Donderski et al. (2021) explained that the lower levels of miR-21 detected in serum samples of individuals with CKD may be as a result of suppression caused by increased fibrosis and TGF–β activity [30]. This is in line with the findings by Sun et al (2018), using a murine kidney fibrosis model, they observed that miR-21 is the main regulator of fibroblasts activation through an auto-regulatory loop between miR-21 and programmed cell death protein 4 and activated protein 1, therefore, suggesting that miR-21 may act as pro- or anti-fibrotic depending on the cell type [106]. It has been suggested that identification of the cellular source of miRNAs would be helpful instead of the biofluid sample to link the miRNAs to the specific disease process [107].

The miR-29 family has been well studied with regard to the TGF-β signalling pathway [104]. Two studies included in our review reported that miR-29b was upregulated in the urine samples of individuals with DKD [50,53], although one study reported no difference in the expression level of miR-29b when quantified in urine supernatant [75]. The lack of difference could be explained by the relatively lower abundance of miR-29b in urine supernatant sample reported in this study [75]. In individuals with HTN, increased expression of miR-29b was positively associated with albuminuria and inversely associated with kidney function [96]. However, these findings are contrary to previous

studies that have reported on the protective role of this miRNA in DKD. Chen et al. (2014) showed that knockdown of miR-29b in diabetic mice was associated with increased albuminuria and TGFβ mediated fibrosis whereas overexpression of miR-29b attenuated kidney fibrosis in DKD through the regulation of TGFβ1/Smad3 pathway [108]. The upregulated expression of miR-29b in DKD observed in the included studies in our review may be due to the compensatory release of miR-29b. Beltrami and colleagues used *in vitro* analyses and observed increased release of miR-29b from glomerular endothelial cells in response to DKD-related cytokines [53]. Regarding the expression profile of miR-29a and miR-29c, contradictory results were observed when quantified in various samples of individuals with DKD. miR-29a was downregulated in plasma samples of individuals with severe DKD [51] and inversely associated with rapid progression to ESKD over a 10-year follow-up period [77], suggesting that this miRNA may have a protective effect against the progression of DKD. However, when quantified in urine supernatant, upregulated expression of miR-29a was observed in individuals with DKD [75]. Studies have previously highlighted that urine supernatant miRNAs inversely reflect intracellular miRNAs [109], which could be the possible reason for the discrepancy, however, tissue expression of miR-29a was not analyzed in this study [75]. On the other hand, Guo et al (2017) [62] analyzed the expression of miR-29c in three different samples of individuals with DKD relative to those without, and found downregulated expression in urinary sediments and kidney tissues but upregulated expression in plasma. Cui and Cui (2020) found that relative to blood, urinary miRNAs highly reflected kidney tissue miRNAs and suggested that urine should be a better sample for kidney miRNAs analysis [110].

MicroRNAs miR-30e and let-7a were consistently downregulated in individuals with DKD relative to those without DKD suggesting that increased expression of these miRNAs may have protective effects in the kidney and therefore may serve as possible diagnostic and prognostic markers of DKD. Accordingly, previous evidence suggests that the let-7 family of miRNAs may be a negative regulator of kidney fibrosis in DKD [111]. Muralidharan and colleagues[112] validated the expression of let-7a in an Alb/TGFβ mouse model of CKD and found that the miRNA was significantly downregulated further suggesting its possible role in the development or progression of CKD. MicroRNAs in the miR-30 family are highly enriched in kidney podocytes cells where they are involved in regulatory roles and are essential for structural and functional homeostasis [113]. In vitro and in vivo experimental studies showed that the expression of miR-30e was significantly decreased in those with DKD whereas overexpression of miR-30e was protective against the development of kidney fibrosis in DKD suggestive the potential role of this miRNA as a therapeutic target.[114]

miR-155 was commonly analyzed in the studies included in this review. Experimental studies have shown that suppressing miR-155 expression in DKD mice protects against kidney damage, attenuates hyperglycaemia-induced kidney damage and downregulates IL-17 expression by enhancing the suppression of cytokine signalling 1 (SOCS1) [115]. In line with these, upregulated expression of miR-155 was observed in the general population with CKD [30,31] and individuals with DKD [53]. However, downregulated expression of miR-155 was commonly reported in individuals with DKD [19,47,52], as well as in the general population with CKD [29,30]. Furthermore, Donderski and colleagues reported on the positive association of miR-155 and eGFR [30]. These findings suggest that miR-155 may also play a role in the development of DKD. Wang et al. (2018) demonstrated that miR-155 is involved in the regulation of the autophagic process in DKD through the regulation of a signalling loop p53/miR-155-5p/Sirt1 and may therefore serve as a therapeutic strategy for DKD [116].

miR-192 has been shown to have a protective effect against kidney fibrosis. Downregulated expression of miR-192 was observed in individuals with DKD [49,70,71], and reduced levels of miR-192 were positively associated with kidney function [71] and inversely associated with kidney damage [70,71], suggesting that miR-192 levels may decrease with the increasing level of albuminuria and a decline in kidney function. Consistently, *in vivo* studies have shown that loss of miR-192 was associated with the development and progression of DKD through exacerbation of kidney fibrosis by enhancing TGF-β1 signalling pathway [117]. These findings suggest that reduced expression of miR-192 in the early stage may be associated with the development of DKD and, therefore may serve as an early indicator of DKD. However, contrary to these findings, increased expression of miR-192 in individuals with DKD relative to controls has been observed in a few studies [64,76]. Jia and colleagues reported that the expression of miR-192 was increased during the early stages of DKD and decreased in the advanced stages of DKD [66]. They further showed that miR-192 was positively correlated with albuminuria and TGF-β1 levels [66], suggesting a profibrotic role of this miRNA. Jenkins et al. (2012) highlighted that miR-192 is pleiotropic, involved in multiple important roles in the kidney, and its role as an antifibrotic or profibrotic factor may be cell dependent [118].

This review provides an overview of miRNA dysregulation in CKD, including diabetic and hypertensive-related CKD in humans. It also highlights miRNAs that are associated with CKD and its clinical indicators. A few miRNAs showed consistent expression patterns in CKD relative to controls, whilst the majority of the frequently studied miRNAs, showed contradictory findings. These discrepancies may be partly explained by the technical and methodological inter-study variabilities, such as the use of different biological samples, sample handling and processing procedures, miRNA extraction protocols, detection and quantification techniques, and normalizing controls [119]. Although the majority of included studies quantified miRNA expression in blood, recent evidence points to the superiority of urine miRNAs to serum/plasma miRNAs for CKD diagnosis with the non-invasive

nature in which urine samples are collected, adding to its preference [120]. Another challenge is the lack of a standardized normalization control for miRNA expression studies. Although a wide range of endogenous and exogenous miRNAs are employed as normalizers, emerging evidence suggests that the use of synthetic spike in controls such as cel-miR-39 is preferable [121]. Therefore, to be able to identify reliable miRNA biomarkers, research findings need to be reproducible and comparable between studies. This can be achieved when normalization controls have been validated, and there is a standardization of robust protocols for sample processing and extraction [122].

4.4.1. Strength and limitations

The main strength of this review is its comprehensive report of miRNAs dysregulated in CKD, their association with CKD as well as clinical markers of CKD in the general population as well as in high-risk individuals with HTN and/or DM for the very first time. The review also provides a list of miRNAs that have been frequently studied in diverse geographical areas and showed consistent expression patterns across studies in CKD and DKD and therefore are worthy for future research.

The studies included in the review had their own shortcomings and, as such, impacted the review's overall quality of evidence. The most important limitation of the review was the inability to perform a pooled meta-analysis of our studies due to various factors, including insufficient raw data on fold changes or relative expression of miRNAs, technical and methodological variabilities between studies, such as the use of different biological samples, normalization control used, and different miRNA quantification techniques. Moreover, due to insufficient data, we could not report on the expression patterns of miRNAs across different stages of CKD. The language restriction on the inclusion criteria may have excluded other relevant studies, thus biasing our findings. Additionally, there were differences in the classification of CKD, wide sample size ranges, variability in participant demographic factors such as age, sex proportion, and race, as well as environmental and regional factors between studies.

4.5. Conclusion

MiRNAs detected in biofluids are promising as potential biomarkers of disease diagnosis and therapeutic targets for future clinical applications. However, understanding their role in CKD pathophysiology and how their expression pattern is regulated is still in its infancy. As such, further research is required to fully elucidate their roles before any extrapolation for clinical use. Prevention and early detection of CKD have been a topic of interest for many researchers and clinicians in the field. This review highlights a number of dysregulated miRNAs that were frequently studied and showed consistent findings across studies in CKD (miR-126 and miR-223) or DKD (miR-21, miR-29b, let-7a and miR-30e) with a potential for clinical application in CKD diagnosis/prognosis in the future. This consistent alteration of miRNAs with CKD/DKD and their stability and detectability in

bodily fluids suggests that these miRNAs are promising potential non-invasive or minimally invasive diagnostic markers for early detection and therapeutic targets of CKD/DKD and warrant further scrutiny in future investigations. Besides these specific miRNAs, miR-155, miR-192, miR-15a-5p, miR-29a and miR-29c, despite their inconsistent expression patterns reported in different studies, were commonly dysregulated in CKD and/or DKD, and therefore may also play an important role in CKD. As such, their further exploration is warranted. Furthermore, it may be worthwhile for future studies to focus on identifying target genes and pathways of these frequently studied miRNAs, to get a complete understanding of their role in the development and progression of CKD, as well as to assess their potential value as diagnostic markers or therapeutic targets.

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4.6. References

1. Foreman KJ, Marquez N, Dolgert A, Fukutaki K, Fullman N, McGaughey M, et al. Forecasting life expectancy, years of life lost, and all-cause and cause-specific mortality for 250 causes of death: reference and alternative scenarios for 2016–40 for 195 countries and territories. Lancet. 2018;392(10159):2052–90.

2. Collaboration GC. Global, regional, and national burden of chronic kidney disease, 1990-

2017: a systematic analysis for the Global Burden of Disease Study 2017 - PubMed [Internet]. 2020 [cited 2022 May 31]. Available from: https://pubmed.ncbi.nlm.nih.gov/32061315/

3. Essue BM, Jha V, John O, Knight J, Jan S. Universal health coverage and chronic kidney disease in India. Bull World Health Organ. 2018;96(7):442.

4. Oguntola SO, Hassan MO, Duarte R, Vachiat A, Manga P, Naicker S. Atherosclerotic vascular disease is more prevalent among black ESKD patients on long-term CAPD in South Africa. BMC Nephrol. 2019;20(1):1–10.

5. Li P, Garcia-Garcia G, Lui S-F, Andreoli S, Fung W, Hradsky A, et al. Kidney health for everyone everywhere–from prevention to detection and equitable access to care. Brazilian J Med Biol Res. 2020;53.

6. Lopez-Giacoman S, Madero M. Biomarkers in chronic kidney disease, from kidney function to kidney damage. World J Nephrol. 2015 Feb;4(1):57–73.

7. Inker LA, Eneanya ND, Coresh J, Tighiouart H, Wang D, Sang Y, et al. New creatinine-and cystatin C–based equations to estimate GFR without race. N Engl J Med. 2021;385(19):1737–49.

8. Bukabau JB, Yayo E, Gnionsahé A, Monnet D, Pottel H, Cavalier E, et al. Performance of creatinine-or cystatin C–based equations to estimate glomerular filtration rate in sub-Saharan African populations. Kidney Int. 2019;95(5):1181–9.

9. Fabian J, Kalyesubula R, Mkandawire J, Hansen CH, Nitsch D, Musenge E, et al. Measurement of kidney function in Malawi, South Africa, and Uganda: a multicentre cohort study. Lancet Glob Heal. 2022;10(8):e1159–69.

10. Al-Rubeaan K, Siddiqui K, Al-Ghonaim MA, Youssef AM, Al-Sharqawi AH, AlNaqeb D. Assessment of the diagnostic value of different biomarkers in relation to various stages of diabetic nephropathy in type 2 diabetic patients. Sci Rep. 2017;7(1):1–9.

11. Levin A, Tonelli M, Bonventre J, Coresh J, Donner JA, Fogo AB, et al. Global kidney health 2017 and beyond: a roadmap for closing gaps in care, research, and policy. Lancet. 2017;390(10105):1888–917.

12. Bonneau E, Neveu B, Kostantin E, Tsongalis GJ, De Guire V. How close are miRNAs from clinical practice? A perspective on the diagnostic and therapeutic market. Electron J Int Fed Clin Chem Lab Med. 2019;30(2):114–27.

13. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009;136(2):215–33.

14. Bhatt K, Mi QS, Dong Z. MicroRNAs in kidneys: Biogenesis, regulation, and pathophysiological roles. Am J Physiol - Ren Physiol. 2011;300(3):602–10.

15. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci. 2008;105(30):10513–8.

16. Chen X, Xie D, Zhao Q, You ZH. MicroRNAs and complex diseases: From experimental results to computational models. Brief Bioinform. 2019;20(2):515–39.

17. Zhu H, Wang G, Zhou X, Song X, Gao H, Ma C, et al. miR-1299 suppresses cell proliferation of hepatocellular carcinoma (HCC) by targeting CDK6. Biomed Pharmacother [Internet]. 2016;83:792–7. Available from: http://dx.doi.org/10.1016/j.biopha.2016.07.037

18. Amiri A, Tehran MM, Asemi Z, Shafiee A, Hajighadimi S, Moradizarmehri S, et al. Role of resveratrol in modulating microRNAs in human diseases: From cancer to inflammatory disorder. Curr Med Chem. 2019;26(March 2020).

19. Wang J, Wang G, Liang Y, Zhou X. Expression profiling and clinical significance of plasma microRNAs in diabetic nephropathy. J Diabetes Res. 2019;2019.

20. Liu Y, Usa K, Wang F, Liu P, Geurts AM, Li J, et al. MicroRNA-214-3p in the kidney contributes to the development of hypertension. J Am Soc Nephrol. 2018;29(10):2518–28.

21. Fourdinier O, Schepers E, Metzinger-Le Meuth V, Glorieux G, Liabeuf S, Verbeke F, et al. Serum levels of miR-126 and miR-223 and outcomes in chronic kidney disease patients. Sci Rep. 2019;9(1):1–12.

22. Fujii R, Yamada H, Munetsuna E, Yamazaki M, Ohashi K, Ishikawa H, et al. Associations of Circulating MicroRNAs (miR-17, miR-21, and miR-150) and Chronic Kidney Disease in a Japanese Population. J Epidemiol. 2019 Apr;30(4):177–82.

23. Motshwari DD, Matshazi DM, Erasmus R, Kengne AP, Matsha TE, George C. MicroRNAs associated with chronic kidney disease in the general population and high-risk subgroups: protocol for a systematic review and meta-analysis. BMJ Open. 2022;12(2):e057500.

24. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol. 2010;25(9):603–5.

25. Guyatt G, Oxman AD, Akl EA, Kunz R, Vist G, Brozek J, et al. GRADE guidelines: 1. Introduction—GRADE evidence profiles and summary of findings tables. J Clin Epidemiol. 2011;64(4):383–94.

26. Levey AS, Coresh J, Greene T, Marsh J, Stevens LA, Kusek JW, et al. Expressing the

Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. Clin Chem. 2007;53(4):766–72.

27. Levey AS, Stevens LA, Schmid CH, Zhang Y, Castro III AF, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604–12.

28. Carmona A, Guerrero F, Jimenez MJ, Ariza F, Agüera ML, Obrero T, et al. Inflammation, Senescence and MicroRNAs in Chronic Kidney Disease. Front cell Dev Biol. 2020;8:739.

29. Chen NX, Kiattisunthorn K, O'Neill KD, Chen X, Moorthi RN, Gattone VH, et al. Decreased microRNA is involved in the vascular remodeling abnormalities in chronic kidney disease (CKD). PLoS One [Internet]. 2013 May 22;8(5):e64558. Available from: https://search.ebscohost.com/login.aspx?direct=true&db=cmedm&AN=23717629&site=ehost-live

30. Donderski R, Szczepanek J, Naruszewicz N, Naruszewicz R, Tretyn A, Skoczylas-Makowska N, et al. Analysis of profibrogenic microRNAs (miRNAs) expression in urine and serum of chronic kidney disease (CKD) stage 1-4 patients and their relationship with proteinuria and kidney function. Int Urol Nephrol. 2021 Jul;

31. Eckersten D, Tsatsanis C, Giwercman A, Bruun L, Pihlsgård M, Christensson A. MicroRNA-155 and Anti-Müllerian Hormone: New Potential Markers of Subfertility in Men with Chronic Kidney Disease. Nephron Extra. 2017;7(1):33–41.

32. Fujii R, Yamada H, Yamazaki M, Munetsuna E, Ando Y, Ohashi K, et al. Circulating microRNAs (miR-126, miR-197, and miR-223) are associated with chronic kidney disease among elderly survivors of the Great East Japan Earthquake. BMC Nephrol. 2019 Dec;20(1):474.

33. Fujii R, Yamada H, Tsuboi Y, Ando Y, Munetsuna E, Yamazaki M, et al. Association between circulating microRNAs and changes in kidney function: A five-year prospective study among Japanese adults without CKD. Clin Chim Acta. 2021 Oct;521:97–103.

34. Lange T, Artelt N, Kindt F, Stracke S, Rettig R, Lendeckel U, et al. MiR-21 is up-regulated in urinary exosomes of chronic kidney disease patients and after glomerular injury. J Cell Mol Med. 2019 Jul;23(7):4839–43.

35. Li H, Qiu FX, Tian F, Shi XZ, Gao AQ, Song L, et al. Changes of miR-155 expression in serum of uremic patients before and after treatment and risk factors analysis. Exp Ther Med. 2020;20(4):3352–60.

36. Liu X, Wang W, Bai Y, Zhang H, Zhang S, He L, et al. Identification of a genome-wide serum microRNA expression profile as potential noninvasive biomarkers for chronic kidney disease

using next-generation sequencing. J Int Med Res. 2020 Dec;48(12):300060520969481.

37. Motshwari DD, George C, Matshazi DM, Weale CJ, Davids SFG, Erasmus RT, et al. Novel Whole Blood MicroRNAs Predicting Chronic Kidney Disease in South Africans with Hypertension and Diabetes Mellitus. Appl Sci. 2021;11(16).

38. Muralidharan J, Ramezani A, Hubal M, Knoblach S, Shrivastav S, Karandish S, et al. Extracellular microRNA signature in chronic kidney disease. Am J Physiol - Ren Physiol. 2017;312(6):F982–91.

39. Rudnicki M, Perco P, D Haene B, Leierer J, Heinzel A, Mühlberger I, et al. Renal microRNA- and RNA-profiles in progressive chronic kidney disease. Eur J Clin Invest. 2016 Mar;46(3):213–26.

40. Sayilar EI, Gullulu M, Tuncel E, Peynirci H, Alemdar A, Tunca B, et al. Biomarker Potential of Urine miR-451 at Different Stages of Diabetic Nephropathy. J Diabetes Metab. 2016;7(2).

41. Shang F, Wang S-C, Hsu C-Y, Miao Y, Martin M, Yin Y, et al. MicroRNA-92a Mediates Endothelial Dysfunction in CKD. J Am Soc Nephrol [Internet]. 2017 Nov;28(11):3251–61. Available from: https://search.ebscohost.com/login.aspx?direct=true&db=cmedm&AN=28696247&site=ehost-live

42. Trojanowicz B, Imdahl T, Ulrich C, Fiedler R, Girndt M. Circulating miR-421 Targeting Leucocytic Angiotensin Converting Enzyme 2 Is Elevated in Patients with Chronic Kidney Disease. Nephron. 2019;141(1):61–74.

43. Ulbing M, Kirsch AH, Leber B, Lemesch S, Münzker J, Schweighofer N, et al. MicroRNAs 223-3p and 93-5p in patients with chronic kidney disease before and after renal transplantation. Bone [Internet]. 2017;95:115–23. Available from: http://dx.doi.org/10.1016/j.bone.2016.11.016

44. Abdelsalam M, Wahab AM, El Sayed Zaki M, Motawea M. MicroRNA-451 as an Early Predictor of Chronic Kidney Disease in Diabetic Nephropathy. Int J Nephrol. 2020;2020.

45. Abdou AE, Anani HAA, Ibrahim HF, Ebrahem EE, Seliem N, Youssef EMI, et al. Urinary IgG, serum CX3CL1 and miRNA-152-3p: As predictors of nephropathy in Egyptian type 2 diabetic patients. Tissue Barriers. 2022;10(3):1994823.

46. Akhbari M, Biglari A, Shahrabi-Farahani M, Khalili M, Bandarian F. Expression Level of Circulating miR-93 in Serum of Patients with Diabetic Nephropathy. TURKISH J Endocrinol Metab. 2018;22(2):78–84.

47. Akhbari M, Khalili M, Shahrabi-Farahani M, Biglari A, Bandarian F. Expression Level of

Circulating Cell Free miR-155 Gene in Serum of Patients with Diabetic Nephropathy. Clin Lab. 2019 Aug;65(8).

48. Al-Kafaji G, Al-Mahroos G, Al-Muhtaresh HA, Skrypnyk C, Sabry MA, Ramadan AR. Decreased expression of circulating microRNA-126 in patients with type 2 diabetic nephropathy: A potential blood-based biomarker. Exp Ther Med. 2016 Aug;12(2):815–22.

49. Al-Kafaji G, Al-Muhtaresh HA. Expression of microRNA-377 and microRNA-192 and their potential as blood-based biomarkers for early detection of type 2 diabetic nephropathy. Mol Med Rep. 2018 Jul;18(1):1171–80.

50. Argyropoulos C, Wang K, McClarty S, Huang D, Bernardo J, Ellis D, et al. Urinary microRNA profiling in the nephropathy of type 1 diabetes. PLoS One. 2013;8(1):e54662.

51. Assmann TS, Recamonde-Mendoza M, Costa AR, Puñales M, Tschiedel B, Canani LH, et al. Circulating miRNAs in diabetic kidney disease: case–control study and in silico analyses. Acta Diabetol [Internet]. 2019;56(1):55–65. Available from: http://dx.doi.org/10.1007/s00592-018-1216-x

52. Barutta F, Tricarico M, Corbelli A, Annaratone L, Pinach S, Grimaldi S, et al. Urinary exosomal microRNAs in incipient diabetic nephropathy. PLoS One [Internet]. 2013 Nov 4;8(11):e73798. Available from: https://search.ebscohost.com/login.aspx?direct=true&db=cmedm&AN=24223694&site=ehost-live

53. Beltrami C, Simpson K, Jesky M, Wonnacott A, Carrington C, Holmans P, et al. Association of Elevated Urinary miR-126, miR-155, and miR-29b with Diabetic Kidney Disease. Am J Pathol [Internet]. 2018;188(9):1982–92. Available from: https://doi.org/10.1016/j.ajpath.2018.06.006

54. Cardenas-Gonzalez M, Srivastava A, Pavkovic M, Bijol V, Rennke HG, Stillman IE, et al. Identification, confirmation, and replication of novel urinary microrna biomarkers in lupus nephritis and diabetic nephropathy. Clin Chem. 2017;63(9):1515–26.

55. Conserva F, Barozzino M, Pesce F, Divella C, Oranger A, Papale M, et al. Urinary miRNA-27b-3p and miRNA-1228-3p correlate with the progression of Kidney Fibrosis in Diabetic Nephropathy. Sci Rep. 2019 Aug;9(1):11357.

56. Delić D, Eisele C, Schmid R, Baum P, Wiech F, Gerl M, et al. Urinary Exosomal miRNA Signature in Type II Diabetic Nephropathy Patients. PLoS One. 2016;11(3):e0150154.

57. Dieter C, Assmann TS, Costa AR, Canani LH, de Souza BM, Bauer AC, et al. MiR-30e-5p and MiR-15a-5p Expressions in Plasma and Urine of Type 1 Diabetic Patients With Diabetic

Kidney Disease. Front Genet. 2019;10:563.

58. Eissa S, Matboli M, Aboushahba R, Bekhet MM, Soliman Y. Urinary exosomal microRNA panel unravels novel biomarkers for diagnosis of type 2 diabetic kidney disease. J Diabetes Complications [Internet]. 2016;30(8):1585–92. Available from: http://dx.doi.org/10.1016/j.jdiacomp.2016.07.012

59. Eissa S, Matboli M, Bekhet MM. Clinical verification of a novel urinary microRNA panal: 133b, -342 and -30 as biomarkers for diabetic nephropathy identified by bioinformatics analysis. Biomed Pharmacother. 2016 Oct;83:92–9.

60. Florijn BW, Duijs JMGJ, Levels JH, Dallinga-Thie GM, Wang Y, Boing AN, et al. Diabetic Nephropathy Alters the Distribution of Circulating Angiogenic MicroRNAs Among Extracellular Vesicles, HDL, and Ago-2. Diabetes. 2019 Dec;68(12):2287–300.

61. Fouad M, Salem I, Elhefnawy K, Raafat N, Faisal A. MicroRNA-21 as an Early Marker of Nephropathy in Patients with Type 1 Diabetes. Indian J Nephrol. 2020;30(1):21–5.

62. Guo J, Li J, Zhao J, Yang S, Wang L, Cheng G, et al. MiRNA-29c regulates the expression of inflammatory cytokines in diabetic nephropathy by targeting tristetraprolin. Sci Rep [Internet]. 2017 May 24;7(1):2314. Available from: https://search.ebscohost.com/login.aspx?direct=true&db=cmedm&AN=28539664&site=ehost-live

63. Han Q, Zhang Y, Jiao T, Li Q, Ding X, Zhang D, et al. Urinary sediment microRNAs can be used as potential noninvasive biomarkers for diagnosis, reflecting the severity and prognosis of diabetic nephropathy. Nutr Diabetes. 2021 Jun;11(1):24.

64. He F, Peng F, Xia X, Zhao C, Luo Q, Guan W, et al. MiR-135a promotes renal fibrosis in diabetic nephropathy by regulating TRPC1. Diabetologia [Internet]. 2014 Aug;57(8):1726–36. Available from: https://search.ebscohost.com/login.aspx?direct=true&db=cmedm&AN=24908566&site=ehost-live

65. Hong Y, Wang J, Zhang L, Sun W, Xu X, Zhang K. Plasma miR-193a-3p can be a potential biomarker for the diagnosis of diabetic nephropathy. Ann Clin Biochem. 2021 Mar;58(2):141–8.

66. Jia Y, Guan M, Zheng Z, Zhang Q, Tang C, Xu W, et al. miRNAs in Urine Extracellular Vesicles as Predictors of Early-Stage Diabetic Nephropathy. J Diabetes Res. 2016;2016:7932765.

67. Khokhar M, Roy D, Bajpai NK, Bohra GK, Yadav D, Sharma P, et al. Metformin mediates

MicroRNA-21 regulated circulating matrix metalloproteinase-9 in diabetic nephropathy: an in-silico and clinical study. Arch Physiol Biochem. 2021 Jun;1–11.

68. Lin M, Song D, Zhang S, Li P. Dysregulation of miR-638 in diabetic nephropathy and its role in inflammatory response. Diabetol Metab Syndr. 2021;13(1):1–8.

69. Liu X, Liu S, Luo D, Huang S, Wang F, Zhang B, et al. Involvement of Circulating Exosomal MicroRNAs in Jian-Pi-Yi-Shen Formula Protection Against Adenine-Induced Chronic Kidney Disease. Front Pharmacol [Internet]. 2021 Feb 2;11:622658. Available from: https://search.ebscohost.com/login.aspx?direct=true&db=cmedm&AN=33603670&site=ehost-live

70. Ma X, Lu C, Lv C, Wu C, Wang Q. The Expression of miR-192 and Its Significance in Diabetic Nephropathy Patients with Different Urine Albumin Creatinine Ratio. J Diabetes Res. 2016;2016:6789402.

71. Milas O, Gadalean F, Vlad A, Dumitrascu V, Gluhovschi C, Gluhovschi G, et al. Deregulated profiles of urinary microRNAs may explain podocyte injury and proximal tubule dysfunction in normoalbuminuric patients with type 2 diabetes mellitus. J Investig Med Off Publ Am Fed Clin Res. 2018 Apr;66(4):747–54.

72. Monjezi A, Khedri A, Zakerkish M, Mohammadzadeh G. Resistin, TNF-α, and microRNA 124-3p expressions in peripheral blood mononuclear cells are associated with diabetic nephropathy. Int J Diabetes Dev Ctries. 2022;42(1):62–9.

73. Motawi TK, Shehata NI, ElNokeety MM, El-Emady YF. Potential serum biomarkers for early detection of diabetic nephropathy. Diabetes Res Clin Pract. 2018 Feb;136:150–8.

74. Park S, Kim O-H, Lee K, Park IB, Kim NH, Moon S, et al. Plasma and urinary extracellular vesicle microRNAs and their related pathways in diabetic kidney disease. Genomics. 2022;114(4):110407.

75. Peng H, Zhong M, Zhao W, Wang C, Zhang J, Liu X, et al. Urinary miR-29 correlates with albuminuria and carotid intima-media thickness in type 2 diabetes patients. PLoS One. 2013;8(12):e82607.

76. Petrica L, Milas O, Vlad M, Vlad A, Gadalean F, Dumitrascu V, et al. Interleukins and miRNAs intervene in the early stages of diabetic kidney disease in Type 2 diabetes mellitus patients. Biomark Med. 2019 Dec;13(18):1577–88.

77. Pezzolesi MG, Satake E, McDonnell KP, Major M, Smiles AM, Krolewski AS. Circulating TGF-β1-Regulated miRNAs and the Risk of Rapid Progression to ESRD in Type 1 Diabetes. Diabetes. 2015 Sep;64(9):3285–93.

78. Prabu P, Rome S, Sathishkumar C, Gastebois C, Meugnier E, Mohan V, et al. MicroRNAs from urinary extracellular vesicles are non-invasive early biomarkers of diabetic nephropathy in type 2 diabetes patients with the “Asian Indian phenotype”. Diabetes Metab. 2019 Jun;45(3):276–85.

79. Regmi A, Liu G, Zhong X, Hu S, Ma R, Gou L, et al. Evaluation of Serum microRNAs in Patients with Diabetic Kidney Disease: A Nested Case-Controlled Study and Bioinformatics Analysis. Med Sci Monit Int Med J Exp Clin Res. 2019 Mar;25:1699–708.

80. Ren HW, Ma XY, Shao Y, Han JY, Yang M, Wang QY. Correlation Between Serum miR-154-5p and Osteocalcin in Males and Postmenopausal Females of Type 2 Diabetes With Different Urinary Albumin Creatinine Ratios. Front Endocrinol (Lausanne). 2019;10.

81. Ren H, Wu C, Shao Y, Liu S, Zhou Y, Wang Q. Correlation between serum miR-154-5p and urinary albumin excretion rates in patients with type 2 diabetes mellitus: a cross-sectional cohort study. Front Med. 2020 Oct;14(5):642–50.

82. Roux M, Perret C, Feigerlova E, Mohand Oumoussa B, Saulnier P-J, Proust C, et al. Plasma levels of hsa-miR-152-3p are associated with diabetic nephropathy in patients with type 2 diabetes. Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc. 2018 Dec;33(12):2201–7.

83. Rovira-Llopis S, Escribano-Lopez I, Diaz-Morales N, Iannantuoni F, Lopez-Domenech S, Andújar I, et al. Downregulation of miR-31 in Diabetic Nephropathy and its Relationship with Inflammation. Cell Physiol Biochem Int J Exp Cell Physiol Biochem Pharmacol. 2018;50(3):1005–14.

84. Shao Y, Ren H, Lv C, Ma X, Wu C, Wang Q. Changes of serum Mir-217 and the correlation with the severity in type 2 diabetes patients with different stages of diabetic kidney disease. Endocrine. 2017 Jan;55(1):130–8.

85. Sham SYZ, Ng CT, Azwar S, Yip WK, Abdullah M, Thevandran K, et al. Circulating miRNAs in Type 2 Diabetic Patients with and without Albuminuria in Malaysia. Kidney Blood Press Res. 2022;47(2):81–93.

86. Su J, Ren J, Chen H, Liu B. MicroRNA-140-5p ameliorates the high glucose-induced apoptosis and inflammation through suppressing TLR4/NF-κB signaling pathway in human renal tubular epithelial cells. Biosci Rep. 2020;40(3):1–13.

87. Xiao Y, Guo S, Zhang Y, Bian Z, Jia L, Hu Y, et al. Diabetic nephropathy: serum miR-9 confers a poor prognosis in and is associated with level changes of vascular endothelial growth factor and pigment epithelium-derived factor. Biotechnol Lett. 2017 Oct;39(10):1583–

90.

88. Xie JX, Fan X, Drummond CA, Majumder R, Xie Y, Chen T, et al. MicroRNA profiling in kidney disease: Plasma versus plasma-derived exosomes. Gene. 2017;627(March):1–8.

89. Zang J, Maxwell AP, Simpson DA, McKay GJ. Differential Expression of Urinary Exosomal MicroRNAs miR-21-5p and miR-30b-5p in Individuals with Diabetic Kidney Disease. Sci Rep. 2019 Jul;9(1):10900.

90. Zhang L, Li R, He J, Yang Q, Wu Y, Huang J, et al. Co-expression analysis among microRNAs, long non-coding RNAs, and messenger RNAs to understand the pathogenesis and progression of diabetic kidney disease at the genetic level. Methods. 2017 Jul;124:46–56.

91. Zhang J, Zhang L, Zha DQ, Wu XY. Inhibition of miRNA-135a-5p ameliorates TGF-beta 1-induced human renal fibrosis by targeting SIRT1 in diabetic nephropathy. Int J Mol Med. 2020;46(3):1063–73.

92. Zhao Y, Shen A, Guo F, Song Y, Jing N, Ding X, et al. Urinary Exosomal MiRNA-4534 as a Novel Diagnostic Biomarker for Diabetic Kidney Disease. Front Endocrinol (Lausanne). 2020;11:590.

93. Zhou J, Peng R, Li T, Luo X, Peng H, Zha H, et al. A potentially functional polymorphism in the regulatory region of let-7a-2 is associated with an increased risk for diabetic nephropathy. Gene. 2013 Sep;527(2):456–61.

94. Berillo O, Huo K-G, Fraulob-Aquino JC, Richer C, Briet M, Boutouyrie P, et al. Circulating let-7g-5p and miR-191-5p are independent predictors of chronic kidney disease in hypertensive patients. Am J Hypertens. 2020;33(6):505–13.

95. Huang Y-Q, Huang C, Li J, Zhang B, Feng Y-Q. The association of miR-29a with proteinuria in essential hypertension. J Hum Hypertens. 2018 Nov;32(11):775–80.

96. Huang C, Huang Y-Q. The correlation of circulating miR-29b and inflammatory markers with albuminuria in hypertensive patients. Clin Exp Hypertens. 2020 Nov;42(8):743–7.

97. Nandakumar P, Tin A, Grove ML, Ma J, Boerwinkle E, Coresh J, et al. MicroRNAs in the miR-17 and miR-15 families are downregulated in chronic kidney disease with hypertension. PLoS One. 2017;12(8):1–13.

98. Perez-Hernandez J, Olivares D, Forner MJ, Ortega A, Solaz E, Martinez F, et al. Urinary exosome miR-146a is a potential marker of albuminuria in essential hypertension. J Transl Med. 2018 Aug;16(1):228.

99. Perez-Hernandez J, Riffo-Campos AL, Ortega A, Martinez-Arroyo O, Perez-Gil D, Olivares

D, et al. Urinary- and Plasma-Derived Exosomes Reveal a Distinct MicroRNA Signature Associated With Albuminuria in Hypertension. Hypertens (Dallas, Tex 1979). 2021 Mar;77(3):960–71.

100. Fish JE, Santoro MM, Morton SU, Yu S, Yeh R-F, Wythe JD, et al. miR-126 regulates angiogenic signaling and vascular integrity. Dev Cell. 2008;15(2):272–84.

101. Zhou J, Li Y-S, Nguyen P, Wang K-C, Weiss A, Kuo Y-C, et al. Regulation of vascular smooth muscle cell turnover by endothelial cell–secreted microRNA-126: role of shear stress. Circ Res. 2013;113(1):40–51.

102. Prattichizzo F, Giuliani A, Ceka A, Rippo MR, Bonfigli AR, Testa R, et al. Epigenetic mechanisms of endothelial dysfunction in type 2 diabetes. Clin Epigenetics. 2015;7.

103. Metzinger-Le Meuth V, Burtey S, Maitrias P, Massy ZA, Metzinger L. microRNAs in the pathophysiology of CKD-MBD: Biomarkers and innovative drugs. BBA - Mol Basis Dis [Internet]. 2017 Jan;1863(1):337–45. Available from: http://10.0.3.248/j.bbadis.2016.10.027

104. Panizo S, Martínez-Arias L, Alonso-Montes C, Cannata P, Martín-Carro B, Fernández-Martín JL, et al. Fibrosis in Chronic Kidney Disease: Pathogenesis and Consequences. Int J Mol Sci. 2021 Jan;22(1).

105. Kölling M, Kaucsar T, Schauerte C, Hübner A, Dettling A, Park J-K, et al. Therapeutic miR-21 Silencing Ameliorates Diabetic Kidney Disease in Mice. Mol Ther [Internet]. 2017 Jan 4;25(1):165–80. Available from: https://search.ebscohost.com/login.aspx?direct=true&db=cmedm&AN=28129112&site=ehost-live

106. Sun Q, Miao J, Luo J, Yuan Q, Cao H, Su W, et al. The feedback loop between miR-21, PDCD4 and AP-1 functions as a driving force for renal fibrogenesis. J Cell Sci [Internet]. 2018 Mar 26;131(6). Available from: https://search.ebscohost.com/login.aspx?direct=true&db=cmedm&AN=29361523&site=ehost-live

107. Sun IO, Lerman LO. Urinary microRNA in kidney disease: Utility and roles. Am J Physiol - Ren Physiol. 2019;316(5):F785–93.

108. Chen H-Y, Zhong X, Huang XR, Meng X-M, You Y, Chung ACK, et al. MicroRNA-29b Inhibits Diabetic Nephropathy in db/db Mice. Mol Ther [Internet]. 2014 Apr;22(4):842–53. Available from: http://10.0.4.14/mt.2013.235

109. Zen K, Zhang C. Circulating microRNAs: a novel class of biomarkers to diagnose and monitor human cancers. Med Res Rev. 2012;32(2):326–48.

110. Cui C, Cui Q. The relationship of human tissue microRNAs with those from body fluids. Sci Rep. 2020;10(1):1–7.

111. Sakuma H, Hagiwara S, Kantharidis P, Gohda T, Suzuki Y. Potential Targeting of Renal Fibrosis in Diabetic Kidney Disease Using MicroRNAs. Front Pharmacol. 2020;11:587689.

112. Muralidharan J, Ramezani A, Hubal M, Knoblach S, Shrivastav S, Karandish S, et al. Extracellular microRNA signature in chronic kidney disease. Am J Physiol Ren Physiol [Internet]. 2017 Jun;312(6):F982–91. Available from: http://10.0.4.128/ajprenal.00569.2016

113. Guo L, Tan K, Luo Q, Bai X. Dihydromyricetin promotes autophagy and attenuates renal interstitial fibrosis by regulating miR-155-5p/PTEN signaling in diabetic nephropathy. Bosn J basic Med Sci [Internet]. 2020 Aug 3;20(3):372–80. Available from: https://search.ebscohost.com/login.aspx?direct=true&db=cmedm&AN=31668144&site=ehost-live

114. Zhao D, Jia J, Shao H. miR-30e targets GLIPR-2 to modulate diabetic nephropathy: in vitro and in vivo experiments. J Mol Endocrinol [Internet]. 2017 Aug;59(2):181–90. Available from: https://search.ebscohost.com/login.aspx?direct=true&db=cmedm&AN=28733476&site=ehost-live

115. Lin X, You Y, Wang J, Qin Y, Huang P, Yang F. MicroRNA-155 deficiency promotes nephrin acetylation and attenuates renal damage in hyperglycemia-induced nephropathy. Inflammation. 2015;38(2):546–54.

116. Wang Y, Zheng ZJ, Jia YJ, Yang YL, Xue YM. Role of p53/miR-155-5p/sirt1 loop in renal tubular injury of diabetic kidney disease. J Transl Med. 2018;16.

117. Krupa A, Jenkins R, Luo DD, Lewis A, Phillips A, Fraser D. Loss of MicroRNA-192 Promotes Fibrogenesis in Diabetic Nephropathy. J Am Soc Nephrol. 2010;21(3):438–47.

118. Jenkins RH, Martin J, Phillips AO, Bowen T, Fraser DJ. Pleiotropy of microRNA-192 in the kidney. Biochem Soc Trans [Internet]. 2012 Aug;40(4):762–7. Available from: https://search.ebscohost.com/login.aspx?direct=true&db=cmedm&AN=22817730&site=ehost-live

119. Witwer KW. Circulating microRNA biomarker studies: pitfalls and potential solutions. Clin Chem. 2015;61(1):56–63.

120. Li J, Ma L, Yu H, Yao Y, Xu Z, Lin W, et al. MicroRNAs as Potential Biomarkers for the Diagnosis of Chronic Kidney Disease: A Systematic Review and Meta-Analysis. Front Med. 2021;8.

121. Roberts TC, Coenen-Stass AML, Wood MJA. Assessment of RT-qPCR normalization strategies for accurate quantification of extracellular microRNAs in murine serum. PLoS One. 2014;9(2):e89237.

122. Zampetaki A, Mayr M. Analytical challenges and technical limitations in assessing circulating miRNAs. Thromb Haemost. 2012;108(10):592–8.

CHAPTER 5

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5. MANUSCRIPT 1

EXPRESSION OF WHOLE BLOOD miR-126-3p, -30a-5p, -1299, -182-5p & -30e-3p IN CHRONIC KIDNEY DISEASE IN A SOUTH AFRICAN COMMUNITY-BASED SAMPLE

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ABSTRACT

The burden of chronic kidney disease (CKD) in Africa remains poorly characterized, due partly to the lack of appropriate diagnostic strategies. Although in recent years the diagnostic and prognostic utility of microRNAs (miRNAs) have gained prominence in the context of CKD, its value has not been evaluated in African populations. We investigated the expression of whole blood miRNAs (miR-126-3p, -30a-5p, -1299, -182-5p & -30e-3p) in people with and without CKD, as well as the association of these miRNAs with prevalent CKD, in a community-based sample of South African adults. We used Reverse Transcription Quantitative Real-Time PCR (RT-qPCR) to analyze miRNA expression in 1449 individuals (13.3% with prevalent CKD). There was an increased expression in whole blood miR-126-3p, -30a-5p, -1299 and -182-5p in individuals with CKD, compared to those without (all $p \leq 0.036$), whereas miR-30e-3p showed no significant difference between the groups ($p=0.482$). Only miR-126-3p, -182-5p and -30e-3p were independently associated with increased risk of CKD (all $p \leq 0.022$). This study showed for the first time that there is a dysregulation of whole blood miR-126-3p, -30a-5p, -1299 and -182-5p in South Africans of mixed-ancestry with CKD. More research is needed to ascertain their role in CKD risk screening in African populations.

KEYWORDS

Chronic kidney disease, Whole blood, miRNAs, eGFR, ACR, Africa

5.1. Introduction

Chronic kidney disease (CKD) is a major health concern globally, affecting approximately 8-16% of the adult population worldwide, with more than three quarters of these people residing in low-to-middle-income countries (LMICs)[1]. Although there is a paucity of data on CKD in LMICs like those in Africa, recent studies suggest an escalation in the prevalence of CKD on the African continent. Indeed, in 2014 the first systematic review on CKD prevalence reported that 13.9% of the general sub-Saharan African population had CKD[2], and recently an increased estimated prevalence of 15.8% was reported for adults living in Africa[3]. The burden of CKD in Africa, which is partly attributable to the high prevalence of hypertension, diabetes and human immunodeficiency virus (HIV)-infection[4], also affects younger individuals and disease progression to end-stage renal disease (ESRD) occurs at an earlier age, compared to other populations in Western countries[5].

Currently, the diagnostic approach used to identify persons with CKD depends mainly on the estimated glomerular filtration rate (eGFR), calculated predominantly by means of the 4-variable Modification of Diet in Renal Disease (MDRD)[6] and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations[7]. There are various limitations to the equations, for example the predominant use of serum creatinine for estimation of glomerular filtration rate which may be affected by factors such as age, sex and body mass[8]. Moreover, these prediction equations, were derived and validated in high income countries with predominately Caucasian populations. Therefore, these equations are biased and need to be validated in African populations for correct estimates of CKD burden. The limitations of these equations have stimulated interest for novel diagnostic markers with improved diagnostic/prognostic accuracy for CKD. Since their discovery in 1993[9], microRNAs (miRNAs) have attracted immense attention in biomedical science as possible biomarkers for early detection and prognosis for various diseases such as cancer[10], cardiovascular diseases (CVDs)[11] and kidney diseases[12]. miRNAs are small non-coding single-stranded transcripts with approximately 19 to 24 nucleotides that regulate gene expression post transcriptionally[13]. They function by binding to the 3‘untranslated region (UTR) of their target messenger RNAs (mRNAs), thereby silencing gene expression either by inhibition of translation and/or by mRNA degradation[12]. To date, more than 2700 miRNAs have been identified in the human genome according to database miRbase, release 22.1[14]. A growing body of evidence suggests that miRNAs are involved in more than 90% of cellular activities, and their expression is tissue and cell type specific[15]. Furthermore, it has been found that these miRNAs are also detectable in biofluids in a highly stable manner[16]. As such, their high extracellular stability, tissue-specific expression, and feasible measurability by current techniques, makes them attractive candidate biomarkers for disease diagnosis and prognosis.

A set of miRNAs are expressed specifically in the human kidney and may be involved in the development, homeostasis, and physiology of the kidney[17]. Emerging evidence from animal models[18]

and in humans[18,19] suggest that dysregulation of miRNAs expressed in the kidney may be associated with the development of CKD. Many of these studies quantified miRNA expression in plasma[20,21] and serum samples[19,22]. The use of plasma or serum is subject to limitations due to a number of factors including, cell lysis bias and pre-analytical analysis which might induce contamination or variation during sample processing because of lower miRNA yield in these samples[23].Despite the fact that Africa has the greatest genetic diversity compared to any other region in the world[24], there are as yet no existing studies that have examined the expression of miRNAs in African populations, thus the present study aimed: (1) to characterize the expression level of whole blood miRNAs, previously associated in the literature with kidney function or kidney disease pathophysiology, namely miR-126-3p19, -30a-5p25, -129926, -182-5p27 and -30e-3p28, and (2) to investigate the association between these miRNAs and prevalent CKD, in a community-based sample of adult South Africans.

5.2. Results

5.2.1. General characteristics of the study population

The general characteristics of the participants by CKD status are summarized in Table 1. Of the 1989 individuals recruited for the VMH study, only 1449 samples were included in the present analysis. The remaining 540 samples were excluded due to missing data required to calculate eGFR. The present study comprised of 13.3% individuals with CKD (stage 1-5) and 26.4% male participants. In the group with CKD, 39.4%, 18.1%, 34.7%, 5.7% and 2.1% were in CKD stages 1 to 5, respectively. The individuals with CKD were on average older (61 vs 49 years, $p<0.0001$), with a greater proportion being over the age of 60 years (53.8% vs. 21.4%, $p<0.0001$). Persons with CKD further had a larger WC (96.0 vs 89.6 cm, $p<0.0001$), HC (104.3 vs 101.4 cm, $p=0.001$), and higher BMI (30.1 vs 27.0 kg/m^2, $p=0.0002$), compared to those without CKD. The levels of FPG, 2-hr glucose, fasting insulin, 2-hr insulin, HbA1c, triglycerides, total cholesterol, CRP, SBP, DBP and PP were significantly higher in participants with CKD compared to participants without CKD (all $p<0.05$). Serum cotinine levels were significantly lower in persons with CKD compared to those without CKD ($p=0.0008$). Individuals with CKD also had higher percentages of overweight, obese, pre-diabetic (IFD/IGT), DM and hypertension (all $p<0.0001$); whereas the percentage of smokers and drinkers were significantly lower in individuals with CKD (all $p<0.0001$).

5.2.2. MiRNA expression

The expression levels of whole blood miRNAs, miR-126-3p, -30a-5p, -1299 and -182-5p, were significantly higher in individuals with CKD compared to those without CKD (all $p\leq0.0364$), with similar levels of miR-30e-3p observed between the two groups ($p=0.482$).

5.2.3. Spearman correlation coefficients

The association between circulating miRNAs (miR-126-3p, -30a-5p, -1299, -182-5p and -30e-3p), anthropometric and biochemical parameters are shown in Table 2. Only miR-126-3p was positively

correlated with eGFR and ACR (r=0.09, p=0.045 and r=0.10, p=0.018, respectively). None of the other miRNAs showed an association with either measure of kidney function (p≥0.063 for all). The miRNAs were also correlated with various clinical parameters. miR-126-3p, -30a-5p, -182-5p and -30e-3p were positively associated with fasting insulin (r=0.10, p=0.021; r=0.10, p=0.021; r=0.14, p=0.001 and r=0.13, p=0.004, respectively) and inversely associated with HbA1c (r=-0.12, p=0.005; r=-0.11, p=0.016; r=-0.12, p=0.004 and r=-0.10, p=0.021, respectively). Moreover, miR-126-3p showed positive correlation with WC and HC (r=0.09, p=0.032 and r=0.11, p=0.015, respectively), with miR-30a-5p positively correlated with total cholesterol (r=0.09, p=0.037). Higher levels of miR-182-5p were correlated with a larger HC (r=0.09, p=0.047). Furthermore, miR-30e-3p showed positive correlation with weight, WC and HC (r=0.10, p=0.024; r=0.10, p=0.030 and r=0.13, p=0.004, respectively) and negative correlation with cotinine (r=-0.10, p=0.030).

5.2.4. Relationship between whole blood miRNAs and prevalent CKD

The crude and adjusted associations between whole blood miRNAs (miR-126-3p, -30a-5p, -1299, -182-5p and -30e-3p) and prevalent CKD are presented in Table 3. Higher expression of miR-126-3p, -182-5p and 30e-3p was associated with an increased prevalence of CKD (p≤ 0.001) (Model 1), and this positive association was independent of age, sex, hypertension, DM, smoking status and drinking status (p≤0.022) (Models 2-4). The miRNAs, miR-30a-5p and -1299 were not associated with prevalent CKD across all models (all p≥ 0.093).

Variables	Without CKD (n=1256)	CKD (n= 193)	p-value
Age (years)	49 (35-58)	61 (49-70)	<0.0001
Age >60 years (n,%)	269 (21.4)	104 (53.9)	<0.0001
Gender (n,% male)	344 (27.4)	38 (19.7)	0.024
Weight (kg)	68.9 (57.9-84.4)	73.2 (62.2-83.3)	0.135
Waist circumference (cm)	89.6 (76.8-102.4)	96.0 (86.8-105.7)	<0.0001
Hip circumference (cm)	101.4 (90.5-112.8)	104.3 (96.3-114.5)	0.0011
Body mass index (kg/m^2)	27.0 (21.8-33.1)	30.1 (25.2- 34.6)	0.0002
Fasting plasma glucose (mmol/l)	4.9 (4.5-5.5)	5.3 (4.8-7.4)	<0.0001
2-hour glucose (mmol/l)	5.8 (4.7-7.3)	6.9 (5.6-8.7)	<0.0001
Fasting insulin (IU/l)	6.5 (4.1-10.6)	7.2 (4.6-12.2)	0.015
2-hour insulin (IU/l)	35.6 (18.3-66.9)	47.0 (24.4-81.4)	0.0028
Glycated haemoglobin (%)	5.7 (5.4-6.1)	6.1 (5.6-7.4)	<0.0001
Triglycerides (mmol/L)	1.2 (0.8-1.7)	1.4 (1.0-2.0)	0.0001
HDL-C (mmol/L)	1.3 (1.1-1.5)	1.3 (1.0-1.5)	0.103
LDL-C (mmol/L)	3.0 (2.4-3.7)	3.2 (2.5-3.8)	0.105
Total cholesterol (mmol/L)	5.0 (4.3-5.8)	5.2 (4.5-6.1)	0.019
C-Reactive protein(mg/L)	3.8 (1.51-8.32)	5.1 (2.62-12.61)	<0.0001
Serum cotinine (ng/mL)	79.7 (10-274)	10.0 (10.0-230.5)	0.0008
Systolic blood pressure (mmHg)	131 (116-149)	146 (126-165)	<0.0001
Diastolic blood pressure (mmHg)	83 (75-93)	88 (79-100)	<0.0001
Pulse pressure (mmHg)	70 (63-79)	73 (63-84)	0.005
Body mass index categories (n, %)			<0.0001
Normal weight	501 (39.9)	48 (25.0)	
Overweight	286 (23.0)	48 (25.0)	
Obese	469 (37.7)	97 (50.5)	
Glucose tolerance categories (n, %)			<0.0001
Impaired fasting glucose/impaired glucose tolerance	181 (14.4)	26 (13.5)	
Diabetes mellitus	200 (15.9)	77 (39.9)	
Hypertension (n, %)	708 (56.4)	169 (87.6)	<0.0001
Smokers (n,%)	651 (53.9)	68 (35.4)	<0.0001
Drinkers (n,%)	398 (31.9)	17 (8.9)	<0.0001
miR-126-3p ($2^{-\Delta Ct}$)	0.85 (0.34-1.71)	1.06 (0.38-2.35)	0.003
miR-30a-5p ($2^{-\Delta Ct}$)**	0.10 (0.03-0.31)	0.15 (0.04-0.36)	0.027
miR-1299 ($2^{-\Delta Ct}$)*	0.08 (0.02-0.26)	0.13 (0.02-0.46)	0.036
miR-182-5p ($2^{-\Delta Ct}$)	0.93 (0.35-2.06)	1.32 (0.40-2.71)	0.005
miR-30e-3p ($2^{-\Delta Ct}$)*	0.23 (0.07-0.67)	0.25 (0.06-1.00)	0.482

Table 1: *General characteristics of the study participants and miRNA expression by chronic kidney disease status*

HDL-C (high-density lipoprotein cholesterol); LDL-C (low-density lipoprotein cholesterol); miR (microRNA). Data are presented as median (25th;75th percentiles) and count and percentages. * and ** represent miRNAs factored by 100 and 10 respectively, as the values were very low.

Variables	miR-126-3p ($2^{-\Delta Ct}$)		miR-30a-5p ($2^{-\Delta Ct}$)		miR-1299 ($2^{-\Delta Ct}$)		miR-182-5p ($2^{-\Delta Ct}$)		miR-30e-3p ($2^{-\Delta Ct}$)	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
Age (years)	-0.02	0.588	-0.07	0.123	0.05	0.250	-0.03	0.493	-0.02	0.6216
Weight (kg)	0.07	0.101	0.05	0.258	-0.07	0.149	0.05	0.266	0.10	0.024
Waist circumference (cm)	0.09	0.032	0.05	0.216	-0.05	0.267	0.08	0.071	0.10	0.030
Hip circumference (cm)	0.11	0.015	0.07	0.114	-0.05	0.284	0.09	0.047	0.13	0.004
Body mass index (kg/m^2)	-0.02	0.423	-0.01	0.693	-0.02	0.412	-0.03	0.371	0.03	0.386
Fasting plasma glucose (mmol/l)	-0.03	0.485	-0.02	0.661	-0.04	0.367	-0.03	0.516	-0.03	0.541
2-hour glucose (mmol/l)	0.02	0.690	0.01	0.761	0.05	0.291	-0.00	0.964	0.03	0.428
Fasting insulin (IU/l)	0.10	0.021	0.10	0.021	-0.01	0.767	0.14	0.001	0.13	0.004
2-hour insulin (IU/l)	0.03	0.488	0.04	0.323	-0.03	0.510	0.07	0.129	0.03	0.521
Glycated haemoglobin (%)	-0.12	0.005	-0.11	0.016	-0.03	0.522	-0.12	0.005	-0.10	0.021
Triglycerides (mmol/L)	0.06	0.163	0.06	0.200	-0.05	0.318	0.06	0.179	0.03	0.520
HDL-C (mmol/L)	0.03	0.468	0.03	0.540	-0.01	0.869	0.00	0.961	0.01	0.859
LDL-C (mmol/L)	0.04	0.343	0.07	0.133	-0.06	0.214	0.04	0.328	0.05	0.307
Total cholesterol (mmol/L)	0.05	0.205	0.09	0.037	-0.05	0.261	0.05	0.248	0.04	0.402
C-reactive protein (mg/L)	0.04	0.330	-0.02	0.684	0.03	0.493	-0.00	0.917	-0.00	0.935
Serum cotinine (ng/mL)	-0.03	0.491	-0.03	0.444	0.05	0.328	-0.04	0.337	-0.10	0.030
Systolic blood pressure (mmHg)	0.02	0.695	0.01	0.820	0.07	0.123	-0.03	0.512	-0.03	0.561
Diastolic blood pressure (mmHg)	0.01	0.754	-0.03	0.569	0.03	0.472	-0.01	0.795	-0.03	0.492
Pulse pressure (BPM)	0.06	0.147	-0.02	0.604	0.05	0.277	0.05	0.211	0.05	0.223
eGFR	0.09	0.045	0.04	0.309	-0.01	0.908	0.08	0.076	0.07	0.094
ACR	0.10	0.018	0.07	0.103	0.09	0.063	0.07	0.115	0.08	0.069

Table 2: *Spearman correlation coefficients for the association between circulating miRNAs and anthropometric and biochemical parameters*

ACR (albumin-to-creatinine ratio); eGFR (estimated glomerular filtration rate); HDL-C (high-density lipoprotein cholesterol); LDL-C (low-density lipoprotein cholesterol); miR (microRNA). Data presented as correlation coefficient (*rho*) and p-value.

	OR	95% Confidence interval	p-value
miR-126-3p ($2^{-\Delta CT}$)			
Model 1	1.33	1.19 to 1.48	<0.0001
Model 2	1.36	1.21 to 1.52	<0.0001
Model 3	1.34	1.20 to 1.50	<0.0001
Model 4	1.34	1.20 to 1.51	<0.0001
miR-30a-5p ($2^{-\Delta CT}$)			
Model 1	1.07	0.84 to 1.35	0.581
Model 2	1.23	0.97 to 1.57	0.093
Model 3	1.19	0.91 to 1.56	0.197
Model 4	1.20	0.91 to 1.58	0.202
miR-1299 ($2^{-\Delta CT}$)			
Model 1	1.10	0.98 to 1.22	0.096
Model 2	1.09	0.97 to 1.22	0.171
Model 3	1.10	0.98 to 1.23	0.095
Model 4	1.10	0.98 to 1.23	0.104
miR-182-5p ($2^{-\Delta CT}$)			
Model 1	1.09	1.03 to 1.15	0.001
Model 2	1.11	1.05 to 1.18	<0.0001
Model 3	1.11	1.04 to 1.17	0.001
Model 4	1.10	1.04 to 1.17	0.001
miR-30e-3p ($2^{-\Delta CT}$)			
Model 1	1.18	1.02 to 1.39	0.039
Model 2	1.25	1.06 to 1.48	0.009
Model 3	1.28	1.08 to 1.52	0.005
Model 4	1.27	1.07 to 1.52	0.007

Table 3: *Logistic regression analyses of whole blood miRNAs for the prediction of prevalent CKD*

miR (microRNA) and OR (odds ratio). Models: Model 1: Crude; Model 2: Model 1 + age + sex; Model 3: Model 2 + hypertension + DM; Model 4: Model 3 + smoking status + drinking status.

5.3. Discussion

The present study aimed to characterize the expression level of miR-126-3p, -30a-5p, -1299, -182-5p and -30e-3p in whole blood samples of individuals with CKD compared to those without CKD for the first time in a mixed-ancestry community in South Africa. In this cross-sectional study, we found that the expression levels of circulating miR-126-3p, 30a-5p, -1299 and -182-5p were significantly elevated in individuals with CKD. Moreover, higher levels of miR-126-3p, -182-5p and 30e-3p were independently associated with increased risk of prevalent CKD.

In accordance with our observations, a study conducted in individuals with diabetic kidney disease (DKD), showed that the plasma levels of various miRNAs, including miR-126-3p, were significantly increased in these individuals, compared to people without DKD, and this increase was positively associated with angiopoietin (Ang)-2/Ang-1 ratio, an indicator of systemic microvascular damage[21]. In line with this finding, studies have shown that the plasma level of miR-126-3p is significantly elevated in various forms of diabetes when compared to normoglycaemic controls[29,30]. The potential link between miR-126-3p and microvascular damage is further supported by a study, albeit in an animal model, which showed that vascular damage as a result of CKD is indeed associated with the upregulation of miR-126-3p in the aorta of mice[31]. Interestingly, a study by Park et al., showed that the plasma levels of miR-126-3p were significantly elevated in individuals with essential hypertension and atherosclerotic renal artery stenosis (ARAS) as compared to healthy controls[32]. Taken together, these findings and ours may suggest that increased expression of miR-126-3p is involved in disease progression and may serve as a potential marker of microvascular damage in CKD. In contrast, other studies have reported that the expression of miR-126-3p is downregulated in individuals with CKD (stages 1–5 and those on RRT)[19,22], DKD[33] and in individuals with ESRD[34]. In support of these reports, an animal study by Bijkerk et al., showed that overexpression of miR-126-3p in the kidneys of mice offered protection against renal ischemia by promoting vascular regeneration[35]. Also, an in vitro study on human cavernous endothelial cells (hCECs) exposed to a diabetic-like environment, showed that overexpression of miR-126-3p ameliorates endothelial dysfunction by regulating the mitogen-activated protein kinase (MAPK) signaling pathway, by reducing the expression of its target gene, Sprouty-related protein with an EVH1 domain (SPRED1)[36]. These findings[35,36] are supported by the observation of highly expressed miR-126-3p in endothelial cells, where it is involved in processes including vascular integrity, angiogenesis and wound repair[37].

The present study provides evidence for the first time that links miR-182-5p with an increased risk of preva-lent CKD. Previous studies have shown a positive association between miR-182-5p and DKD[38] as well as acute kidney injury (AKI)[39]. Ming et al., reported that the expression of miR-182-5p was upregulated in the podocytes of individuals with DKD, compared to non-diabetic controls. According to their findings, increased expression of miR-182-5p was associated with the downregulation of CD2-associated protein (CD2AP); which is a protein involved in podocyte apoptosis and the development of CKD in diabetics[38]. Likewise, Wilflingseder et al., found that the patients who developed AKI after kidney transplant had an increased expression level of miR-182-5p as compared to those who didn't develop AKI post-transplant[39]. Our findings are further corroborated by the results obtained from animal studies of AKI, where inhibition of miR-182-5p in kidney tissue, improved kidney function and facilitated cell proliferation, metabolism, and angiogenesis[40]. Given that miR-182-5p is involved in a variety of processes including apoptosis, proliferation, metabolism, and angiogenesis, upregulation of this miRNA could indicate increased cell death. Taken together, these

findings suggest that miR-182-5p may play a role in the pathogenesis of CKD and may serve as a potential prognostic marker and a possible therapeutic target for CKD. However, contrary to our findings, others observed that reduced expression of miR-182-5p was associated with disease development, including DKD[41] and human renal carcinoma cells (RCC) via activation of AKT/FOXO3 signalling pathway[42].

miR-30e-3p, which belongs to the miR-30 family (miR-30a to -e), is abundantly expressed in human glomerular podocytes cells[43]. The involvement of this family of miRNAs has been reported in processes related to podocyte injury, glomerulosclerosis, and proteinuria[43]. In the present study, we for the first time observed a positive association between miR-30e-3p and increased risk of CKD. A previous study also found plasma levels of miR-30e-3p were significantly increased, however in individuals with Contrast-Induced AKI (CI-AKI) compared to those without CI-AKI[44]. Contrary, others have reported reduced expression of miR-30e-3p in individuals with DKD[45], with the reduced expression being associated with the transforming growth factor beta 1 (TGF-β1)-mediated epithelial–mesenchymal transition and kidney fibrosis[46]. miR-30a-5p, another member of the miR-30 family and miR-1299 showed no association with increased risk of prevalent CKD in the current study. However, their expression level was upregulated in CKD individuals as compared to those with normal kidney function. Even though the upregulation of miR-30a-5p has been reported in plasma samples of individuals with CI-AKI[44], other studies reported the downregulation of this miRNA, in glomeruli and proximal tubules of DKD[41] and in RCC[25]. In line with our findings, Jeong et al.[26] also reported that miR-1299 was not associated with CKD progression. Thus, these findings taken collectively infer that miR-30-5p and -1299 may not be involved in the development of CKD.

Contradictory findings regarding the expression of miR-126-3p, miR-182-5p and miR-30e-3p in CKD from different studies may be attributable to several factors, including the differences in cohorts. Several of these studies have been evaluated in either adults of Asian or European descent, with none conducted in African populations. Studies have shown that differences in demographic factors including ethnicity[47], age and sex[48] influence the expression of certain miRNAs. Moreover, in the present study we included individuals from an industrialized area and even though we adjusted for smoking, we did not consider the effects of environmental factors such as air pollution. In a review by Vrijens et al., the expression of certain miRNAs was found to change as a result of exposure to smoking and air pollution[49]. Another contributing factor may have been due to the varying sample types used to quantify miRNA expression. In the present study we quantified miRNA expression in whole blood samples which comprise different blood cell populations; potentially confounding the true miRNA profile specific for CKD[50]. However, whole blood samples are superior to plasma or serum samples in that it has a high miRNA yield and is not affected by pre-analytical analysis or cell lysis bias and therefore the results may be more reliable[23]. A study by Pascut et al., showed that there

were more miRNAs expressed in whole blood than serum[51]. Additionally, others have reported that certain miRNAs are highly expressed in serum as compared to plasma, which may be as a result of RNA molecules released during coagulation in serum[52]. Thus, these findings suggest that results of miRNA expressions quantified in different blood fractions are not necessarily comparable. Moreover, the lack of a validated normalization control remains a major challenge that affects the interpretability of results and may partly be the reason behind the variations and lack of comparability between studies[53]. Furthermore, some studies included individuals who were on medication, although there is evidence that corticosteroids[54] reduce whereas metformin[55] increases the expression of certain miRNAs. In the present study we did not adjust for any medication taken and steroids were not measured, as this was beyond the scope of our study. As such, we cannot rule out their possible effect on the observed miRNA expressions. Furthermore, our study has a high prevalence of DM, hypertension and obesity and multiple studies have shown that these conditions are independently associated with circulatory miRNAs[30,32,37]. However, this is unlikely to be the rea-son for the lack of comparability between the current study and others as we have adjusted for these variations. While some studies have shown that miRNA expression decreased with the severity of kidney disease[19,20], we were unable to verify this due to the small sample size of CKD individuals which prevented stratification into the various stages of CKD. Therefore, further mechanistic studies are warranted for elucidation of the exact role of these whole blood miRNAs in CKD.

The present study has other limitations. The cross-sectional design of the study did not allow exploration of the causal relationship between miRNA expression and CKD. We used whole blood samples for miRNA quantification, without normalizing for different blood cell populations. The majority of the individuals were females; although this is a common observation in South African studies. Furthermore, a once-off creatinine measure was used for the estimation of GFR, although the KDIGO international guidelines suggest periodic measurements over a period of 3 months. Also, the kidney function was measured indirectly using eGFR which lacks precision and accuracy instead of the direct measurement of GFR which is more accurate. Moreover, due to the limited sample number of individuals with CKD in the present study we were unable to stratify the CKD group into various stages to see if the miRNAs could discriminate between the stages. However, a strength of the present study is CKD was classified using eGFR incorporated with ACR, as recommended by the KDIGO guidelines. Moreover, the present study included individuals in the early and advanced stages of CKD which serves as a further strength.

5.4. Conclusion

To the best of our knowledge, the current study present evidence for the first time in an African population demonstrating dysregulation of whole blood miRNAs in CKD. Taken together, the findings of this study suggest that miR-126-3p, -182-5p and -30e-3p may be implicated in the

development/progression of CKD and may serve as potential independent prognostic markers for CKD in this population. However, the findings of our study are still exploratory and the conflicting findings in literature suggest that CKD is heterogeneous, and the role of these miRNAs in CKD remains elusive. Large studies in various ethnic groups are warranted to validate our findings, explore molecular mechanisms underlying whole blood miRNA expression in CKD, and elucidate the potential relevance of these miRNAs as diagnostic and/or prognostic markers of CKD in African population before further exploration of their applicability in clinical settings.

5.5. Materials and methods

5.5.1. Study setting and participants

This study which was of a cross-sectional design, involved participants of mixed-ancestry recruited for the Vascular and Metabolic Health (VMH) study[56]. A total of 1989 individuals were recruited between 2014 and 2016 from the Bellville South area, which is located within the northern sub-urbs of Cape Town, South Africa. A detailed description of the study setting, and population has been reported previously[57]. Briefly, individuals aged 20 years or older who gave consent to genetic analysis were included in the current study. The exclusion criteria were ongoing pregnancy, acute illnesses and active communicable diseases.

5.5.2. Questionnaire and physical examination

A standard questionnaire was used to gather information about the participants' demographics. Participants were classified a "drinker" if they self-reported to regularly consume alcohol. The anthropometric measurements including body weight, height, waist circumference (WC) and hip circumferences (HC) were taken by trained personnel, using standardized methods. Body mass index (BMI) was calculated as weight in kilograms, divided by the square of height in meters (kg/m^2). Participants were classified as normal weight, overweight and obese, if BMI was between 18.5–24.9 kg/m^2, 25.0–29.99 kg/m^2 and ≥ 30.0 kg/m^2, respectively.

Blood pressure (BP) measurements were performed according to the World Health Organisation (WHO) guidelines[58], using an automated digital BP monitor (Omron M6 Comfort-preformed Cuff Blood Pressure Moni-tor, Omron), with the participants sitting quietly in a relaxed position. Three BP readings, systolic blood pressure (SBP) and corresponding diastolic blood pressure (DBP), at one-minute intervals, were taken and the lowest read-ing was chosen as the participants' BP. Pulse pressure (PP) was determined by subtracting the DBP from the SBP. Hypertension was defined as SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg or those on antihypertensive medication.

5.5.3. Biochemical analysis

All biochemical analysis was conducted by an ISO 15189 accredited pathology practice (PathCare Laboratory, Cape Town, South Africa). The following biochemical parameters were measured in all

participants: plasma glucose concentrations were measured using the enzymatic hexokinase method (Beckman AU, Beckman Coulter, South Africa), glycated haemoglobin (HbA1c) was measured using high performance liquid chromatography (HPLC) (Biorad Variant Turbo, South Africa), serum insulin was measured using a paramagnetic particle assay (Chemiluminescence), low-density lipoprotein cholesterol (LDL-C) was measured using an Enzymatic Selective Protection –Endpoint assay (Beckman AU, Beckman Coulter, South Africa), high-density lipoprotein cholesterol (HDL-C) using an Enzymatic Immuno-inhibition-Endpoint assay (Beckman AU, Beckman Coulter, South Africa), triglycerides were estimated using a glycerol phosphate oxidase in the presence of peroxidase (GPO-POD) Endpoint assay (Beckman AU, Beckman Coulter, South Africa), ultrasensitive C-reactive protein (CRP) was measured by Latex Particle immunoturbidimetry and serum cotinine was measured by Competitive Chemiluminescent (Immulite 2000, Siemens, South Africa). Participants were classified a "current smoker", if serum cotinine levels were > 15 ng/mL[59]. All participants, excluding those who self-reported their diabetic status (confirmed by either participant medical card record or use of diabetic medication), underwent a 75 g oral glucose tolerance test (OGTT) after an overnight fast, according to WHO guidelines[60]. The OGTT glucose levels were used to group participants according to WHO criteria[61] as: (1) normal glucose tolerance [fasting plasma glucose (FPG) <6.1 mmol/l and 2 hour postprandial glucose (2-hr glucose) <7.8 mmol/l]; (2) pre-diabetes including impaired fasting glucose (IFG, 6.1≤FPG< 7.0 mmol/l), impaired glucose tolerance (IGT, 7.8<2-hr glucose<11.1 mmol/l) and the combination of both; and (3) diabetes mellitus (DM) (FPG≥7.0 mmol/l and/or 2-hr glucose≥11.1 mmol/l). In addition to the screen detected DM, those with a history of previously diagnosed DM were also grouped as DM.

5.5.4. Classification of kidney function

Urinary albumin levels were measured using the colorimetric (using bromocresol purple) method (Beckman AU, Beckman Coulter, South Africa) and serum creatinine and urinary creatinine were measured by the modified Jaffe-Kinetic method (Beckman AU, Beckman Coulter, South Africa). The participants were classified into CKD stages 1-5 using eGFR incorporated with staging based on three levels of albumin-to-creatine ratio (ACR) as recommended by the international guidelines by the Kidney Disease: Improving Global Outcomes (KDIGO) 2012[6]. The GFR was estimated using the MDRD equation[6], without the correction factor for African ethnicity. Findings were mostly similar in secondary analyses based on CKD-EPI equation estimated GFR (data not shown). CKD stages were classified as follows: without CKD (eGFR ≥ 90 mL/minute per 1.73 m^2 and ACR < 3 mg/mmol); CKD stage 1 (eGFR ≥ 90 mL/minute per 1.73 m^2 and ACR > 3 mg/mmol), CKD stage 2 (eGFR = 60-89 mL/minute per 1.73 m^2 and ACR > 3 mg/mmol), CKD stage 3 (eGFR = 30-59 mL/minute per 1.73 m^2), CKD stage 4 (eGFR = 15-29 mL/minute per 1.73 m^2) and CKD stage 5 (eGFR < 15 mL/minute per 1.73 m^2). As a collective, CKD was defined as an eGFR <90 ml/min/1.73 m^2 and/or ACR >3 mg/mmol).

5.5.5. RNA extraction

During the survey, whole blood samples were collected in Tempus RNA tube and stored at – 20 degrees Celsius for miRNAs extraction and analysis. The MagMAX^{TM} for Stabilized Blood Tubes RNA Isolation Kit was used for extraction of total RNA, including miRNAs as per manufacturer's specifications (Life Technologies, South Africa). RNA yield and quality were determined using Nanodrop spectrophotometry (Nanodrop one C, Thermo Fisher Scientific, USA). The concentration of RNA sample was determined by measuring its absorbance at 260nm and A260/A280 ratio was used to determine the quality of RNA. RNA samples with concentration > 20 ng/ul and 260/280 value >1.8 were used for miRNA quantification.

5.5.6. Quantitative Reverse transcription PCR (qRT-PCR)

Following total RNA isolation, miRNAs were converted to cDNA before further quantitative analysis. The subsequent reverse transcription was achieved using the TaqMan^{TM} Advanced cDNA Synthesis Kit, and in accordance with the manufacturer's specifications (Applied Biosystems, 2015). Quantification of miRNA expression was then performed using the Taqman Advanced miRNA assay protocol as per manufacturer's instructions, on a QuantStudio 7 Flex (Life Technologies, USA). miR-16 was identified as the most stable endogenous reference gene for miRNA studies in samples derived from individuals with CKD[62]. The delta Ct ($2^{-\Delta Ct}$) method was used to assess the microRNA expression level in each sample whilst the relative miRNA expression between samples was calculated using the delta delta Ct ($2^{-\Delta\Delta CT}$) method[63].

5.5.7. Ethics consideration

Ethical clearance for the VMH study was granted by the Research Ethics Committees of the Cape Peninsula University of Technology (CPUT) and Stellenbosch University (NHREC: REC-230 408-014 and N14/01/003, respectively). The present study was separately approved by the CPUT Faculty of Health and Wellness Sciences Research Ethics Committee (CPUT/HW-REC 2020/H11). Participants were informed about their rights and the procedures were fully explained in the language of their choice. Written informed consent were obtained from all participants. Permission was sought from the relevant authorities in this community. All information about the participants and aspects of the study is kept confidential. Research was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

5.5.8. Statistical analysis

Due to the non-Gaussian distribution of most variables, the general participants' characteristics were presented as median (25^{th}–75^{th} percentiles) or count and percentages. Wilcoxon rank-sum tests (continuous variables) and chi-square tests (categorical variables) were used for comparisons between individuals with CKD and those without CKD. Spearman correlation coefficients (*rho*, r) were used to

assess the association between whole blood miRNAs and anthropometric and biochemical parameters. Logistic regression models were used to analyse the ability of circulating miRNAs to predict prevalent CKD. The models used were as follows: Model 1: Crude; Model 2: Model 1 + age + sex; Model 3: Model 2 + hypertension + DM; Model 4: Model 3 + smoking status+ drinking status.

5.6. References

1. Jha, V. et al. Chronic kidney disease: Global dimension and perspectives. Lancet 382(9888), 260–272. https:// doi. org/ 10. 1016/ S0140- 6736(13) 60687-X (2013).
2. Stanifer, J. W. et al. The epidemiology of chronic kidney disease in sub-Saharan Africa: A systematic review and meta-analysis. Lancet Glob. Health 2(3), e174–e181. https:// doi. org/ 10. 1016/ S2214- 109X(14) 70002-6 (2014).
3. Kaze, A. D., Ilori, T., Jaar, B. G. & Echouffo-Tcheugui, J. B. Burden of chronic kidney disease on the African continent: A systematic review and meta-analysis. BMC Nephrol. 19(1), 1–11 (2018).
4. George, J. A. et al. Kidney damage and associated risk factors in rural and urban sub-Saharan Africa (AWI-Gen): A cross-sectional population study. Lancet Glob. Health 7(12), e1632–e1643 (2019).
5. Arogundade, F. A. & Barsoum, R. S. CKD prevention in Sub-Saharan Africa: A call for governmental, nongovernmental, and community support. Am. J. Kidney Dis. 51(3), 515–523 (2008).
6. Levin, A. et al. Kidney disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int. Suppl. 3(1), 1–150 (2013).
7. Levey, A. S. et al. A new equation to estimate glomerular filtration rate. Ann. Intern. Med. 150(9), 604–612 (2009).
8. Rysz, J., Gluba-Brzózka, A., Franczyk, B., Jabłonowski, Z. & Ciałkowska-Rysz, A. Novel biomarkers in the diagnosis of chronic kidney disease and the prediction of its outcome. Int. J. Mol. Sci. 18(8), 1702 (2017).
9. Lee, R. C., Feinbaum, R. L. & Ambros, V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complemen-tarity to lin-14. Cell 75(5), 843–854 (1993).
10. Zheng, H., Liu, J.-Y., Song, F.-J. & Chen, K.-X. Advances in circulating microRNAs as diagnostic and prognostic markers for ovar-ian cancer. Cancer Biol. Med. 10(3), 123 (2013).
11. Tijsen, A. J., Pinto, Y. M. & Creemers, E. E. Circulating microRNAs as diagnostic biomarkers for cardiovascular diseases. Am. J. Physiol. Circ. Physiol. 303(9), H1085–H1095 (2012).
12. Ramanathan, K. & Padmanabhan, G. MiRNAs as potential biomarker of kidney diseases: A review. Cell Biochem. Funct. 38(8), 990–1005 (2020).

13. Bhatt, K., Mi, Q. S. & Dong, Z. MicroRNAs in kidneys: Biogenesis, regulation, and pathophysiological roles. Am. J. Physiol. Ren. Physiol. 300(3), 602–610 (2011).
14. Kozomara, A., Birgaoanu, M. & Griffiths-Jones, S. MiRBase: From microRNA sequences to function. Nucleic Acids Res. 47(D1), D155–D162 (2019).
15. Londina, E. et al. Analysis of 13 cell types reveals evidence for the expression of numerous novel primate—And tissue-specific microRNAs. Proc. Natl. Acad. Sci. U.S.A. 112(10), E1106–E1115 (2015).
16. Weber, J. A. et al. The microRNA spectrum in 12 body fluids. Clin. Chem. 56(11), 1733–1741 (2010).
17. Sun, Y. et al. Development of a micro-array to detect human and mouse microRNAs and characterization of expression in human organs. Nucleic Acids Res. 32(22), e188 (2004).
18. Tian, Z., Greene, A. S., Pietrusz, J. L., Matus, I. R. & Liang, M. MicroRNA–target pairs in the rat kidney identified by microRNA microarray, proteomic, and bioinformatic analysis. Genome Res. 18(3), 404–411 (2008).
19. Fourdinier, O. et al. Serum levels of miR-126 and miR-223 and outcomes in chronic kidney disease patients. Sci. Rep. 9(1), 1–12 (2019).
20. Chen, N. X. et al. Decreased microRNA is involved in the vascular remodeling abnormalities in chronic kidney disease (CKD). PLoS ONE 8(5), e64558 (2013).
21. Bijkerk, R. et al. Circulating MicroRNAs associate with diabetic nephropathy and systemic microvascular damage and normalize after simultaneous pancreas-kidney transplantation. Am. J. Transplant. 15(4), 1081–1090 (2015).
22. Fujii, R. et al. Circulating microRNAs (miR-126, miR-197, and miR-223) are associated with chronic kidney disease among elderly survivors of the Great East Japan Earthquake. BMC Nephrol. 20(1), 1–7 (2019).
23. Grasedieck, S. et al. Circulating microRNAs in hematological diseases: Principles, challenges, and perspectives. Blood 121(25), 4977–4984 (2013).
24. Choudhury, A., Aron, S., Sengupta, D., Hazelhurst, S. & Ramsay, M. African genetic diversity provides novel insights into evolu-tionary history and local adaptations. Hum. Mol. Genet. 27(R2), R209–R218 (2018).
25. Jiang, L., Liu, Y., Ma, C. & Li, B. MicroRNA-30a suppresses the proliferation, migration and invasion of human renal cell carcinoma cells by directly targeting ADAM9. Oncol. Lett. 16(3), 3038–3044 (2018).
26. Jeong, S., Oh, J. M., Oh, K. H. & Kim, I. W. Differentially expressed miR-3680-5p is associated with parathyroid hormone regula-tion in peritoneal dialysis patients. PLoS ONE 12(2), 1–13 (2017).
27. Kulkarni, P. et al. Elevated miR-182-5p associates with renal cancer cell mitotic arrest through diminished MALAT-1 expression. Mol. Cancer Res. 16(11), 1750–1760 (2018).

28. Wang, D. et al. MicroRNA-30e-3p inhibits cell invasion and migration in clear cell renal cell carcinoma by targeting snail1. Oncol. Lett. 13(4), 2053–2058 (2017).
29. Seyhan, A. A. et al. Pancreas-enriched miRNAs are altered in the circulation of subjects with diabetes: A pilot cross-sectional study. Sci. Rep. 6(1), 1–15 (2016).
30. Ghaneh, T., Zeinali, F., Babini, H., Astaraki, S. & Hassan-Zadeh, V. An increase in the expression of circulating miR30d-5p and miR126-3p is associated with intermediate hyperglycaemia in Iranian population. Arch. Physiol. Biochem. https:// doi. org/ 10. 1080/ 13813 455. 2020. 18391 05 (2020).
31. Taïbi, F. et al. Possible involvement of microRNAs in vascular damage in experimental chronic kidney disease. Biochim. Biophys. Acta Mol. Basis Dis. 1842(1), 88–98. https:// doi. org/ 10. 1016/j. bbadis. 2013. 10. 005 (2014).
32. Park, M. Y. et al. Circulating and renal vein levels of microRNAs in patients with renal artery stenosis. Nephrol. Dial Transplant. 30(3), 480–490 (2015).
33. Al-Kafaji, G. et al. Decreased expression of circulating microRNA-126 in patients with type 2 diabetic nephropathy: A potential blood-based biomarker. Exp. Ther. Med. 12(2), 815–822 (2016).
34. Wang, H. et al. Circulating levels of inflammation-associated mir-155 and endothelial-enriched mir-126 in patients with end-stage renal disease. Braz. J. Med. Biol. Res. 45(12), 1308–1314 (2012).
35. Bijkerk, R. et al. Hematopoietic MicroRNA-126 protects against renal ischemia/reperfusion injury by promoting vascular integrity. J. Am. Soc. Nephrol. 25(8), 1710–1722 (2014).
36. Lei, H. et al. Icariside II ameliorates endothelial dysfunction by regulating the MAPK pathway via miR-126/SPRED1 in diabetic human cavernous endothelial cells. Drug Des. Dev. Ther. 12, 1743 (2018).
37. Wang, S. et al. The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev. Cell. 15(2), 261–271 (2008).
38. Ming, L., Ning, J., Ge, Y., Zhang, Y. & Ruan, Z. Excessive apoptosis of podocytes caused by dysregulation of microRNA-182-5p and CD2AP confers to an increased risk of diabetic nephropathy. J. Cell Biochem. 120(10), 16516–16523 (2019).
39. Wilflingseder, J. et al. Molecular pathogenesis of post-transplant acute kidney injury: Assessment of whole-genome mRNA and miRNA profiles. PLoS ONE 9(8), e104164 (2014).
40. Wilflingseder, J. et al. miR-182-5p inhibition ameliorates ischemic acute kidney injury. Am. J. Pathol. 187(1), 70–79 (2017).
41. Baker, M. A. et al. Tissue-specific microRNA expression patterns in four types of kidney disease. J. Am. Soc. Nephrol. 28(10), 2985–2992 (2017).
42. Xu, X. et al. Downregulation of microRNA-182-5p contributes to renal cell carcinoma

proliferation via activating the AKT/FOXO3a signaling pathway. Mol. Cancer 13(1), 1–11 (2014).

43. Wu, J. et al. Downregulation of microRNA-30 facilitates podocyte injury and is prevented by glucocorticoids. J. Am. Soc. Nephrol. 25(1), 92–104 (2014).
44. Sun, S. Q. et al. Circulating microRNA-188, -30a, and -30e as early biomarkers for contrast-induced acute kidney injury. J. Am. Heart Assoc. https:// doi. org/ 10. 1161/ JAHA. 116. 004138 (2016).
45. Wang, J., Wang, G., Liang, Y. & Zhou, X. Expression profiling and clinical significance of plasma micrornas in diabetic nephropathy. J. Diabetes Res. 2019, 1–12 (2019).
46. Jiang, L. et al. A microRNA-30e/mitochondrial uncoupling protein 2 axis mediates TGF-β1-induced tubular epithelial cell extra-cellular matrix production and kidney fibrosis. Kidney Int. 84(2), 285–296. https:// doi. org/ 10. 1038/ ki. 2013. 80 (2013).
47. Gong, Z. et al. Differences in microRNA expression in breast cancer between women of African and European ancestry. Carcino-genesis 40(1), 61–69 (2019).
48. Kwekel, J. C., Vijay, V., Desai, V. G., Moland, C. L. & Fuscoe, J. C. Age and sex differences in kidney microRNA expression during the life span of F344 rats. Biol. Sex Differ. 6(1), 1–16 (2015).
49. Vrijens, K., Bollati, V. & Nawrot, T. S. MicroRNAs as potential signatures of environmental exposure or effect: A systematic review. Environ. Health Perspect. 123(5), 399–411 (2015).
50. Sohel, M. H. Extracellular/circulating microRNAs: Release mechanisms, functions and challenges. Achiev. Life Sci. 10(2), 175–186 (2016).
51. Pascut, D. et al. A comparative characterization of the circulating miRNome in whole blood and serum of HCC patients. Sci. Rep. 9(1), 1–11 (2019).
52. Wang, K. et al. Comparing the microRNA spectrum between serum and plasma. PLoS ONE 7(7), e41561 (2012).
53. Madadi, S., Schwarzenbach, H., Lorenzen, J. & Soleimani, M. MicroRNA expression studies: Challenge of selecting reliable refer-ence controls for data normalization. Cell Mol. Life Sci. 76(18), 3497–3514 (2019).
54. Igaz, I. et al. Analysis of circulating microRNAs in vivo following administration of dexamethasone and adrenocorticotropin. Int. J. Endocrinol. 2015, 1–6 (2015).
55. Witkowski, M. et al. Metformin is associated with reduced tissue factor procoagulant activity in patients with poorly controlled diabetes. Cardiovasc. Drugs Ther. 35, 1–5 (2020).
56. Matsha, T. E. et al. MicroRNA profiling and their pathways in South African individuals with prediabetes and newly diagnosed type 2 diabetes mellitus. Oncotarget 9(55), 30485–30498 (2018).
57. Matsha, T. E. et al. Genome-wide DNA methylation in mixed ancestry individuals with diabetes and prediabetes from South Africa. Int. J. Endocrinol. 2016, 1–11 (2016).

58. Chalmers, J. et al. 1999 World Health Organization-International Society of Hypertension. Guidelines for the management of hypertension Guidelines sub-committee of the World Health Organization. Clin. Exp. Hypertens. (New York) 21(5–6), 1009–1060 (1999).
59. Pirkle, J. L. et al. Exposure of the US population to environmental tobacco smoke: The Third National Health and Nutrition Examination Survey, 1988 to 1991. JAMA 275(16), 1233–1240 (1996).
60. Alberti, K. G. M. M. & Zimmet, P. Z. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. Diabet. Med. 15(7), 539–553 (1998).
61. World Health Organization. Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycaemia: Report of a WHO/IDF Consultation (WHO, 2006).
62. Lange, T. et al. Identification of miR-16 as an endogenous reference gene for the normalization of urinary exosomal miRNA expression data from CKD patients. PLoS ONE 12(8), 1–13 (2017).
63. Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 25(4), 402–408 (2001)

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Authors contribution

Study conceptualization (TEM, RTE, APK, AEZ), funding acquisition (TEM, RTE, APK), laboratory analysis (DMM, CJW), data analysis (CG, DDM, SFGD), data interpretation (DDM, CG), manuscript draft (DDM), critical revision of content and final approval of manuscript (all authors).

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Competing interest statement

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Additional information

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CHAPTER 6

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6. MANUSCRIPT 2

Novel Whole Blood MicroRNAs Predicting Chronic Kidney Disease in South Africans with Hypertension and Diabetes Mellitus

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Abstract: The asymptomatic nature and lack of effective early-stage diagnostic tools in CKD, predisposes individuals to the risk of end-stage CKD and related complications. Whole blood microRNAs (miRNAs) have the potential for CKD risk screening. We evaluated the expression profile of six novel whole blood miRNAs as well as their ability to predict prevalent CKD in individuals with hypertension and/or diabetes. We included 911 individuals with hypertension and/or diabetes, of which 18.8% had prevalent CKD. miRNA expression was analyzed using quantitative reverse transcription PCR (RT-PCR). Five of the six miRNAs, namely hsa-miR-novel-chr1_36178, hsa-miR-novel-chr2_55842, hsa-miR-novel-chr7_76196, hsa-miR-novel-chr5_67265 and hsa-miR-novel-chr13_13519, were significantly increased in people with CKD (all $p<0.028$). Only the increased expression of hsa-miR-novel-chr2_55842 and hsa-miR-novel-chr7_76196 were independently associated with reduced estimated glomerular filtration rate (eGFR) (both $p\leq 0.038$), while all the analyzed miRNAs were positively associated with prevalent CKD (all $p\leq 0.038$). All the blood miRNAs were acceptable predictors of CKD (C-statistic> 0.7 for all), with similar predictive capacity ($p= 0.202$). However, hsa-miR-novel-chr13_13519 added to CKD prediction beyond

conventional factors (p=0.040). Novel whole blood miRNAs showed an acceptable discriminative power to predict prevalent CKD; thereby suggesting the potential use of these miRNAs, particularly hsa-miR-novel-chr13_13519, in clinical practice as screening tool for CKD in high-risk individuals.

Keywords: MicroRNAs; chronic kidney disease; hypertension; diabetes mellitus; predictive value

6.1. Introduction

The burden of chronic kidney disease (CKD) has increased substantially over the past three decades, progressing from the 36th ranked cause of death in 1990 to the 12th ranked cause of death worldwide in 2017 [1]. Globally, around 10% of the general adult population have CKD, with an estimated 16% for the African population [2]. It has been suggested that the high prevalence of CKD observed in Africa is partly attributable to the high prevalence of hypertension (HTN) and increasing incidence of diabetes mellitus (DM) [2,3] as a result of urbanization, sedentary lifestyles and longevity. DM, which is the leading cause of end-stage renal disease (ESRD), accounts for ap-proximately 11% to 83.7% of CKD cases in Africa [4], depending on the method of diagnosis used for CKD. This is concerning as according to the International Diabetes Federation IDF), of the approximate 9.3% (463 million) of people with DM, 50% are unaware of their condition and the highest proportions of the undiagnosed DM population are found in the African region (59.7%) [5]. HTN, which is another independent modifiable risk factor of CKD development and progression to ESRD [6], is strongly associated with cardiovascular disease (CVD) and a leading cause of premature death [7]. Among the leading risk factors of CKD, HTN is the most prevalent, affecting approximately 31% (1.39 billion) of the adult population globally, with highest proportions observed in the low-and-middle-income countries [8]. A high prevalence of 57% has been reported in the older adults of Africa [9].

The high incidences of CKD due to DM and HTN will result in significant social and economic ramifications particularly in Africa due to the limited and inadequate health resources. Early identification of CKD will enable early initiation of risk reducing therapies which may subsequently prevent or delay progression to advanced CVDs complications or ESRD that requires costly renal replacement therapy (RRT) [10]. However, due to its asymptomatic nature in the early stages, CKD is frequently diagnosed only during the advanced stages of the disease when prevention interventions are less likely to be effective. Therefore, screening individuals at high-risk of developing CKD, particularly those with HTN and DM, may prevent or halt the development or progression of CKD.

The expression profiles of microRNAs (miRNAs) in biofluids have been associated with diseases such as cancer [11], neurodegenerative disease [12] and CVDs [13], therefore suggesting the potential utility of these miRNAs as minimally invasive biomarkers for disease diagnosis, monitoring and as therapeutic targets. miRNAs are a family of small non-coding transcripts that regulate gene expression post-transcriptionally, by inhibiting mRNA translation or triggering mRNA degradation,

thereby preventing the synthesis of certain proteins [14]. miRNAs have been shown to have important regulatory function such as proliferation, cell differentiation, development, apoptosis, and metabolism [15]. Therefore, dysregulation of miRNAs may result in impaired cellular function and disease development [16]. In addition to their intracellular function, studies have shown that miRNAs are also secreted by cells into the blood and other body fluids, although the mechanism remains unclear [17]. Unlike RNA, miRNAs in blood and other body fluids are very stable, as they are released into circulation bound to proteins and/or encapsulated in microvesicles, thereby protecting them from degradation by ribonucleases [17,18].

Several studies have shown that the expression of certain miRNAs is tissue specific and may be involved in the development, homeostasis, and physiology of the kidney [14,19]. Although it has been demonstrated that miRNAs exhibit functional dysregulation in various diseases, including CKD [19], HTN [20], DM [21], as well as HTN and DM-related kidney diseases [22,23], minimal evidence exists on the predictive ability of these miRNAs, in relation to CKD, and none in sub-Saharan Africa. We have previously identified novel whole blood miRNAs via deep sequencing, which were significantly dysregulated in HTN (hsa-miR-novel-chr1_36178 and hsa-miR-novel-chr15_18383) [24], pre-diabetes (hsa-miR-novel-chr2_55842) [25] and DM (hsa-miR-novel-chr7_76196, hsa-miR-novel-chr5_67265 and hsa-miR-novel-chr13_13519) (unpublished data). The current study aimed to: 1) evaluate the expression profile of these miRNAs in high-risk individuals (HTN and/or DM) with and without CKD; 2) determine the diagnostic ability of these six novel whole blood miRNAs to discriminate between individuals with CKD and those without; and 3) determine whether these miRNAs offer additional diagnostic advantage above and beyond conventional CKD risk factors.

6.2. Materials and methods

6.2.1. Study Design and Procedures

The current study, which is based on data collected between 2014 and 2016, forms part of the ongoing Vascular and Metabolic Health (VMH) study, an extension of the Cape Town Bellville South study, previously described in details [26]. Ethical clearance for the VMH study was granted by the research ethics committees of Cape Peninsula University of Technology (CPUT) and Stellenbosch University (NHREC: REC—230,408– 014 and N14/01/003, respectively). For the current study, ethical clearance was granted by the Faculty of Health and Wellness Sciences Research Ethics Committee of the CPUT (CPUT/HW-REC 2020/H11). The participants gave signed written consent after they were informed about their rights and the procedures were fully explained in the language of their choice. Research was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). A total of 1989 individuals of mixed-ancestry were recruited between 2014 and 2016 as part of the VMH Study and of these 911 individuals had HTN and DM, with age range between 20 and 91, a median age of 56 years and were thus selected for the present analysis (18.8%

with CKD and 22 % males). Of the total sample 6.4%, 3.6%, 7.1%, 1.2% and 0.4% of the individuals were in CKD stages 1-5 respectively.

The detailed data collection procedures using standardized questionnaires and physical examination have been explained elsewhere [25]. Briefly, clinical measurements including body weight, height, hip circumference (HC), waist circumference (WC) and blood pressure (BP) were taken by trained personnel by standardized methods. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m2). Participants were classified as underweight (BMI <18.5 kg/m2), normal weight (BMI 18.5–24.9 kg/m2), overweight (BMI 25.0–29.99 kg/m2) and obese (BMI ≥ 30.0 kg/m2) respectively. BP measurements were performed according to WHO guidelines [27], using an automatic digital BP monitor (Omron M6 Comfort-preformed Cuff Blood Pressure Monitor, Omron), with the participants sitting quietly in a relaxed position for at least 5 minutes. Two BP readings, including systolic BP (SBP) and diastolic BP (DBP), at three-minute intervals, were taken and the lowest SBP and the corresponding DBP were chosen as the participant's BP. Pulse pressure (PP) was estimated by subtracting the DBP from the SBP. HTN was defined as SBP ≥140mmHg and/or DBP ≥90mmHg or self-reported ongoing use of antihypertensive medications.

All biochemical analysis was conducted by an ISO accredited pathology practice (PathCare Laboratory, Cape Town, South Africa). All participants, excluding those who self-reported diabetes mellitus (confirmed by either participant medical card record or use of diabetic medication), underwent a 75g oral glucose tolerance test (OGTT) after an overnight fast, according to WHO guidelines [28]. DM was defined as fasting blood glucose (FBG) ≥7.0 mmol/L and/or 2-hour postprandial glucose (Glucose 2 HR) ≥11.1 mmol/L, or ongoing use of glucose control medications. Plasma glucose concentrations were measured using the enzymatic hexokinase method (Beckman AU, Beckman Coulter, Brea, CA, USA), glycated haemoglobin (HbA1c) was measured using high performance liquid chromatography (HPLC) (Biorad Variant Turbo, BioRad, Hercules, CA, USA), urine albumin levels were measured using the colorimetric (using bromo-cresol purple) method (Beckman AU, Beckman Coulter, Brea, CA, USA) and serum and urinary creatinine was measured by the modified Jaffe-Kinetic method (Beckman AU, Beckman Coulter, Brea, CA, USA). For serum samples, fasting and 2-hour blood samples were collected in a plain tube (with no clotting factors) and this was centrifuged using a Beckman General Purpose centrifuge (Beckman Coulter Inc., CA, USA) to obtain serum and for urine samples, the urine was collected in specimen containers supplied by the laboratory and all samples were transported daily in an ice-box for processing at pathology practice (PathCare Laboratory, Cape Town, South Africa).

The level of kidney function was measured using estimated glomerular filtration rate (eGFR) which was calculated using the 4-variable Modification of Diet in Renal Disease (MDRD) (eGFR= 175 x (SCr)-1.154 x (age)-0.203 x 0.742 [if female]) [29], and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations (eGFR=141 x min(SCr/κ, 1)α x max(SCr /κ, 1)-1.209 x 0.993Age x 1.018 [if female], where SCr was defined as serum creatinine, κ was 0.7 (females) or 0.9 (males), α was -0.329 (females) or -0.411 (males), min indicated the minimum of SCr/κ or 1 and max indicated the maximum of SCr/κ or 1. The correction factor for African American ethnicity was not included as recommended for our study population [30]. Only the MDRD data are shown as similar results were obtained for both equations. CKD was defined as eGFR <90 ml/min/1.73 m2 and/or albumin-to-creatinine ratio (ACR) >3 mg/mmol as recommended by the current Kidney Disease: Improving Global Outcomes (KDIGO) guidelines [29]. Participants were classified a "current smoker", if serum cotinine levels were >15 ng/mL [31] and a "drinker" if they self-reported to consuming alcohol.

6.2.2. MicroRNA analysis

The detailed methodology of whole blood miRNA extraction and analysis was de-scribed previously [25]. Briefly, whole blood samples were collected in Tempus RNA tubes and stored at – 20 degrees for circulating miRNA extraction and analysis. The samples were sent to Arraystar Inc (Rockville, USA) for small RNA library construction, deep sequencing, and data processing. The identified miRNAs that showed dysregulation between the groups were selected for validation in a larger study sample by quantitative reverse transcription polymerase chain reaction (RT-qPCR) to assess the reproducibility of the results. Following isolation, using the MagMAX^{TM} for Stabilized Blood Tubes RNA Isolation Kit (Life Technologies, Waltham, MA, USA), total RNA was reverse transcribed into complementary DNA (cDNA) using the TaqMan^{TM} Advanced cDNA Synthesis Kit, and in accordance with the manufacturer's specifications (Applied Biosystems, 2015). Following reverse transcription, quantification of miRNA expression was then performed using TaqMan^{TM} miRNA assay protocol as per manufacturer's instructions, with Quantum Studio 7 (Life Technologies, Waltham, MA, USA). The delta Ct (2−ΔCt) method was used to assess the miRNA expression level in each sample by normalization to endogenous control (miR-16-5p) expression levels. The relative miRNA expression between samples was calculated using the delta-delta Ct (2−ΔΔCT) method [32].

6.2.3. Statistical analysis

The Shapiro-Wilk W test was used to check the data distribution. Due to the non-Gaussian distribution of most variables, the general participant characteristics were presented as median (25th–75th percentiles) or count and percentages. Wilcoxon rank-sum tests (continuous variables) and chi-square tests (categorical variables) were used for comparisons between individuals with CKD and those without CKD. Robust linear regression models, unadjusted and adjusted for age, gender,

smoking status, drinking status, HTN and DM status were used to assess the association between whole blood miRNAs and eGFR. The models used were as follows: Model 1: Crude; Model 2: Model 1 + age + gender; Model 3: Model 2 + smoking status + drinking status; Model 4: Model 3 + HTN status + DM status. Logistic regression models with similar level of adjustment were used to analyse the ability of whole blood miRNAs to predict prevalent CKD. The Area under the Receiver Operating Characteristics (ROC) curve (AUC) was used to determine the discriminatory power of each miRNA (hsa-miR-novel-chr1_36178, hsa-miR-novel-chr15_18383, hsa-miR-novel-chr2_55842, hsa-miR-novel-chr7_76196, hsa-miR-novel-chr5_67265 and hsa-miR-novel-chr13_13519), alone and as a collective, and alongside conventional risk factors to distinguish participants with CKD from those without. Statistical significance was defined as a p-value of < 0.05.

6.3. Results

6.3.1. General characteristics of the study population

A total of 1989 individuals of mixed-ancestry were recruited between 2014 and 2016 as part of the VMH Study and of these 911 individuals had HTN and DM, and were thus selected for the present analysis (18.8% with CKD and 22 % males). The clinical characteristics of the study participants by CKD status are summarized in Table 1. Of the total sample 6.4%, 3.6%, 7.1%, 1.2% and 0.4% of the individuals were in CKD stages 1-5 respectively. Individuals with CKD were significantly older (63 vs 55 years, $p<0.0001$), had a larger waist circumference (98.5 vs. 94.4 cm, $p=0.023$), higher fasting plasma glucose (5.3 vs 5.2, $p=0.0026$), 2-hour glucose (7.1 vs 6.4, $p= 0.0047$), HbA1c (6.2 vs 5.9, $p<0.0001$), 2-hour insulin (51.5 vs 41.8, $p=0.032$), SBP levels (149 vs 146, 0.047) and a higher proportion of DM (45 vs 27%, $p<0.001$) compared to those without CKD. Conversely, participants without CKD had higher proportion of smokers (47.7 vs 31.2%) and alcohol consumers (28.5 vs 7.6%) than those with CKD ($p<0.0001$ for both).

6.3.2. Relative expression levels of whole blood miRNAs

The relative expression levels of the six novel whole blood miRNAs are shown in Figure 1 (A-F). The expression levels of whole blood miRNAs (hsa-miR-novel-chr1_36178, hsa-miR-novel-chr2_55842, hsa-miR-novel-chr7_76196, hsa-miR-novel-chr5_67265 and hsa-miR-novel-chr13_13519) were significantly higher in individuals with CKD as compared to those without CKD (all $p<0.028$), whereas the expression of hsa-miR-novel-chr15_18383 showed no differences between the two groups ($p=0.197$)

6.3.3. Relationship between whole blood miRNAs, eGFR and prevalent CKD

In robust linear regression models (Table 2), increased expression of blood miRNAs (hsa-miR-novel-chr2_55842, hsa-miR-novel-chr7_76196) were significantly associated with reduced eGFR, independent of age, gender, smoking status, drinking status, DM and HTN status (Models 1-5,

p<0.038 for all). hsa-miR-novel-chr5_67265 and hsa-miR-novel-chr13_13519 showed significant association with lower eGFR levels, in-dependent of age and gender (Models 1-2, all p≤0.05), but not after further adjustment for smoking status, alcohol consumption, DM and HTN status (Models 3-4, p<0.062). The miRNAs, hsa-miR-novel-chr1_36178 and hsa-miR-novel-chr15_18383, showed no association with eGFR (p≥ 0.311 for all). Table 3 presents the odds ratios (ORs) with 95% confidence intervals (CIs) from logistic regression analysis of whole blood miRNAs for the prediction of CKD. All the whole blood miRNAs were positively associated with CKD in people with HTN and/or DM even after adjustment for a range of confounders (p≤ 0.038, for all).

6.3.4. Diagnostic value of whole blood miRNAs to predict CKD

Figure 2 presents the ROC curves for the discriminatory ability of whole blood miRNAs to identify people with CKD. All the whole blood miRNAs had acceptable discriminatory power for prevalent CKD in people with HPT and/or DM (all AUC> 0.7), however AUC comparison showed no significant difference between miRNAs in predicting prevalent CKD (p=0.202). Moreover, when comparing the standard model (including only age, gender, and smoking, drinking, HTN and DM status) with the models containing the miRNAs, only the model with hsa-miR-novel-chr13_13519 was significantly different to the standard model (p= 0.0397).

Table 1: *General characteristics of the study participants at risk of developing CKD, categorized by CKD status*

Variables	Total (n=911)	Without CKD (n=740)	CKD (n=171)	p-value
Age (years)	56 (47-64)	55 (47-62)	63 (53-70)	<0.0001
Gender (n, % male)	203 (22.3)	168 (22.7)	35 (20.5)	0.517
Weight (kg)	74.5 (61.9-87.5)	74.5(61.5-87.6)	74.7 (63.9-85.3)	0.859
Waist circumference (cm)	95.3 (84.3-106.7)	94.4 (83.4-106.8)	98.5 (88.3-106.4)	0.023
Hip circumference (cm)	104.5 (94.5-115.9)	104.5 (94.0-116.0)	104.8 (96.7-115.0)	0.313
Body mass index (kg/m^2)	29.7 (24.5-35.2)	29.5 (24.2-35.1)	30.5 (25.7-35.6)	0.175
Serum cotinine (ng/mL)	10.0 (10.0-257.0)	10.0 (10.0-263.0)	10.0 (10.0-208.00)	0.250
Fasting plasma glucose (mmol/l)	5.2 (4.7-6.2)	5.2 (4.7-6.0)	5.3 (4.8-7.8)	0.003
2-hour glucose (mmol/l)	6.5 (5.3-8.3)	6.4 (5.3-8.2)	7.1 (5.8-8.8)	0.005
Fasting insulin (IU/l)	7.4 (4.7-12.1)	7.3 (4.6-11.9)	7.5 (4.9-12.5)	0.292
2-hour insulin (IU/l)	43.5 (23.2-78.0)	41.8 (22.6-76.5)	51.5 (30.3-90.7)	0.035
Glycated haemoglobin (%)	5.9 (5.5-6.6)	5.9 (5.5-6.4)	6.2 (5.8-7.7)	<0.0001
Systolic blood pressure (mmHg)	147 (132-162)	146 (132-161)	149 (132-169)	0.047
Diastolic blood pressure (mmHg)	91 (82-100)	92 (82-99)	90 (80-103)	0.656
Pulse pressure (mmHg)	72 (64-81)	72 (64-80)	74 (64-84)	0.079
Body mass index categories (n, %)				0.085
Normal weight	247 (27.4)	212 (29.0)	35 (20.6)	
Overweight	216 (24.0)	172 (23.5)	44 (25.9)	
Obese	438 (48.6)	347 (47.5)	91 (53.5)	
Diabetes mellitus (n, %)	277 (30.4)	200 (27.0)	77 (45.0)	<0.0001
Hypertension (n, %)	878 (96.4)	709 (95.8)	169 (98.8)	0.057
Current smokers (n, %)	395 (44.5)	342 (47.7)	53 (31.2)	<0.0001
Current drinkers (n, %)	222 (24.6)	209 (28.5)	13 (7.6)	<0.0001

Data is presented as median (25^{th} - 75^{th} percentiles) and count and percentages

Abbreviations: CKD (chronic kidney disease); miR (microRNA)

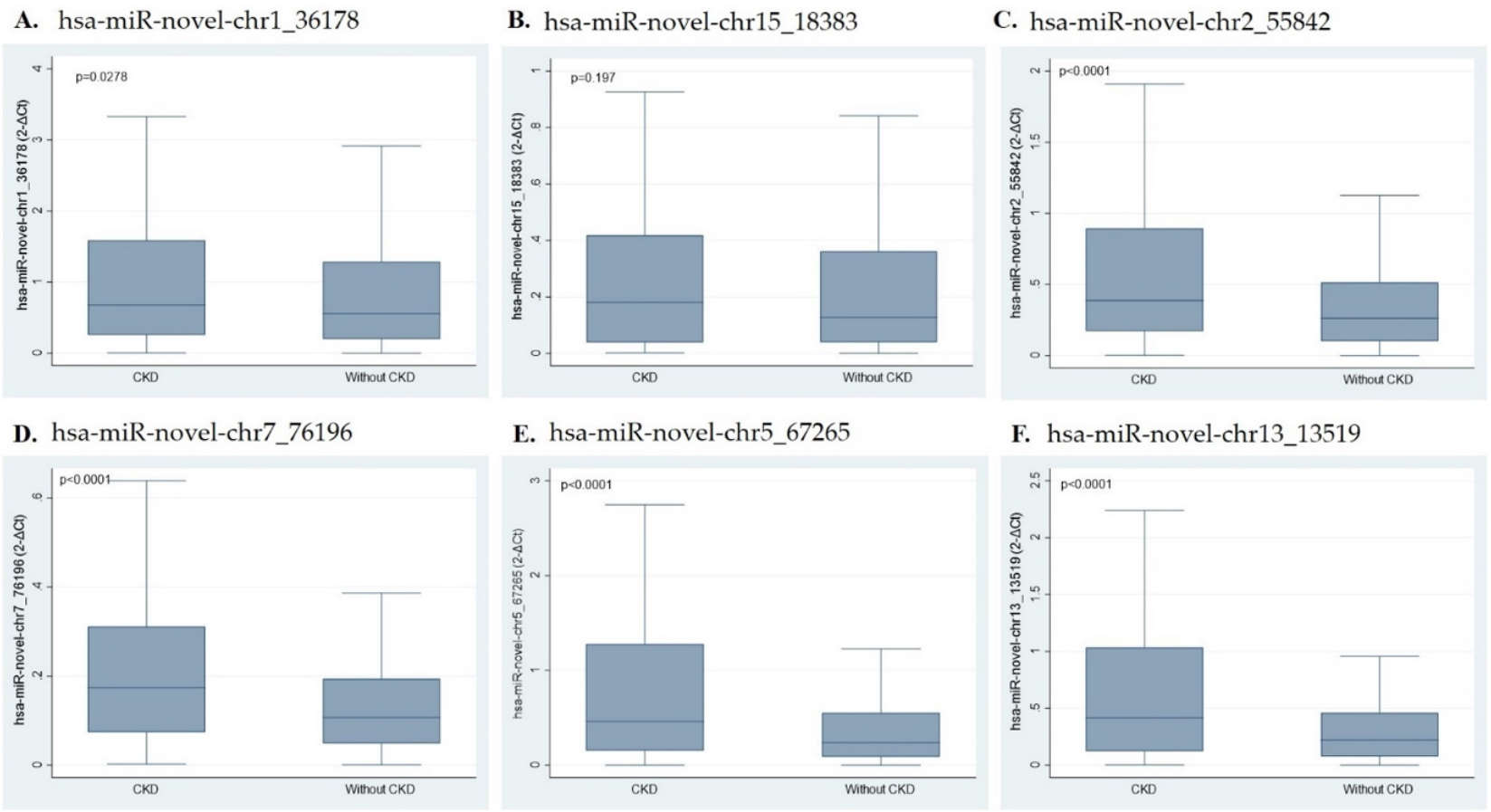

Figure 1: *Box and Whisker plots showing miRNA expression.* A) hsa-miR-novel-chr1_36178, B) hsa-miR-novel-chr15_18383**, C) hsa-miR-novel-chr2_55842, D) hsa-miR-novel-chr7_76196**, E) hsa-miR-novel-chr5_67265*, F) hsa-miR-novel-chr13_13519*. * Represent miRNAs factored by 10 and ** represent miRNAs factored by 100 as the values were very low. Abbreviations: CKD (chronic kidney disease); miR (micro-RNA).

Table 2: *Robust linear regression models for the association between whole blood miRNAs and eGFR*

	β	**95% CI**	**p-value**
hsa-miR-novel-chr1_36178			
Model 1	-0.52	-1.71 to 0.67	0.395
Model 2	-0.45	-1.45 to 0.55	0.381
Model 3	-0.52	-1.52 to 0.49	0.311
Model 4	-0.49	-1.49 to 0.52	0.343
hsa-miR-novel-chr15_18383			
Model 1	1.46	-1.84 to 4.76	0.386
Model 2	0.36	-2.42 to 3.14	0.802
Model 3	-0.10	-2.87 to 2.68	0.942
Model 4	-0.05	-2.83 to 2.74	0.973
hsa-miR-novel-chr2_55842			
Model 1	-5.51	-8.01 to -3.02	<0.0001
Model 2	-2.74	-4.85 to -0.64	0.011
Model 3	-2.64	-4.77 to -0.52	0.015
Model 4	-2.70	-4.82 to -0.57	0.013
hsa-miR-novel-chr7_76196			
Model 1	-10.1	-18.05 to -2.15	0.013
Model 2	-7.07	-13.73 to -0.41	0.038
Model 3	-7.34	-14.00 to -0.68	0.031
Model 4	-7.39	-14.05 to -0.72	0.030
hsa-miR-novel-chr5_67265			
Model 1	-2.87	-4.59 to -1.15	0.001
Model 2	-1.62	-3.07 to -0.17	0.029
Model 3	-1.35	-2.81 to 0.12	0.071
Model 4	-1.40	-2.88 to 0.07	0.062
hsa-miR-novel-chr13_13519			
Model 1	-4.05	-6.70 to -1.41	0.003
Model 2	-2.19	-4.42 to 0.00	0.050
Model 3	-1.73	-3.97 to 0.51	0.131
Model 4	-1.76	-4.02 to 0.49	0.126

Models: Model 1: Crude; **Model 2**: Model 1 + age + gender; **Model 3**: Model 2 + smoking status + drinking status; **Model 4**: Model 3 + DM status + HTN status.

Abbreviations: miR (MicroRNA) and β (beta-coefficient)

Table 3: *Logistic regression analysis of blood miRNAs for prediction of CKD*

	OR	**95% CI**	**p-value**
hsa-miR-novel-chr1_36178			
Model 1	1.20	1.09 to 1.32	<0.0001
Model 2	1.22	1.10 to 1.35	<0.0001
Model 3	1.23	1.10 to 1.38	<0.0001
Model 4	1.22	1.10 to 1.37	<0.0001
hsa-miR-novel-chr15_18383			
Model 1	1.33	1.02 to 1.74	0.038
Model 2	1.39	1.06 to 1.83	0.019
Model 3	1.46	1.11 to 1.93	0.007
Model 4	1.44	1.09 to 1.89	0.009
hsa-miR-novel-chr2_55842			
Model 1	1.70	1.40 to 2.07	<0.0001
Model 2	1.66	1.36 to 2.04	<0.0001
Model 3	1.65	1.34 to 2.04	<0.0001
Model 4	1.65	1.33 to 2.05	<0.0001
hsa-miR-novel-chr7_76196			
Model 1	4.40	2.37 to 8.19	<0.0001
Model 2	4.34	2.27 to 8.30	<0.0001
Model 3	4.64	2.37 to 9.08	<0.0001
Model 4	4.89	2.48 to 9.64	<0.0001
hsa-miR-novel-chr5_67265			
Model 1	1.20	1.05 to 1.37	0.007
Model 2	1.17	1.02 to 1.33	0.027
Model 3	1.38	1.19 to 1.61	<0.0001
Model 4	1.37	1.17 to 1.60	<0.0001
hsa-miR-novel-chr13_13519			
Model 1	1.96	1.56 to 2.46	<0.0001
Model 2	1.93	1.52 to 2.45	<0.0001
Model 3	1.83	1.44 to 2.32	<0.0001
Model 4	1.79	1.40 to 2.28	<0.0001

Models: Model 1: Crude; **Model 2**: Model 1 + age + gender; **Model 3**: Model 2 + smoking status + drinking status; **Model 4**: Model 3 + DM status + HTN status.

Abbreviations: β (Beta coefficient) and OR (odds ratio)

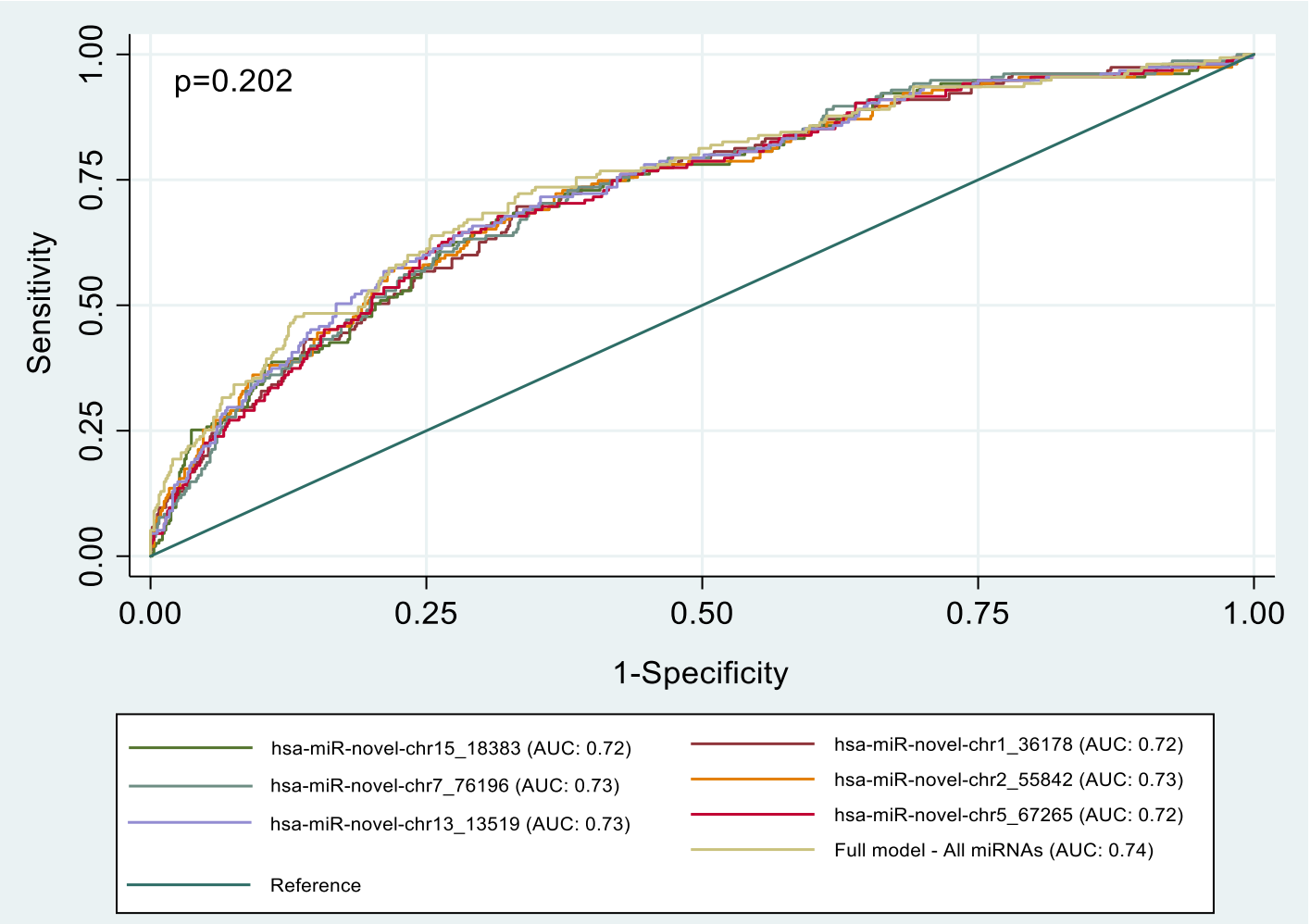

Figure 2: *Area under the Receiver Operating Characteristics (ROC) curves (AUC) illustrating the diagnostic ability of six novel whole blood miRNAs to discriminate between individuals with CKD and those without in a group of high-risk individuals.*

Models: miRNA + age + gender + smoking status + drinking status + DM status + HTN status. Full model includes all six miRNAs. **Abbreviation:** miRNA (microRNA).

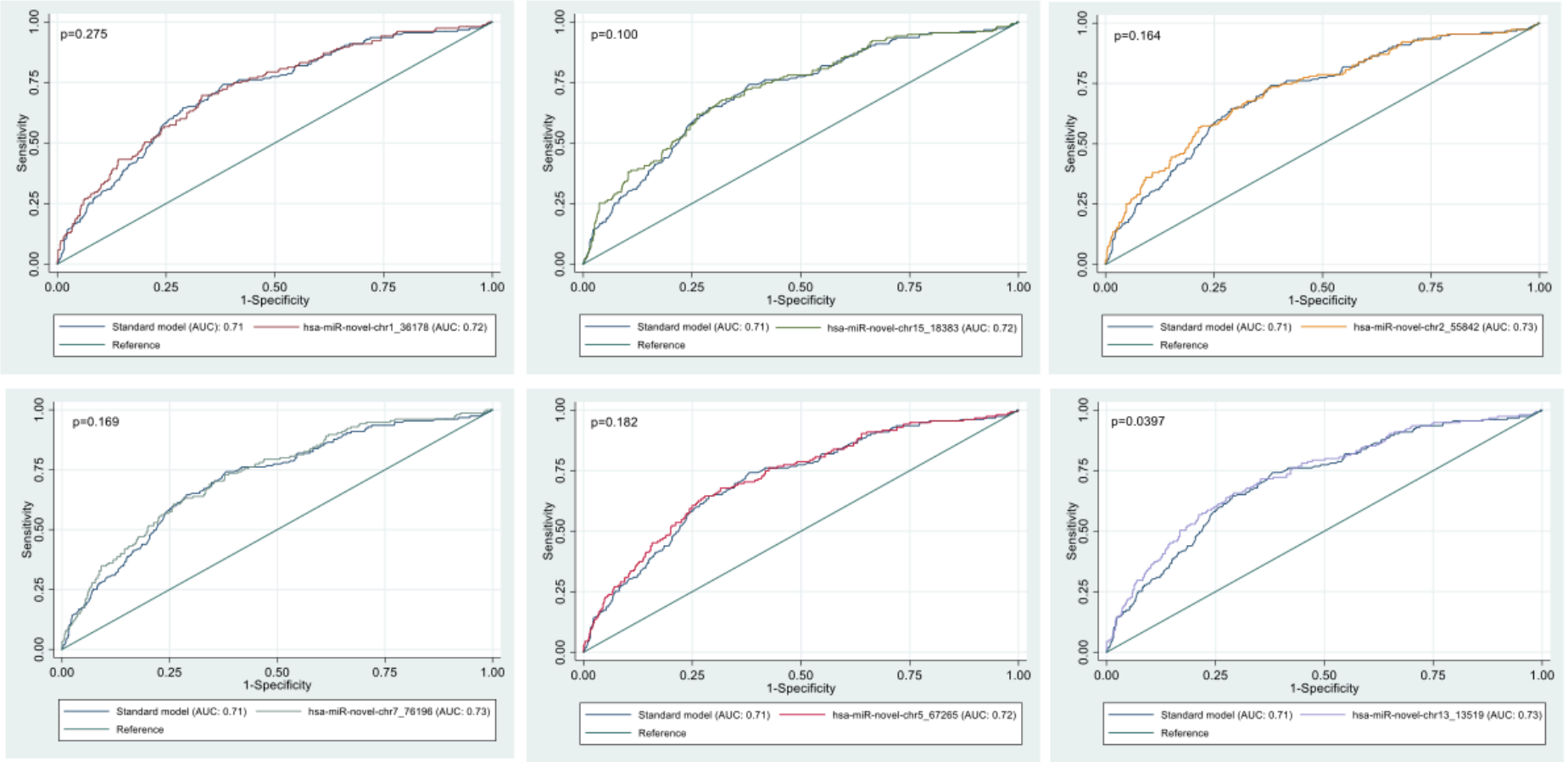

Figure 3: *Area under the Receiver Operator Characteristic (ROC) curves (AUC) illustrating the illustrating the diagnostic advantage of six novel whole blood miRNAs to predict CKD in a group of high-risk individuals above that of conventional risk factors.*

Standard model: age + gender + smoking status + drinking status + diabetes status + hypertension status. **Model for each miRNA separately:** miRNA + age + gender + smoking status + drinking status + diabetes status + hypertension status. miRNA, microRNA

6.4. Discussion

The key findings of this study are that all six novel whole blood miRNAs were significantly associated with prevalent CKD, independent of conventional risk factors. The expression profile of miRNAs (hsa-miR-novel-chr1_36178, hsa-miR-novel-chr2_55842, hsa-miR-novel-chr7_76196, hsa-miR-novel-chr5_67265 and hsa-miR-novel-chr13_13519) were significantly higher in CKD individuals as compared to those with normal kidney function. While all the miRNAs had acceptable and comparable discriminatory power for prevalent CKD, only hsa-miR-novel-chr13_13519 added to predictions beyond conventional risk factors. The findings of the current investigation suggest that these novel whole blood miRNAs have a potential to contribute to CKD risk screening in people with HTN and/or DM.

Various studies have reported on the dysregulated patterns of miRNAs in CKD in individuals with HTN and DM. Kato and colleagues were among the firsts to demonstrate the role of miRNAs in the development of diabetic kidney disease (DKD) [21,33]. They showed that the expression of miR-192 was significantly elevated in the glomeruli of a diabetic mouse model [21], and in a further study showed that miR-192 induced the transforming growth factor beta 1 (TGF-β1) signalling, accelerating the progression of DKD [33]. Another study explored the expression profile and clinical significance of plasma miRNAs in Chinese individuals with DKD and found that the expression profiles of miR-150-5p, miR-155-5p, miR-30e, miR-320e, and miR-3196 were significantly de-creased during the early stages of DKD [23]. A study performed in Egyptians with type 2 DM showed that levels of miR-451 were elevated and reduced in plasma and urine samples respectively in different stages of DKD [34]. Studies by Liu and colleagues demonstrated that downregulation of miR-214-3p may be associated with the development of chronic kidney injury in HTN, and showed that upregulation of this miRNA may offer a protective role in the kidneys [35,36]. A study by Lu and colleagues showed that serum and urine derived miR-103a-3p were significantly higher in individuals with hypertensive nephropathy and hypertensive mice infused with angiotensin II hormone compared to normal controls. The authors found that this hormone induced kidney injury via activation of the SNRK /NF-κB /p65 signalling pathway and this was positively as-sociated with increased levels of miR-103a-3p [37]. Berillo and colleagues recently examined the expression profile of let-7g-5p and miR-191-5p in platelet-poor plasma and found that the decreased levels of these miRNAs were independently associated with CKD among individuals with HTN. Therefore, suggesting that let-7g-5p and miR-191-5p may be involved in the pathophysiology of CKD and may serve as potential biomarkers for disease diagnosis [38].

In the current study we observed that increased expression of hsa-miR-novel-chr2_55842 and hsa-miR-novel-chr7_76196 were independently associated with reduced eGFR independent of confounding variables, however, the association between hsa-miR-novel-chr5_67265 and hsa-miR-

novel-chr13_13519 and eGFR was influenced by smoking. Previous studies, like the one by Yokoyama and colleagues too found that exposure to cigarette smoke mediated the regulation of certain miRNAs. In their case miR-155 and miR-21 were upregulated and the expression of miR-126-3p was downregulated [39]. Moreover, the current study showed that all six novel miRNAs were positively associated with prevalent CKD in individuals with HTN and/or DM, independent of conventional risk factors, like age and gender. Furthermore, all six novel miRNAs had an acceptable ability to predict CKD (AUC>0.7). However, the prediction model containing hsa-miR-novel-chr13_13519 offered additional advantage in predicting CKD, above that of conventional risk factors (age, gender, smoking status, drink status, DM and HPT status). These findings demonstrate that although the studied miRNAs are all acceptable predictors of CKD, only hsa-miR-novel-chr13_13519 seems to offer an additional advantage.

Contrary to our study, previous studies that have explored the predictive value of miRNAs analyzed for CKD in individuals with HTN or DM, quantified their expression profile in plasma [23,34], platelet poor plasma [38], serum [37] and urine [34,37] and in the current study we used whole blood. As reviewed by Witwer in 2015 [40], the establishment of an accurate and reliable circulating miRNA biomarker for disease has proven to be quite challenging as it may be affected by pre-analytical factors such as the starting material of biofluid, processing and the type of normalization miRNA used. Although platelet-poor plasma is not widely biased by coagulation due to lack of platelets [41], similarly to plasma and serum, the concentration of total miRNA is reduced after ex-traction [42] and may be affected by pre-analytical processes such as sample handling and bias due haemolysis [13,41]. Urine sample also present with some shortcomings in particular to the use of normalization control, some studies showing that the inclusion of a normalization control might reduce the predictive value of urine miRNAs whereas the exclusion of it might affect the accuracy of the results [43]. In the current study, we analysed miRNA expression in whole blood samples which comprises of multiple different blood cell types with their own specific miRNA expression profile which might have contributed to the profile of miRNAs observed. Individuals with CKD generally have lower levels of red blood cell (RBC) count due to anaemia, compared to their control counterparts [44]. It has been reported that RBC-derived miRNAs constitute the majority of miRNAs expressed in whole blood [45]. However, we found high levels of miRNA expression in CKD individuals supposedly with low RBC count, therefore it likely that our novel miRNAs are not highly expressed in RBCs and our results were not affected. Furthermore, Keller and colleagues performed a statistical evaluation for the effect of different blood cell count on miRNA expression profile for different human diseases to test for disease-specific alterations and found that they only partly affect the profile of miRNAs and that they do not significantly affect the feasibility to associate miRNA profile and human disease and therefore support the use of whole blood for miRNA profile analysis as the basis for detection of

disease [46]. Moreover, whole blood has high concentration of total miRNA after extraction and is not affected by pre-sample analysis and cell lysis [47].

The current study had few limitations which need to be taken into consideration when interpreting the findings. The cross-sectional design of the study limits the exploration of a causal relationship between the whole blood miRNAs and CKD, therefore future longitudinal analysis is recommended to elucidate the causal relationship between studied miRNAs and the pathogenesis of CKD in individuals with HTN and/or DM. Although the present study had a large study sample, we had a small number of individuals with CKD in the various stages of the disease, thereby not allowing for the exploration of miRNA profiles in various stages of CKD. CKD was diagnosed based on eGFR estimated from a single time point creatinine measurement, which is not ideal. However, we included levels of ACR which are important for interpretation when eGFR levels are above 60 ml/min/1.73m2 as recommended by the KDIGO guidelines [29]. HTN was diagnosed with BP measurements taken on one visit, although the International Society of Hypertension recommends that diagnosis should be made based on measurements taken at two or more visits separated by a period of one week [48]. Moreover, we did not exclude individuals on antihypertensive/antidiabetic medication and take into consideration the duration of the disease, therefore we cannot exclude the possibility that these might have influenced the findings of this study. However, we adjusted for some of the common risk factors associated with CKD as well as miRNAs thereby eliminating their confounding bias. Moreover, the fact that in the present study we explored the differentially expressed miRNAs that were identified in our population and evaluate their potential role as screening tools for CKD especially in high-risk individuals with HTN and or DM considering their reported high incidences serves as a strength for this study. Our findings form basis to potential pathophysiological importance of whole blood miRNAs in CKD.

6.5. Conclusion

Taken together, the findings of the present study demonstrate that hsa-miR-novel-chr13_13519 that was positively associated with CKD prevalent, with further research may be used as a potential screening tool for CKD risk screening particularly in people with HTN and DM for whom early initiation of treatment may prevent the onset or halt the progression of CKD. However, these miRNAs are still novel, they have only been reported in our study population, therefore, future studies are necessary to validate our findings in large study sample and most importantly explore the origin of these miRNAs, elucidate their physiological roles and target genes and pathways.

Author Contributions: Conceptualization, Rajiv Erasmus, Andre Kengne and Tandi Matsha; Data curation, Don Matshazi, Cecil Weale and Saarah Davids; Formal analysis, Dipuo Motshwari, Cindy George and Saarah Davids; Funding acquisition, Rajiv Erasmus, Andre Kengne and Tandi Matsha;

Investigation, Dipuo Motshwari, Don Matshazi and Cecil Weale; Methodology, Dipuo Motshwari, Don Matshazi and Cecil Weale; Project administration, Rajiv Erasmus, Andre Kengne and Tandi Matsha; Resources, Tandi Matsha; Software, Cindy George; Supervision, Cindy George and Tandi Matsha; Validation, Cindy George; Visualization, Dipuo Motshwari; Writing – original draft, Dipuo Motshwari; Writing – review & editing, Dipuo Motshwari, Cindy George, Don Matshazi, Cecil Weale, Rajiv Erasmus, Andre Kengne and Tandi Matsha.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the CPUT Faculty of Health and Wellness Sciences Research Ethics Committee reference number (CPUT/HW-REC 2020/H11).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.
Data Availability Statement: The dataset used for this study is available from the corresponding author with reasonable request.

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Conflicts of Interest: The authors declare no conflict of interest.

6.6. References

1. Bikbov, B.B.; Purcell, C.A.; Levey, A.S.; Smith, M.; Abdoli, A.; Abebe, M.; Adebayo, O.M.; Afarideh, M.; Agarwal, S.K.; Agudelo-Botero, M.; et al. Global, regional, and national burden of chronic kidney disease, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet. 2020, 395, 709-733.

2. Kaze, A.D.; Ilori, T.; Jaar, B.G.; Echouffo-Tcheugui, J.B. Burden of chronic kidney disease on the African continent: A systematic review and meta-analysis. BMC Nephrol. 2018,19, 1–11.

3. George, J.A.; Brandenburg, J.T.; Fabian, J.; Crowther, N.J.; Agongo, G.; Alberts, M.; Ali, S.; Asiki, G.; Boua, P.R.; Gómez-Olivé, F.X.; et al. Kidney damage and associated risk factors in rural and urban sub-Saharan Africa (AWI-Gen): a cross-sectional population study. Lancet Glob Heal. 2019, 7, 1632–43.

4. Noubiap, J.J.N. Diabetic nephropathy in Africa: A systematic review. World J Diabetes. 2015, 6, 759.

5. IDF 9th edition. International Diabetes Federation.The Lancet. 2020.266, 134–137.
6. Anderson, A.H.; Yang, W.; Townsend, R.R.; Pan, Q.; Chertow, G.M.; Kusek, J.W.; Charleston, J.; He, J; Kallem, R.; Lash, J.P.; et al. Time-updated systolic blood pressure and the progression of chronic kidney disease: a cohort study. Ann Intern Med. 2015,162, 258–65.
7. Mills, K.T.; Stefanescu, A.; He, J. The global epidemiology of hypertension. Nat Rev Nephrol. 2020, 16, 223–37, doi:10.1038/s41581-019-0244-2.
8. Mills, K.T.; Bundy, J.D.; Kelly, T.N.; Reed, J.E.; Kearney, P.M.; Reynolds, K.; Chen, J.; He, J. Global disparities of hypertension prevalence and control. Circulation. 2016, 134, 441–50.
9. Bosu, W.K.; Reilly, S.T.; Aheto, J.M.K.; Zucchelli, E. Hypertension in older adults in Africa: a systematic review and meta-analysis. PLoS One. 2019,14, e0214934.
10. USRDS, U. Annual data report: atlas of chronic kidney disease and end-stage renal disease in the United States. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. 2013, 2014.
11. Yuan, H.L.; Wang. T.; Zhang, K.H. MicroRNAs as potential biomarkers for diagnosis, therapy and prognosis of gastric cancer. Onco Targets Ther. 2018, 11, 3891.
12. Danborg, P.B.; Simonsen, A.H.; Waldemar, G.; Heegaard, N.H.H. The potential of microRNAs as biofluid markers of neurodegenerative diseases–a systematic review. Biomarkers. 2014, 19, 259–68.
13. Felekkis, K.; Papaneophytou, C. Challenges in using circulating micro-RNAs as biomarkers for cardiovascular diseases. Int J Mol Sci. 2020, 21, 561.
14. Bhatt, K;. Mi, Q.S.; Dong, Z. MicroRNAs in kidneys: Biogenesis, regulation, and pathophysiological roles. Am J Physiol - Ren Physiol. 2011, 300, 602–10.
15. Brodersen, P.; Voinnet, O. Revisiting the principles of microRNA target recognition and mode of action. Nat Rev Mol Cell Biol. 2009, 10, 141–8.
16. Abdellatif, M. Differential expression of microRNAs in different disease states. Circ Res. 2012, 110, 638–50.
17. Keller, S.; Ridinger, J.; Rupp, A.K.; Janssen, J.W.G. Altevogt, P. Body fluid derived exosomes as a novel template for clinical diagnostics. J Transl Med. 2011,9, 1–9.
18. Mitchell, P.S.; Parkin, R.K.; Kroh, E.M.; Fritz, B.R.; Wyman, S.K.; Pogosova-Agadjanyan, E.L.; Peterson, A.; Noteboom, J.; O'Briant, K.C.; Allen, A.; et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci. 2008, 105, 10513–8.
19. Chandrasekaran, K.; Karolina, D.S.; Sepramaniam, S.; Armugam, A.; Wintour, E.M.; Bertram, J.F.; Jeyaseelan, K. Role of microRNAs in kidney homeostasis and disease. Kidney Int. 2012, 81, 617–27, doi:10.1038/ki.2011.448
20. Marques, F.Z.; Campain, A.E.; Tomaszewski, M.; Zukowska-Szczechowska, E.; Yang, Y.H.J.; Charchar, F.J; Morris, B.J. Gene expression profiling reveals renin mRNA overexpression in human hypertensive kidneys and a role for microRNAs. Hypertension. 2011, 58, 1093–8.

21. Kato, M.; Zhang, J.; Wang, M.; Lanting, L.; Yuan, H.; Rossi, J.J.; Natarajan, R. MicroRNA-192 in diabetic kidney glomeruli and its function in TGF-β-induced collagen expression via inhibition of E-box repressors. Proc Natl Acad Sci. 2007, 104, 3432–7.
22. Wang, G.; Kwan, B.C.H.; Lai, F.M.M.; Choi, P.C.L.; Chow, K.M.; Li, P.K.T.; Szeto, C.C. Intrarenal expression of miRNAs in patients with hypertensive nephrosclerosis. Am J Hypertens. 2010, 23, 78–84, doi:10.1038/ajh.2009.208.
23. Wang, J.; Wang, G.; Liang, Y.; Zhou, X. Expression profiling and clinical significance of plasma microRNAs in diabetic nephropathy. J Diabetes Res. 2019, 2019.
24. Matshazi, D.M.; Weale, C.J.; Erasmus, R.T.; Kengne, A.P.; Davids, S.F.; Raghubeer, S.; Davison, G.M.; Matsha, T.E.Two novel microRNAs and their association with absolute blood pressure parameters in an urban South African community. Mol Biol Rep. 2021, 1–8.
25. Matsha, T.E.; Kengne, A.P.; Hector, S.; Mbu, D.L.; Yako, Y.Y.; Erasmus, R.T. MicroRNA profiling and their pathways in South African individuals with prediabetes and newly diagnosed type 2 diabetes mellitus. Oncotarget. 2018, 9, 30485–98.
26. Erasmus, R.T.; Soita, D.J.; Hassan, M.S.; Blanco-Blanco, E.; Vergotine, Z.; Kengne, A.P.; Matsha, T.E. High prevalence of diabetes mellitus and metabolic syndrome in a South African coloured population: Baseline data of a study in Bellville, Cape Town. South African Med J. 2012, 102, 841–4.
27. Chalmers, J.O.H.N.; MacMahon, S.; Mancia, G.; Whitworth, J.; Beilin, L.; Hansson, L.; Neal, B.; Rodgers, A.; Mhurchu, N.; Clark, T. World Health Organization-International Society of Hypertension Guidelines for the management of hypertension.

Guidelines sub-committee of the World Health Organization. Clin Exp Hypertens. 1999, 21, 1009–60.

28. Alberti, K.G.M.M.; Zimmet, P.Z. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. Diabet Med. 1998, 15, 539–53.
29. Levin, A.; Stevens, P.E.; Bilous, R.W.; Coresh, J.; De Francisco, A.L.; De Jong, P.E.; Griffith, K.E.; Hemmelgarn, B.R.; Iseki, K.; Lamb, E.J.; et al. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int Suppl. 2013, 3, 1–150.
30. Holness, J.L.; Bezuidenhout, K.; Davids, M.R.; and Warwick, J.M. Validation of equations to estimate glomerular filtration rate in South Africans of mixed ancestry. S Afr Med J. 2020, 110, 229–34.
31. Pirkle, J.L.; Flegal, K.M.; Bernert, J.T.; Brody, D.J.; Etzel, R.A.; Maurer, K.R. Exposure of the US population to environmental tobacco smoke: the Third National Health and Nutrition Examination Survey, 1988 to 1991. Jama. 1996, 275, 1233–40.
32. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. methods. 2001, 24, 402–8.

33. Kato, M.; Arce, L.; Wang, M.; Putta, S.; Lanting, L.; Natarajan, R. A microRNA circuit mediates transforming growth factor-β1 autoregulation in renal glomerular mesangial cells. Kidney Int. 2011, 80, 358–68.
34. Abdelsalam, M.; Wahab, A.M.; El Sayed;. Zaki, M.; Motawea, M. MicroRNA-451 as an Early Predictor of Chronic Kidney Disease in Diabetic Nephropathy. Int J Nephrol. 2020, 2020.
35. Liu, Y.; Usa, K.; Wang, F.; Liu, P.; Geurts, A.; Li, J.; Williams, A.M.; Regner, K.R.; Kong, Y.; Liu, H.; Nie, J.; et al. MicroRNA-214-3p in the kidney contributes to the development of hypertension. J Am Soc Nephrol. 2018, 29, 2518–28.
36. Cheng, Y.; Wang, D.; Wang, F.; Liu, J.; Huang, B.; Baker, M.A.; Yin, J.; Wu, R.; Liu, X.; Regner, K.R.; Liu, Y.J. Endogenous miR-204 protects the kidney against chronic injury in hypertension and diabetes. Am Soc Nephrol. 2020, 31, 1539–54.
37. Lu, Q.; Ma, Z.; Ding, Y.; Bedarida, T.; Chen, L., Xie, Z., Song, P. and Zou, M.H.. Circulating miR-103a-3p contributes to angiotensin II-induced renal inflammation and fibrosis via a SNRK/NF-κB/p65 regulatory axis. Nat Commun. 2019, 10, 1-4.
38. Berillo, O.; Huo, K.G.; Fraulob-Aquino, J.C.; Richer, C.; Briet, M.; Boutouyrie, P.; Lipman, M.L.; Sinnett, D.; Paradis, P.; Schiffrin, E.L. Circulating let-7g-5p and miR-191-5p are independent predictors of chronic kidney disease in hypertensive patients. Am J Hypertens. 2020, 33, 505–13.
39. Yokoyama, Y.; Mise, N.; Suzuki, Y.; Tada-Oikawa, S.; Izuoka, K.; Zhang, L.; Zong, C.; Takai, A.; Yamada, Y.; Ichihara, S. MicroRNAs as potential mediators for cigarette smoking induced atherosclerosis. Int J Mol Sci. 2018, 19, 1097.
40. Witwer, K.W. Circulating microRNA biomarker studies: pitfalls and potential solutions. Clin Chem. 2015, 61, 56–63.
41. Rodriguez-Rius, A.; Martinez-Perez, A.; López, S.; Sabater-Lleal, M.; Souto, J.C.; Soria, J.M. Expression of microRNAs in human platelet-poor plasma: analysis of the factors affecting their expression and association with proximal genetic variants. Epigenetics. 2020, 15, 1396–406.
42. Sunderland, N.; Skroblin, P.; Barwari, T.; Huntley, R.P.; Lu, R.; Joshi, A.; Lovering, R.C.; Mayr, M. MicroRNA biomarkers and platelet reactivity: the clot thickens. Circ Res. 2017;120(2):418–35.
43. Cochetti, G.; Cari, L.; Nocentini, G.; Maulà, V.; Suvieri, C.; Cagnani, R.; De Vermandois, J.A.R.; Mearini, E. Detection of urinary miRNAs for diagnosis of clear cell renal cell carcinoma. Sci Rep. 2020, 10, 1–13.
44. George, C.; Matsha ,T.E.; Erasmus, R.T.; Kengne, A.P. Haematological profile of chronic kidney disease in a mixed-ancestry South African population: A cross-sectional study. BMJ Open. 2018, 8, e025694.
45. Sun, L.; Yu, Y.; Niu, B.; Wang, D. Red Blood cells as potential repositories of microRNAs in

the circulatory system. Front Genet. 2020, 11, 442.

46. Keller, A.; Leidinger, P.; Bauer, A.; ElSharawy, A.; Haas, J.; Backes, C.; Wendschlag, A.; Giese, N.; Tjaden, C.; Ott, K.; Werner, J. Toward the blood-borne miRNome of human diseases. Nat Methods. 2011,8, 841–3.

47. Grasedieck, S.; Sorrentino, A.; Langer, C.; Buske, C.; Döhner, H.; Mertens, D.; Kuchenbauer, F. Circulating microRNAs in hematological diseases: Principles, challenges, and perspectives. Blood. 2013;121(25):4977–84.

48. Unger, T.; Borghi, C.; Charchar, F.; Khan, N.A.; Poulter, N.R.; Prabhakaran, D.; Ramirez, A.; Schlaich, M.; Stergiou, G.S.; Tomaszewski, M.; et al. 2020 International Society of Hypertension global hypertension practice guidelines. Hypertension. 2020;75(6):1334–57.

CHAPTER 7

7. OVERALL DISCUSSION

This chapter summarizes the main findings of our study, their implications, and highlights the limitations and recommendations for future studies.

MicroRNAs associated with chronic kidney disease in the general population and high-risk subgroups: protocol for a systematic review and meta-analysis

The systematic review identified all published miRNAs that were found to be associated with prevalent CKD and/or measures of kidney function and kidney damage, as well as their expression patterns, in the general population and in high-risk subgroups (HTN and DM). Observations revealed that there is dysregulation in the expression pattern of miRNAs with CKD in the general population and high-risk subgroups. Moreover, some of the miRNAs were frequently studied in the general population with CKD (miR-126, miR-223, miR-155, miR-21) and in individuals with DKD (miR-155, miR-126, miR-192, miR-21, miR-15a-5p, miR-29a, miR-29b and miR-29c). Of these, miR-126 and miR-223 were commonly downregulated in prevalent CKD whilst miR-21 and miR-29b were consistently upregulated in DKD. The findings from this review will inform the choice of miRNAs that should be focused on in future research, particularly in studies investigating the clinical value of miRNAs in CKD pathophysiology.

Expression of whole blood miR-126-3p, -30a-5p, -1299, -182-5p & -30e-3p in chronic kidney disease in a South African community-based sample

Using RT-qPCR, we quantified the expression level of five known miRNAs (miR-126-3p, -30a-5p, -1299, -182-5p and -30e-3p) that were previously shown to be associated with kidney function and/or kidney disease pathophysiology and investigated their association with prevalent CKD for the first time in an African setting. We showed a dysregulated pattern of these miRNAs in CKD relative to controls and of note miR-126-3p, miR -182-5p and miR-30e-3p were independently associated with increased risk of CKD. This study provides the basis for future investigations in the clinical relevance of these miRNAs as possible markers for the diagnosis or prognosis of CKD in our population.

Novel Whole Blood MicroRNAs Predicting Chronic Kidney Disease in South Africans with Hypertension and Diabetes Mellitus

Herein, we characterized the expression profile of novel whole blood miRNAs (hsa-miR-novel-chr1_36178, hsa-miR-novel-chr15_18383, hsa-miRnovel-chr2_55842, hsa-miR-novel-chr7_76196, hsa-miR-novel-chr5_67265, and hsa-miR-novel-chr13_13519) previously identified by our research team using a high throughput next generation sequencing (NGS) in a high-risk population with DM and/or HTN in a South African cohort. This study revealed an upregulated pattern of the novel whole blood miRNAs that are associated with CKD in high-risk individuals unique to our study population. Furthermore, increased expression of hsa-miR-novel-chr2_55842 and hsa-miR-novel-chr7_76196

were inversely associated with eGFR, suggesting that these miRNAs may reflect kidney function impairment in our population. Whilst all the novel whole blood miRNAs were acceptable predictors of CKD, hsa-miR-novel-chr13_13519 had an additional advantage for CKD prediction beyond conventional factors. The findings of the current investigation suggest that these novel whole blood miRNAs have a potential to contribute to CKD risk screening in people with HTN and/or DM, particularly hsa-miR-novelchr13_13519 and as such, warrant further research into its role and target pathways.

7.1. Clinical significance

Chronic kidney disease is a silent condition, with the majority of affected individuals unaware of their diagnosis, particularly in the early stages, due to a lack of obvious clinical symptoms [1]. Moreover, the currently used diagnostic tools for CKD present with major limitations. Indeed, eGFR and albuminuria, which are measures frequently used to detect CKD, cannot inform on the cause of CKD [2]. Kidney biopsies which are used when confirmation of CKD diagnosis is required, are quite invasive and costly. Although there are various therapies to slow CKD progression, they cannot completely halt or reverse disease progression [3]. Consequently, this is associated with catastrophic health expenditure, particularly in poor-resourced countries [4]. Therefore, new personalized approaches for CKD management, including more precise and reliable biomarkers to allow for early identification of CKD, particularly in high-risk individuals, as well as targeted therapeutic interventions, are vital to improving disease outcomes.

MiRNAs are promising as potential biomarkers of a multitude of diseases, including CKD, that are more precise and reliable and can be utilized in both clinical and personalized medicine [5–8].
A number of miRNAs have been shown to have the ability to reflect and regulate CKD-specific pathophysiologic pathways[9–11], therefore suggesting that these miRNAs are of biological relevance in CKD and associated complications. A recent review highlighted a number of altered podocytes miRNAs that contribute to the progression of DKD through regulation of signalling pathways such as transforming growth factor beta (TGF-β), advanced glycation end products, insulin signalling pathway and oxidative stress during the pathogenesis of DKD [11]. These findings bring insights into the mechanisms underlying DKD progression and the potential use of podocytes-specific miRNAs as biomarkers for diagnosis and prognosis of DKD.

Experimental studies have shown that miR-126 regulates pathways that are critical for vascular homeostasis and vascular inflammation, and as a result, dysregulation of this miRNA may result in the development of vascular dysfunction which is very common in the early-stage CKD, suggesting miR-126 as a potential marker for early detection of CKD [12,13]. Animal models of DM showed that inhibition of miR-93-5p was associated with the upregulation of vascular endothelial growth factor,

which led to increased levels of collagen and fibronectin, and subsequently, DKD [14]. Whilst increased expression of miR-124 was associated with adhesive capacity damage of podocyte observed in DKD [15]. Furthermore, a diabetic animal and cell model study provided evidence indicating that miR-154-5p promotes fibrosis in DKD through the regulation of TGFβ1/Smads pathway [16]. Mechanistic studies revealed that increased miR-103a-3p expression is associated with angiotensin II-induced HTN-associated CKD through the activation of SNRK/nuclear factor-κB/p65 regulatory axis, therefore, suggesting that miR-103a-3p may inform on the pathogenesis of CKD in HTN [17].

Knockdown of miR-30 in CKD rats was associated with the development of cardiac hypertrophy, a CVD complication through the activation of the calcineurin/NFATc3 signalling, therefore, suggesting a potential therapeutic for CKD with CVD-associated complications [18]. MiR-21-based therapy, anti miR-21 drug called lademirsen (SAR339375), is currently in phase II of a clinical trial for the treatment of Alport nephropathy and efficacy of this drug in reducing kidney function decline [19]. A study by Peters and colleagues highlighted four miRNAs (miR-103a-3p, miR-192-5p, the miR-29 family and miR-21-5p) that have been widely studied and have shown potential in clinical application of CKD. They concluded that antagonism of miR-21-5p in DKD and miR-103a-3p in HTN-associated CKD may serve as a therapeutic intervention strategy to improve disease outcome, whilst miR-192-5p involved in the development and progression of DKD may serve as a potential diagnostic and prognostic marker of DKD and miR-29 family may be of beneficial effects in the regulation of fibrosis in DKD [20]. These findings provide clues on the mechanisms underlying CKD development and suggest a critical role of these miRNAs in the innovation of new CKD therapies.

The upregulated expression pattern of known whole blood miRNAs and novel whole blood miRNAs studied in our population with CKD relative to controls present evidence for the first time in an African population. These findings suggest that these miRNAs may be able to discriminate between individuals with and without CKD in our study population. Moreover, the observed association between miRNAs and CKD as well as its clinical indicators, is indicative of the possible role of these miRNAs in the pathogenesis of CKD and therefore suggests that these miRNAs may be of clinical values in CKD prediction. Of interest, hsa-miR-novel-chr13_13519 showed to have added advantage beyond that of conventional factors for CKD prediction, therefore, suggesting a potential screening value of this miRNA in individuals at high risk of developing CKD in our population. However, although these miRNAs can discriminate between individuals with and without CKD, their clinical value remains to be validated. The consistent alteration of miRNAs miR-126, miR-223, miR-21 and miR-29b in CKD and DKD observed in our systematic review suggests that these miRNAs may be implicated in the pathogenesis of CKD and may present possible diagnostic markers for early detection and therapeutic targets of CKD and warrant further scrutiny in future investigations.

7.2. Strength and limitations

The main strength of our study is that we performed a systematic review to provide a comprehensive report of miRNAs dysregulated in CKD, associated with prevalent CKD, as well as its clinical markers in the general population and high-risk individuals in various geographic locations for the very first time. The prominent miRNAs identified can be targeted in future studies investigating the clinical value of miRNAs in CKD. We reported on the pattern of miRNAs in CKD that have previously been reported in other populations for the first time in an African population. Moreover, the novel miRNAs investigated in our study were previously identified in our own population by high throughput NGS techniques therefore, these are specific to our population and may be of clinical value for CKD management, further research into their roles and origin is essential. Furthermore, confounding variables, including age, sex, smoking status, drinking status, HTN status and DM status, were adjusted for when evaluating the association between the studied miRNAs and CKD or its clinical indicators in our population as they may have influenced our findings, therefore, adding strength to our findings.

The major drawback of our systematic review was that we were unable to perform a pooled meta-analysis due to several variabilities across studies in relation to the quantification of miRNAs, sample type used and disease outcome measures, and as a result, opted for a narrative synthesis of evidence. In our miRNA characterization study, the main limitation was the cross-sectional single-centre study design, including only individuals of mixed-ancestry, which does not allow exploration of the causal relationship between the studied miRNAs and CKD as well as the limited generalizability of our findings in other African populations. Moreover, although we had a large study population, the number of individuals with CKD was relatively small therefore, we could not evaluate miRNA patterns at various stages of CKD. There were inconsistencies in the data reported by our study compared to other published studies on the same miRNA signatures in CKD. However, variabilities in the source of miRNA, sampling methods, handling and processing of samples, different detection methods and normalization controls and the difference in CKD cohort sizes may have contributed to the discrepancies. For example, our study used whole blood sample for miRNA quantification in CKD, and previously published studies mostly used serum or plasma samples. Moreover, several studies have reported that urine is a superior sample for CKD, as miRNAs detected in urine correlated strongly with those in kidney tissue [21]. For the quantification of miRNAs, we used the RT-qPCR technique, which requires a normalization control, and in our study, we used miR-16-5p as an endogenous control. However, due to the lack of standardized controls, various controls are employed by different studies, which potentially further contributes to the discrepancies in findings.

7.3. Future prospects

To establish the potential clinical applications of the studied miRNAs in the diagnosis, treatment or prognosis of CKD in our study population, large multicentre follow-up studies are essential for validation of our findings and exploration of the causal relationship between miRNAs and CKD as well as their effect on CKD prognosis. Bioinformatic tools should be employed for miRNA target predictions and pathway analysis to yield additional mechanistic insights and contributions of miRNAs to the pathophysiology of CKD. Future investigations on the clinical value of miRNAs should also focus on the standardization of protocols for pre-analysis of miRNAs in terms of the type of sample used for miRNA extraction, collection, storage and optimization of techniques for miRNA detection to improve reproducibility and comparability of literature on same miRNA signatures [22]. Moreover, future studies should confirm the stability of all chosen normalization miRNAs in each experiment and where possible more than one normalizer involving both an exogenous or spike in control and endogenous reference gene should be used for more accurate miRNA results [23].

7.4. Conclusion

Our study provides evidence of miRNA dysregulation in CKD for the first time in a South African setting. We anticipate that the findings of our study will form the basis for future investigations on the clinical value of miRNAs in CKD early diagnosis, prognosis and therapeutic pathways, particularly in Africa. However, we do acknowledge that our study had a number of limitations and should be interpreted with caution as validations are still required. Future studies are also required to explore the functional significance and contribution of these miRNAs to CKD pathophysiology. Although the understanding of the clinical value of miRNAs in the pathophysiology of CKD is still in its infancy, miRNAs are promising as valuable diagnostic tools and targets for therapeutic interventions for CKD in the future. Collective efforts between researchers and clinicians are therefore critical to facilitate the transition of these miRNAs from bench side to clinical practice.

7.5. Reference

1. Bidin MZ, Shah AM, Stanslas J, Seong CLT. Blood and urine biomarkers in chronic kidney disease: An update. Clin Chim Acta. 2019;495:239–50.

2. Al-Rubeaan K, Siddiqui K, Al-Ghonaim MA, Youssef AM, Al-Sharqawi AH, AlNaqeb D. Assessment of the diagnostic value of different biomarkers in relation to various stages of diabetic nephropathy in type 2 diabetic patients. Sci Rep. 2017;7(1):1–9.

3. Stanton RC. Clinical challenges in diagnosis and management of diabetic kidney disease. Am J kidney Dis. 2014;63(2):S3–21.

4. Essue BM, Jha V, John O, Knight J, Jan S. Universal health coverage and chronic kidney disease in India. Bull World Health Organ. 2018;96(7):442.

5. Chen X, Xie D, Zhao Q, You ZH. MicroRNAs and complex diseases: From experimental results to computational models. Brief Bioinform. 2019;20(2):515–39.

6. Zhu H, Wang G, Zhou X, Song X, Gao H, Ma C, et al. miR-1299 suppresses cell proliferation of hepatocellular carcinoma (HCC) by targeting CDK6. Biomed Pharmacother [Internet]. 2016;83:792–7. Available from: http://dx.doi.org/10.1016/j.biopha.2016.07.037

7. Amiri A, Tehran MM, Asemi Z, Shafiee A, Hajighadimi S, Moradizarmehri S, et al. Role of resveratrol in modulating microRNAs in human diseases: From cancer to inflammatory disorder. Curr Med Chem. 2019;26(March 2020).

8. Wang J, Wang G, Liang Y, Zhou X. Expression profiling and clinical significance of plasma microRNAs in diabetic nephropathy. J Diabetes Res. 2019;2019.

9. Taïbi F, Metzinger-Le Meuth V, M'Baya-Moutoula E, Djelouat M seif el I, Louvet L, Bugnicourt JM, et al. Possible involvement of microRNAs in vascular damage in experimental chronic kidney disease. Biochim Biophys Acta - Mol Basis Dis [Internet]. 2014;1842(1):88–98. Available from: http://dx.doi.org/10.1016/j.bbadis.2013.10.005

10. Metzinger-Le Meuth V, Burtey S, Maitrias P, Massy ZA, Metzinger L. microRNAs in the pathophysiology of CKD-MBD: Biomarkers and innovative drugs. Biochim Biophys Acta - Mol Basis Dis [Internet]. 2017;1863(1):337–45. Available from: http://dx.doi.org/10.1016/j.bbadis.2016.10.027

11. Wonnacott A, Denby L, Coward RJM, Fraser DJ, Bowen T. MicroRNAs and their delivery in diabetic fibrosis. Adv Drug Deliv Rev. 2021;114045.

12. Fish JE, Santoro MM, Morton SU, Yu S, Yeh R-F, Wythe JD, et al. miR-126 regulates angiogenic signaling and vascular integrity. Dev Cell. 2008;15(2):272–84.

13. Zhou J, Li Y-S, Nguyen P, Wang K-C, Weiss A, Kuo Y-C, et al. Regulation of vascular smooth muscle cell turnover by endothelial cell–secreted microRNA-126: role of shear stress. Circ Res. 2013;113(1):40–51.

14. Long J, Wang Y, Wang W, Chang BHJ, Danesh FR. MicroRNA-29c is a signature microRNA under high glucose conditions that targets Sprouty homolog 1, and its in vivo knockdown prevents progression of diabetic nephropathy. J Biol Chem [Internet]. 2011 Apr 1;286(13):11837–48. Available from: https://search.ebscohost.com/login.aspx?direct=true&db=cmedm&AN=21310958&site=ehost-live

15. Panizo S, Martínez-Arias L, Alonso-Montes C, Cannata P, Martín-Carro B, Fernández-Martín JL, et al. Fibrosis in Chronic Kidney Disease: Pathogenesis and Consequences. Int J Mol Sci.

2021 Jan;22(1).

16. Bian C, Luan Z, Zhang H, Zhang R, Gao J, Wang Y, et al. miR-154-5p Affects the TGFβ1/Smad3 Pathway on the Fibrosis of Diabetic Kidney Disease via Binding E3 Ubiquitin Ligase Smurf1. Oxid Med Cell Longev. 2022;2022.

17. Lu Q, Ma Z, Ding Y, Bedarida T, Chen L, Xie Z, et al. Circulating miR-103a-3p contributes to angiotensin II-induced renal inflammation and fibrosis via a SNRK/NF-κB/p65 regulatory axis. Nat Commun. 2019;10(1).

18. Bao J, Lu Y, She Q, Dou W, Tang R, Xu X, et al. MicroRNA-30 regulates left ventricular hypertrophy in chronic kidney disease. JCI insight. 2021;6(10).

19. Study of Lademirsen (SAR339375) in Patients With Alport Syndrome - Full Text View - ClinicalTrials.gov [Internet]. [cited 2022 Jul 26]. Available from: https://clinicaltrials.gov/ct2/show/NCT02855268

20. Peters LJF, Floege J, Biessen EAL, Jankowski J, van der Vorst EPC. MicroRNAs in Chronic Kidney Disease: Four Candidates for Clinical Application. Int J Mol Sci. 2020 Sep;21(18).

21. Li J, Ma L, Yu H, Yao Y, Xu Z, Lin W, et al. MicroRNAs as Potential Biomarkers for the Diagnosis of Chronic Kidney Disease: A Systematic Review and Meta-Analysis. Front Med. 2021;8.

22. Witwer KW. Circulating microRNA biomarker studies: pitfalls and potential solutions. Clin Chem. 2015;61(1):56–63.

23. Madadi S, Schwarzenbach H, Lorenzen J, Soleimani M. MicroRNA expression studies: challenge of selecting reliable reference controls for data normalization. Cell Mol life Sci. 2019;76(18):3497–514.

8. APPENDIX

Appendix A: Permission for reuse of figure

Copy rights for Figure 2.2 on miRNA biogenesis (Hajarnis et al., 2015)

Appendix B: Supplementary data for systematic review

Supplementary Table S 5 Medline (PubMed) search strategy (from inception to 31 October 2021)

Search	Query	Number of hits
#1	(Chronic kidney disease) OR (chronic kidney failure) OR (chronic renal disease) OR (chronic renal failure) OR (end-stage renal disease) OR (end-stage renal failure) OR (diabetic kidney disease) OR (diabetic nephropathy) OR (hypertensive nephrosclerosis) OR albuminuria OR proteinuria OR (HIV associated nephropathy) OR HIVAN OR (HIV-associated kidney disease) OR (HIV-associated renal disease)	277,388
#2	(Serum creatinine) OR (serum cystatin C) OR (estimated glomerular filtration rate) OR (urinary albumin excretion rate) OR (albumin-to-creatinine ratio) OR (urinary albumin)	95,064
#3	microRNAs OR miRNA OR miRNAs	133,454
#4	animal OR rat OR mouse OR (cell-line)	7,721,509
#5	cancer OR (acute kidney injury)	4,215,872
#6	#1 OR #2	333,540
#7	#6 AND #3	1,627
#8	#7 NOT #	683

#9	#8 NOT #5	577

Supplementary Table S 6 Web of science search strategy (from inception to 31 October 2021)

Search	**Query**	**Number of hits**
#1	(Chronic kidney disease) OR (chronic kidney failure) OR (chronic renal disease) OR (chronic renal failure) OR (end-stage renal disease) OR (end-stage renal failure) OR (diabetic kidney disease) OR (diabetic nephropathy) OR (hypertensive nephrosclerosis) OR albuminuria OR proteinuria OR (HIV associated nephropathy) OR HIVAN OR (HIV-associated kidney disease) OR (HIV-associated renal disease)	244,799
#2	(Serum creatinine) OR (serum cystatin C) OR (estimated glomerular filtration rate) OR (urinary albumin excretion) OR (albumin-to-creatinine ratio) OR (urinary albumin)	87,982
#3	microRNAs OR miRNA OR miRNAs	117,582
#4	animal OR rat OR mouse OR (cell-line)	4,402,334
#5	cancer OR (acute kidney injury)	3,752,456
#6	(ALL= ((Chronic kidney disease) OR (chronic kidney failure) OR (chronic renal disease) OR (chronic renal failure) OR (end-stage renal disease) OR (end-stage renal failure) OR (diabetic kidney disease) OR (diabetic nephropathy) OR (hypertensive nephrosclerosis) OR albuminuria OR proteinuria OR (HIV associated nephropathy) OR HIVAN OR (HIV-associated kidney disease) OR (HIV-associated renal disease)) OR ALL= ((Serum creatinine) OR (serum cystatin C) OR (estimated glomerular filtration rate) OR (urinary albumin excretion) OR (albumin-to-creatinine ratio) OR (urinary albumin))	296,928
#7	((ALL=((Chronic kidney disease) OR (chronic kidney failure) OR (chronic renal disease) OR (chronic renal failure) OR (end-stage renal disease) OR (end-stage renal failure) OR (diabetic kidney disease) OR (diabetic	1,554

	nephropathy) OR (hypertensive nephrosclerosis) OR albuminuria OR proteinuria OR (HIV associated nephropathy) OR HIVAN OR (HIV-associated kidney disease) OR (HIV-associated renal disease)) OR ALL=((Serum creatinine) OR (serum cystatin C) OR (estimated glomerular filtration rate) OR (urinary albumin excretion) OR (albumin-to-creatinine ratio) OR (urinary albumin)) AND ALL=(microRNAs OR miRNA OR miRNAs)	
#8	(((ALL=((Chronic kidney disease) OR (chronic kidney failure) OR (chronic renal disease) OR (chronic renal failure) OR (end-stage renal disease) OR (end-stage renal failure) OR (diabetic kidney disease) OR (diabetic nephropathy) OR (hypertensive nephrosclerosis) OR albuminuria OR proteinuria OR (HIV associated nephropathy) OR HIVAN OR (HIV-associated kidney disease) OR (HIV-associated renal disease)) OR ALL=((Serum creatinine) OR (serum cystatin C) OR (estimated glomerular filtration rate) OR (urinary albumin excretion) OR (albumin-to-creatinine ratio) OR (urinary albumin)) AND ALL=(microRNAs OR miRNA OR miRNAs)) NOT ALL=(animal OR rat OR mouse OR (cell-line))	913
#9	((((ALL=((Chronic kidney disease) OR (chronic kidney failure) OR (chronic renal disease) OR (chronic renal failure) OR (end-stage renal disease) OR (end-stage renal failure) OR (diabetic kidney disease) OR (diabetic nephropathy) OR (hypertensive nephrosclerosis) OR albuminuria OR proteinuria OR (HIV associated nephropathy) OR HIVAN OR (HIV-associated kidney disease) OR (HIV-associated renal disease)) OR ALL=((Serum creatinine) OR (serum cystatin C) OR (estimated glomerular filtration rate) OR (urinary albumin excretion rate)) OR (albumin-to-creatinine ratio) OR (urinary albumin)) AND ALL=(microRNAs OR miRNA OR miRNAs)) NOT ALL=(animal OR rat OR mouse OR (cell-line))) NOT ALL=(cancer OR (acute kidney injury))	669

Supplementary Table S 7 Scopus search strategy (from inception to 31 October 2021)

Search	Query	Number of hits
#1	"Chronic kidney disease" OR "chronic kidney failure" OR "chronic renal disease" OR "chronic renal failure" OR "end-stage renal disease" OR "end-stage renal failure" OR "diabetic kidney disease" OR "diabetic nephropathy" OR "hypertensive nephrosclerosis" OR albuminuria OR proteinuria OR “HIV associated nephropathy” OR HIVAN OR “HIV-associated kidney disease” OR “HIV-associated renal disease”	626,284
#2	"Serum creatinine" OR "serum cystatin C" OR "estimated glomerular filtration rate" OR " urinary albumin excretion rate" OR "albumin-to-creatinine ratio" OR "urinary albumin"	139,458
#3	microRNAs OR miRNA OR miRNAs	309,549
#4	animal OR rat OR mouse OR "cell-line"	13,190,253
#5	cancer OR "acute kidney injury"	7,436,396
#6	("Chronic kidney disease" OR "chronic kidney failure" OR "chronic renal disease" OR "chronic renal failure" OR "end-stage renal disease" OR "end-stage renal failure" OR "diabetic kidney disease" OR "diabetic nephropathy" OR "hypertensive nephrosclerosis" OR albuminuria OR proteinuria OR “HIV associated nephropathy” OR HIVAN OR “HIV-associated kidney disease” OR “HIV-associated renal disease”) OR ("Serum creatinine" OR "serum cystatin C" OR "estimated glomerular filtration rate" OR " urinary albumin excretion rate" OR "albumin-to-creatinine ratio" OR "urinary albumin")	682,499
#7	(("Chronic kidney disease" OR "chronic kidney failure" OR "chronic renal disease" OR "chronic renal failure" OR "end-stage renal disease" OR "end-stage renal failure" OR "diabetic kidney disease" OR "diabetic nephropathy" OR "hypertensive nephrosclerosis" OR albuminuria OR proteinuria OR “HIV associated nephropathy” OR HIVAN OR “HIV-associated kidney disease” OR “HIV-associated renal disease”) OR ("Serum creatinine" OR "serum cystatin C" OR "estimated glomerular filtration rate" OR "urinary albumin excretion	13,330

	rate" OR "albumin-to-creatinine ratio" OR "urinary albumin")) AND (microRNAs OR miRNA OR miRNAs)	
#8	((("Chronic kidney disease" OR "chronic kidney failure" OR "chronic renal disease" OR "chronic renal failure" OR "end-stage renal disease" OR "end-stage renal failure" OR "diabetic kidney disease" OR "diabetic nephropathy" OR "hypertensive nephrosclerosis" OR albuminuria OR proteinuria OR “HIV associated nephropathy” OR HIVAN OR “HIV-associated kidney disease” OR “HIV-associated renal disease”) OR ("Serum creatinine" OR "serum cystatin C" OR "estimated glomerular filtration rate" OR " urinary albumin excretion rate" OR "albumin-to-creatinine ratio" OR "urinary albumin")) AND (microRNAs OR miRNA OR miRNAs)) AND NOT (animal OR rat OR mouse OR "cell-line")	1,505
#9	(((("Chronic kidney disease" OR "chronic kidney failure" OR "chronic renal disease" OR "chronic renal failure" OR "end-stage renal disease" OR "end-stage renal failure" OR "diabetic kidney disease" OR "diabetic nephropathy" OR "hypertensive nephrosclerosis" OR albuminuria OR proteinuria OR “HIV associated nephropathy” OR HIVAN OR “HIV-associated kidney disease” OR “HIV-associated renal disease”) OR ("Serum creatinine" OR "serum cystatin C" OR "estimated glomerular filtration rate" OR " urinary albumin excretion rate" OR "albumin-to-creatinine ratio" OR "urinary albumin")) AND (microRNAs OR miRNA OR miRNAs)) AND NOT (animal OR rat OR mouse OR "cell-line")) AND NOT (cancer OR "acute kidney injury")	568

Supplementary Table S 8 EBSCOhost search strategy (from inception to 31 October 2021)

Search	**Query**	**Number of hits**
#1	(Chronic kidney disease) OR (chronic kidney failure) OR (chronic renal disease) OR (chronic renal failure) OR (end-stage renal disease) OR (end-stage renal failure) OR (diabetic kidney disease) OR (diabetic nephropathy) OR (hypertensive nephrosclerosis) OR albuminuria OR proteinuria OR (HIV associated nephropathy) OR HIVAN OR (HIV-associated kidney disease) OR (HIV-associated renal disease)	430,313
#2	(Serum creatinine) OR (serum cystatin C) OR (estimated glomerular filtration rate) OR (urinary albumin excretion rate) OR (albumin-to-creatinine ratio) OR (urinary albumin)	127,450
#3	microRNAs OR miRNA OR miRNAs	219,791
#4	animal OR rat OR mouse OR (cell-line)	11,974,642
#5	cancer OR (acute kidney injury)	5,633,922
#6	#1 OR #2	506,221
#7	#6 AND #3	2,460
#8	#7 NOT #4	1,205
#9	#8 NOT #5	839

Appendix C: Ethics Certificate

Cape Peninsula University of Technology

HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE (HWS-REC)
Registration Number NHREC: REC- 230408-014

P.O. Box 1906 • Bellville 7535 South Africa
Symphony Road Bellville 7535
Tel: +27 21 959 6917
Email: simonsy@cput.ac.za

1 February 2022
REC Approval Reference No:
CPUT/HW-REC 2020/H11 (renewed)

Faculty of Health and Wellness Sciences

Dear Ms DD Motshwari,

Re: APPLICATION TO THE HWS-REC FOR ETHICS CLEARANCE

Approval was granted by the Health and Wellness Sciences-REC to **Ms DD Motshwari** for ethical clearance. This approval is for research activities related to research for **Ms DD Motshwari** at Cape Peninsula University of Technology.

TITLE: **Non-coding RNA modifications in chronic kidney disease individuals with and without hypertension**

Supervisors: Prof. TE Matsha
Dr C George

Comment:

Approval will not extend beyond 2 February 2023. An extension should be applied for 6 weeks before this expiry date should data collection and use/analysis of data, information and/or samples for this study continue beyond this date.

The investigator(s) should understand the ethical conditions under which they are authorized to carry out this study and they should be compliant to these conditions. It is required that the investigator(s) complete an **annual progress report** that should be submitted to the HWS-REC in December of that particular year, for the HWS-REC to be kept informed of the progress and of any problems you may have encountered.

Kind Regards

Ms Carolynn Lackay
Chairperson – Research Ethics Committee
Faculty of Health and Wellness Sciences