Prevalence of *APOL1* risk variants in HIV-positive compared to HIV-negative individuals with evidence of kidney disease.

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Abstract

Background:

Various types of kidney diseases, including HIV-associated nephropathy (HIVAN), focal segmental glomerulosclerosis (FSGS), chronic kidney disease (CKD), and end-stage renal disease (ESRD), have been associated to variants in the *Apolipoprotein1* (*APOL1*) gene. *APOL1* high risk (G1 and G2) variants are exclusively found in individuals of African ancestry, because of this, *APOL1* has been associated with kidney disease only in those with recent African ancestry. However, there is little data on the prevalence and implications of the *APOL1* variation in HIV-positive patients in the South African population.

Aim:

The aim was to determine whether the *APOL1* gene variants are present and whether genetic susceptibility along with HIV infection contributes to the development of kidney diseases, in a South African population, treated in Bloemfontein.

Methods:

This was a case-control study that included two hundred and twenty (n=220) participants consisting of four groups, namely: HIV positive with kidney disease indicators (n=55); HIV positive without kidney disease (n=55); HIV negative with kidney disease indicators (n=55) and HIV negative without kidney disease (n=55). The participant samples were selected from archived material according to the inclusion and exclusion criteria. Genotyping analysis using qPCR was performed to detect the *APOL1* G1 and G2 variants. Sanger sequencing was used for confirmation.

Results and Discussion:

The frequencies of the *APOL1* high risk genotypes revealed that the G2 variant was more prevalent than G1 in the entire study population. The frequencies were 0.0045 for *APOL1* G1/G1, the 1000 Genome Africa found the frequency of 0.080. For G1/G2 (compound heterozygote) the frequency was 0.014. The frequency was higher for G2/G2 for this study (0.0455) compared to the 1000 genome Africa at

0.017. The prevalence of *APOL1* G2 was higher than G1; this does not collaborate with 1000 Genome project. The p-value for *APOL1* G2 was 0.752. There are no statistically significant associations found between the genotypes categorized by risk and either HIV or CKD.

Conclusion:

The study identified the presence of all the tested *APOL1* risk variants linked to chronic kidney disease (CKD) within a specific demographic (individuals of African descent in the South African population). The prevalence of the *APOL1* G2 variant was more than that of the *APOL1* G1 variant. This study did not discover any statistical significance linking *APOL1* high-risk genotypes with the occurrence of chronic kidney disease in either HIV-positive or negative individuals. Despite this, the established connection between these factors highlights the importance of screening patients who are of African ancestry, HIV-positive, and have CKD for potential personalized treatment options. Further studies with larger samples sizes and kidney biopsies are recommended to confirm whether variants of *APOL1* contribute to the development of kidney disease in HIV positive individuals.

Keywords

CKD

HIV

APOL1

Genotype

Risk variants

African ancestry

Human African trypanosomiasis

HIVAN

Genotype

Kidney disease markers

Declaration Masters Student

I, Madingaka Dorah Notani declare that the Master's Degree research dissertation I herewith submit for the Master's Degree qualification M.Sc. Genetics at the University of the Free State is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

29/11/2023

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Student's Signature Date

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List of Abbreviations

A	Adenine
ACEi	Angiotensin-converting enzyme inhibitors
ACR	Albumin-to-creatinine-ratio
AER	Albumin excretion rate
ADPKD	Autosomal dominant polycystic kidney disease
AG II	Angiotensin II
AIDS	Acquired Immune Deficiency Syndrome
AKI	Acute kidney Injury
APOL1	*Apolipoprotein L1*
APOLLO	*APOL1* long-term kidney transplantation outcomes network
ARBs	Angiotensin II receptor blockers
ARPKD	Autosomal recessive polycystic kidney disease
ART	Antiretroviral therapy
AV	Average
B	BH3 domain
BMI	Body mass index
Bp	Base pair
C	Cytosine
CALLA	Common acute lymphoblastic leukaemia antigen
CASTp	Computed Atlas of Surface Topography of proteins
CCR5	Cysteine-Cysteine Chemokine Receptor 5
$CD4^+$ T	Cluster of differentiation 4 Helper T cell
CDC25A	Cell division cycle 25A
CKD	Chronic kidney disease
Cl	Chlorine
CI	Confidence interval
C-Myc	Cellular Myelocytomatosis
Cq	Quantification cycle
CRF	Chronic renal failure
CX3CR4	CX3C motif chemokine receptor 4

Cys	Cystanine
DCAL	Death-censored allograft loss
DNA	Deoxyribonucleic acid
DILS	Diffuse infiltrative lymphocytosis syndrome
E. coli	Escherichia coli
EDTA	Ethylene diamine tetra acetic acid
eGFR	Estimated glomerular filtration rate
eGFRcr	Estimated glomerular filtration rate- creatinine
eGFRcys	Estimated glomerular filtration rate- cystatine C
ERK	Extracellular signal-regulated kinase
ESKD	End stage kidney disease
ESRD	End stage renal disease
FAM	Fluorescein amidite
FAT1	FAT atypical cadherin 1
FGF	Fibroblast growth factor 2
FSGS	Collapsing focal segmental glomerulosclerosis
G	Guanine
GFR	Glomerular filtration rate
H_2O	Dihydrogen oxide
HAART	Highly Active Antiretroviral Therapy
HAT	Human African trypanosomiasis
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDL	High density lipoprotein
HEK	Human Embryonic Kidney
HIV	Human immunodeficiency virus
HIVAN	Human immunodeficiency virus- associated neuropathy
HIVCK	HIV associated immune complex kidney disease
HSREC	Health Science Research Ethics Committee
HUS	Haemolytic Uremic Syndrome
LMIC	Low-income and-middle-income countries
HWE	Hardy-Weinberg equilibrium
IDT	Integrated DNA Technologies

Inc RNAs	Long non-coding RNAs
IRIS	Immune reconstruction inflammatory syndrome
K	Potassium
KDIGO	Kidney disease: Improving global outcomes
M	Molar
MAD	Membrane addressing domain
MAF	Minor allele frequency
MAP	Motigen-activated protein
Mg/dl	Milligram per decilitre
MGB	Minor grove binder
Mi RNAs	Micro RNAs
MiR193a	Micro RNA 193a
MRA	Mineralocorticoid receptor antagonist
MYH9	Myosin heavy chain 9
N	Number of samples
Na	Sodium
NALs	Number of alleles
NAN	No real number
NC	Negative control
NCBI	National centre for Biotechnology information
NDRD	Non-diabetic renal disease
Nef	Negative factor
Neph1 (KIRREL1)	kirre like nephrin family adhesion molecule 1
NFQ	Non-fluorescent quenchers
ng/μl	Nanograms per microliter
NK cells	Natural killer cells
NLRP3	Nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3
NOS	Not otherwise specified
NSAIDs	Non-steroidal anti-inflammatory drugs
NTC	No template control
NTDs	Neglected tropical diseases
NTP	No template control

OR	Odds ratio
P27	Protein 27
P57	Protein 57
PASSer	Protein Allosteric Sites Server
PCR	Polymerase chain reaction
PFD	Pore-forming domain
pH	Potential of hydrogen
PKD 1	Polycystin-1
PKD 2	Polycystin-2
PKHD1	Polycystic Kidney and Hepatic Disease 1
PLA2R	M-type phospholipase A2 receptor
RAAS	Renin-Angiotensin-Aldosterone System
REV	Virion expression
RhoA	Ras homolog family member A
RNA	Ribonucleic acid
RRT	Renal replacement therapy
Rpm	Revolutions per minute
S	Secretory protein
SA	South Africa
SADTR	Statistics of the South African Dialysis and Transplant Registry
SAPK	Stress-activated protein kinases
Scr	Serum creatinine
SLC	Solute carriers
SLE	Systemic lupus erythematosus
SIV	Simian immunodeficiency viruses
SNP	Single nucleotide polymorphism
SRA	Serum resistance-associated
STAT	Signal transducer and activator of transcription
T	Thymine
T-lymphocytes	Thymus lymphocytes
TAE	Tris-acetate-EDTA
TAT	Trans-Activator of Transcription
TE	Tris-EDTA

Tm	Annealing temperature
TRPC6	Transient receptor potential cation channel, subfamily C, member 6
Tris	Tris hydro methyl aminomethane
UMOD	Uromodulin
qPCR	Quantitative polymerase chain reaction
UFS	University of the Free State
UK	Unites Kingdom
USA	United States of America
VIC	Fluorescent dye is proprietary to ABI (now Life Technologies) and its Chemical structure is currently not publicly available. Wavelength 551nM
VIF	Virion infectivity factor
Vpr	Viral Protein R
Vpu	Viral protein U
Vpx	Viral protein X
UV	Ultraviolet
WHO	World Health Organization
WT1	Wilms tumour 1
w/v	Weight in volume
µl	Microliter

Table of Contents **Page**

List of tables **Page**

List of figures **Page**

CHAPTER 1

Problem statement, aims and objectives of the study.

1.1. Introduction

Variants in the *Apolipoprotein L1 (APOL1)* gene have been linked with a variety of kidney diseases including HIV-associated nephropathy (HIVAN), focal segmental glomerulosclerosis (FSGS), chronic kidney disease (CKD) and end stage renal disease (ESRD). The association between the *APOL1* gene and kidney disease is prevalent in people of African ancestry (Friedman and Pollak, 2020) as *APOL1* G1 and G2 variants are only found in individuals with recent African ancestry (Friedman and Pollak, 2021). The prevalence of *APOL1* variants differ extensively by the geographic region in the African continent, the highest variants are reported in West Africans (30%-40%) in Igbo- and Yoruba-speaking Nigerians individuals and Asante-speaking people from Ghana. It has significantly lower rates (5%–12%) in South and East Africa with virtually complete lack in those from the horn of Africa (Ethiopia) (Hung, *et al.,* 2022). The prevalence in the South African population specifically has also been scarcely investigated with only one published study to date with many articles referencing data from this one study from (Kasembeli, *et al*., 2015).

1.2. Problem statement

The susceptibility to *APOL1*-induced nephropathy typically occurs among individuals possessing two *APOL1* gene risk variants, a condition primarily observed in individuals of recent African ancestry. Whether the development of kidney disease necessitates a second risk factor remains unknown. In the context of this study, a secondary factor under consideration is a chronic viral infection, such as HIV.

The frequency of specific genotypes between different groups, such as HIV-positive individuals with kidney disease and HIV-negative individuals, aid in identifying whether certain genotypes are more or less prevalent in the presence of HIV and kidney disease. Populations of African ancestry, including those in South Africa, exhibit a higher prevalence of *APOL1* risk variants. Understanding the distribution

and impact of these variants in such populations is essential for targeted healthcare interventions and public health strategies.

We will examine the presence of *APOL1* gene variants in South African participants and explore their potential contribution to the development of kidney disease in HIV-positive patients.

1.3. Aim

The aim was to determine whether the *APOL1* gene variants are present and whether genetic susceptibility along with HIV infection contributes to the development of kidney diseases, in a South African population, treated in Bloemfontein.

1.4. Specific objectives

- Objective 1: To determine the frequency of *APOL1* G1 and/or G2 gene risk variants in HIV-positive and HIV-negative individuals with and without evidence of kidney disease.
- Objective 2: Determine the prevalence of high-risk kidney disease *APOL1* genotypes in the context of four phenotype categories: HIV+ CKD+, HIV+ CKD-, HIV- CKD+ and HIV- CKD-.
- Objective 3: Compare the no-risk, low-risk and high-risk *APOL1* genotypes in these four groups.
- Objective 4: Assessing the potential association between the *APOL1* risk genotype and individuals suffering from CKD in conjunction with a positive HIV status.

1.5. Structure of the dissertation

The dissertation's structure commences with an introductory chapter (**Chapter 1**) that provides a concise overview of the subject, outlines the study's aim and objectives. According to the author's perspective, this framework establishes the focus for the subsequent literature review and the entirety of the thesis.

Chapter 2, the literature review, provides a comprehensive overview of kidney disease, highlighting associated genes and environmental factors, notably focusing on HIV. This section elaborates on the rationale behind the selected gene of interest and the study population for this study.

Chapter 3 describes the methods used to detect the *APOL1* gene variants. Furthermore, the study design, sample size, inclusion criteria and exclusion criteria as well as methodologies used for data analysis methodology are described in this chapter.

In **Chapter 4,** the traditional results and discussion chapters are combined into one. Recently this has become common practice in the field of Natural Sciences driven by international journals using this structure, including PLOS ONE and Nature communications. This amalgamation also facilitates smoother reading as it eliminates the need for readers to flip back and forth between result tables and the discussion section.

The final chapter (**chapter 5**), concludes the study, lists limitations and the potential impact and recommendations.

CHAPTER 2

Literature Review

Introduction

2.1. A history of kidney disease

The founding father of kidney disease or renal failure is considered to be Richard Bright (Zulkarnaev, 2017). Although this is the case, it must be mentioned that back in the V-IV century BC Hippocrates of Kos also known as Hippocrates II, the father of modern medicine noticed that some symptoms like; bubbles appearing on the surface of urine indicated disease of the kidneys and seemed to prolong illness, (Dunea, 2017) this was linked to kidney disease. Rufus of Ephesus, a disciple of Hippocrates, published a treatise on Diseases of the Bladder and Kidneys. He said that kidney sclerosis causes oedema but may also occur painlessly (Eknoyan, 2002). Avicenna associated kidney sclerosis with chronic disease too in I-II centuries (Zulkarnaev, 2017).

In 1527, Paracelsus saw that urine coagulated after the addition of vinegar or wine and the urine of some patients produced a creamy layer (Cameron, 2003). Frederick Dekkers in 1694 found that urine produces a precipitate when exposed to heat and acetic acid (Medical Annotations, 1873); he associated this with proteinuria (Armstrong, 2007). He also described hydrosarca in some patients, but he did not associate it with changes in urine (Zulkarnaev, 2017). In 1764, nearly seventy years later, Domenico Cotugno described a 28-year-old patient presenting with fever as well as hydrosarca. He found albuminuria in a case of oedema, which is the swelling of soft tissues brought on by the retention of excess water (Schena, 1994). He too found that by boiling urine, it would evaporate into a white mass such as egg albumen (Black, 1980). Concurrently, Rosen von Rosenstein defined the course of scarlet fever as preceding oedema and haematuria (Hektoen International, 2021). When Richard Bright founded nephrology, there was some knowledge of kidney diseases, but it was just not systematic. Bright's most notable contribution to nephrology was carried out using the renal tissues of deceased patients (Zulkarnaev, 2017). He systematically compared and collected the clinical signs and how the

patient presented with histological changes in the renal tissues. This system differentiates between different types of pathology of the renal parenchyma (Zulkarnaev, 2017). In his reports on medical cases in 1827, Bright defined acute nephritis, nephrosis (nephrotic syndrome), uraemia, and small contracted and large swollen kidneys. He described the clinical picture of kidney disease and its clinical features and anatomic illustrations (Goodrich, 2010). He also reported on the changes in lab indicators identified by the protein in blood and urine (Figure 2.1). What was known as Bright's disease is currently known as nephrotic syndrome. Today, nephrology is not just limited to the classic Bright's disease but comprises a spectrum of kidney diseases and the systemic processes including acute kidney injury and hypertension. This spectrum in nephrology was a result of histological studies conducted in deceased patients with oedema and albuminuria. The outcome was heterogeneous, hence the conclusion that different diseases may cause similar clinical symptoms.

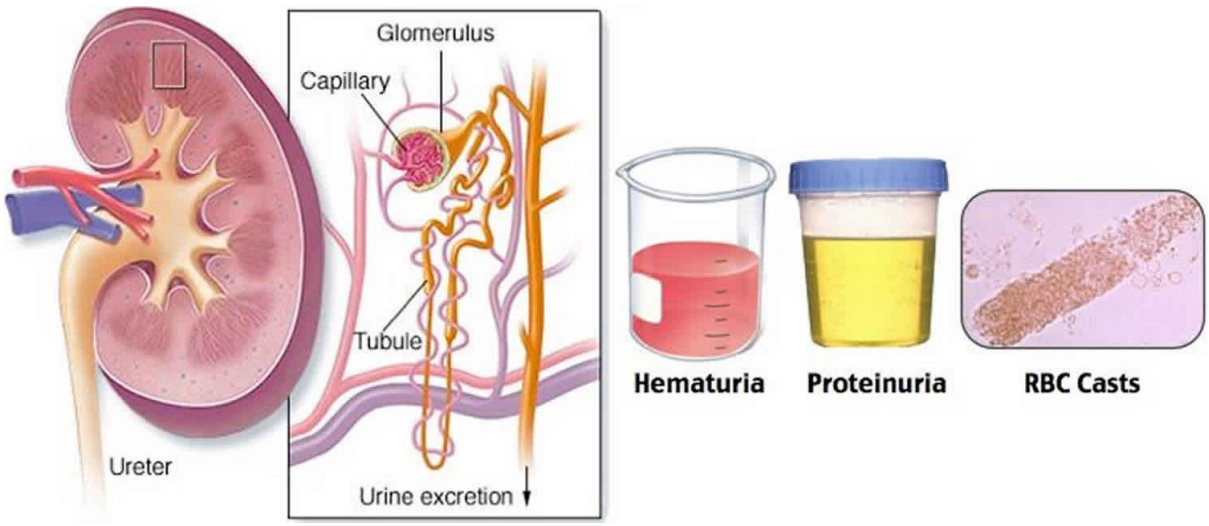

Figure 2.1 Description of Bright's disease (Health Jade Team, 2019)

- Pink or cola colour in urine from red blood cells is called haematuria.
- Foamy urine due to elevated albumin is a sign of albuminuria.
- Red blood cell casts mean there is a microscopic amount of bleeding from the glomerulus.
- All are signs of damaged kidneys and are seen in various kidney diseases.

2.2. Characterization of Kidney disease

Kidneys play a large role in keeping the major physiological functions of the body in balance. They remove nitrogenous wastes and toxins as well as excess water from the bloodstream and then excrete them through urine (Work and Health, 2022). Kidneys are vital as they synthesize erythropoietin which is a hormone that stimulates the production of red blood cells and one alpha hydroxylase which is responsible to convert vitamin D into its usable active form in the body (Santoro, *et al*., 2015). There are uncontrollable life processes such as aging that causes kidney diseases in that as we age, nephrons are lost, and kidney function may decline. Lifestyle choices such as smoking can increase the risk of kidney disease or worsen existing problems especially in individuals with diabetes and hypertension. Obesity is another risk factor for developing kidney diseases by either a direct effect of glomerular hyperfiltration followed by focal segmental glomerulosclerosis or indirectly by contributing to development of metabolic syndrome (Patschan and Müller, 2016). Kidney failure can either result from acute kidney failure/injury (AKI) or progressive chronic kidney failure/disease (CKD) (Bindroo, *et al*., 2023).

AKI is a sudden, rapid, and often reversible loss of kidney function that occurs within hours and may last for up to 3 months (Goyal, *et al*., 2023). It can be caused by a variety of factors that may either compromise the perfusion pressure of the kidneys or cause direct damage to the glomerulus, tubulointerstitial compartment or the vasculature within the kidneys. AKI may also result from anything that obstructs the urine flow in the urinary tract. The common causes of AKI include severe dehydration, blood loss, heart and liver failure, infections, medication toxicity, kidney trauma, severe allergic reactions, and obstruction due to kidney stones or tumours of the urinary tract and surrounding structures (Normandin, 2017; Goyal, *et al*., 2023; Makris and Spanou, 2016). Its symptoms include, decreased urine output, swelling in legs, ankles, or face, fatigue, confusion, nausea, and vomiting (Holland, 2023). AKI is characterized further into different categories. Pre-renal AKI occurs when there is a problem in the blood supply to the kidneys, such as low blood pressure or dehydration. Secondly there is intrinsic AKI which results from direct damage to the kidney tissues, often due to medications, toxin overload, or infections, a lack of oxygen to the kidneys (ischaemia), as well as direct damage to the kidneys, such as

a physical blow or an accident. Lastly, post-renal AKI, occurring when there is an obstruction in the urinary tract, preventing the flow of urine out of the kidneys or the presence of kidney stones (Makris and Spanou, 2016).

Haemolytic Uraemic Syndrome (HUS) is another cause of acute kidney failure resulting from an infection by a specific E. coli (Bhandari and Sedhai, 2023). Nephrotic Syndrome is a condition characterized by the leakage of large amounts of protein into the urine (greater than 40 mg/m^2 per hour), leading to oedema, hypoalbuminemia, and lipid abnormalities (Tapia and Bashir, 2023).

Chronic Kidney disease (CKD) on the other hand is a long-term condition where the kidneys gradually and progressively lose their ability to function accurately. CKD is often characterized by a progressive decline in kidney function (Vaidya and Aeddula, 2023). The baseline GFR category and albuminuria category induces the decline in kidney function (KDIGO, 2012).

Typically, CKD is categorized into stages using the CGA classification based on the cause (C), estimated glomerular filtration rate (G) and the presence of kidney damage represented by the degree of albuminuria (A) (Perez-Gomez, *et al*., 2019). The stages range from Stage 1 which is mild to Stage 5 representing end-stage kidney disease. Common causes of CKD include hypertension (high blood pressure), diabetes mellitus, glomerulonephritis (inflammation of the kidney's filtering units), polycystic kidney disease (genetic disorder leading to cyst formation in the kidneys), autoimmune diseases affecting the kidneys (lupus) (Veeraish, 2021). It can also result from long-term exposure to certain medications or toxins. There may also be a genetic component to the risk of CKD. For instance, the risk of CKD may be increased by double in individuals with sickle cell trait and two *APOL1* risk alleles, both of which are more prevalent in people with African heritage than European ancestry (Chen, *et al*., 2019). Symptoms of CKD include, fatigue, swelling in the legs, ankles, or face, shortness of breath, persistent itching, high blood pressure and changes in urination patterns which could be either increased or decreased urine output (Malkina, 2023; Vaidya and Aeddula, 2023).

Because CKD is gradual and progressive, it can lead to End-Stage kidney Disease (ESKD). ESKD is the final stage of CKD where the kidneys can no longer function

well enough to meet the requirements of daily life (Hashmi, *et al*, 2023; Vaidya and Aeddula, 2023; DiMaria, 2022). Treatment options include dialysis to remove waste products and excess fluids from the blood and kidney transplant to replace a failed kidney with a healthy kidney from a donor (Okorie, *et al*., 2018).

2.3. Kidney disease diagnosis

It is important to note that kidney disease can be asymptomatic in its early stages (Fraser and Blakeman, 2016) but one of the early signs of detection is proteinuria. Proteinuria, also known as albuminuria, is a condition in which there is an elevated amount of protein in the urine (Haider and Aslam, 2023). Proteinuria/Albuminuria is not a disease but a symptom of diseases such as kidney disease (Hopkinsmedicine.org, 2019). It can be diagnosed at the bedside by doing a urine dipstick and be further quantified by calculating a urine albumin-to-creatinine ratio (ACR). Measuring urinary ACR in a spot urine sample is the suggested approach for assessing albuminuria. ACR is derived by dividing the urine albumin concentration in milligrams by creatinine concentration in grams. According to KDIGO, albuminuria is classified according to stages; stage A1 which represents normal to mild albuminuria (urine ACR <30 mg/g), A2 which was previously known as micro-albuminuria represents the moderately increased albuminuria (30–300 mg/g), and finally severely increased albuminuria or macro-albuminuria (>300 mg/g) which is stage A3 (KDIGO, 2012). A diagnosis of CKD is confirmed when there is decreased eGFR and other markers of kidney damage, there is a review of previous measurements, past history of kidney disease and the duration of CKD markers is more than three months (KDIGO, 2012; Chen, *et al*., 2019).

Estimated Glomerular Filtration Rate (eGFR) is the most commonly used indicator of general kidney function in clinical practice. When there is no other indication of kidney disease, an estimated glomerular filtration rate of 60-89 ml/min/1.73 m2 does not suggest chronic renal disease or the need for additional testing (Traynor, *et al*., 2006). The glomerular filtration rate (GFR) is a measure of the flow of filtered fluid through the kidney's glomerular capillaries. Urinary clearance of inulin under continuous intravenous infusion is considered the "gold standard" approach for

calculating eGFR (Speeckaert, *et al*., 2021; Rizk, *et al*., 2018). Creatinine is a waste product that is produced during creatine metabolism and its measurement in the serum can be used to estimate GFR in the clinical setting. Since creatinine is strongly correlated with muscle mass, it might be deceiving in certain situations like spinal cord injury, sarcopenia, or at extremes of body physical build. Age, sex, serum creatinine levels and body size all affect the normal GFR, which also decreases with advancing years. eGFR is determined based on filtration markers and creatinine (eGFRcr) is the most widely used filtration marker (KDIGO, 2017). Cystatin C is an alternative endogenous filtration marker that is more reliable to estimate GFR under steady state, but it is not widely used in clinical practice due to its price (National Institute for Health and Care Excellence (NICE), 2021; Shardlow, *et al*., 2017; KDIGO, 2017).

Chronic kidney disease is characterized by advancing damage to the kidneys and their loss of function indicated by a GFR of less than 60 mL/min per 1·73 m2 (Chen, *et al*., 2019). The progression of CKD and ultimately death is indicated by the presence of proteinuria. Histological evidence of CKD can also be shown by glomerular sclerosis, tubular atrophy, and interstitial fibrosis in kidney biopsies (Webster, 2017). There are five stages of kidney disease of which end-stage renal disease (ESRD) is the final stage and is fatal without artificial filtering (dialysis) or kidney transplantation (https://www.kidneyfund.org). The stages of kidney disease are indicated in Table 2.1.

Table 2.1 kidney disease stages. Adapted from KDIGO, 2012

Prognosis of CKD by GFR and albuminuria categories				Albuminuria categories Descriptions and range		
				A1	**A2**	**A3**
				Normal to moderately increased	Moderately increased	Severely increased
				<30 mg/g <3 mg/mmol	30-299 mg/g 3–29 mg/mmol	≥300 mg/g ≥30 mg/mmol
GFR categories (ml/min/1.73 m^2) Description and range	**G1**	Normal or high	≥90			
	G2	Mildly decreased	60-90			
	G3a	Mildly to moderately decreased	45-59			
	G3b	Moderately and severely decreased	30-44			
	G4	Severely decreased	15-29			
	G5	Kidney failure	<15			

Green: Low risk (if no other markers of kidney disease, no CKD); Yellow: Moderately increased risk; Orange: High risk; Red: Very high risk

2.4. Kidney disease prevalence

It is estimated that chronic kidney disease affects about one in seven American adults (chronic kidney disease in the United States, 2023). There are no reliable statistics for any African countries including South Africa, but it is estimated that the prevalence is at least three to four times higher than in developed countries (Naicker, 2009). Statistics of the South African Dialysis and Transplant Registry (SADTR) are available in South Africa, but they are not reliable as they only include individuals who qualify for renal replacement therapy (RRT) and dialysis and do not accurately reflect the aetiology of chronic renal failure (CRF) as many patients present too late to be evaluated with a kidney biopsy (Naicker, 2003). This fact is reflected in almost all developing countries and some developed ones. However, two studies estimated the prevalence of CKD in the South African population. The prevalence of CKD in

schoolteachers in Cape Town was reported by Adeniyi, *et al*., 2017 at 6%, and Matsha, *et al*., 2013 found CKD in 17% of the mixed-ancestry South African population in Cape Town (Jardine and Davids, 2020). Patterns of renal disease have been reviewed in South Africa and the frequencies of glomerulonephritis have been found to be the highest in the African ancestry population, lesser in individuals with Indian descent, and much lower in Caucasians (Naicker, 2003).

2.5. Common Causes and Comorbidities of CKD

CKD has a multifaceted aetiology, and it is often influenced by various factors, including both common causes and comorbidities. These contributing factors may intertwine, leading to the development and progression of CKD in individuals who are at risk. One of the primary contributors to CKD is the presence of comorbid conditions such as hypertension, diabetes, (Erfanpoor, *et al*., 2020) and cardiovascular disease (MacRae, *et al*., 2021; Jankowski, *et al*., 2021). These conditions frequently co-exist with CKD and are known risk factors for its development. The complex interaction between CKD and these diseases can aggravate the progression of both the primary condition and CKD. Individuals with hypertension and diabetes are more likely to develop CKD, and CKD, in turn, can worsen these conditions (Barhum, 2023).

In addition to the diseases mentioned above, CKD can also be a consequence of other underlying conditions, such as HIV infection and other systemic diseases such as autoimmune diseases. CKD in the context of HIV infection is associated with major morbidity and mortality (Heron, *et al*., 2020). The burden of HIV on healthcare systems, particularly in the African continent, is well-documented and contributes adversely to outcomes of CKD. HIV can directly impact kidney function, causing conditions such as HIV-associated nephropathy (HIVAN) and other renal complications (International Association of Providers of AIDS Care, 2021). Moreover, some antiretroviral medications such as tenofovir disoproxil fumarate which are used in the treatment of HIV are nephrotoxic (Wearne, *et al*., 2020).

It is critical to recognize that many CKD patients show a complex interaction of factors that contribute to the causation and progression of the disease. In some instances, the exact cause may remain elusive, particularly at an advanced stage

where the patients present with small-sized kidneys, presenting a challenge of performing a kidney biopsy to determine the exact aetiology.

This highlights the need for a multidisciplinary approach to healthcare management and enforcement of screening campaign to the population groups at risk for early detection and prevention of CKD (Whaley-Connell, et al., 2011). The management of CKD in individuals with multiple contributing factors requires a thorough understanding of the various conditions playing a role, as well as a tailored and comprehensive approach to provide optimal care and treatment. This may involve addressing comorbidities or coexisting disorders, managing the underlying causes, and mitigating the potential interactions between medications used to treat these conditions. This is especially important for HIV involvement in CKD.

2.6. Human Immunodeficiency Virus

Human immunodeficiency virus (HIV) has been one of the world's most serious illnesses and presents a major risk to public health. Researchers have determined that there are two types of this lentivirus; HIV-1 and HIV-2 (German Advisory Committee Blood (Arbeitskreis Blut), 2016), of which the former is more virulent and spreads easily and is the reason for the HIV global pandemic (Esbjörnsson, *et al.*, 2019). HIV-1 still is not just one virus, it encompasses four different lineages; namely group M, N, O, and Group P, and Group M is the source of the pandemic (Sharp and Hahn, 2011). The virus was identified as the cause of Acquired Immune Deficiency Syndrome (AIDS). Although scientists agree that the origin of the virus was identified to be in West Africa (Cameroon specifically) and is closely correlated to the simian immunodeficiency viruses (SIVs), evaluations in stored blood of early HIV-1 that transmitted from non-humans to humans and of old cases of AIDS in Central Africa, have led many scientists to the conclusion that HIV-1 group M was probably further south in the Democratic Republic of the Congo and not Cameroon and that is where it spread to other parts of the world (Theaidsinstitute.org, 2019).

The HIV species belongs to the genus Lentivirus, subfamily Orthoretrovirinae and family Retroviridae (Lopez, *et al.,* 2010). HIV is classified into type 1 and 2, according to genetic characteristics and differences its viral antigens (German Advisory Committee Blood (Arbeitskreis Blut), 2016). The genetic material of HIV

consists of two identical copies of single stranded RNA. Reverse transcriptase is an enzyme necessary for the translation of RNA into double stranded DNA in the human host cell (Aiken and Rousso, 2021). It is estimated that HIV reproduces 100 million to 100 billion virions each day (Munoz, *et al.,* 2022). HIV affects immune cell function and causes immunological insufficiency by infecting and replicating in $CD4^+$ T cells, dendritic cells, and macrophages (Balasubramaniam, *et al.,* 2019). The virus gradually reduces tissue-based and circulating $CD4^+$ T lymphocytes over time (Deeks, *et al.,* 2015). CD4 T lymphocytes form part of the human T-lymphocyte cells that fight against bacteria, viruses, and other organisms. In the absence of antiretroviral therapy, the degree of viremia is linked to the speed at which $CD4^+$ T cells deplete, and AIDS development occurs (Patterson, *et al*., 1999). Adults and adolescents typically have between 500 and 1500 cells per mm^3 of blood as their absolute CD4 count, which declines as the HIV illness worsens (Ratnam, *et al.,* 2018). Even though it could take months, an effective combination of antiretroviral medication typically results in an increase in CD4 levels, a decrease in HIV RNA levels, and prolongs AIDS-free survival (Ray, *et al.,* 2010).

HIV is transmitted from a host via different routes, including unprotected sexual intercourse with an infected individual. Through drug-related sharing of needles between infected and uninfected individuals receiving a blood transfusion from an infected person. Another way is a mother passing on the virus to her unborn child during pregnancy through maternal cell contamination, during birth, or through breastfeeding after the child is born. The most prevalent route of transmission is sexual transmission in which HIV infects mucosal macrophages and dendritic cells (Gonzalez*, et al*., 2019).

The latest statistics released in 2021 estimated that 37.7 million people were living with HIV; about 1.5 million of these were new infections in 2020, and 25.7 million of these individuals living in Africa (World Health Organization, 2019). South Africa has the largest HIV epidemic in the world, with an estimated 7.7 million people living with HIV at present (Avert, 2020). Free State Province has the second highest percentage (25.5%) after KwaZulu Natal (27%) (Hansoti, *et al*., 2019).

2.7. Background on HIV-associated kidney disease

HIV-positive individuals are at increased risk of developing kidney disease (Swanepoel, *et al.,* 2018). The different types of kidney diseases include HIV-associated nephropathy, non-collapsing focal segmental glomerulosclerosis, immune-complex kidney disease, and comorbid kidney disease (Salifu, 2023). There is also a type of kidney injury that is a result of an elongated exposure to antiretroviral therapy where medications like tenofovir disoproxil fumarate, indinavir, and atazanavir can induce acute and/or chronic kidney injury (Kalyesubula and Perazella, 2011) through mitochondrial toxicity and tubular damage or intra-tubular crystallization (Ross, 2014). Then there is kidney disease from opportunistic infections and other infections that often co-exist in the context of HIV infection such as hepatitis B and C infection and syphilis. With the effective anti-retroviral therapy (ART) roll-out, the lifespan of HIV-positive people has since increased and they live long enough to develop other metabolic diseases such as diabetes mellitus type 2 and hypertension (Rajagopaul and Naidoo, 2021) as well as other kidney diseases that are not pathogenically related to HIV infection such as glomerulonephritis.

HIV-associated nephropathy (HIVAN) is one of the many renal diseases affecting the outcome of HIV-infected persons. It is a specific kidney condition that is closely linked to uncontrolled HIV infection and disproportionately affects individuals of African descent, particularly those who carry specific genetic variants known as *APOL1* risk variants (Husain, *et al.,* 2018). Before the widespread availability of ART, HIVAN was the most common type of kidney disease in HIV-positive individuals (McCulloch and Ray, 2008; Diana, *et al.,* 2022). This remains the case in delayed HIV diagnosis and treatment naïve patients. The fact that ART retards the progression of HIVAN to end-stage renal disease (https://www.aidsmap.com/news/nov-2010/hiv-and-kidneys) indicates that there is a direct relationship between HIV infection, renal epithelial cells, and how the viral genes are expressed in the kidney (Blasi and Klotman, 2023). This relationship was established through studies of human biopsies and animal models and depicts the dysregulation of host genes involved in cell differentiation and host signalling pathways (Rednor and Ross, 2018).

One significant aspect of HIVAN is its association with specific genetic risk factors, known as *APOL1* risk variants. These variants are more prevalent in people of African descent. When individuals with these genetic variants become infected with HIV and the virus is not adequately controlled, they are at a higher risk of developing HIVAN. In short, the most unique features of HIVAN are the clinical symptoms, its preference for individuals with African ancestry, and renal enlargement (Husain, *et al*., 2018). Healthcare professionals treating kidney disease in HIV-positive patients must comprehend the relationship between uncontrolled HIV infection, genetic susceptibility, and the occurrence of HIVAN. Knowing these interactions is vital as it assists in early and effective HIV treatment to reduce the risk of HIVAN. It might also play a role in individualized care based on a patient's genetic background and medical history.

HIVAN has received substantial attention due to its rapid development of irreversible chronic kidney failure. HIVAN was identified in patients with AIDS in New York and Miami in 1984 (Pardo, *et al*., 1984; Rao*, et al.,* 1984). It was initially named AIDS-associated nephropathy but was changed to HIVAN when people who were asymptomatic of AIDS had the same clinical and renal histological features as those who had AIDS (Ray, 2012). The existence of HIV-related glomerulopathy was thought to have evolved from intravenous drug use but in the late 1980s, HIVAN was detected in children who obtained HIV-1 via vertical transmission (Pardo, *et al.,* 1987). Some HIV-infected children acquired a childhood variation of HIVAN characterized by proteinuria, together with mesangial hyperplasia and microcystic tubular dilatation (Ray, *et al.,* 2021). These were considered the early stages of HIVAN. The kidneys of individuals with HIVAN are typically larger than those with other types of kidney diseases (Wyatt, *et al.,* 2008). The renal enlargement is due to a spectrum of renal glomerular lesions ranging from focal and diffuse mesangial hyperplasia (Ray, *et al.,* 2021). HIVAN clinically presents with a syndrome of nephrotic range proteinuria which may progress to loss of kidney function and the classical histological feature of HIVAN is collapsing focal segmental glomerulosclerosis (FSGS) (Rosenberg, *et al.,* 2015). Table 2.2 represents the different types of HIV-related kidney diseases and how they are pathologically classified. This also includes all other diseases related to it.

Table 2.2 Pathologic classification of HIV-related kidney diseases. (Swanepoel, *et al*., 2018).

Pathologic classification of HIV-related kidney diseases	
I. Glomerular-dominant	
a. Podocytopathies (all characterized by extensive foot process effacement)	I. Classic HIVAN ii. FSGS (NOS) in the setting of HIV iii. Minimal change disease in the setting of HIV iv. Diffuse mesangial hypercellularity in the setting of HIV v. Other podocytopathy in the setting of HIV
b. Immune complex-mediated glomerular disease	I. IgA nephropathy in the setting of HIV ii. Lupus-like glomerulonephritis in the setting of HIV iii. Lupus nephritis in the setting of HIV iv. Membranous nephropathy in the setting of HIV • Indicate whether HBV positive, HCV positive, PLA2R positive (should not preclude workup for other secondary causes) v. Membranoproliferative pattern glomerulonephritis in the setting of HIV • Indicate whether HCV positive (should not preclude workup for other secondary causes) vi. Endocapillary proliferative and exudative glomerulonephritis in the

	setting of HIV • Post-streptococcal, staphylococcal-associated, and other vii. Fibrillary or immunotactoid glomerulonephritis in the setting of HIV viii. Other immune complex disease in the setting of HIV
II. Tubulointerstitial-dominant	
a. Tubulointerstitial injury in the setting of classic HIVAN	I. Hyaline droplet tubulopathy ii. Tubular microcysts iii. Tubulointerstitial inflammation
b. Acute tubular injury or acute tubular necrosis	I. Ischemic ii. Toxic (associated with ART vs. other)
c. Drug-induced tubulointerstitial nephritis (other than ART)	I. Antibiotics ii. Proton pump inhibitors iii. NSAIDs iv. Other
d. Direct renal parenchymal infection by pathogens (bacterial, viral, fungal, protozoal, etc.)	
e. Immunologic dysfunction-related tubulointerstitial inflammation	I. Diffuse infiltrative lymphocytosis syndrome (DILS) ii. Immune reconstitution inflammatory syndrome (IRIS) f. Other tubulointerstitial inflammation in the setting of HIV
III. Vascular-dominant	
a. Thrombotic microangiopathy in the setting of HIV	
b. Arteriosclerosis	

IV. Other, in the setting of HIV infection	
a. Diabetic nephropathy	
b. Age-related nephrosclerosis	

ART, antiretroviral therapy; HBV, hepatitis B virus; HCV, hepatitis C virus; FSGS, focal segmental glomerulosclerosis; HIVAN, HIV-associated nephropathy; NOS, not otherwise specified; NSAID, nonsteroidal anti-inflammatory drug; PLA2R, M-type phospholipase A2 receptor.

Indicates likelihood of HIV causality.

Indicates association with *APOL1* risk allele genotype

2.7.1. HIV-associated immune complex glomerulonephritis

HIV-associated immune complex glomerulonephritis (HIVICK) is a type of kidney disease that occurs in individuals living with HIV, even in those who are virally suppressed and on antiretroviral therapy. It is predominantly found in individuals of African ancestry (Foy, *et al*., 2013). It is characterized by the creation of immune complexes in the glomeruli of the kidneys, leading to inflammation and impairment. On microscopy and immunofluorescence, it presents with “lupus-like” features (Kofotolios, *et al.,* 2023). Notably, HIVICK often exhibits in patients who have well-controlled viral loads, emphasizing the complex interaction between the immune system, viral infection, and kidney function. Unlike HAVAN, individuals with HIVICK exhibit viral loads that are lower and a high CD4 including eGFR values that are higher (Kawakita, *et al.,* 2019). Some cases of HIVICK have displayed a positive response to corticosteroid therapy (Kofotolios, *et al.,* 2023) indicating the potential benefit of immunosuppressive treatment in managing this disorder. As the understanding of the complex relationship between HIV and kidney diseases continues to evolve, targeted interventions may offer valued therapeutic possibilities for improving the renal health in individuals living with HIV.

2.7.2. Mechanisms of HIV-Associated Kidney Disease

HIV transfer in the kidney happens through podocyte infection and/or tubular epithelial infection (Rednor and Ross, 2018). Podocyte infection is associated with

podocyte injury and dedifferentiation as well as a more rapid loss of kidney function (Lu, *et al.,* 2019). This is due to HIV causing immune deficiency and direct podocyte injury. Reports have confirmed the presence of HIV nucleic acids in renal epithelial cells, providing proof that HIV directly infects renal cells. However, classical HIV-1 receptors such as CD4, CXCR4, and CCR5 show no expression on renal cells (Hughes, *et al*., 2020). This absence indicates the presence of an alternate viral entry mechanism that is non-receptor mediated. In the quest to identify HIV entry into human podocytes, an unorthodox route of viral entry was hypothesized to be via lipid rafts (Mañes, *et al*., 2000). Lipid rafts consists of cholesterol and as podocyte cholesterol decreases, lipid raft integrity diminishes and this leads to easy entry of HIV into podocytes (Mikulak and Singhal, 2010). Another mechanism is through dynamin-mediated endocytosis (Miyauchi, *et al.,* 2009). Endocytosis is a process by which cells engulf substances from their external environment and dynamin is a protein involved in the pinching off of vesicles during endocytosis. The suggestion is that HIV could be internalized by podocytes via this endocytic process.

These alternative mechanisms for HIV entry into podocytes, if confirmed, could have implications for understanding the pathogenesis of HIV-associated kidney diseases. This is an area of ongoing research, and further studies are required to validate these hypotheses and clarify the specific mechanisms involved in HIV entry into podocytes. On the other hand, drug toxicity causes tubular damage resulting from mitochondrial abnormalities and tubulointerstitial inflammation (Ross, 2014). Both these mechanisms lead to kidney failure and are depicted in figure 2.2.

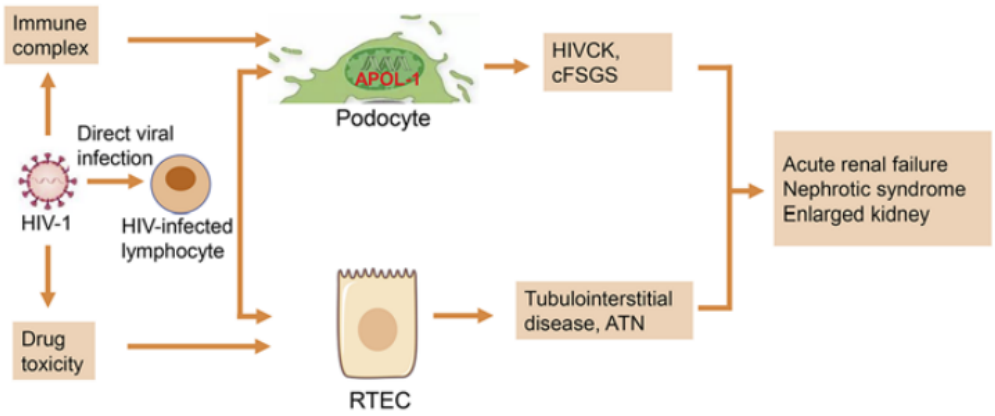

Figure 2.2 Mechanisms leading to kidney disease. (Chen, *et al.,* 2021)

Direct infection of kidney disease by HIV causes HIV-associated kidney either by depletion of immune complex, or through drug toxicity. Cell-to-cell transmission from virus-infected lymphocytes to tubular epithelial cells leads to HIV-associated immune complex kidney disease (HIVCK). HIVCK is the immune-mediated glomerular nephritis in HIV-infected patients and is caused by immune complex deposits in the glomeruli. Antiviral drugs such as tenofovir can also cause nephrotoxicity in HIV patients. Pathologic features of HIVAN include cFSGS and tubular dilatation with microcystins. Clinically, these patients present with renal impairment, nephrotic range proteinuria, and enlarged kidneys. The pathologic and clinical findings of HIVCK are similar to those of other glomerulonephritis, on the other hand, drug-induced nephrotoxicity manifests as acute tubular necrosis, acute interstitial nephritis, or Fanconi syndrome.

2.8. Impact of HIV infection on kidney function

HIV infection can directly impact the kidneys, leading to a range of clinical manifestations and kidney-related complications (HIV and Kidney Disease | NIH, n.d.-b) (Wyatt, *et al*., 2008). The clinical features of kidney damage caused by HIV include; proteinuria accompanied decline in eGFR and development of secondary hypertension. This elevated blood pressure further accelerates kidney damage and contributes to overall cardiovascular risk and adverse outcomes. Kidney dysfunction may result in electrolyte abnormalities of potassium, calcium, and phosphate levels and acid-base disturbances. Renal Insufficiency occurs in more severe cases of HIV-related kidney damage, with a reduced ability to regulate fluid and electrolyte balance and excrete waste products (AIDS Working Group, 2014). Impaired kidney function can lead to fluid retention and the development of oedema. Lastly individuals with HIV may have a higher risk of developing kidney stones due to altered urine composition and electrolyte imbalances, this can also occur as a result of ART precipitation in the tubules (Hamada, *et al.,* 2012).

It is important for healthcare providers to be aware of these clinical manifestations in individuals with HIV, as kidney complications can occur at any stage of the disease. Management of kidney damage in HIV-positive individuals involves screening for

incident kidney disease at the time of HIV diagnosis and monitoring kidney function through regular blood and urine tests, adjusting ART regimens if necessary, and addressing underlying risk factors such as high blood pressure and diabetes. Early detection, prevention and intervention are crucial in managing kidney issues associated with HIV.

2.8.1. Podocyte injury and dysfunction

Podocytes (figure 4) are a type of epithelial cells that are extremely specialized and enclose capillaries (Reiser and Altintas, 2016). They are post-mitotic and are characterized by their foot processes (May, *et al.,* 2014). The foot processes are known as pedicels that sprout from the podocytes and wrap around the glomerulus' capillaries to create the filtering slits. They provide glomerular capillaries with more surface area, which makes ultrafiltration more effective (Reiser and Altintas, 2016). Glomerular filtration has a three-way barrier in which the podocytes are an essential cellular component, providing coverage on the exterior of the glomerular capillary (Krtil, *et al.,* 2007). In between foot processes, there is an assembly of plasma membrane proteins called the slit-diaphragm which functions as a molecular filter (Voinova, *et al*., 2019) to provide mechanical stimuli and size selectivity for ultrafiltration. The slit diaphragm consists of nephrin as its core and functions as a modulator of pro-survival signaling in podocytes (Martin and Jones, 2018). Expression of slit-diaphragm proteins corresponds with podocyte differentiation and maturation. When nephrin or any other slit diaphragm components such as Neph1, podocin, TRPC6, and FAT1 are impaired, the result is podocyte dysfunction which manifests as albuminuria and often FSGS and subsequent decline in glomerular filtration rate (Nagata, 2016). Mechanisms of proteinuria in HIVAN are depicted in figure 2.3.

The classic characteristic of HIVAN is the collapsing FSGS lesion. The glomerular tuft collapses as a result of podocyte dedifferentiation and hyperplasia. The podocyte fails to differentiate due to many reasons. There is a loss of expression of podocyte maturity markers such as WT1 and p27, p57, cyclin D1, cyclin A, and Ki-67 (Barisoni, *et al.,* 2000). Another mechanism in which podocytes lose their function is through a pathologic indicator known as foot process effacement (Ichimura, *et al*.,

2019). There are multiple mechanisms triggering podocyte death, they include detachment-related cell death, namely anoikis and entosis, programmed apoptotic cell death called apoptosis, programmed nonapoptotic cell death autophagy, and podoptosis, immune-reactive cell death such as pyroptosis, additionally, there is necroptosis and mitotic catastrophe-induced cell death (Yin, *et al.,* 2021). Lastly, the loss of podocytes is accelerated by heavy proteinuria (Liapis, *et al.,* 2013).

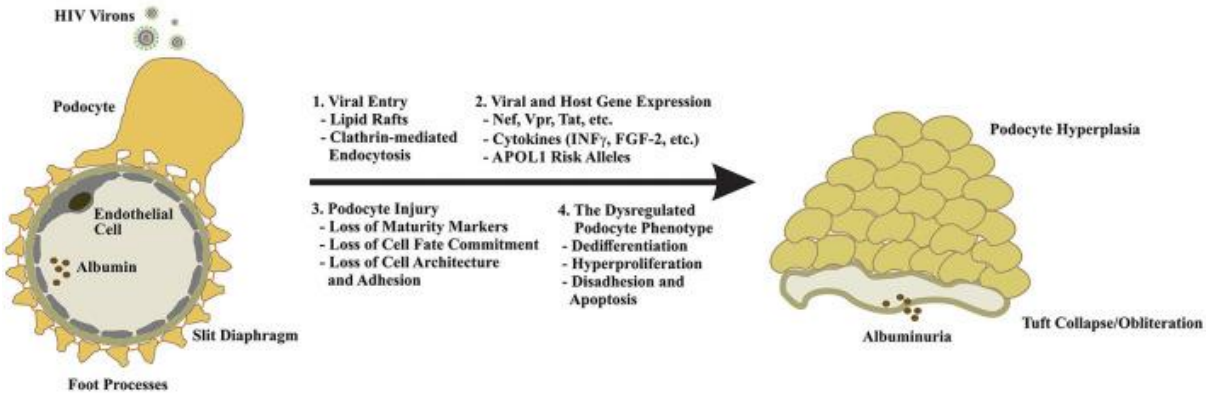

Figure 2.3 Mechanism of proteinuria in HIVAN. (Hall and Wyatt, 2021)

HIV-induced podocyte dysfunction manifests in a variety of ways. The entry of viral particles via lipid rafts and clathrin-mediated endocytosis has been postulated because podocytes lack receptors for classical viral entry. The expression of viral genes and other innate immune mediators after virus entry aids in the promotion of podocyte cytotoxicity. Expression of the high-risk *APOL1* alleles may enhance the likelihood of getting HIVAN among people of African heritage. Loss of maturation markers (such as nephrin, synaptopodin, WT1, CALLA, etc.), loss of cell fate tendency, hyperplasia, and loss of cytoarchitecture and adhesion are all signs of podocyte injury.

2.8.2. Tubular epithelial cell damage

HIV infection of lymphocytes is more efficient through direct contamination of healthy lymphocytes by disease-ridden lymphocytes (Dufloo, *et al.,* 2018). The HIV proviral DNA becomes incorporated into the lymphocyte DNA and they become one (Figure 2.4). The transfer of HIV RNA and expression of HIV genes thereafter is higher in tubular epithelial cells following direct infection of lymphocytes. It is more likely that HIV infection of tubular epithelial cells is instigated through leukocytes (Chen, *et al*.,

2016). The virus is transferred from T-lymphocytes to tubular epithelial cells regardless of CD4 expression (Chen, *et al*., 2011). Expression of proinflammatory mediators controlled by nuclear factor kappa B becomes increased when renal tubular epithelial cells are infected with HIV (Ross, 2014) this is due to the upregulation of kappa B-regulated proinflammatory mediators. Chronic inflammation within the kidney can lead to a cascade of events that includes oxidative stress, impaired renal tubular function, and altered renal hemodynamic. These changes can collectively contribute to kidney injury and dysfunction (Imig and Ryan, 2013).

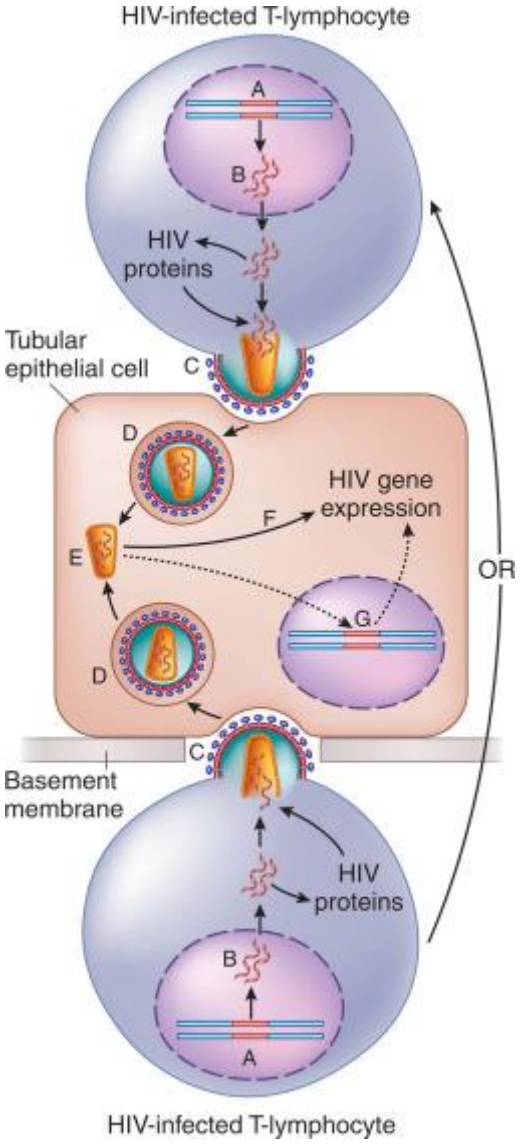

Figure 2.4 Proposed mechanism of transfer of HIV from lymphocytes to tubular epithelial cells. (Ross, 2014)

2.8.3. Role of viral infection and comorbidities

HIV infection plays a role in podocyte pathology through a range of intracellular and systemic processes (Hall and Wyatt, 2021). Within the HIV genome (figure 2.5), there are different accessory proteins expressed, Nef, Vpr, Vif, and Vpu, with regulator proteins, Tat, and Rev (Faust, *et al.,* 2017). The expression of these proteins is cytotoxic to the podocytes. Using HIVAN experimental models it was established that a dysregulated podocyte phenotype and the collapsing FSGS lesion are induced by podocyte-restricted expression of viral proteins (Zhong, *et al.,* 2005). Specific HIV proteins that are said to be the driving force of podocyte dysregulation are Nef, Vpr, and Tat (Bruggeman and Nelson, 2009).

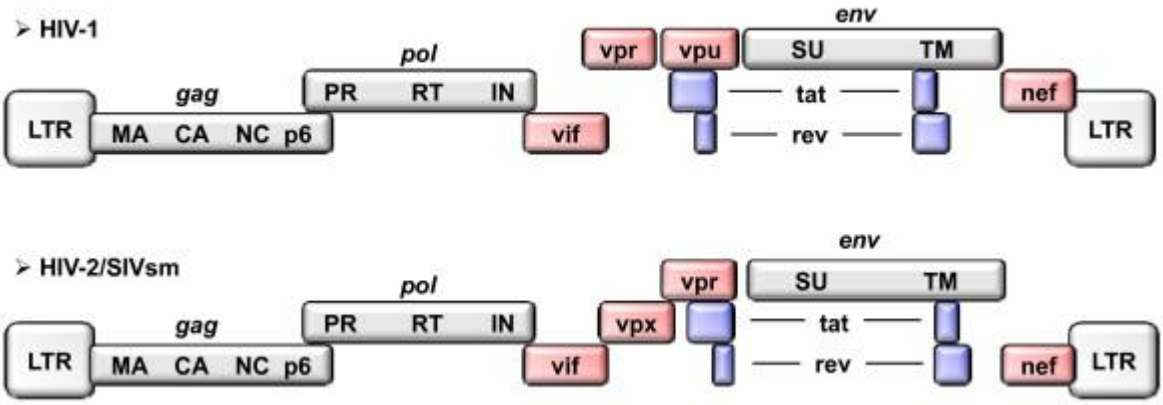

Figure 2.5 Schematic representations of HIV-1 and HIV-2 genomes.
Pink boxes denote accessory genes, blue boxes regulatory genes, and grey boxes structural genes (Ayinde, *et al.,* 2010).

Nef protein was previously described as a negative regulatory factor of HIV viral replication. However, it was later proven to exert a modest effect on viral replication (Basmaciogullari and Pizzato, 2014) by making virions ineffective (Schaeffer, *et al.,* 2001). It is deleterious on podocyte physiology and function. When Nef is expressed, there is a loss of maturity markers (synaptopodin and Wilms Tumour 1(WT1)), expression of the proliferation marker Ki-67, and anchorage-independent growth in podocytes (Hall and Wyatt, 2021). Nef expression also induces activation of signal transducer and activator of transcription (STAT) 3 signalling. STAT3 is hypothesized to play a major role in podocyte proliferation regulation in HIVAN (Gu*, et al.,* 2013). By regulating molecules that are responsible for cell-cycle re-entry and proliferation

such as C-Myc, Cyclin D-1, CDC25A, and anillin. Deletion of STAT3 or a reduction of expression and activity promotes proteinuria, glomerulosclerosis, and tubulointerstitial injury. Nef protein disrupts the shape of the podocyte through various intermolecular interactions using actin (Mathieson, 2012). It also interacts with clathrin to disrupt endocytic trafficking at the plasma membrane (Burtey, *et al*., 2007).

Another HIV accessory protein, Viral Protein R (Vpr), was shown to have an impact on kidney injury in HIVAN. Expression of Vpr is enough to cause glomerular collapse and tubulointerstitial disease (Hall and Wyatt, 2021). In animal models, expression of both Vpr and Nef cooperatively induced the whole spectrum of podocyte injury, glomerular collapse, and tubulointerstitial diseases seen in human HIVAN. Vpr induces cell cycle arrest at the G2/M phase and causes dysregulation of cytokinesis in renal tubular epithelial cells (Rednor and Ross, 2018). It also induces apoptosis through the activation of ERK MAP kinase and the upregulation of the ubiquitin-like protein. Anillin is a regulatory cytokinesis protein and pro-proliferative signalling molecule that becomes upregulated when Vpr is expressed. Anillin is a driver of irregular cellular proliferation in several cancers.

Trans-Activator of Transcription (Tat) is another HIV regulatory protein contributing to podocyte dysfunction in HIVAN. It is essential for an increased rate of transcription of the HIV gene. Tat exposure induces hyperplasia, loss of maturity markers, cytoskeletal dysregulation, and impairment of permselectivity (Hall and Wyatt, 2021). It also reduces nephrin (Doublier, *et al.,* 2007). Tat targets lipid rafts, where it drives RhoA, matrix metalloproteinase-9 expression, and FGF-2-mediated pro-proliferative signalling (Xie, *et al.,* 2014).

2.9. Novel therapeutic approaches for HIV-associated kidney disease

Therapeutic approaches for HIVAN include combination antiretroviral therapy (cART), Renin–angiotensin–aldosterone system (RAAS) blockade, and renal replacement therapy in ESKD (Menez, *et al.,* 2018). Patients with HIVAN who developed ESKD should be offered renal replacement therapy in form of either dialysis or kidney transplantation. Corticosteroid therapy was investigated for the

management of HIVAN before the cART without evidence of benefit (Smith*, et al*., 1994). In addition to raising the risk of other infections, steroid use carries a risk of avascular necrosis in HIV-positive patients (Lucas, *et al.,* 2014).

For many years, the mainstays of treatment to halt the progression of CKD have been drugs that block the RAAS (Chávez-Íñiguez and Rifkin, 2022). The usage of RAAS-blocking agents has the potential to improve albuminuria and decelerate GFR loss. RASS blockade therapeutics includes angiotensin-converting enzyme inhibitors (ACEi) and angiotensin II (AG II) receptor blockers (ARBs).
cART remains the most beneficial treatment option for HIVAN. It slows the progression of ESKD and may reduce the need for renal replacement therapy and /or renal transplantation if started in time. There was a decrease of HIVAN from 75 to 29% in the United States from 1995 – 2007 due to the usage of cART (Lescure, *et al*., 2012; Wearne and Okpechi, 2016). cART was also demonstrated to reduce mortality in patients with HIVAN (Wearne, *et al*., 2012). Table 2.3 shows different treatment options and their effectiveness throughout the years.

Presented below is a comprehensive summary of the studies performed on treatment mechanisms for HIV- associated kidney diseases. The impacts of Renin-Angiotensin-Aldosterone System (RAAS) blockade, steroid therapy, and Antiretroviral Therapy (ART) on the progression of HIVAN were investigated. A retrospective study involving 18 African Americans with biopsy-confirmed HIVAN, evaluated the efficacy of Captopril three times a day versus no treatment (Kimmel, *et al*., 1996). The treated group exhibited a significantly lower progression of kidney disease. Another retrospective study on 20 biopsy-confirmed HIVAN cases in homosexual men, explored the effects of Eosinophil 10 mg daily versus no treatment (Burns, *et al.,* 1997). The Eosinophil group demonstrated significantly lower serum creatinine (Scr) and proteinuria at 12 and 24 weeks. Wei, *et al*., 2003, in a prospective study of 44 HIVAN patients with normal blood pressure and Scr ≤ 2 mg/dL, investigated the impact of Eosinophil 10 mg daily versus no treatment. The Eosinophil group exhibited higher survival rates, with a significantly lower progression to dialysis.

A retrospective study in 2004 by Szczech, *et al.,* involving 89 HIV patients with kidney biopsy, compared the outcomes of patients with and without HIVAN receiving ART and ACEI/ARBs. Progression of kidney disease was slower in HIVAN patients receiving ART. Those receiving ACEI/ARBs had a slower progression to dialysis compared to others. Moving to steroid therapy, Smith, *et al.,* 1994 and Smith, *et al.,* 1996, in case series and a prospective study, respectively, explored the effects of prednisolone in HIVAN patients. Steroid therapy significantly improved Scr in all patients in the case series, and in the prospective study, Scr and proteinuria/24h significantly improved.

In a retrospective study of 21 biopsy-confirmed HIVAN cases, compared the effects of prednisolone versus no treatment (Eustace, *et al.,* 2000). The treatment group exhibited significantly lower progression to dialysis with azotaemia, lower Scr, and proteinuria. Finally, studies on ART by Atta, *et al*., 2006 and Kalayjian, *et al*., 2008, investigated the impact of cART on HIVAN. A retrospective study of 53 HIV-1 patients with biopsy-proven HIVAN showed significantly higher renal survival in the ART group (Atta*, et al.,* 2006). Kalayjian, *et al.,* 2008 performed a prospective multicentre study involving 1776 HIV-1 patients treated with ART; this study demonstrated a significant association between a decrease in HIV viral load and an increase in GFR. Collectively, these studies provide valuable insights into the potential benefits of RAAS blockade, steroid therapy, and ART in managing HIVAN.

2.10. Apolipoprotein L1 (*APOL1*)

The human apolipoprotein L (APOL) family of proteins is mainly involved in lipid transport and metabolism (Vanhollebeke and Pays, 2006). In the APOL family, *APOL1* is the only gene expressed that exhibits extracellular activity and has intracellular functions (Pays, 2021). *APOL1* contains 398 amino acids and has several functional protein domains required for the secretion of APOL into plasma (Beckerman and Susztak, 2018). The domains are the secretory signal (S), membrane addressing domain (MAD), BH3 domain (B), pore-forming domain (PFD), and serum resistance-associated binding domain (SRA) (Madhavan and O'Toole, 2014). The *APOL1* gene encodes a protein called apolipoprotein L1, which is found in human serum and plays a role in the regulation of lipid metabolism. The *APOL1*

gene was identified in 1997, initiating extensive research aimed at unravelling its intricate characteristics. Synthesized by hepatocytes, the APOL1 protein serves a crucial function in the circulation of the high-density lipoprotein (HDL) complex (Beckerman and Susztak, 2018). Its expression extends across various cell types such as macrophages, kidneys, brain, and pancreas.

The *APOL1* gene is situated on chromosome 22, encompassing a region of 14,461 nucleotides with seven exons and six introns. Two common variants named the G1 variant which encodes two missense mutations and G2, a six base pair deletion from the last exon of the gene, are situated in exon seven. The G2 mutation results in the removal of two amino acids from the last section of the polypeptide chain (An, *et al.,* 2019). These two variants (G1 and G2) are on opposing chromosomes and are therefore in negative disequilibrium (Kopp, *et al*., 2011; An, *et al.,* 2019). Due to their proximity, they have not undergone recombination (Dummer, *et al.,* 2015). The *APOL1* gene variants play a role in innate immunity and are up-regulated by interferons and other inflammatory cytokines (An, *et al.,* 2019). When the variant G1 and G2 genotypes are expressed, it protects humans against being infected by the African parasite *Trypanosoma brucei gambiense* leading to the African sleeping sickness disease (elaboration on this topic to follow in section 2.10.1).

When in a mutated or variant state, the *APOL1* gene has an effect on a spectrum of disorders referred to as *APOL1*-associated nephropathy, encompassing non-diabetic renal diseases (NDRD) such as lupus nephritis, hypertensive nephrosclerosis, primary FSGS, and HIVAN (Sepahi*, et al*., 2019). These conditions underscore its involvement in adapting to parasitic diseases and contributing to the pathogenesis of kidney disorders. The discovery of *APOL1* gene variants in 2010 significantly enhanced the comprehension of the prevalence of non-diabetic kidney disorders specifically in persons with African ancestry (Daneshpajouhnejad, *et al*., 2022).

2.10.1. Human African trypanosomiasis (HAT) and *APOL1*

The parasitic disease known as sleeping sickness, or human African trypanosomiasis (HAT), is spread by vectors (https://www.who.int/news-room/fact-sheets/detail/trypanosomiasis-human-african-(sleeping-sickness)). Its name is derived from the symptoms seen such as disrupted sleep patterns and confusion.

Two distinct species of parasites are responsible for causing Human African Trypanosomiasis, each exhibiting a variance in disease advancement. *Trypanosoma brucei gambiense* prompts a chronic and gradual progression of symptoms, whereas *Trypanosoma brucei rhodesiense* leads to an acute and rapid advancement of the disease (Bouteille and Buguet, 2012). It is brought on by parasites belonging to the *Trypanosoma* genus, transmitted to humans via the bites of tsetse flies (*Glossina* species) that have acquired the infection from humans or animals (Papagni, *et al*., 2023). HAT is part of Neglected Tropical Diseases (NTDs) according to US Centers for Disease Control and Prevention and World Health Organization, It was once the leading cause of death within the vector's geographical distribution in 36 Sub-Saharan African countries (https://www.who.int/news-room/fact-sheets/detail/trypanosomiasis-human-african-(sleeping-sickness)). In the context of HAT, *APOL1* gene variants have received significant attention due to their protective role, particularly against *Trypanosoma brucei gambiense*, responsible for the chronic form of the disease, while *Trypanosoma brucei rhodesiense* is resistant to the *APOL1* variants (Cooper, *et al*., 2017). However, *APOL1* has another critical function when concerning HAT. Due to its antitrypanosomal activity, it can kill the *Trypanosoma brucei* parasites responsible for HAT (Cayla, *et al*., 2019). *Trypanosoma brucei gambiense* has developed a resistance mechanism against *APOL1*, allowing it to survive in the bloodstream and evade the host's immune system (Stijlemans, *et al*., 2016). This resistance involves a protein called Serum Resistance-Associated (SRA) protein, which neutralizes *APOL*1 variant expressed protein's trypanolytic activity (Pays and Nolan, 2021). Although *APOL1* has high risk variants (G1 and G2) that protect the body from HAT, these variants lead to an increased risk of developing kidney disease. In areas where Human African Trypanosomiasis (HAT) is prevalent, there is a hypothesis suggesting that the occurrence of *APOL1* gene variants confers a selective advantage against sleeping sicknesses but adds a risk to develop *APOL1* associated kidney diseases. This contributes to the increase in *APOL1* gene variants (Kasembeli, *et al.,* 2015), much like the relationship observed between sickle cell anaemia and the Plasmodium parasite in mosquitoes.

The allele frequencies of G1G (rs73885319), G1M (rs60910145), and G2 (rs71785313) according to the 1000 Genomes Project Phase 3 provide valuable

insights into the distribution of these *APOL1* risk variants across different populations. The regions involved were Africa (with at least nine sub-populations, America, East Asia, Europe, and South Asia (figures: 2.6 to 2.8 and 2.10). The African sub-populations are highlighted due to their diversity and because Africa was the region of this particular study.

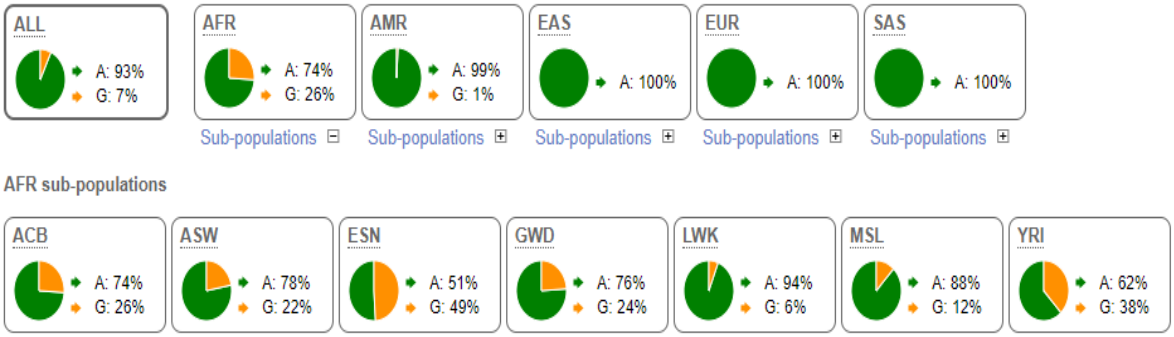

Figure 2.6 Missense variant **G1^{G}:rs73885319** allele frequencies according to the 1000 genome project phase 3. Alleles: A/G. (ensemble.org)

AFR – Africa; AMR – America; EAS – East Asia; EUR – Europe; SAS – South Asia

African sub-populations**:** ACB - African Carribean in Barbados; ASW - American's of African Ancestry in SW USA; ESN - Esan in Nigeria; GWD – Mandinka in Gambia, LWK - Luhya in Webuye, Kenya;MSL - Mende in Sierra Leone; YRI - Yoruba in Ibadan, Nigeria

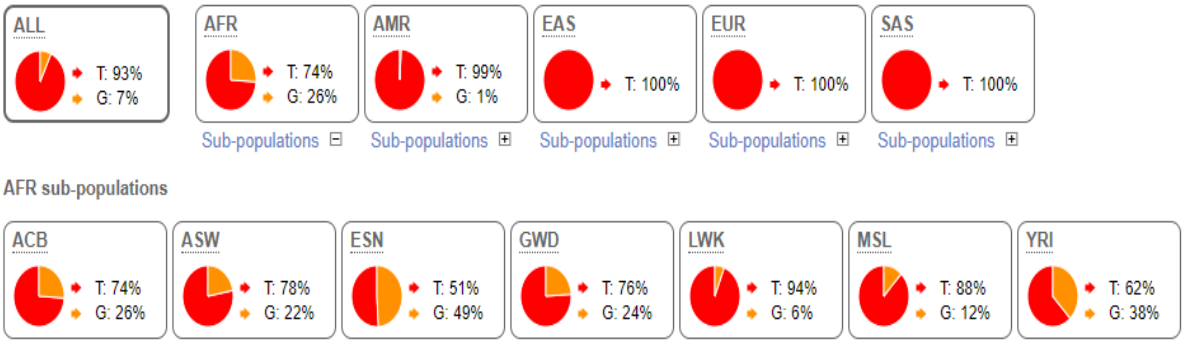

Figure 2.7 Missense variant **G1^{M} rs60910145** allele frequencies according to the 1000 genome project phase 3. Alleles: T/G. (ensemble.org)

AFR – Africa; AMR – America; EAS – East Asia; EUR – Europe; SAS – South Asia

African sub-populations**:** ACB - African Carribean in Barbados; ASW - American's of African Ancestry in SW USA; ESN - Esan in Nigeria; GWD – Mandinka in Gambia, LWK - Luhya in Webuye, Kenya;MSL - Mende in Sierra Leone; YRI - Yoruba in Ibadan, Nigeria

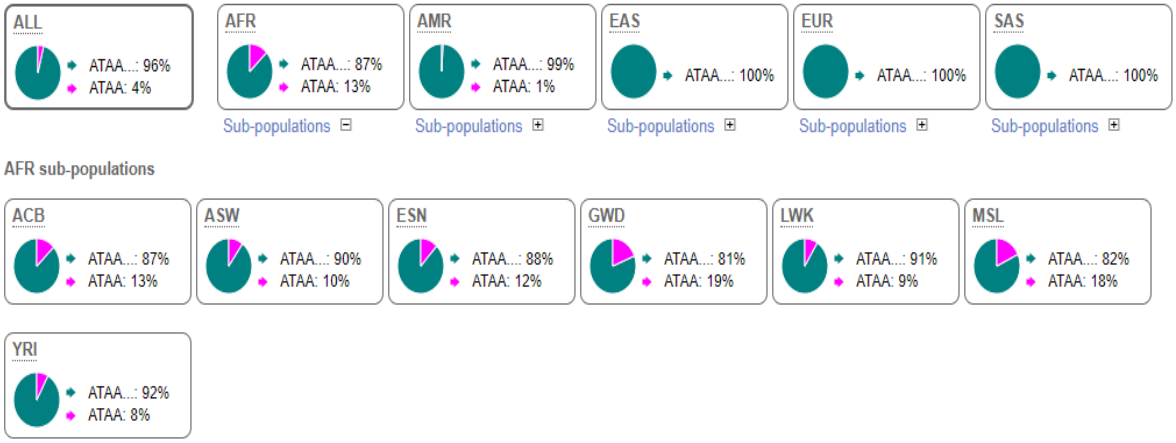

Figure 2.8 In frame deletion **G2: rs71785313** allele frequencies according to the 1000 genome project phase 3. Alleles: ATAATTATAA/ATAA. (ensemble.org)

AFR – Africa; AMR – America; EAS – East Asia; EUR – Europe; SAS – South Asia

African sub-populations**:** ACB - African Carribean in Barbados; ASW - American's of African Ancestry in SW USA; ESN - Esan in Nigeria; GWD – Mandinka in Gambia, LWK - Luhya in Webuye, Kenya;MSL - Mende in Sierra Leone; YRI - Yoruba in Ibadan, Nigeria

2.10.2. Differential susceptibility to kidney disease among carriers of *APOL1* variants

The *APOL1* C-terminal variants G1 and G2 trigger overexpression of the gene (gain of function mutation) which is toxic to the cells and can kill *T. rhodesiense*, but these variants also induce kidney podocyte dysfunctions, leading to chronic kidney disease in the presence of other second hit factors. High-risk variants are defined as carriage of two risk alleles with genotype G1/G1, G1/G2, or G2/G2 (An, *et al.,* 2019). Low-risk variants are defined by the carriage of one or none of the risk alleles (An, *et al.,* 2019). The frequency of the risk alleles varies in different geographical regions with the G1 allele being more prevalent than the G2 allele (Kasembeli, *et al*., 2015). The exact mechanism for the development of *APOL1*-associated kidney diseases is poorly understood (Zhang, *et al.,* 2018). However, it is proposed that the G1 variant specifically stimulates toxicity through K-current or initiation of the stress-activated protein kinases (SAPK) pathway (Olabisi, *et al*., 2016). They form permeable pores made up of cations that lead to swelling of lysosomes and ultimately death of the podocyte. The swelling is induced when SAPKs are activated and this causes toxicity and cell death through downstream effectors that control the flow of Na, Cl,

and H_2O. This is due to the resulting potassium depletion. The intracellular potassium efflux also induces proinflammatory cytokine expression and activates the NLRP3 inflammasome and cellular pyroptosis (Jha, *et al.*, 2020).

APOL1 risk variants also affect the mitochondria by inducing mitochondrial fission (Ma, *et al.,* 2020), reducing its respiratory capacity, respiration rate, and membrane potential. When expressed, the G1 and G2 variants induce dysregulation of endosomal trafficking and lysosomal acidification in yeast cells (*Saccharomyces cerevisiae)*. Moreover, *APOL1* high-risk variants enhance the expression of miR193a which is a negative regulator of autophagy. Using HEK-293 cells Wen, *et al.,* 2018, showed that overexpression of the *APOL1* risk alleles induces endoplasmic reticulum stress, confirming impairment in autophagy. When upregulated, miR193a damages adherence complex stability, disrupt actomyosin cytoskeletal organization, reduce nephrin expression, and promote dedifferentiation in podocytes. There is evidence that shows that *APOL1* risk alleles interact with genetic or environmental factors to cause cell death in renal cells (Reidy, *et al.,* 2018).

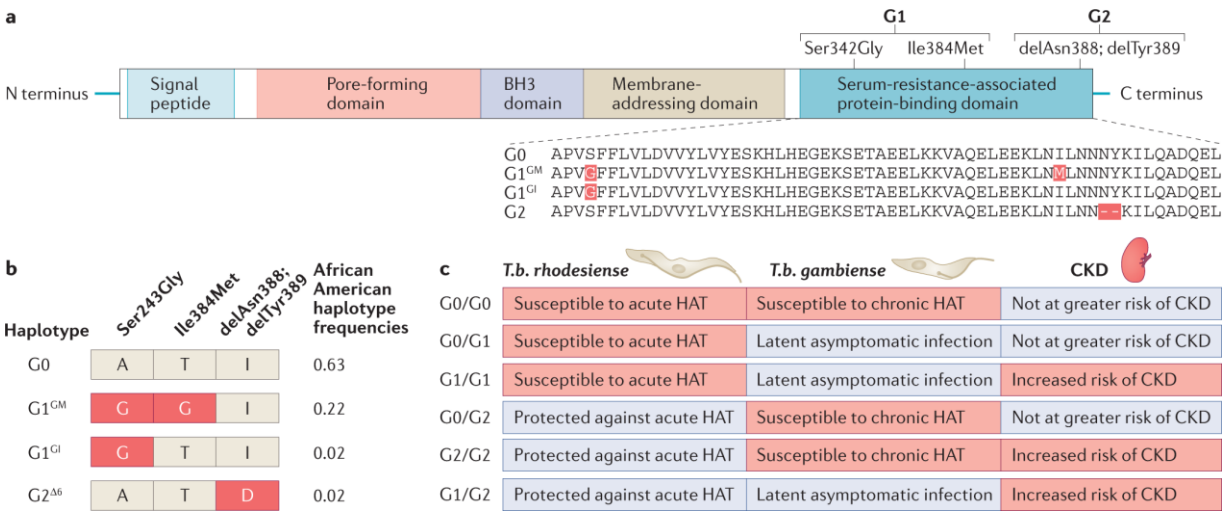

Figure 2.9 *APOL1* domains and variants. (Daneshpajouhnejad, *et al.,* 2022)

Figure 2.9 above shows the *APOL1* domains (A), its variants (B) as well as its suitability and protection from acute human African trypanosomiasis and CKD (C). A. Shows that there are four functional domains and a signal peptide in APOL1 protein. The G1 and G2 variants are primarily responsible for *APOL1*-mediated chronic

kidney disease. The G1 and G2 variants are situated in the nucleotide sequence encoding the serum resistance-associated protein-binding domain of *APOL1*. G1 is a result of the presence of two missense mutations Ser342Gly and Ile384Me, while G2 is due to a 6 base-pair deletion that results in the loss of two amino acids delAsn388 and delTry389. B. Is an illustration of the three kidney risk variants, they create four haplotypes.

In C, there is susceptibility and resistance (indicated by red and blue shading, respectively), to acute human African trypanosomiasis (HAT) caused by *Trypanosoma brucei rhodesiense* and chronic HAT caused by *T.b. gambiense*, and risk of *APOL1*-associated CKD. The susceptibility varies in accordance with particular *APOL1* haplotypes. Heterozygous or homozygous carriers of the G0 (wild-type) allele do not have an increased risk of developing kidney disease. The mechanism behind this protective connection is unknown; however, carriers of 1 or 2 copies of the G1 allele are more prone to contracting *T.b. gambiense* infection. By effectively lysing *T.b. rhodesiense* in vitro, the G2 variant protein prevents infection in G2 carriers. People with the genotypes G1/G1, G2/G2, and G1/G2 have a higher chance of developing CKD. G1/G0 persons may also be at an elevated risk of CKD in some unusual circumstances (for instance, in people with untreated HIV infection).

2.10.3. *APOL1* gene variant studies in African populations

Various studies have been conducted in different populations to determine the prevalence of *APOL1* risk variants. In a study done by O'Seaghdha, *et al.,* 2011 it was determined that variants in the *MYH9* region rather than the *APOL1* region cause the risk for kidney disease in populations of European ancestry (O'Seaghdha, *et al.,* 2011). Twelve to fourteen percent of African Americans carry two of the variants and this leads to a 17 to 29-fold increased risk of developing HIVAN whereas the prevalence in West Africa is approximately 25% (An, *et al.,* 2019). To the best of my knowledge to date, there was only one study done to determine the prevalence of the *APOL1* gene variants concerning HIVAN and HIV in South Africa, on a study population of 228 individuals (Kasembeli, *et al.,* 2015). They found that 79% of participants with HIVAN carry two copies of *APOL1* risk alleles compared to 2% in the general population (Kasembeli, *et al.,* 2015). However, looking at HIV and

kidney disease, they found the frequency to be much lower than that in West Africa and in African Americans at 2-4%. According to Rednor and Ross, this (*APOL1*) introduces an 89-fold increased risk for South Africans to develop HIVAN (Rednor and Ross, 2018). In Sub Saran Africa, with populations from Cameroon, Senegal, Burkina Faso, in a study of 369 individuals, the prevalence of *APOL1* risk alleles were 4.9% in Senegal and Burkina Faso, and 3.4% in Cameroon (Kabore, *et al.,* 2021). In East Africa, particularly Tanzania (n=481), the frequencies of the high-risk alleles were reported to be between 7 to 11% (Stanifer, *et al.,* 2017). Conducting a similar study in Northern Nigeria, Wudil,*et al.,*2021 found the frequency of 6.2% in a study of 2 458. Geographic distributions *APOL1* variants along with that of *Trypanosomiasis* are depicted in Figure 2.10.

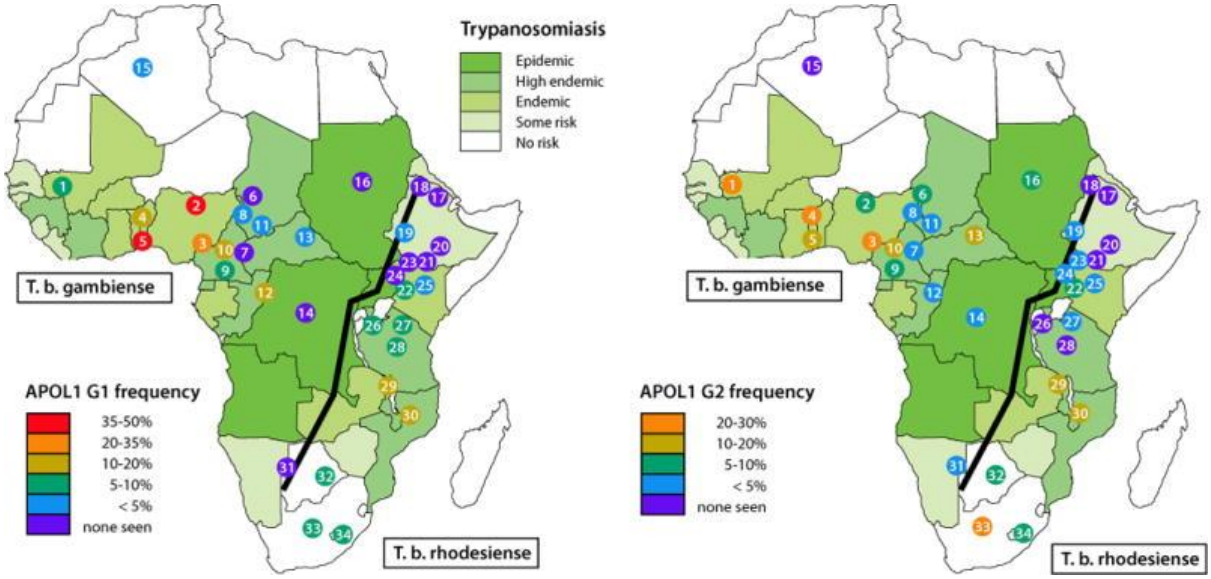

Figure 2.10 Geographic distributions of *Trypanosoma brucei* subspecies and *APOL1* risk alleles.

The G1 and G2 allele distributions among population groups, primarily in sub-Saharan Africa, are displayed along with the population ranges for *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* (Limou, *et al.,* 2014).

2.10.4. The association between *APOL1* risk variants and kidney disease

One South African study showed a dominant expression of the G1 variant in patients with HIVAN (Kasembile, *et al.*, 2015), this was an exception because there is

overwhelming evidence of loss of function or gain of dysfunction (Daneshpajouhnejad, *et al*., 2022). *APOL1* risk variants have been shown to have a recessive pattern of inheritance and promote cytotoxicity (Bruggeman, *et al.,* 2019). Recessive alleles are often linked to a loss of gene function; despite this, there is evidence indicating that *APOL1* variants do not follow this; they follow a gain-of-dysfunction mechanism.

The *APOL1* gene, which encodes a protein involved in normal kidney function and the immune system, is also associated with high-risk alleles that can lead to altered protein function. These alterations result in direct cytotoxic effects on renal cells. Figure 2.11 illustrates the mechanism of the mutated *APOL1* gene leading to kidney injury.

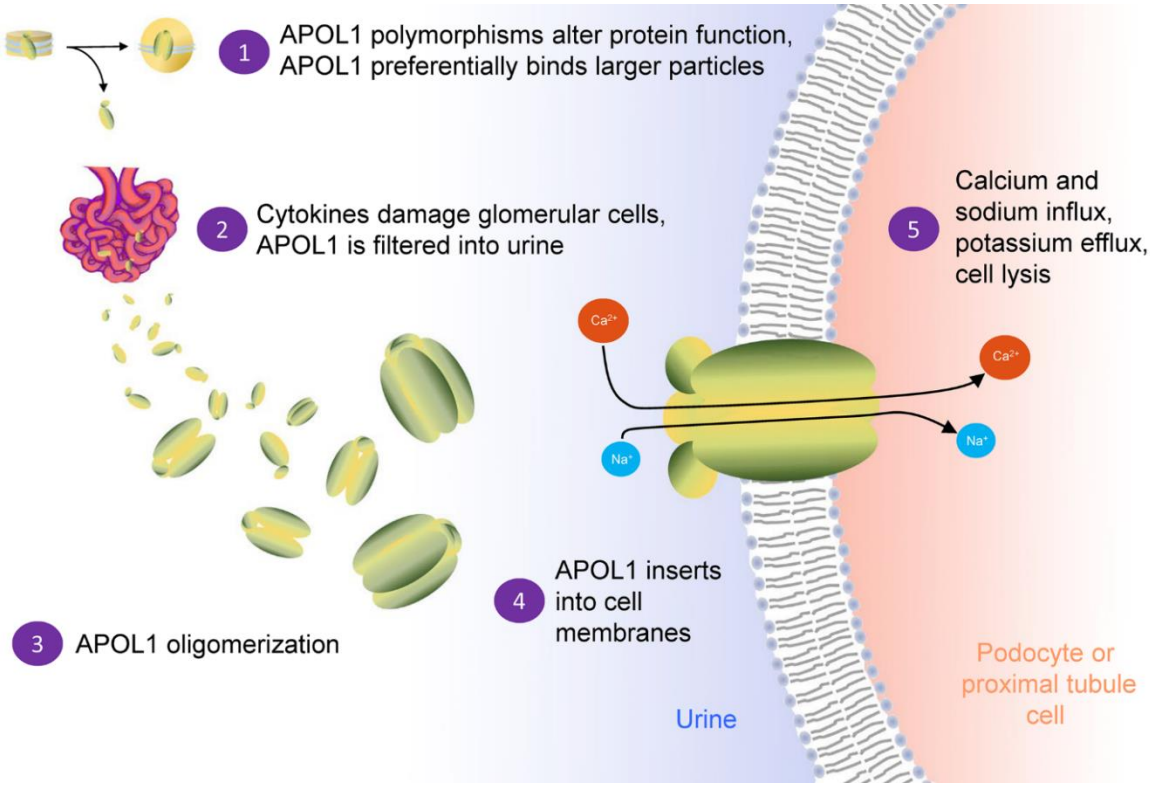

Figure 2.11 A speculative model for the role of circulating *APOL1* in kidney injury (Andrews, *et al.,* 2022).

Step 1- In comparison to *APOL1*-G0, the high-risk coding variations show different biochemical characteristics in plasma and are linked to bigger particles. Similar to albumin, *APOL1* leaking into the Bowman space enables the creation of *APOL1* multimers that (Step 2), after the pH of the glomerular filtrate is made acidic (Step 3), can reach the plasma membrane of podocytes or proximal tubule cells

(Step 4). Step 5- The ensuing potassium efflux and inflow of calcium and sodium cause the death of proximal tubule cells, aggravating renal damage.

Despite *APOL1* gene variants being linked to the development of kidney disease, the molecular mechanisms through which these variants impact the onset, as well as the progression of CKD, are still unknown (Mayanja, *et al.,* 2022). Most research in the prediction of the active site and allosteric site on the APOL1 protein is done using computer simulations. One study used the Computer Atlas of Surface Topography of Proteins (CASTp) and the Protein Allosteric Sites Server (PASSer) (Mayanja, *et al.*, 2022) indicating the particular structural disturbances in the 3D structures of *APOL1* variants using molecular dynamics simulations. The G0 wild-type protein when compared to G1 and G2 has a higher overall stability.

When comparing the prevalence of *APOL1* risk variants in HIV-positive individuals with evidence of kidney disease to HIV-negative individuals with kidney disease, the prevalence is expected to be higher in the HIV-positive group. However, it is important to note that not all HIV-positive individuals will develop kidney disease, and among those who do, not all will carry the *APOL1* risk variants (Goyal and Singhal, 2021).

2.11. Clinical Implications and Management

2.11.1. *APOL1* testing and risk stratification in HIV-infected patients

Recent years have seen an increased interest in *APOL1* genetic testing due to its possible role in determining the risk of developing kidney disease, particularly in people with African ancestry. While *APOL1* gene variants are primarily associated with kidney disease, there is also some research exploring the role in HIV-infection. Studies are aimed at identifying genetic contributors to the significant racial discrepancy in rates of kidney disease discovered the *APOL1* locus and the specific variations linked to kidney disease (Pollak and Friedman, 2023).

Although high-risk variants of *APOL1* are contributing to kidney disease in African individuals; it is wise to note they are not the sole contributor. Kidney disease is found at higher rates in this population also due to socio-economic status and other risk factors such as malaria infection. A study conducted by Hung, *et al.,* 2022 was a cross-sectional study examining the relationship between the *APOL1* renal risk alleles and kidney illness in HIV-positive adults of African descent in the UK. It was called the Gen-Africa study. They found G0/G0 in 1406 (49.1%), G0/G1 or G0/G2 in 1104 participants (38.5%), and G1/G1, G1/G2 or G2/G2 in 354 (12.4%). The overall allele frequencies for G0 were 68.4% for G1 was 19.8%, and G2 was 11.9% (Hung, *et al*., 2022). A similar study was conducted in the US and found that approximately 13% of African Americans have two high-risk variants (G1/G1; G1/G2; or G2/G2), 87% have *APOL1* low-risk genotypes; 39% have one risk variant (G0/G1 or G0/G2) and 48% have no risk variant (G0/G0) (Malone, 2021). Both studies were conducted on individuals of African ancestry, 18 years and above. Not on the general population but on individuals who are HIV positive on renal replacement therapy (dialysis or kidney transplantation) at 15 HIV clinics and 3 dialysis/kidney transplantation centres. This indicates that the *APOL1* renal risk alleles are not the major contributor to kidney disease in patients from African descent.

APOL1 high-risk genotypes have been found to be present in about 70% of those with HIVAN (Hung, *et al*., 2022). This was an average between two major studies. In a case-control study by Kasembeli, *et al.*, 2015 conducted on 228 (cases and controls included) individuals of African ancestry from Johannesburg, South Africa, the percentage was 79%. Kopp, *et al,* 2011 was a larger study with 271 African American cases, 168 European American cases, and 939 controls; the probability for high-risk genotypes was 68%. It is important to note that this was in confirmed cases of HIVAN, hence the high frequencies. When looking at just HIV and the general population, the frequencies of high-risk genotypes in the South African population is 2-4%. The frequencies of high-risk genotypes are much lower in South Africans compared to Nigerians and African Americans. ART usage has been successful in presenting the development of HIVAN, without ART; individuals with high-risk genotypes have an increased risk of developing HIVAN. Studies that have reported an association between *APOL1* high-risk genotypes and kidney disease have raised the prospect that even a single variant poses a risk of developing kidney disease.

The G1 variant has been found to pose a higher risk than G2 (Kasembeli, *et al.,* 2015).

The prevalence of the high-risk variants can be distinguished by geographic location. Individuals from West Africa, specifically Nigeria, have a higher prevalence (Aliyu, *et al.,* 2019) than those from East, Central, and Southern Africa. The G1 and G2 variations are better characterized as ancestry-associated than race-associated now that the specific variants affecting illness risk have been found (Pollak and Friedman, 2023). The distribution of *APOL1* alleles by regions in Africa is represented by Figure 2.12.

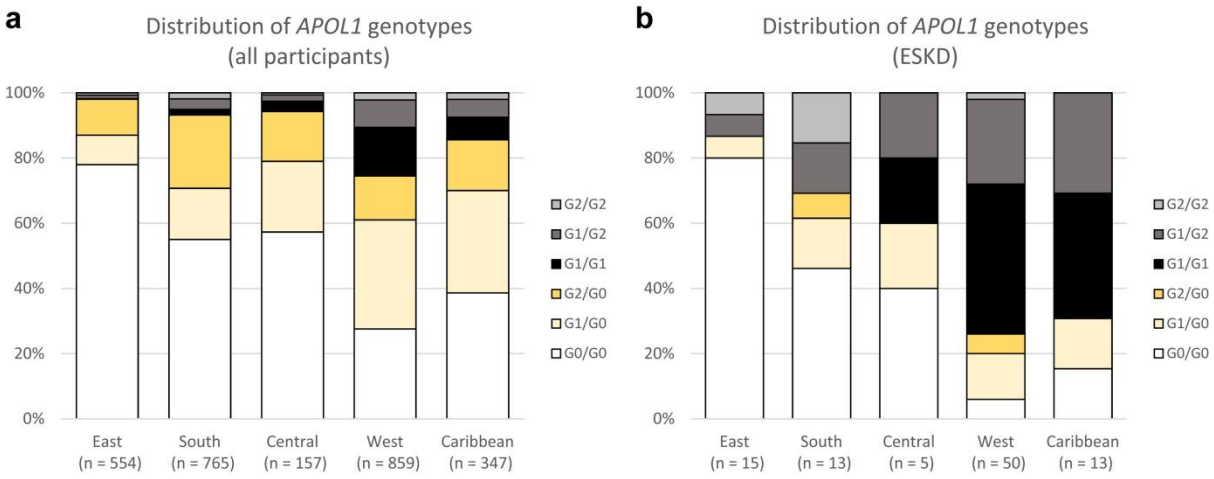

Figure 2.12 Distribution of *APOL1* alleles by region of African ancestry

In (a) all participants and (b) those with end stage kidney disease (ESKD). East, South, Central, and West refer to regions within sub-Saharan Africa; ESKD includes participants with estimated glomerular filtration rate (eGFR). HIVAN (Hung, *et al*., 2022).

In addition to HIVAN, *APOL1* risk alleles have been implicated in several other kidney diseases that primarily affect individuals of African ancestry (figure 2.13). These risk alleles have been linked to an increased susceptibility to non-diabetic kidney diseases, particularly focal segmental glomerulosclerosis (FSGS) (Goyal and Singhal, 2021) and hypertension-attributed nephropathy (Robinson and Freedman, 2019). FSGS is a progressive kidney disorder that affects the filtering units of the kidneys, called glomeruli, leading to scarring and impaired kidney function. Studies have shown that individuals with *APOL1* risk variants have a higher risk of

developing FSGS, which can progress to end-stage renal disease (ESRD) (Fine, *et al*., 2012). Hypertension-attributed nephropathy, on the other hand, is characterized by kidney damage due to uncontrolled high blood pressure. *APOL1* risk alleles have been associated with an increased risk of developing this form of kidney disease, contributing to health disparities among populations of African Ancestry. Additionally, there is Lupus nephritis as a kidney disorder that can occur as a complication of systemic lupus erythematosus (SLE), which is an autoimmune disease (Lupus and Kidney Disease - Lupus Nephritis, 2023). Research has shown that individuals of African descent who carry *APOL1* risk variants may be at an increased risk of developing aggressive lupus nephritis that tends to progress to kidney failure (Friedman and Pollak, 2021).

It is essential to note that the association between *APOL1* risk alleles and DKD is an area of on-going research, and the mechanisms behind this link are not fully understood. However, the impact of these genetic variants in DKD may involve pathways related to kidney inflammation and fibrosis. The interplay between diabetes-related factors and genetic susceptibility may exacerbate kidney damage in individuals who carry *APOL1* risk alleles. This increased susceptibility to kidney diseases in individuals with *APOL1* risk alleles, primarily observed in individuals of African ancestry, underscores the importance of understanding genetic factors in the context of health disparities, as these individuals may be at higher risk for kidney diseases and related complications. Further research in this area is crucial to improve diagnosis, prevention, and management of these kidney diseases, particularly in populations at greater risk.

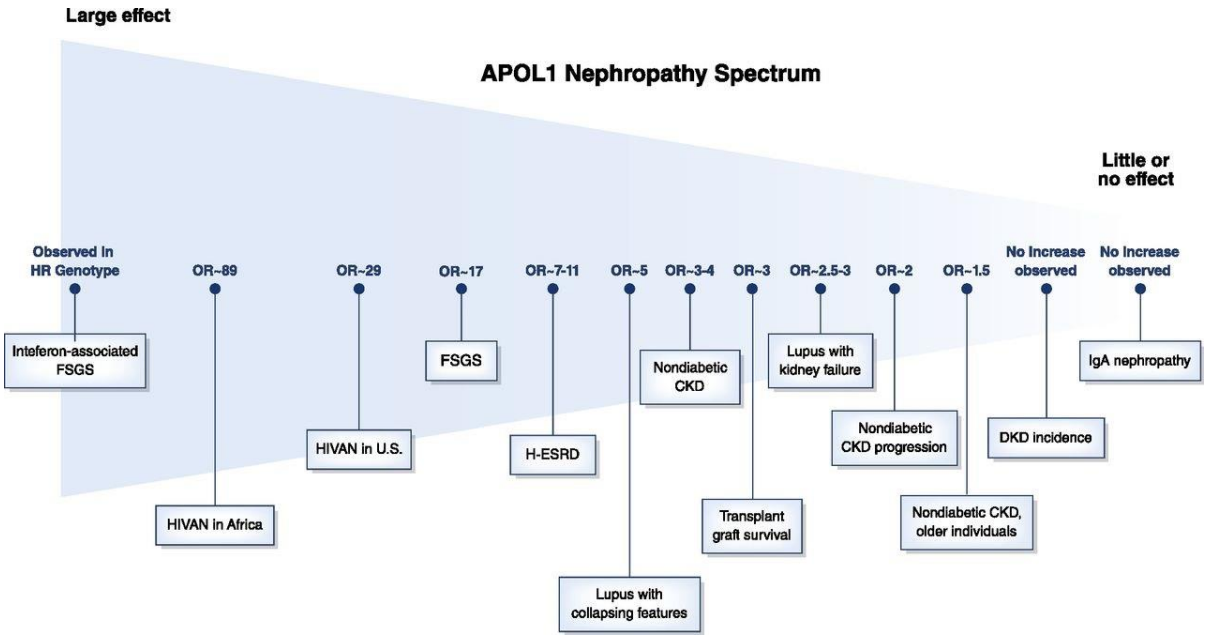

Figure 2.13 Persons with African ancestry are more susceptible to many forms of kidney diseases due to *APOL*1 risk variations.

The incidence of hypertension-attributed ESKD (H-ESKD; 7–11 fold increased), FSGS (17–17 fold increased), HIV-associated nephropathy (HIVAN; 29–89 fold increased), nondiabetic CKD (2–4 fold increased), and various kidney disease presentations is increased by *APOL1* risk variations. (Friedman and Pollak, 2021).

2.11.2. Considerations for kidney transplantation in *APOL1* high-risk individuals

Before 2008, most government-run hospitals in South Africa where access to kidney replacement therapy is rationed due to limited resources, did not allow individuals with HIV into their chronic dialysis programs because they believed transplantation would result in unfavourable outcomes. This policy was examined in 2008 and the rules were updated after the SA renal and transplant societies agreed that HIV-positive patients could not be denied care based solely on their HIV status. (South African Renal Society, SATS, Southern African HIV Clinicians Society, 2008).

Patients with HIV of African descent who appear with persistent severe proteinuria and deteriorating renal impairment should be treated with a high index of suspicion for HIVAN (Rivera, *et al.,* 2022). The presence of advanced HIV infection with a high viral load and low CD4 count, nephrotic range proteinuria, and big echogenic kidneys

on ultrasonography are common characteristics of HIVAN. Patients with those signs are candidates for *APOL1* genetic testing, not yet in clinical practice but offered for kidney donors and recipients. This test is not offered in the clinical platforms in South Africa yet. Families of such patients should also be counselled and tested for carrier status. Kidney transplantation is suggested as the preferred manner of care for people with ESKD, including those with *APOL1*-associated nephropathy (Freedman and Moxey-Mims, 2018).

When considering kidney transplantation doctors frequently request genetic testing to assess potential live kidney donors whose families suffer from Mendelian diseases such as Fabry disease, Alport syndrome, inherited podocytopathies, and polycystic kidney disease. Donors with causative variants are deemed to be not suitable living kidney donors as they are at risk of developing kidney diseases. However, there are presently no recommendations for *APOL1* risk allele genetic testing in HIV patients because the genotype's current understanding would not alter clinical management. This presents a challenge to clinical management due to wide variation in eGFR in individuals with *APOL1* high-risk genotypes. It is also more complex as *APOL-1* risk alleles are not directly causal and only seem to increase the risk of CKD in the presence of other environmental factors. However, a link was discovered between T cell-mediated rejection and *APOL1* risk alleles (Zhang, *et al.,* 2018), this is clinically significant as such that patients may need more regular monitoring and severe immunosuppression causing the patient to change their way of life (Kopp and Winkler, 2020).

Although there is a clinical challenge relating to *APOL1* high-risk genotypes, studies have proven that two *APOL1* risk alleles in donor kidneys have a detrimental effect on kidney allograft survival shown in Figure 2.14. Therefore, individuals with high-risk genotypes may not be considered viable donors. *Retrospective* data analysis showed that genotypes of deceased donors were linked to quicker failure of kidney allografts from African American donors irrespective of recipient ethnicity (Freedman, *et al.,* 2017). It is however important to clarify that not all individuals with high-risk genotypes end up developing kidney disease; this is likely due to the absence of a second hit.

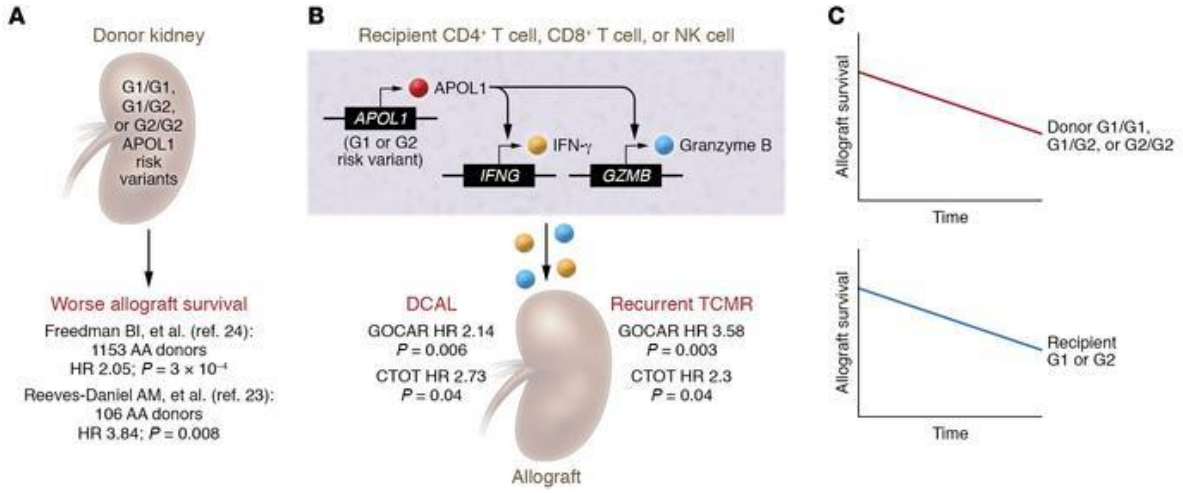

Figure 2.14 *APOL1* risk variants influence kidney transplantation outcomes via intrinsic and extrinsic mechanisms (Malone, 2021).

(A) Previous research revealed that donors with two *APOL1* risk factors had lower allograft survival. The intrinsic actions of *APOL1* risk variants within the kidney result in dose-dependent podocyte damage.

(B) Zhang, *et al.,* 2021 demonstrated a correlation between death-censored allograft loss and recurrent T cell-mediated rejection when the recipient had *APOL1* G1 or G2. Through an extrinsic, immune-mediated mechanism, *APOL1* risk mutations cause kidney damage by activating T and NK cells. T cell-mediated rejection is also known as T cell-censored allograft loss or DCAL.

(C) When recipients of kidney transplants carry the risk variations or receive a transplant from an *APOL1* donor; the risk variants have a long-term unfavourable impact on allograft survival.

APOL1 genetic testing may not be just required for the donors but for the recipients as well. The association between kidney allograft failure and *APOL1* risk variants of the recipients was reported by Zhang, *et al.,* 2018. So far, no genetic studies have been conducted for both donor and recipient at the same time. In the effort to increase the number of African kidney donors, as well as reduce the fear of early deterioration of kidneys from donors of African descent, the National Institute of Health initiated *APOL1* Long-Term Kidney Transplantation Outcomes Network (APOLLO) (Freedman and Moxey-Mims, 2018). This initiative aims to determine whether kidney donors with high-risk genotypes survive in the long run. Although this study is confined to the centres in the United States and Puerto Rico, its success can be extrapolated to African countries. Knowing the *APOL1* genotypes of kidney donors with recent African ancestry may help predict kidney transplant outcomes more accurately.

2.11.3. Therapeutic interventions targeting *APOL1*-related pathways

There is currently no proven curative medication to halt the progressive loss of kidney function caused by *APOL1*- related nephropathies. To successfully treat *APOL1* nephropathies, exact deregulated cellular pathways need to be identified. *APOL1*-associated toxicity is heterogeneous.

APOL1 is a newly discovered and rapidly evolving gene, the only one of the family of six *APOL* genes that is expressed in human primates. Because of this reason, *APOL*1 might not be so vital in kidney function (Pollak and Friedman, 2023). Therefore, completely silencing it or reducing its expression might not be detrimental to kidney function. It should be noted though that this is only viable in parts of the world or among Africans where trypanosomes are absent (Daneshpajouhnejad, *et al.,* 2022). Another method of targeting the gain of function of the G1 and G2 alleles is to alter their expression into acting like the wild-type allele. Since treatments targeting the expression of the alleles themselves might be trickier, *APOL1*-targeted therapeutics have been aimed at the kidney itself (Daneshpajouhnejad, *et al.,* 2022).

As mentioned before, podocytes, endothelial cells, and proximal tubule epithelial cells all express *APOL1*, but podocytes are thought to be the primary target of *APOL1*-mediated actions in the kidney (Chun, *et al.,* 2019). It would be fair to assume that the best course of targeting *APOL1* would be to target podocytes. Yang, *et al.,* 2022 demonstrated this in their study. They dispensed antisense oligonucleotides (ASO) to podocyte G2 mice targeting *APOL1*. Their observations included a significant reduction of *APOL1* expression, and protection from albuminuria, glomerulosclerosis, tubulointerstitial fibrosis, and renal failure (Yang, *et al.,* 2022).

Two clinical studies are also testing novel substances against *APOL1* levels and activity. Antisense oligonucleotide targeting *APOL1* RNA expression and reducing proteinuria in transgenic mice (Aghajan, *et al.,* 2019). The drug was licensed to AstraZeneca and carried out a first human trial but was discontinued because the sponsor decided that there was enough evidence to move on with additional research. Another phase II study by Vertex Pharmaceuticals used inhibited *APOL1*

activity with an oral molecule drug called VX-147 (Daneshpajouhnejad, *et al.,* 2022). This study is still on-going.

2.12. Future Directions and Research Gaps

2.12.1. Genetic determinants beyond *APOL1*

Monogenic disorders, resulting from mutations in a single gene leading to the disease, are less common compared to multifactorial disorders triggered by numerous genes (polygenic) and influenced by environmental factors. The multifactorial aetiology of kidney diseases suggests an unlikelihood of them arising from monogenic causes. Instead, a more probable scenario involves polygenic disorders wherein multiple genes play a role (Hildebrandt, 2010), potentially with epigenetic influences. Kidney diseases can be inherited in a recessive fashion, an example of this is Autosomal Recessive Polycystic Kidney Disease (ARPKD), which is caused by a mutation in a kidney-building block protein called fibrocystin. The gene responsible for this is *Polycystic Kidney and Hepatic Disease 1 (PKHD1*) and is the only one known to cause this disease; this mutation is found in almost 90% of people with ARPKD.

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is an example of a disease showing a dominant pattern of inheritance. ADPKD is caused by a mutation in one of two kidney-building block proteins; polycystin 1 (PKD1) and polycystin 2 (PKD2) (Genetics and Kidney Disease. UNC Kidney Center, 2022). Penetrance and expressivity play a role in these types of inheritance patterns (Hildebrandt, 2010). This shows the broad and complex range of the influence of genetic factors on kidney disease.

Köttgen, *et al*., identified several kidney susceptibility genes that showed a correlation with reduced kidney function (Köttgen, *et al.,* 2010). One of these genes is the *UMOD* gene associated with a risk gene for chronic kidney disease (Köttgen*, et al.,* 2010). Mutations in several genes of the *SLC* (solute carrier) gene family have also been associated with kidney disease (Hildebrandt, 2010). *SLC7A9* and *SLC3A1*

are both associated with cystinuria whereas *SLC4A1* is associated with distal renal tubular acidosis (Köttgen, *et al*., 2010). In 2010 the *MYH9* gene was associated with an increased risk of developing focal segmental glomerulosclerosis (Hildebrandt, 2010). The *MYH9* locus was later also associated with other kidney diseases including end-stage renal disease and HIVAN (O'Seaghdha, *et al.,* 2011).

2.12.2. Epigenetic mechanisms in kidney disease

Epigenetics represents another influential factor in the onset and advancement of kidney diseases. Multiple epigenetic elements contribute to kidney disease, including DNA methylation, a process involving the addition of methyl groups to specific cytosine residues within the DNA molecule. Irregular DNA methylation patterns can prompt changes in gene expression, thereby contributing to the progression of kidney disease. Elevated methylation levels of genes responsible for regulating renal function may result in reduced expression and impaired kidney functionality (Rysz, *et al.,* 2022). The other epigenetic mechanism is histone modification such as acetylation, methylation, phosphorylation, and ubiquitination; they influence gene expression by either opening or closing chromatin structure on genes that play a role in kidney disease (Wing*, et al.,* 2013). Dysregulation of histone modifications can affect the expression of genes involved in inflammation, fibrosis, and renal function.

Non-coding RNAs represent an additional category of epigenetic alterations observed in renal disease. These encompass microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), which serve as epigenetic regulators by binding to messenger RNAs (mRNAs) and influencing their translation or stability. The disruption of miRNAs and lncRNAs has been associated with kidney diseases, impacting pathways associated with inflammation, fibrosis, and oxidative stress (Liu, *et al*., 2022a). Aging signifies the accumulation of epigenetic alterations within the kidneys over time and is linked to modifications in DNA methylation patterns and histone modifications (Liu, *et al*., 2022b). These age-related epigenetic alterations may contribute to the decline in kidney function observed in older individuals. On the other hand, foetal or developmental origins represent epigenetic changes occurring during foetal development that can impact an individual's vulnerability to kidney disease later in life. The foetal programming concept suggests that

unfavourable conditions experienced during pregnancy, such as maternal undernutrition or exposure to toxins, can induce epigenetic modifications in the developing kidneys in utero (Argeri, *et al.,* 2020), thereby heightening the risk of kidney diseases in adulthood. Understanding the epigenetic factors involved can provide insights into disease mechanisms and potential therapeutic targets. Epigenetic therapies, such as drugs that target DNA methylation or histone modifications, are being explored as potential treatments for kidney diseases.

2.13. Conclusion

2.13.1. Summary of the current understanding of *APOL1* involvement in HIV-associated kidney disease

APOL1 gene variants have been associated with kidney disease, but since not everyone with high-risk variants ends up developing kidney disease, a second hit theory has been proposed as a plausible explanation. HIV infection has been identified as one of the second hits that increase the risk of developing kidney disease in genetically predisposed individuals. The exact mechanisms are still unknown, but several avenues have been proposed and are being investigated. In individuals who are HIV positive and carry two *APOL1* high-risk alleles, it needs to be identified whether *APOL1* directly impacts renal epithelial cells and causes glomerular damage or if it assists HIV in causing damage to podocytes. HIV infection is associated with heightened immune activation and elevated levels of proinflammatory cytokines, including interferons (Shebl, *et al*., 2012). These cytokines play a significant role in the body's defence against viral infections (Klimpel, 1996), including HIV, by activating immune responses to target and clear infected cells. However, this heightened immune activation, especially in the context of chronic HIV infection, can have various downstream effects on different genes and pathways, including the upregulation and expression of the *APOL1* gene.

Since *APOL1* is a part of the innate immune system and is involved in protecting cells from a variety of pathogens, including certain types of African trypanosomes that cause sleeping sickness. It had been suggested to play a role in protecting

against other intracellular pathogens as well (Friedman and Pollak, 2021). The actual mechanisms through which interferons and other immune factors upregulate the *APOL1* gene and how this induces kidney diseases, are still being researched.

In the context of HIVAN, the overexpression of *APOL1* may be linked to the kidney damage observed in some HIV-positive individuals who carry high-risk *APOL1* alleles. The interplay between HIV infection, immune activation, and *APOL1* gene expression underscores the complexity of the relationship between genetic factors and kidney diseases are also an area of on-going research.

2.13.2. Importance of further research and clinical implementation of *APOL1* gene variant screening

The *APOL1* Long-Term Kidney Transplantation Outcomes Network (APOLLO) is a national observational study with the aim of testing kidney donors and kidney transplant recipients for variants of the *APOL1* (Freedman and Moxey-Mims, 2018). APOLLO is a step in the right direction in the treatment of kidney disease in HIV-positive individuals, but there needs to be a South African database for kidney disease and not only for individuals qualifying for renal replacement therapy. There is a lot of scepticism regarding the benefits of *APOL1* testing because there is not sufficient information to extrapolate the risk into the wide population. It has been established that *APOL1* is ancestry-related and not race-related; just testing Africans without knowing their country of origin may not answer the question. A complete study of an individual's origin (ethnicity) should include a complete or somewhat complete family history (region of origin of both parents) before testing. One proposed treatment for *APOL1* risk variant carrying patients, involves the silencing of the *APOL1* gene, this would be detrimental to Africans where trypanosomes are present, hence the need for a complete family history. Regional exposure has to be taken into consideration if the silencing of the gene is considered as a treatment option.

In 2014, the Clinical Practice Guidelines for the Care of HIV-Positive Patients with Chronic Kidney Disease were published. They highlighted the need to initiate cART when HIVAN is suspected, this aims to slow the progression of ESKD. Adding

APOL1 testing to that may add value to patient care, more personalized medication can be administered as well as early intervention, and affected family members (carriers) may be given a chance to adjust their way of life. *APOL1* testing may assist in the treatment of patients of African descent by guiding in the selection of viable kidneys for donation and mitigate the long-term risk of developing CKD in those who are genetically predisposed. There are concerns that *APOL1* testing of donors and recipients may marginalise the people of colour who are on the waiting list and decrease their likelihood of being selected to receive a kidney for transplantation. Certified laboratories are offering rapid *APOL1* genotyping in deceased black kidney donors and recipients before transplantation.

Therefore, given the intricate relationship between the *APOL1* gene, kidney function, and immune response outlined in this literature, this study is essential to further elucidate the mechanisms underlying the cytotoxic effects of high-risk *APOL1* alleles on renal cells. By investigating the prevalence of these alleles and their association with kidney disease in specific populations, such as HIV-positive individuals in in South Africa, this research aims to provide valuable insights into the pathogenesis of kidney disease and inform targeted interventions for prevention and management.

CHAPTER 3

Materials and methods

3.1. Introduction

This chapter discusses the research methodology, which includes the study design, demographic, sample selection (inclusion and exclusion criterion), materials, methods, and procedures employed in the study.

3.1.1. Study design

This was a case-control study that included two hundred and twenty (n=220) participants consisting of four groups, namely: HIV positive with kidney disease indicators (n=55); HIV positive without kidney disease (n=55); HIV negative with kidney disease indicators (n=55) and HIV negative without kidney disease (n=55). The participant samples were selected from archived material according to the inclusion and exclusion criteria.

3.2 Ethics conduct

This study was conducted with ethics approval (UFS-HSD2023/0249/2305) obtained at the University of the Free State, Health Sciences Research Ethics Committee, represented by Appendix A. Participant blood and urine samples were collected under the study performed from 2018-2022 with ethics approval number HSREC C19/2017. The principal investigator of that study provided permission, shown in Appendix B, for the usage of the samples. A new research application was submitted and approved for the study in this thesis (UFS-HSD2023/0249/2305). Participants were not required to provide additional blood for the new study since archived samples, delinked from all personal information, were used for all procedures in this study. Care was taken to assure that all participant samples used in this study provided informed consent for genetic studies to be conducted on their blood (HSREC C19/2017).

3.3. Summary of study procedure

The flow of the study is represented by Figure 3.2.1. After ethics approval was obtained, samples were selected from archived samples based on inclusion and exclusion criteria as stipulated in the sampling to follow. Genetic tests were performed at the Faculty of Natural and Agricultural Sciences, Department of Genetics, at the University of the Free State to determine the association between *APOL1* risk variants with kidney disease in HIV-positive and HIV-negative participants. Sanger sequencing was performed to verify qPCR controls as well as verification of genotypes in 45% of the participant samples.

The percentage of genotypes confirmed by Sanger sequencing in studies utilizing qPCR for genotyping can vary depending on several factors, including the specific genetic markers being analyzed, the quality of the qPCR assay, and the criteria for confirmation. In general, it is common practice for studies to confirm a subset of qPCR results using Sanger sequencing to validate the accuracy of the genotyping method. However, there isn't a fixed or standard percentage across all studies. Some studies may aim to confirm a high percentage of genotypes to ensure accuracy, while others may only confirm a smaller subset due to resource constraints or other considerations, and not all studies use Sanger sequencing to confirm qPCR genotype results (Almeida *et al*., 2010).

This study is not aimed at clinical diagnostics or diagnostic method validation; thus, the diagnostic criteria referred to by the MIQE guidelines (Bustin *et al*., 2009) are not required for this study. According to the MIQE guidelines for research applications, the sequence confirmation of a subset of the study population is not required. But it states that all positive controls were sequence confirmed along with outliers; this was adhered to, and additionally, the sequence confirmation of heterozygote genotypes and a subset of the homozygous population for all three SNP’s were performed. Since no discrepancy was found within comparing the genotyping results with sequence results in 45% of samples and the fact that qPCR genotyping assays have been proven in the literature to be extremely accurate, it was decided to save laboratory resources and abandon sequencing for the rest of the study population.

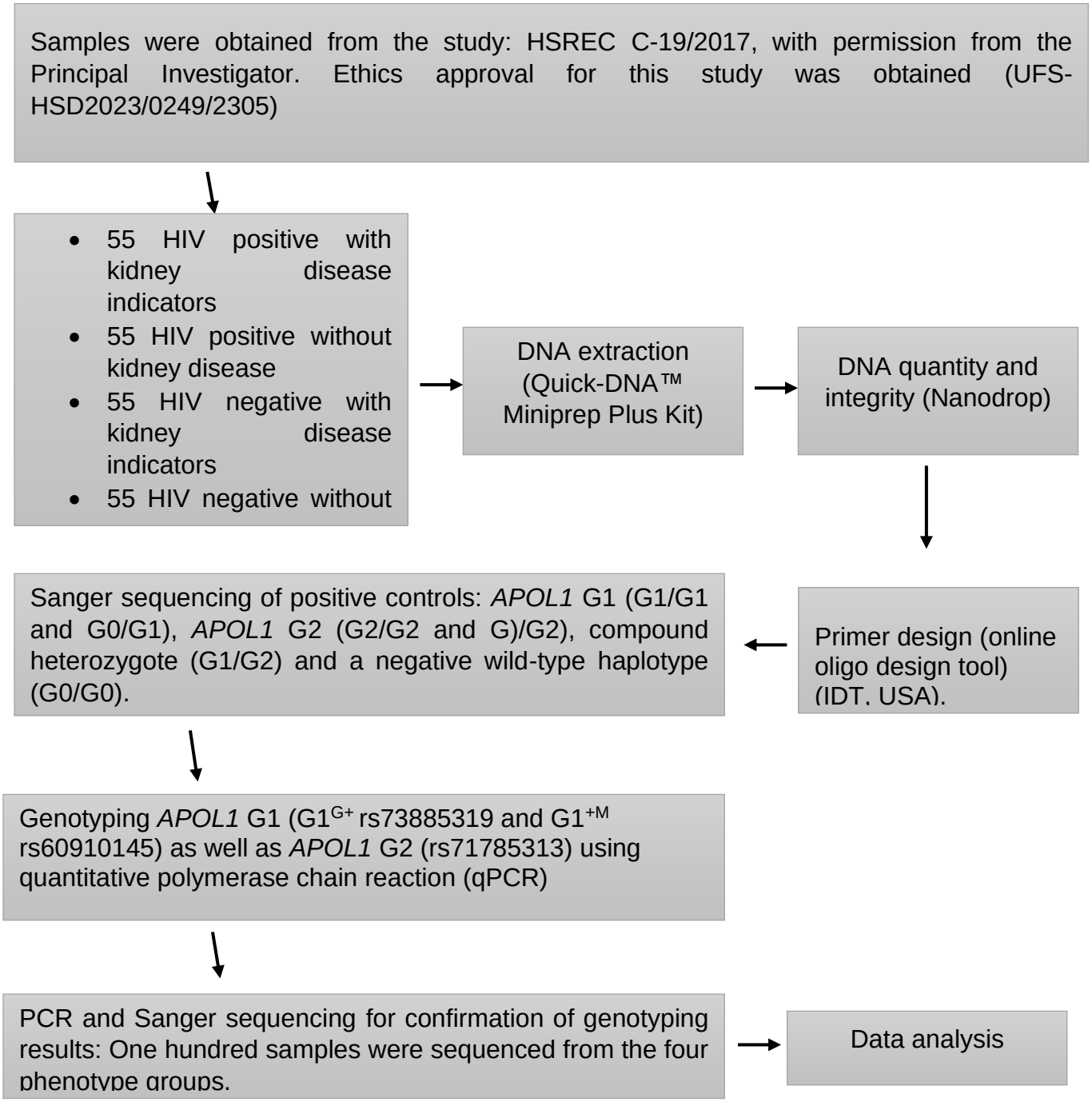

Figure 3.1 Flow diagram of the study.

3.4. Sampling

The study population were recruited from patients attending the voluntary HIV counselling and testing facility at the Polyclinic at the Pelonomi Regional Hospital, Bloemfontein, Mangaung Metropolitan Municipality, Free State, South Africa. All participants were from a similar socio-economic background, low to middle income households and at least 80% spoke Sesotho or Setswana. More than 50% of the participants were unemployed, informally employed or delf employed. About 70% were single or unmarried.

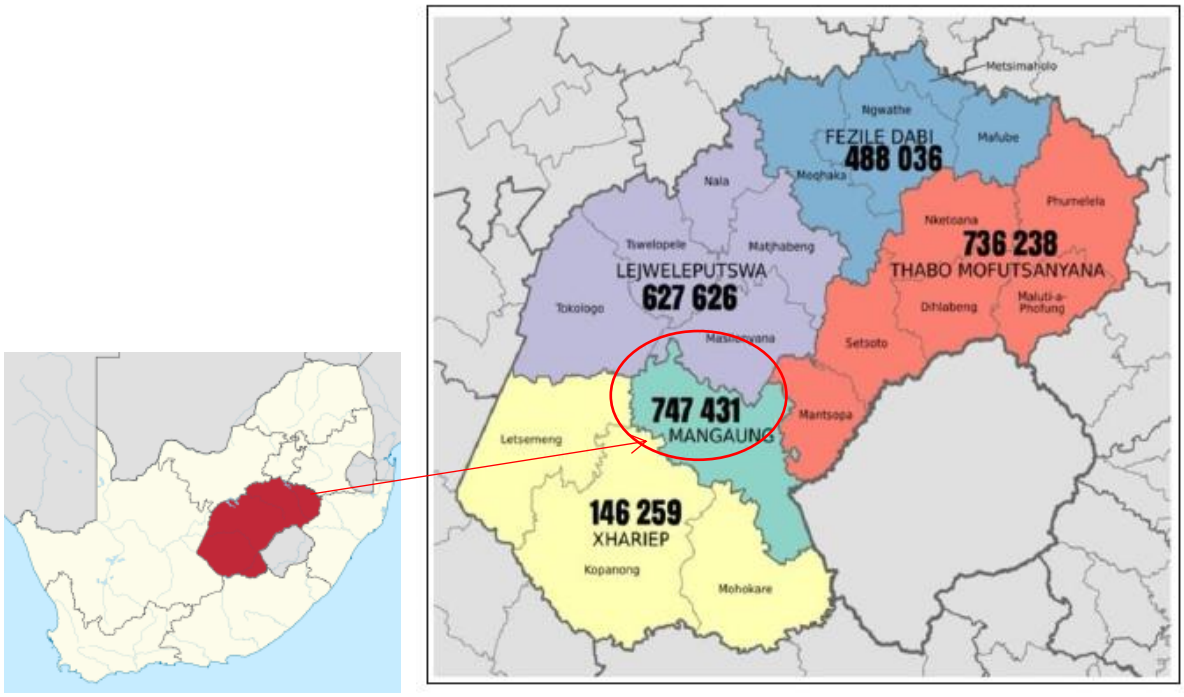

Figure 3.2 Map of the four districts in Free State and the Mangaung metro. The most densely populated metro in the Free State province of South Africa (Pitso, *et al.,* 2019).

As part of the original study the following parameters were tested, and results recorded in the study file of each patient. Kidney disease markers such as creatinine, proteinuria, and albumin were tested for using urine dipsticks and blood tests on the same day the blood was drawn from the participants. Rapid HIV tests were performed to determine the status of participants. A general medical history was recorded to determine the inclusion and exclusion criteria.

Anthropometry was utilized to obtain physical measurements of individuals, with a focus on weight, height, and BMI (body mass index). Weight, representing the actual body weight measured at the time of examination, was recorded using a periodically calibrated scale to ensure accuracy. Subjects were weighed while wearing light clothing and without shoes, with the weight recorded to the nearest 0.1 kg (VanWormer, *et al*., 2012). Height was measured with participants standing flat (Hammond and Litchford 2012), without shoes, and their feet together, ensuring heels were against the measuring board. During measurement, participants stood upright, maintaining a straight posture, and looking straight ahead without tilting their heads. The top of the ear and outer corner of the eye were aligned parallel to the floor. A ruler was positioned perpendicular to the wall, touching the top of the head to obtain the correct height measurement, which was recorded to the nearest 0.1 centimetre (De Bruyne et al. 2012). The BMI was calculated with the following formula: (weight in Kg/ height in m2). BMI standards encompass a variety of guidelines and criteria used to interpret and classify an individual's BMI measurement. These standards are typically classified based on the BMI value ranges and the associated categories of weight status. BMI classifications according to KDIGO are as follows: Underweight: BMI less than 18.5 kg/m², normal weight: BMI 18.5 to 24.9 kg/m², overweight: BMI 25.0 to 29.9 kg/m² and obesity: Class I: BMI 30.0 to 34.9 kg/m², Class II: BMI 35.0 to 39.9 kg/m², Class III (Severe or Morbid Obesity): BMI 40.0 kg/m² and above. These standardized procedures were followed to ensure consistency and accuracy in the anthropometric measurements obtained.

A total of 510 individuals were recruited as part of the original study, 259 were HIV positive and 261 were HIV negative. A local registered nurse administered an HIV rapid antigen test to each participant. Some participants dropped out before commencement, others were excluded due to incomplete information in the study file. We subsequently evaluated results from the remaining participants and divided them into four groups. The following exclusion and inclusion criteria were followed:

Exclusion criteria:

Participants with comorbidities that predispose them to kidney disease were excluded from this study. These include:

- Hypertension Systolic mmHg less than 120 and Diastolic mmHg less than 80
- Age below 18.
- Diabetes
- Cardiovascular disease
- History of kidney transplantation
- Pregnant
- Unable to give consent

Inclusion criteria:

HIV-positive participants with kidney disease indicators:

- HIV-positive rapid test results
- Proteinuria shown with a urine dipstick test: Trace to + and + to greater
- Abnormal urine albumin: creatinine ratio (30-299 and >300 mg/g)
- Abnormal protein: creatinine ratio, values 30 mg/mmol and above
- Elevated serum creatinine (0.6-1.2 mg/dl in male, 0.5-1.1 mg/dl in females)
- Males and females above the age of 18

HIV-negative participants with kidney disease indicators:

- HIV-negative rapid test results
- Proteinuria shown with a urine dipstick test: Trace to + and + to greater
- Abnormal urine albumin: creatinine ratio (30-299 and >300 mg/g)
- Abnormal protein: creatinine ratio values 30 mg/mmol and above
- Abnormal serum creatinine (0.6-1.2 mg/dl in male, 0.5-1.1 mg/dl in females)
- Males and females above the age of 18

Samples were regarded as being positive for CKD markers when the protein in urine (mg/dL) was trace to + (positive), and + (positive) to greater, they were regarded as negative when the protein was negative. Under the new KDIGO classification, albuminuria ≥30 mg/24 hours may be used as a substitute for a urinary total protein or albumin excretion rate above the normal threshold for age (KIDGO, 2017). However, at the time of the study, the protein in urine dipstick was used to determine CKD.

Table 3.1 KDIGO classification equivalent indicating the kidney markers used for inclusion and exclusion criteria. (KDIGO, 2017)

KDIGO CLASSIFICATION EQUIVALENT			
	Normal to Mildly Increased (A1)	**Moderately Increased (A2)***	**Severely Increased (A3)†**
AER			
µg/min	<20	20–200	>200
mg/24 hours	<30	30–300	>300
ACR			
mg/g	<30	30–299	>300
mg/mmol	<3	3–30	>30
PER (mg/24 hours)	<150	150–500	>500
PCR			
mg/g	<150	150–500	>500
mg/mmol	<15	15–50	>50
Protein reagent strip	Negative to trace	Trace to +	+ or greater

The conversions are rounded; for an exact conversion from mg/g of creatinine to mg/mmol of creatinine, multiply by 0.113. ACR, urinary albumin-to-creatinine ratio; AER, albumin excretion rate; KDIGO, Kidney Disease: Improving Global Outcomes; PCR, protein-to-creatinine ratio; PER, protein excretion rate.

*

Relative to young adult level

†

Including nephrotic syndrome (albumin excretion usually >2,200 mg/24 hours [ACR >2,220 mg/g; >220 mg/mmol]).

3.5. DNA isolation and quantification

3.5.1. DNA extraction

Peripheral blood was collected in Ethylene diamine tetra acetic acid (EDTA) tubes (BD Vacutainer, Reference no: 368861, Becton Dickinson, South Africa) during the initial study (HSREC C19/2017). The blood was stored in an access-controlled laboratory between -15°C and -25°C until required for extraction. Genomic DNA was isolated from the peripheral blood using the Quick-DNA™ Miniprep Plus Kit (Zymo research, cataloque number: D4069). It combines enzymatic and chemical extraction procedures to rapidly and effectively remove DNA from biological material. In a 1.5ml Eppendorf, 20 µl proteinase K and 200 µl of blood were mixed thoroughly by

pipetting to achieve a homogenous mixture. Biofluid and Cell Buffer were added to the mixture and were mixed by vortex. The sample was then incubated at 55°C for 10 min and briefly centrifuged after. For sample digestion, a Genomic Binding buffer was added, and the samples were vortexed and then briefly centrifuged. The material was put in a collection tube with a Zymo-Spin^{TM} IIC-XLR Column (Zymo research, cataloque number: C1102) and centrifuged at 12 000 x g for one minute. A clean 2 ml collection tube was used to hold the Zymo-Spin^{TM} IIC-XLR Column (Zymo research, cataloque number: C1102), and the filtrate-containing tube was discarded. DNA pre-wash buffer was added, and the tube was centrifuged again. Contents in the collection tube were emptied and a g-DNA wash buffer was added to the column and the sample was centrifuged. A second wash was done using the g-DNA wash buffer. The collecting tube with the flow through was discarded once the spin column was moved to a clean micro-centrifuge tube. The DNA was eluted with 50µl of DNA Elution Buffer directly to the matrix and was allowed to sit for 5 minutes at room temperature. The samples were centrifuged at the fastest speed possible (21200 x g) and stored between -15°C and -25°C until genetic tests were performed**.**

3.5.2. DNA quantity and quality determination

The quantity and quality of the extracted DNA were determined by making use of the NanoDrop 2000 Spectrophotometer (Thermo Scientific, USA) according to the manufacturer's instructions. The quantity of the samples was expressed as ng/µl. The quality of the DNA was determined by two values at the wavelengths; ($A_{260/280}$) and ($A_{230/260}$). The ratio of absorbance at 260 nm and 280 nm (ideally ~1.8) was used to determine the presence of contaminants such as proteins or phenol. The ratio of absorbance at 230 nm and 260 nm (ideally ~2.0-2.2) was used to determine the presence of contaminants that absorb at 230 nm. Before measuring the actual DNA samples, a blank sample was subjected to the Spectrophotometer using the same TE buffer that the DNA samples were dissolved in. A blank is used as a reference to calibrate the instrument and account for any background absorbance or interference that may affect the accuracy of the measurements; overall, it ensures the reliability and accuracy of the spectrophotometric analysis.

3.6. Primer design

3.6.1. Real-time Polymerase chain reaction (PCR) single nucleotide polymorphism (SNP) genotyping)

Custom TaqMan® SNP Genotyping Assays (Thermo Fisher Scientific, Waltham, MA) were used to detect *APOL1* G1 (both variants) and G2 alleles. The G1 alleles consisted of rs73885319 (p.S342G) (Assay ID: C__98253221_10) and rs60910145 (p.I384M) (Assay ID: C__89555688_30), for G2 it was rs71785313 (Assay ID: C_102754756_10) depicted by Table 3.2. The positive controls for each variant used in the assays were sequence confirmed by Sanger sequencing.

Table 3.2 Assay design details

Assay ID	**Genotype**	**NCBI SNP Reference**	**Context Sequence**
C__89555688_30	*APOL1* $G1^{+M}$	rs60910145	AGGAGCTGGAGGAGAAGCTAAACAT[T/G*]CTCAACAATAATTATAAGATTCTGC
C__98253221_10	*APOL1* $G1^{G+}$	rs73885319	CAAGCTCACGGATGTGGCCCCTGTA[A/G*]GCTTCTTTCTTGTGCTGGATGTAGT
C_102754756_10	*APOL1* G2	rs71785313	AGCTAAACATTCTCAACAATAA[TTATAA/*]GATTCTGCAGGCGGACCAAGAAC

*Indicates the minor allele

3.6.2. SNP Genotype Sanger sequencing

Sanger sequencing was performed to confirm major and minor alleles to serve as positive controls in qPCR genotyping. Sequencing primers for *APOL1* G2 (rs71785313), *APOL1* $G1^{G+}$ (rs73885319) and *APOL1* $G1^{+M}$ (rs60910145) were designed using the oligo design tool (IDT, USA). Using this program; parameters such as the melting temperature, hairpin loop, and self-dimerization were calculated. The oligonucleotide parameters for *APOL 1* G1 and *APOL1* G2 are illustrated in Tables 3.3 and 3.4, respectively.

Table 3.3 Forward and reverse primer oligonucleotide parameters (*APOL1* G1 ($G1^{G+}$ rs73885319 and $G1^{+M}$ rs60910145)

Forward Primer: 5’ CTTCAGTCAGTACCGCATGC 3’	
Length:	20 bp
GC Content	55 %
Tm	59
Hairpin formation	0
Self-Dimer	-5.34
Hetero Dimer	-2.15
Reverse Primer: 5’ GGCATATCTCTCCTGGTGGC 3’	
Length:	20 bp
GC Content	60 %
Tm	57.6
Hairpin formation	0.81 (3’ end)
Self-Dimer	-3.91 (internal)
Hetero Dimer	-4.7 (internal)

bp-base pair; T-thymine; A-adenine; G-guanine; C-cytosine; Tm-annealing temperature.

Below is a DNA sequence of the leading strand showing the *APOL1* G1 ($G1^{G+}$ rs73885319 and $G1^{+M}$ rs60910145) SNPs:

GGAACCTGGGATGGAGTTGGGAATCACAGCCGCTTTGACCGGGATTACCAGCA
GTACCATGGACTACGGAAAGAAGTGGTGGACACAAGCCCAAGCCCACGACCTG
GTCATCAAAAGCCTTGACAAATTGAAGGAGGTGAGGGAGTTTTTGGGTGAGAA
CATATCCAACTTTCTTTCCTTAGCTGGCAATACTTACCAACTCACACGAGGCATT
GGGAAGGACATCCGTGCCCTCAGACGAGCCAGAGCCAATCTTCAGTCAGTACC
GCATGCCTCAGCCTCACGCCCCCGGGTCACTGAGCCAATCTCAGCTGAAAGCG
GTGAACAGGTGGAGAGGGTTAATGAACCCAGCATCCTGGAAATGAGCAGAGGA
GTCAAGCTCACGGATGTGGCCCCTGTAAGCTTCTTTCTTGTGCTGGATGTAGTC
TACCTCGTGTACGAATCAAAGCACTTACATGAGGGGGCAAAGTCAGAGACAGC
TGAGGAGCTGAAGAAGGTGGCTCAGGAGCTGGAGGAGAAGCTAAACATTCTCA
ACAATAATTATAAGATTCTGCAGGCGGACCAAGAACTGTGACCACAGGGCAGG
GCAGCCACCAGGAGAGATATGCCTGGCAGGGGCCAGGACAAAATGCAAACTTT
TTTTTTTTTCTGAGACAGAGTCTTGCTCTGTCGCCAAGTTGGAGTGCAATGGTG
CGATCTCAGCTCACTGCAAGCTCTGCCTCCCGTGTTCAAGCGATTCTCCTGCCT
TGGCCTCCCAAGTAGCTGGGACTACAGGCGCCTACCACCATGCCCAGCTAATT
T

Green highlight (A) – missense mutation G1 rs73885319 (A > G); Red highlight (T) – missense mutation G1 rs60910145 (T > G); Blue highlight- Forward primer binding site; Pink highlight- Reverse primer binding site

Table 3.4 Forward and reverse primer oligonucleotide parameters (*APOL1* G2 rs71785313).

Forward Primer: 5' AAGCACTTACATGAGGGGGC 3'	
Length:	20 bp
GC Content	55 %
Tm	57.5
Hairpin formation	1.04 (Internal)
Self-Dimer	-5.38 (Internal)
Hetero Dimer	-4.67(Internal)
Blast	2 Hits
Reverse Primer: 5' GGCATATCTCTCCTGGTGGC 3'	
Length:	20 bp
GC Content	60 %
Tm	57.6
Hairpin formation	0.81 (3' end)
Self-Dimer	-3.91 (Internal)
Hetero Dimer	-4.67(Internal)
Blast	1 Hit

bp-base pair; T-thymine; A-adenine; G-guanine; C-cytosine; Tm-annealing temperature.

Below is a DNA sequence of the leading strand showing the *APOL1* G2 (rs71785313) SNPs:

TGGAAATGAGCAGAGGAGTCAAGCTCACGGATGTGGCCCCTGTAAGCTTCTTT
CTTGTGCTGGATGTAGTCTACCTCGTGTACGAATCAAAGCACTTACATGAGGGG
GCAAAGTCAGAGACAGCTGAGGAGCTGAAGAAGGTGGCTCAGGAGCTGGAGG
AGAAGCTAAACATTCTCAACAATAATTATAAGATTCTGCAGGCGGACCAAGAAC
TGTGACCACAGGGCAGGGCAGCCACCAGGAGAGATATGCCTGGCAGGGGCCA
GGACAAAATGCAAACTTTTTTTTTTTTCTGAGACAGAGTCTTGCTCTGTCGCCAA
GTTGGAGTGCAATGGTGCGATCTCAGCTCACTGCAAGCTCTGCCTCCCGTGTT
CA

Green highlight- Forward primer binding site, Red highlight- *APOL1* G2 deletion position, Blue highlight- Reverse primer binding site

3.7. Conventional PCR optimization

For qPCR optimization and to enable DNA sequencing, two samples were chosen at random (HAART 508 and HAART 272). The Amplitaq® Gold 360 Polymerase reagents (Applied Biosystems, cataloque number: 439881, lot number: 01254421) were used for these reactions, as described by the manufacturer, with 12.5 ng template DNA added per 20 μl reaction (Table 3.5). Two different annealing temperatures were tested during the PCR cycle namely 58°C and 62°C (Table 3.6). Conventional PCR was performed on the SimpliAmp Thermal cycler (Applied Biosystems by Life Technologies, USA) to determine which of the two was the optimal annealing temperature for the assays. Both samples were tested on the two temperatures. This optimization step tested for the two annealing temperatures as well as the designed primers for sequencing. All test subjects passed. It was determined that the extraction method worked, the two temperatures were determined to be optimal, and the primers also worked. The annealing temperature that was selected for the remainder of the experiments was 62°C.

Table 3.5 Conventional PCR reaction mixture

Reagent	**Volume (μl)**
Amplitaq Gold 360 Mastermix	10
Nuclease free water	4
Forward primer (10 μM)	2
Reverse primer (10 μM)	2
DNA (12.5 ng)	2

Table 3.6 Conventional PCR Cycling conditions

Temperature	**Time**
95°C denaturation	10 seconds
58 and 62°C annealing	1 min
72°C elongation	1 min

3.7.1. Gel electrophoresis

PCR products were run on a 2% (w/v) agarose gel (Cleaver scientific, cataloque number: 9012-36-6, lot number: 14180928), set with TAE buffer (cataloque number: 51235, lot number: 0000637709) together with a size standard (Hyperladder II, Sigma-Aldrich®, cataloque number: 17-11-00050, lot number: 07110830.2) to determine DNA fragmentation. GelRed® Biotium Inc (Fermont, USA, cataloque number: 41001) was used to visualize the DNA when loading it into the agarose gel wells. The gel was run for 30 minutes at 120V and visualized using UV light. Figure 3.3 is an example of PCR gel electrophoresis, indicating amplicon fragments of 356 bp for samples that was optimized, and different temperatures tested for.

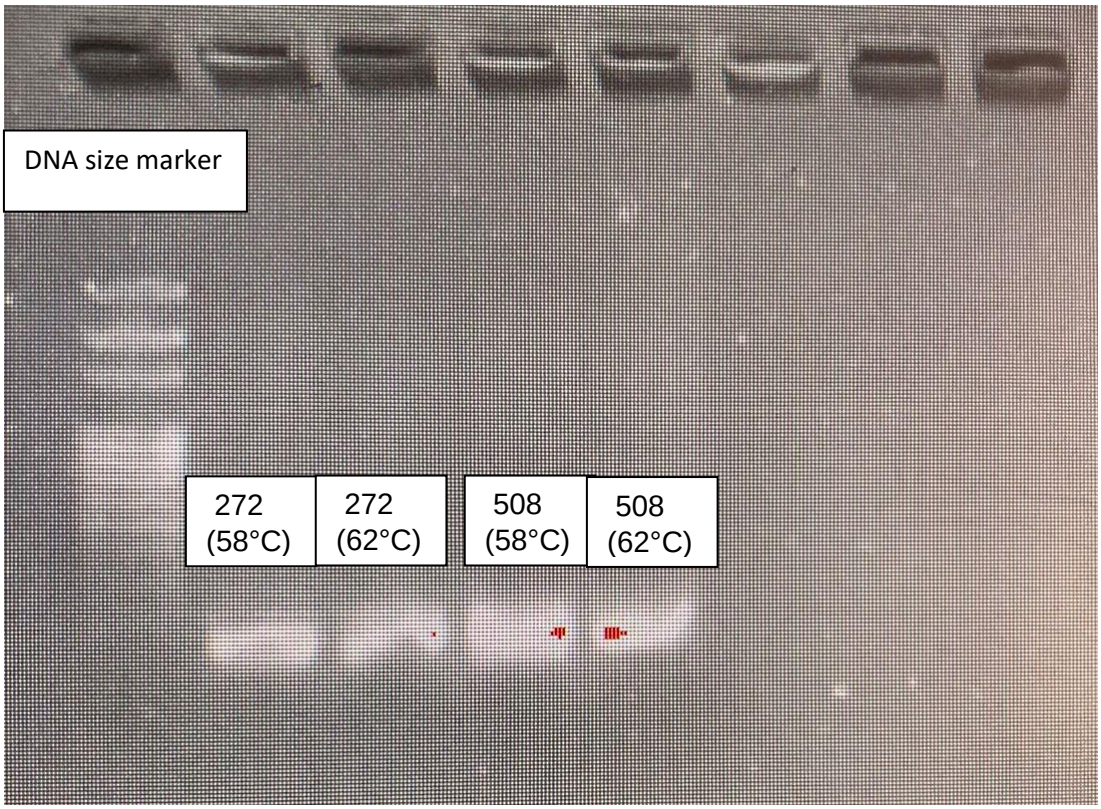

Figure 3.3 Gel electrophoresis images of PCR amplification products before performing Sanger sequencing.

A 2% (v/w) TAE agarose gel was electrophoresed and visualized by GelRed® stain. Lane 1: 500 bp DNA molecular weight marker (Hyperladder II Invitrogen, USA); lane 2 - HAART 272 at 58°C, lane 3 HAART 272 at 62°C, lane 4 - HAART 508 at 58°C and lane 5 - HAART 508 at 62°C.

3.7.2. PCR clean up

The PCR amplicon was cleaned up before submitting it to the Sanger sequencing PCR, using 2 μl of ExoSAP-IT® Express PCR product Clean up (Affymetrix USB, USA, cataloque number: 75000.200.UL, lot number: 01244584) added to 5 μl of PCR amplicon. ExoSAP is a combination of patented Exonuclease 1 and Shrimp Alkaline Phosphatase (SAP) enzymes that remove excess primers and nucleotides from PCR amplification product. The amplicon and ExoSAP mixture was incubated at 37°C for 4 minutes and had further incubation at 80°C for 1 minute for enzyme inactivation.

3.7.3. Sequencing PCR

For sequencing, the Big Dye Terminator V3.1 cycle sequencing kit (Applied Biosystems by Life Technologies, USA, cataloque number: 4336917, lot number: 01021333) was used. Cleaned PCR product that served as template (2 μl) was mixed with 2 μl of 5x sequencing buffer and 1 μl of premix reagent which contains dye terminators, deoxynucleotide triphosphates, AmpliTaq DNA Polymerase, FS, rTth pyrophosphatase, magnesium chloride and buffer. In the reaction was also 1 μl of forward/reverse primer (3.2 pmol) and 4 μl nuclease free water making up 10 μl reaction. As suggested by the manufacturer kit inserts. Table 3.7 represents sequencing cycling conditions.

Table 3.7 Sequencing PCR Cycling conditions

Temperature	**Time**
96°C	1 minute
96°C	10 seconds
65°C	5 seconds
60°C	4 minutes
4°C	∞

25 cycles

3.7.4. Purification of the sequencing product

For pre- sequencing clean-up of the PCR product, a 2 ml collection tube containing a filter column was filled with 10 μl of sequencing PCR product and 240 μl of sequencing binding buffer (PCR purification kit, Zymo Research, cataloque number: D405, lot number: 221427). The sample was centrifuged at 13000 rpm for 30 seconds. Sequencing wash buffer was added, and it was centrifuged at 13 000 rpm for 30 seconds. After moving the filter column to a fresh 1.5 ml tube, 20 μl of Hi Dye injection buffer was added, and the tube was centrifuged once more at 13 000 rpm for 30 seconds**.** The sample was denatured at 95°C for 5 minutes, followed by 5 minutes of snap cooling on ice. Finally, the sample was loaded onto the ABI Genetic Analyzer 3500 (Applied Biosystems by Thermo Fisher Scientific) for sequence analysis.

3.7.5. Electropherogram and Sequence analysis

Chromas (Technelysium, Pty Ltd) was used to visually inspect and analyse the generated sequences on the electropherogram. The sequences of study participants with and without the *APOL1* G2 deletion (rs71785313) were aligned against the most recent human reference sequence GRC38.p14 using LALIGN online software. The reference allele was 5'-ATAATTATAA-3' and the variation allele: 5'-ATAA-3'. The human reference sequence from NCBI and Ensemble reference databases were used for comparison with the study participants. A 6 bp deletion indicated a positive result. Varsome was used to determine the pathogenicity of this variant using the African population frequencies and was found to be benign as there is conflicting evidence regarding its pathogenicity. Apart from employing Varsome, ClinVar was utilized, revealing that rs71785313 has not been documented within ClinVar's database. The same methods were followed for *APOL1* G1 ($G1^{G+}$ rs73885319 and $G1^{+M}$ rs60910145). For *APOL1* $G1^{G+}$ rs73885319; the reference allele is 5'-CCTGTA**A**GCTTC-3' and the variant is 5'-CCTGTA**G**GCTTC-3', a single base pair change from A to G indicates the SNP. For $G1^{+M}$ SNP (rs60910145) the reference allele is 5'-AAACAT**T**CTCAAC-3' while the variant is represented by a single nucleotide change from T to G (5'-AAACAT**G**CTCAAC3-').

3.8. qPCR SNP genotyping

The genotype detection of the *APOL1* G1 and G2 deletion variations were performed using the QuantStudio 5 Real Time PCR system (Applied Biosystems). The Applied Biosystems™ TaqMan® SNP Genotyping Assays using TaqMan® 5' nuclease chemistry were used to amplify and identify the *APOL1* variants in pure genomic DNA samples. Each assay contained both forward and reverse primers which are sequence specific and two TaqMan® minor groove binder (MGB) probes with non-fluorescent quenchers (NFQ). The probes consist of a VIC™labeled probe that detects Allele 1 sequence and a FAM™labeled probe that detects Allele 2 sequence. The wavelengths of excitation and detection for VIC™ are 470±10 nm and 510±5 nm, respectively, and for FAM™; 530±5 nm for excitement and 557±5 nm for detection. The assay context sequence was employed for SNP detection, representing the nucleotide sequence surrounding the SNP region. This sequence aligns with the positive genome strand orientation as per the National Centre for Biotechnology Information (NCBI) reference genome. The order of the SNP alleles corresponds to the association with probe reporter dyes, where, where [Allele 1 = VIC™ (yellow) dye / Allele 2 = FAM™ (green) dye]. The SNP alleles are always enclosed in square brackets. Allele to dye associations for the G1 allele is represented by Table 3.8.

Table 3.8 *APOL1* G1 Allele-to-dye associations

Context sequence clip	**NCBI SNP Reference**	SNP alleles	VIC™ associated allele	FAM™ associated allele
AGGAGCTGGAGGAGAAGCTAAACAT[T/G]CTCAACAATAATTATAAGATTCTGC	rs60910145	[T/G]	T	G
CAAGCTCACGGATGTGGCCCCTGTA[A/G]GCTTCTTTCTTGTGCTGGATGTAGT	rs73885319	[A/G]	A	G
AGCTAAACATTCTCAACAATAA[TTATAA/-]GATTCTGCAGGCGGACCAAGAAC	rs71785313	[TTATAA/*]	ATAATTATAA	ATAA-----

3.9. Assay set up conditions.

For assay setup conditions, 40X TaqMan® SNP Genotyping assays (cat. 4351379) with 300 reactions were used. These assays were initially diluted to a 20X working stock using 1X TE buffer and subsequently stored at -20 to -15°C, ensuring protection from exposure to light. In preparing the reaction mix, TaqMan® SNP Genotyping assay (20X) was combined with TaqMan® Genotyping master mix. Following this, DNA was incorporated into the reaction mix, as outlined in Table 3.9 and Table 3.10. The final reaction volume was 25 µl, and the mixture was thoroughly vortexed. Subsequently, standard mode thermal cycling conditions were applied, as detailed in Table 3.11.

Table 3.9 Recommended reaction types for each assay

Reaction type	**Component**
Test sample	DNA samples with unknown genotype at the polymorphism of interest
No template control	DNase free water
(Optional) Positive control	DNA sample with known genotype at the polymorphism of interest

Table 3.10 SNP genotyping reaction mixture

Reagent	**Volume (µl)**
TaqMan® Genotyping Master Mix	12.5
20X Assay Working Stock (containing the primer and probe sets)	1.25
Nuclease free H_2O	9.25
DNA (5 ng)	2
Total	25

Table 3.11 Cycling conditions

Step	Predesigned TaqMan® SNP Genotyping Assays		
	Temperature	Time	Cycle
Polymerase activation	95°C	10 minutes	Hold
Denaturation	95°C	15 minutes	40
Annealing/Extension	60°C	1 minute	

3.10. Data analysis

3.10.1. SNP genotyping

The visualization and analysis of genotype calling were conducted using the QuantStudio™ Design and Analysis Software V4.2 from Applied Biosystems by Thermo Fisher Scientific. The default analysis call settings of the software were used as follows: for Data analysis: data was analysed from pre-PCR read and post-PCR read, with auto caller enabled and a quality value of 95.0.

Additionally, the raw run files underwent analysis through the Design and Analysis 2 Software (DA2), accessible on the Thermo Fisher Connect Platform; previously called the Thermo Fisher Cloud. This software has been updated from the QuantStudio™ Design and Analysis Software as issued on qPCR instruments and utilizes algorithms known for generating more precise genotype calls and superior quality control capabilities. Using this software Data analysis settings used was Analyse Real-time dRn data. This option shows the data when the baseline has already been subtracted, but also shows the traces showing the migration patterns for each reaction.

3.11. Study population Statistics

Statistical analysis of data in this study was performed by calculating allele frequencies and gene frequencies using the 2x2 contingency table (VassarStats). Medians, means, standard deviations, and t-tests were used to compare variables. Statistical significance was set at p-values less than 0.05. A comparison of the frequencies of between males and females was also conducted as well as the average age of individuals with high-risk genotypes. To compare these frequencies studies conducted on the South African population as well as 1000 genome were studied. The 1000 Genomes Project is an international research effort that that was initiated in 2007 which aims to catalogue human genetic variations at the genomic level from a large number of individuals from different populations around the world. The primary objectives of the project include identifying common and rare genetic variants, understanding their distribution across populations, and exploring their implications for health and disease (Devuyst, 2015).

3.12. Genotype Statistics

Allele and genotype frequencies were calculated for the study population and different phenotypic groups. The Hardy-Weinberg equilibrium (HWE) was piloted to determine if the genotypic frequencies of selected SNPs adhered to the assumptions of the HWE model. The HWE entails the following assumptions: infinite population; discrete generations; no selection; random mating; no new mutations; no migration in and out of the population and equal initial genotype frequencies in the two sexes. The observed genotypic frequencies were compared with the expected genotypic frequencies using the chi-square statistical method. A statistically significant difference between the observed and expected genotype frequencies was indicated by a p-value of less than 0.05 and used to indicate if the SNP adhered to the HWE model. HWE calculations are as follows: $(\text{Observed} - \text{Expected})^2 / (\text{Expected})$ (Court 2005-2008). Allele and genotypic frequencies were determined using calculation tools in Microsoft Excel (https://www.xlstat.com/en/). The study population, HIV positive with kidney disease markers was compared to the controls. The association between *APOL1* risk genotype, CKD, and HIV status was determined using contingency tables with significance set at $p < 0.05$.

Chapter 4

Results and discussion

4.1. Study Population

The study included 220 adult individuals of African descent residing in Mangaung, Bloemfontein, South Africa. Characteristics of all the participant samples tested, including the HIV status, sex, age, protein mg/dl (indication of CKD) and BMI have been captured in Appendix C. Archived participant samples were initially divided into two groups: 110 of those with HIV and 110 without. The two groups were further divided into sub-groups of 55 with CKD and 55 without. Samples were regarded as being positive for CKD markers when the protein in urine (mg/dL) was trace to + (positive), and + (positive) to greater, they were regarded as negative when the protein was negative. Under the new KDIGO classification, albuminuria ≥30 mg/24 hours may be used as a substitute for a urinary total protein or albumin excretion rate above the normal threshold for age (KIDGO, 2017). However, at the time of the study, the protein in urine dipstick was used to determine CKD.

The average age of all participants was 41.59 years, with the youngest being a 28-year-old male who was HIV negative and had kidney disease markers. The oldest participant was a 77-year-old female with neither HIV nor CKD. The average age ranged from 38.55 as the lowest and 45.20 being the highest. There was no major difference in age between the HIV positive group and the control group. The average BMI for the study population was 25.37.

It is common knowledge that as we age, so do our kidneys and GFR decreases with age. Age-related changes in kidney function and structure, combined with the presence of comorbidities and medication use, increase the susceptibility of older adults to kidney disease and its complications. There is decreased renal function, exacerbated progression of CKD due to reduced renal reserve and a decline in immune function, which may impair the kidneys' ability to defend against infections and autoimmune diseases that can damage renal tissue.

4.2. Study Population phenotype

4.2.1. HIV status

Focusing on HIV, an analysis of the study population was conducted to compare various demographic data (BMI, age, and sex) and clinical characteristics between individuals with a positive HIV status (n=110) and those without (n=110). Table 4.1 demonstrates the classifications of BMI (World Health Organization, 2000). A statistical table was generated to present these comparisons. Table 4.2 includes p-values that assess the significance of differences in age between the two sexes with a p-value of 0.681 in female participants and an average mean age of 41.5 with HIV and 42.3 without HIV. Comparison of the males indicated no differences with a p-value of 0.684 and an average mean age of 41.3 with HIV and 42.1 without. As expected, the average BMIs differed significantly (Hurley, *et al*., 2011; Puoane, *et al*., 2002; Wandai, *et al*., 2020) between females (28.15) and males (21), irrespective of the HIV status. Comparing the BMIs between HIV positive and negative irrespective of sex, the BMIs were 22.8 and 26.5, respectively. A study by Phalane, *et al*., 2018 conducted on a South African population about metabolic syndrome and renal function in African individuals infected by HIV, found the BMI of 22.8 in HIV positive individuals (n=114) and 27.4 in unaffected individuals (n=114). The BMI is similar to the results we obtained; the average BMI of HIV negative individuals is higher than HIV positive individuals. The mean BMI between females with HIV (25.4) was lower than that of the female group without HIV (30.9), however not statistically significant with a p-value of 0.069. The mean BMI between males with a positive HIV status (19.9) and with a negative HIV status (22.1) was statistically significant with a $p<0.0001$. The number of female participants with HIV was slightly higher (53.6%) than that of males (46.4%). A study researching obesity and HIV with 88 HIV positive ART naïve Africans from Durban KwaZulu Natal, females were found to be overweight with an average BMI of 29.9 (n=77), while males had a normal average BMI of 24.4 (n=7), only seven males were included in the study (Biggs and Spooner, 2018). The HIV positive females in our study also had an average BMI that placed them in the overweight category (25.4), with the HIV positive males having a normal average BMI of 19.9. Participants without HIV had the same amounts of female and male participants. However, it must be remembered that the sample size was selected to fit into two groups with equal amount of HIV positive and negative status,

thus not giving any indication of HIV status of the rest of the populations residing in the Mangaung area.

Table 4.1 BMI Classification according to the World Health Organization (World Health Organization, 2000).

BMI (Kg/m^2)	**Classification**
<18.5	Underweight
18.5-24.9	Normal
25-30	Overweight
>30	Obese

Table 4.2 Summary of data distribution and p-values (statically significant at p<0, 05) of HIV positive samples (Females n=59, Males n=51) and HIV negative samples (Females n=59, Males n=51).

Variable		**HIV+**				**HIV-**				
		Min	**Max**	**Median**	**Mean (SD)**	**Min**	**Max**	**Median**	**Mean (SD)**	**P-value**
BMI Kg/m^2	**F**	15.8	41.8	24.3	25.4 (7.8)	17.5	44.4	30.5	30.9 (6.5)	0.069
	M	14.5	35.5	19.3	19.9 (5.6)	16.5	36.2	20.8	22.1 (7.00)	<0.0001
AGE years	**F**	30	64	40	41.5 (8.5)	29	77	41	42.3 (10.8)	0.681
	M	30	56	42	41.3 (6.8)	28	72	38	42.1 (11.3)	0.684

F= Female, M= Male, SD= Standard deviation

4.2.2. CKD indicators

Various demographic data (BMI, age, and sex) and clinical characteristics between individuals with positive CKD markers (n=110) and those without (n=110) were analysed. Table 4.3 indicates the average BMI as well as age for males and females as well as the statistical significance using the p-values, providing valuable insights into potential relationships and differences within the study population. The p-values for the average age for males were 0.066 and 0.681 for females. The overall mean age of participants being 40.1. The percentage of female participants was slightly higher than males at 52.7%.

Two South African studies that have reported on the prevalence of CKD. Both were conducted in the Western Cape and included representatives of the major races in South African. In a study conducted by Matsha, *et al*., 2013, the overall mean age of participants with CKD was 52.9 years with females constituting 75.3% of participants, aimed at classifying kidney disease in different classes. Looking only at individuals with kidney disease, the average mean age in our study was lower at 40.1 years with 40.5 years for females and 39.7 years average age for males. The average BMIs followed the same pattern in this study with 23.15 (normal BMI), males having an average of 18.8 and females at 27.5. In the Matsha, *et al.* study from 2013, it was observed that the average BMI stood at 29.9, indicating that females had an average BMI classifying them as obese (31.0), while males were categorized as overweight (26.6) on average. They did not report on the prevalence of HIV.

The study conducted through a cross-sectional survey about CKD status (Adeniyi, *et al.,* 2017) the mean age of participants was 46.3 and females again being the major constituents at 70.3%. The prevalence of HIV was also not reported by this study. This study differs from the two conducted in the Western Cape by specifically selecting archived patient samples that were categorized into four equally sized groups. The average participant age was lower compared to the studies in the Western Cape. The balance in sex was achieved in this study by matching equal numbers of male and female participants across the four study groups, and considerations were made for their HIV statuses.

Table 4.3 Summary of data distribution and p-values (statically significant at p<0,05) of CKD positive samples (Females n=58, Males n=52) and CKD negative samples (Females n=60, Males n=50)

Variable		CKD+				CKD-				p-value
		Min	Max	Median	Mean (SD)	Min	Max	Median	Mean (SD)	
BMI	F	15.3	44.4	27.7	27.5 (8.1)	15.8	42	28.8	28.8 (7.3)	0.352
	M	14.5	33.3	19.7	18.8 (7.2)	15.1	36.2	20.8	22.1 (6.2)	0.013
AGE	F	29	73	40	40.5 (8.5)	29	77	42	43.1 (10.6)	0.146
	M	28	72	39	39.7 (9.3)	30	60	42	43.2 (9.3)	0.066

F= Female, M= Male, SD= Standard deviation

4.3. HIV and CKD population summary

The study population was chosen from the larger study to fit into four groups of equal participants (n=55 in each group), the participants were sex matched. The groups were as follows: HIV+ CKD+; HIV+ CKD-; HIV- CKD+ and HIV- CKD-. Table 4.4 indicates the age and sex of the four phenotypic groups. The average BMIs for each phenotypic group are as follows: HIV- CKD+ was 21.14 for male and 25.74 for females, HIV- CKD- males, the BMI was 19.86 and 29.89 for female. In the HIV+ CKD+, the average BMI for males was 17.89 and was the lowest in the whole study and for females it was 24.17, for HIV+ CKD- males the average BMI was 21.14 and 25.74 for females. HIV negative individuals were more on the overweight side that HIV positive individuals. The demographics (age, BMI, and sex) between all study groups did not have major differences (figure 4.1). The reason behind the miniscule differences in age and sex is because the study groups were sex matched and samples from adults were used for study and the ages ranged from 28 to 77. The p-values for the BMI between the four phenotype groups, HIV+ CKD+; HIV+ CKD-; HIV- CKD+ and HIV- CKD- were 0.013 for males and for females was 0.352. The group without HIV and CKD had the highest BMI and the group with a positive HIV

status and with CKD markers had the lowest BMI. This is to be expected since the burden of disease for both HIV and CKD may cause muscle and weight loss (Grinspoon and Mulligan, 2003) (Mak, *et al*., 2011)

Table 4.4 Sex, average age, and the average BMI in each phenotypic group

Sex	**HIV+ CKD+**			**HIV+ CKD-**			**HIV- CKD-**			**HIV- CKD-**		
	n	**Av BMI**	**Av AGE**	**n**	**Av BMI**	**Av AGE**	**N**	**Av BMI**	**Av AGE**	**n**	**Av BMI**	**Av AGE**
Male	26	17.89	40.81	25	21.14	41.44	26	19.86	38.65	25	23.70	45.2
Female	29	24.17	38.55	30	25.74	44.03	29	29.89	42.17	30	31.51	38.65

n= number of samples

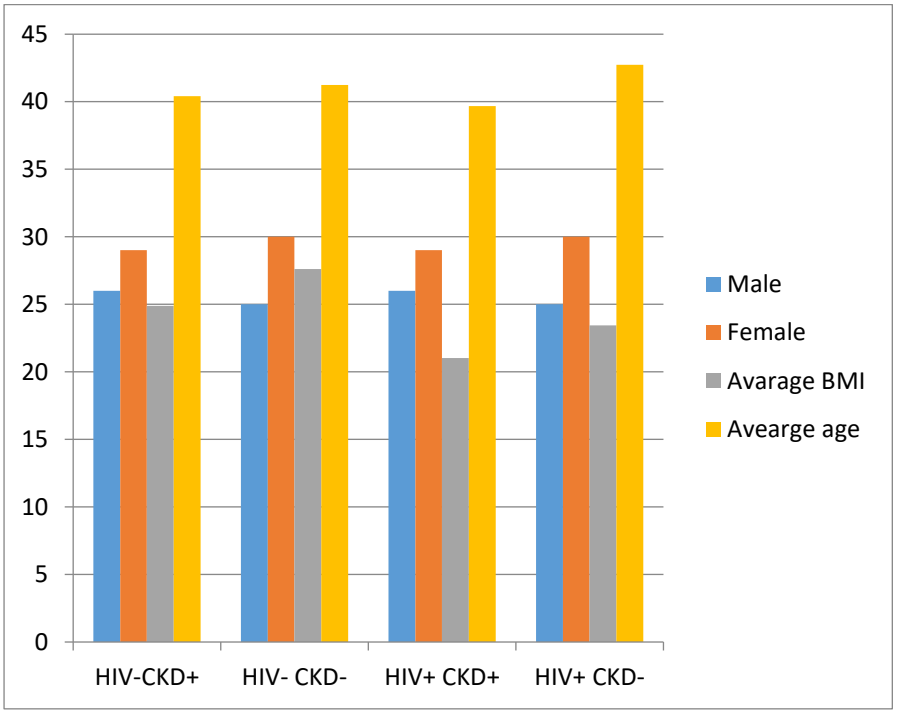

Figure 4.1 Sex, age and the average BMI plotted against each phenotypic group.

4.4. Genotype results

The study population were screened for the three SNPs previously described to be associated with the development kidney disease in individuals of African descent (Hung, *et al*., 2022; Pollak and Friedman, 2023; Abdu, *et al*., 2022). Custom TaqMan® SNP Genotyping Assays (Thermo Fisher Scientific, Waltham, MA) were used to detect *APOL1* gene variants. For clinical significance, the SNPs are denoted as G1, encompassing rs73885319 (p.S342G) (Assay ID: C__98253221_10) and rs60910145 (p.I384M) (Assay ID: C__89555688_30), while G2 specifically refers to rs71785313 (Assay ID: C_102754756_10). The results are represented as genotypes. The genotypes were generated using the Quantstudio 5^{TM} (Applied Biosystems by Thermo Fisher Scientific) qPCR instrument and visualized on the Allelic discrimination plot graph. Allelic discrimination plots are as a result of the fluorescence released by the fluorophore bound to the particular probe when it is separated from its quenching molecule during PCR amplification. The qPCR instrument, which shows the variation in PCR cycle number that amplification curves cross the baseline value, depicts the fluorescence produced during PCR in amplification plots. The junction of an amplification curve and a threshold line is known as the threshold cycle, or Cq (quantification cycle) value (Bustin, *et al.,* 2009). It measures a PCR reaction's relative target concentration.

An allelic discrimination plot of genotypes for *APOL1* G1^{G+} is shown in figure 4.2 as an example of the allele determination as generated by the instrument. The three genotypes are represented as three distinct clusters on the data plot. These clusters show how samples with similar genotypes aggregate together. A VIC™ labelled probe indicate the Allele 1 SNP sequence that is displayed by the red colour representing a homozygous wild type genotype (G0/G0). A FAM™-labelled probe that detects the Allele 2 SNP sequence is displayed by the blue colour and represents a homozygous mutant genotype (G1/G1). The green colour represents the heterozygous genotype (G0/G1) due to signals from both FAM™-labelled probe and VIC™ labelled probes. Finally, a black block represents the no template control, which is a reaction that contains nuclease free water instead of template DNA.

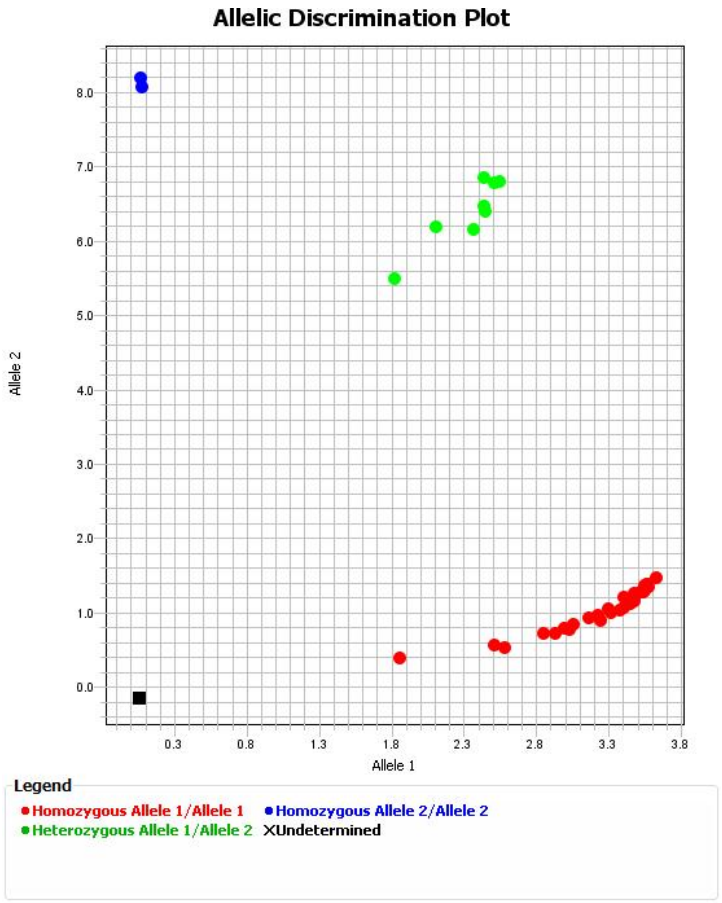

Figure 4.2 qPCR allelic discrimination plot for *APOL1* G1^{G+}**,** rs73885319 (p.S342G) (Assay ID: C__98253221_10).

NC= Negative control, PC= Positive control, NTC= No template control.

Genotypes of the participants are represented as follows: Red colour = (AA) homozygous wild type / common allele genotype [013, 033, 038, 054, 055, 056, 098, 113, 119, 122, 143, 146, 150, 152, 162, 463, 176, 181, 186, 193, 219, 220, 233, 234, 265, 279, 298, 337, 437, NC1, NC2]; the green colour = (AG) heterozygous genotype [030, 037, 039, 140, 151, 224, PC3, PC4]; the blue colour = (GG) homozygous mutant / rare allele genotype [PC1, PC2]; and the black colour = No template control [NTC].

An example of the *APOL1* G1^{+M} genotypes is displayed in Figure 4.3. The probes are labelled the same for all assays in this study with the VIC™labeled probe detecting the homozygous wild type genotype (G0/G0) indicted in red and the FAM™labeled probe detecting the homozygous mutant genotype (G1/G1) in blue and green dots indicating the heterozygous genotype (G0/G1). The black blocks represent the no template control, which is reaction done using nuclease free water instead of template DNA. Within this figure, most genotypes form a cluster represented by the colour blue, signifying the mutant or variant genotype. However,

it is important to note that this depiction serves as an example. The wild-type allele's frequency predominates across the study population, as detailed in Table 4.5.

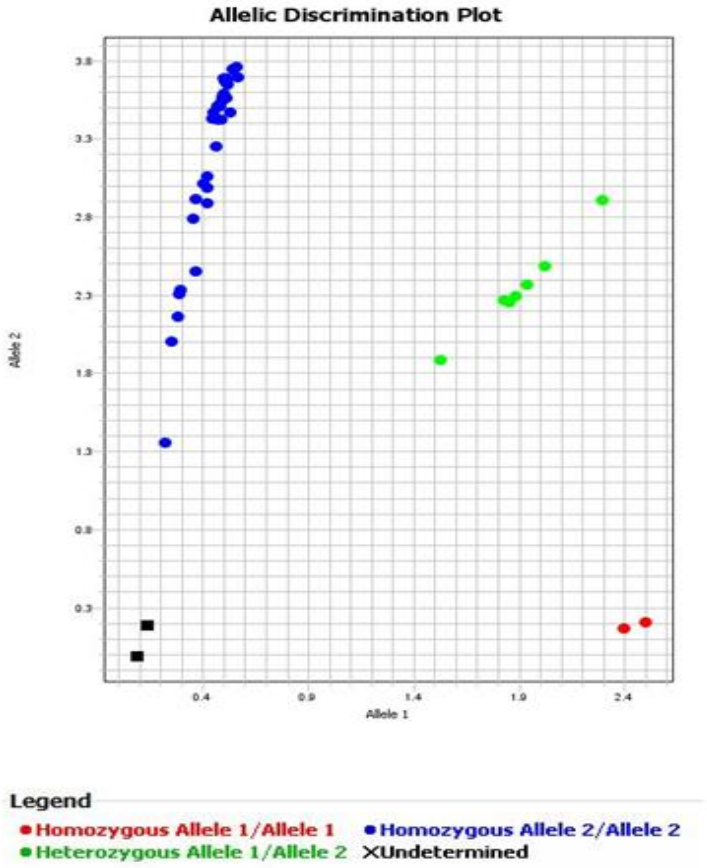

Figure 4.3 *APOL1* G1$^{+M,}$ rs60910145 (p.I384M) (Assay ID: C__89555688_30).

NC= Negative control, PC= Positive control, NTC= No template control. Genotypes of the participants are represented as follows: Blue colour = (TT) homozygous wild type / common allele genotype [013, 030, 033, 038, 054, 055, 056, 098, 113, 119, 122, 143, 146, 150, 152, 162, 463, 176, 181, 186, 193, 219, 220, 233, 234, 265, 279, 298, 337, 437, NC1, NC2]; the green colour = (TG) heterozygous genotype [037, 039, 140, 151, 224, PC3, PC4]; the red colour = (GG) homozygous mutant / rare allele genotype [PC1, PC2]; and the black colour = No template control [NTC1, NTC2]

Genotypes for *APOL1* G2 which consists of a 6–base pair in-frame deletion, rs71785313, an example of one of the reaction data is depicted in Figure 4.4. The VIC™labeled probe detects the Allele 1 sequence that is displayed by the red colour representing a homozygous wild type genotype (G0/G0). A FAM™labeled probe detects the Allele 2 deletion sequence is displayed by the blue colour and represents a homozygous mutant genotype (G2/G2). The green colour indicates the heterozygous genotype (G0/G2) with one 6 bp deletion allele and one wild type

allele. Due to the design of the assay and probe binding positions, the plot looks different for the SNP assay plots. However, three distinct clusters are present indicating the three genotypes. Furthermore, with each run two sequence verified homozygous deletion genotype and homozygous wild type controls are added and taken into consideration by the instrument algorithm calling the genotypes.

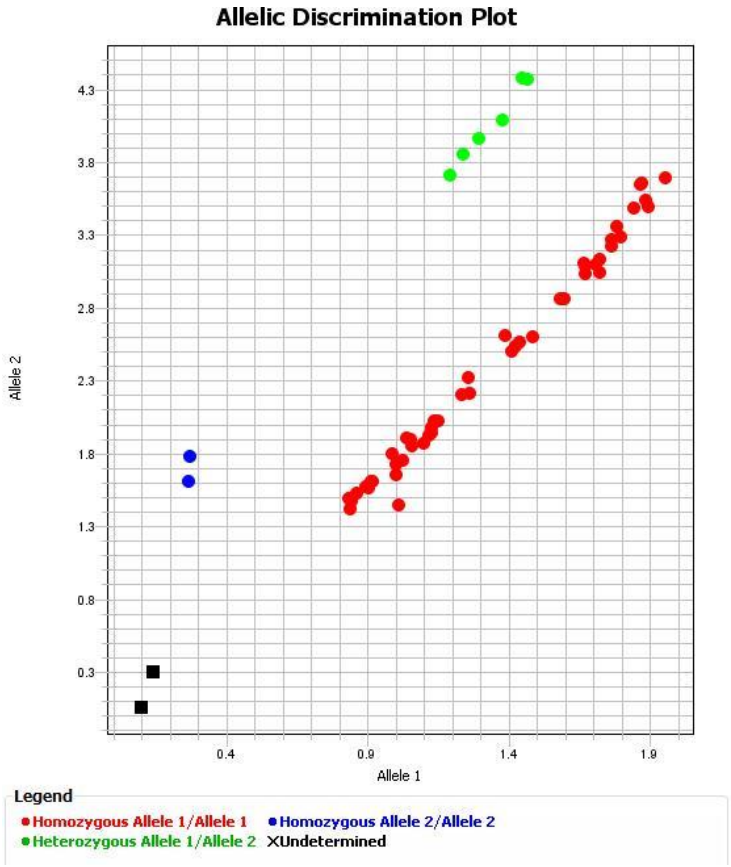

Figure 4.4 *APOL1* G2 rs71785313 (Assay ID: C_102754756_10).

I: Insertion; D: Deletion; H: Heterozygous

NC= Negative control, PC= Positive control, NTC= No template control.

Genotypes of the participants are represented as follows: Red colour = (II) homozygous wild type / insertion [003, 005, 015, 016, 023, 036, 040, 041, 046, 047, 052, 053, 058, 061, 068, 069, 074, 116, 131, 167, 176, 256, 259, 278, 285, 291, 305, 330, 336, 351, 364, 365, 383, 390, 397, 410, 412, 421, 431, 458, 468, 469, 485, 502, 506, NC1, NC2]; the green colour = (ID) heterozygous genotype [PC1, PC2, 010, 025, 175, 235]; the blue colour = (DD) homozygous mutant / deletion [PC3,PC4]; and the black colour = No template control [NTC1, NTC2].

4.4.1. Allele frequencies of the *APOL1* variants

Allele frequencies depicting the major and minor alleles for the three SNPs of the *APOL1* gene, rs73885319, rs60910145 and rs71785313, as well as the HWE are indicated for the study population in table 4.5. HWE stands as a fundamental principle in population genetics, disclosing the anticipated relationship between allele and genotype frequencies within a population assuming no evolutionary changes occur. It is based on the assumption that there are no mutations, and the genetic makeup of the population remains unchanged. The second assumption is that there is no migration; no entering or leaving the population and introducing new genetic material. There has to be a large population size leaving no room for random chance to introduce changes to allele frequencies. There is random mating and no natural selection. HWE calculations are as follows: $(\text{Observed} - \text{Expected})^2$ / (Expected) (Court, 2005-2008). When a population is in Hardy-Weinberg equilibrium (HWE), the expected p-value for a goodness-of-fit test examining whether observed genotype frequencies match the expected frequencies should ideally be greater than 0.05 (or 5%). A p-value greater than 0.05 suggests that the observed genotype frequencies do not significantly deviate from the frequencies expected under Hardy-Weinberg equilibrium, supporting the assumption that the population is indeed in equilibrium for the examined genetic loci.

The minor (G) allele frequency for SNP rs73885319 was 0.0886. This is closer to the global allele frequency (0.070) reported by the 1000 genomes Project (Peng, *et al*., 2017), but lower than that reported for the African population (0.259) (Wudil, *et al*., 2021). The same pattern was also observed for the major allele frequency. The minor allele (G) frequency for rs60910145 was 0.0864; this too was lower than that reported by 1000 genomes for the African population at 0.259 and the global allele frequency at 0.069. This SNP also had almost similar major allele frequency as the previous mentioned. The minor allele frequency for the deletion polymorphism rs71785313 was higher at 0.1432 than both the 1000 genomes Africa (0.129) and globally at 0.035. The allele frequencies in this study do not follow what is expected for the African population for all three SNPs. This is to be expected due the small sample size and the fact the participant samples were selected based on phenotype (HIV and CKD status) equal to health non-HIV and CKD participants that is not representative of the entire African population. *APOL1* G1 is expected to be higher

than G2 according to African and Global allele frequencies; however, the study results indicate a higher allele frequency of the deletion than the minor alleles for the G1 genotype. Closer to what has been recorded for the 1000 genomes in Africa. Moreover, when considering the Hardy-Weinberg equilibrium (HWE), both rs73885319 and rs60910145 exhibit p-values of 0.0886 and 0.0864, respectively, and adheres to HWE. However, rs71785313 shows a p-value of 0.0026, suggesting departure from HWE. This deviation might be attributed to the study population's selection criteria, which involves an equal representation of CKD and non-CKD cases, a circumstance typically not encountered in populations where HWE is applicable.

Table 4.5 Allele frequencies for *APOL1* gene, rs73885319 rs60910145 and rs71785313 including data from the ENSEMBL of allele frequencies in the African region and globally as well as HWE.

SNP	**Major allele**	**Minor allele**	**HWE (p-value)**	**1000 genomes (Global)**		**1000 genomes (Africa)**	
				Major allele	**Minor allele**	**Major allele**	**Minor allele**
$G1^{G+}$ (rs73885319)	A:0.9114 (n=200) NALs=401	G:0.0886 (n=20) NALs=39	0.543	A:0.930 (n=4659)	G:0.070 (n=349)	A:0.741 (n=979)	G:0.259 (n=343)
$G1^{+M}$ (rs60910145)	T:0.9136 (n=201) NALs=402	G:0.0864 (n=19) NALs=38	0.584	T:0.931 (n=4660)	G:0.069 (n=348)	T:0.741 (n=980)	G:0.259 (n=342)
G2 (rs71785313)	I:0.8568 (n=188) NALs=377	D:0.1432 (n=32) NALs=63	0.0026	I:0.965 (n=4832)	D:0.035 (n=176)	I:0.871 (n=1152)	D:0.129 (n=170)

NALs: Number of alleles.

A-adenine; C-cytosine; G-guanine; T-thymine; I-insertion; D-deletion; MAF- Minor allele frequency; HWE- Hardy Weinberg Equilibrium.

4.4.2. Genotype frequencies of the *APOL1* variants

Genotype frequencies of participant samples for this study, alongside data from the 1000 genomes Project on a global scale and specifically for the African population, are presented comprehensively in table 4.6. This comparison provides a robust understanding of the genetic variations within our studied cohort concerning broader population data, shedding light on both commonalities and disparities across different demographic groups. The homozygous genotype consisting of the major alleles is clinically regarded as a no-risk genotype. The percentages of variants with no risk [AA =82.73% (rs73885319, TT=83.18 (rs60910145) and Insertion/Insertion = 75.90 (rs71785313)] were the highest in this population. Genotype frequencies for the *APOL1* G1 SNP rs73885319 associated with low and high risk were 0.1681 for A/G and 0.0045 for G/G, respectively. The frequencies for the same SNP reported in 1000 genomes globally database was 0.097 for A/G and for G/G it was 0.021. The genotype frequencies were higher in 1000 genomes Africa database with 0.359 for A/G and 0.080 for G/G. Regarding the no risk genotype (AA), the frequency was 0.8272 for this study and a similar frequency (0.882) was reported in 1000 genomes globally database while 1000 genomes Africa database reported a lower frequency of 0.561.

The *APOL1* G1(M) SNP rs60910145 showed a prevalence of 0.1636 for the T/G genotype, classified as low risk, while the high-risk G/G genotype exhibited a frequency of 0.0045. Comparatively, within the 1000 genomes global database, the frequency observed for T/G was notably lower at 0.097, whereas the G/G genotype displayed a frequency of 0.021. In the 1000 genomes Africa database, a substantial difference was observed with a frequency of 0.357 for T/G and 0.080 for G/G, signifying distinctive allele distributions in the African population compared to the global dataset.

Geographical factors account for the similarities observed between the frequencies of no risk, low risk, and high risk in this study and those found in the global data from the 1000 genomes Project. The 1000 genomes global database included a wider population comprising of not only individuals from areas with *Trypanosomiasis.* It included individuals from East Asia, South Asia, and Europe; all with the frequency of 0.00. America was represented by four sub-populations: Colombia, Mexico, and

Peru with a frequency of 0.00, the last subpopulation Puerto Rico had a frequency of 3%. Individuals from Africa also formed a part of this, but this also includes regions outside the *Trypanosomiasis* belt. Our study follows this same model for *APOL* G1 (both SNPs), and this attributed to the fact that South Africa also falls outside the *Trypanosomiasis* belt. The 1000 genomes Africa database reported higher frequency because Africa is the hot spot for *APOL1*. There are numerous studies performed on different African populations according to geographic regions mainly focusing on regions with *Trypanosoma brucei gambiense,* parasite which is found in 24 countries of west and central Africa. This is supported by *APOL1* statistics in Africa. The prevalence of *APOL1* variants differ extensively by African geographic region with the highest variants reported in West Africans (30%–40%) in Igbo-speaking and Yoruba-speaking Nigerians and Asante-speaking Ghanaians. It has significantly lower rates (5%–12%) in South and East Africa with virtually complete lack in those from the horn of Africa (Ethiopia) (Hung, *et al.,* 2022).

The *APOL1* G2 deletion variant rs71785313 exhibited a frequency of 0.1955 for the low-risk allele ATAA/ATAATTATAA (I/D), while the high-risk allele ATAA/ATAA (D/D) displayed a frequency of 0.0455. The frequency for the low-risk genotype in 1000 genomes global was 0.062 and 0.004 for the high-risk genotype. In the 1000 genomes Africa, the frequencies were 0.224 for the low-risk genotype and 0.017 for the high-risk genotype.

The low-risk frequencies in this study for *APOL1* G1 (both SNPs) were lower than those in 1000 genomes Africa database but higher than those reported globally. However, the high-risk frequencies for *APOL1* G1 were lower than those reported in the 1000 genomes project databases. On the other hand, the *APOL1* G2 high risk genotypes had a higher frequency in this study than those reported by the 1000 genomes project databases. The frequency of the low-risk genotype in this study was greater compared to the global data from the 1000 genomes Project, yet it closely resembled the frequency observed in the 1000 genomes Project's African population.

Regarding both the variants of *APOL1*, it is observed in this study that the *APOL1* G2 variant or mutant allele is more prevalent than that of *APOL1* G1. This does not correlate with 1000 genomes projects which comprised of 2504 participants from 26

populations; the participants were divided into four groups: Asia, Africa, Europe, and admixed populations. The data also deviates from the Gen-Africa study (Hung, *et al*., 2022), which stated that *APOL1* G1 is more prevalent than *APOL1* G2 in 1104 participants. However, this follows the same disease model displayed in a study with 228 individuals conducted by Kasembeli, *et al.,* 2015 on the African population residing in Johannesburg South Africa. The *APOL1* G1 variant has been reported to be more prevalent that G2 variants by multiple studies and this are mainly observed in West and North Africa where *Trypanosoma brucei gambiense* is rampant. The *APOL1* G1 variant is documented to provide protection against *Trypanosoma brucei gambiense*, responsible for approximately 92% of reported cases of Human African Trypanosomiasis (HAT) or sleeping sickness, according to the World Health Organization (World Health Organization, 2023). In contrast, *APOL1* G2 offers protection against *Trypanosoma brucei rhodesiense*, which is commonly found in Eastern and Southern African countries, contributing to about 8% of Human African Trypanosomiasis (HAT) cases, as indicated by the World Health Organization (World Health Organization, 2023).

We hypothesis that the reason behind the study having low frequencies for the *APOL1* G1 genotype is due to the absence of HAT in South Africa as it falls outside the *Trypanosoma brucei gambiense* affected region. The same reason also applies to why the *APOL1* G2 variant is so high in this study (figure 4.5). The *APOL1* G2 protects against the *Trypanosoma* found in Southern Africa. Though *Trypanosoma brucei rhodesiense* is absent in South Africa, we have to take into account the population dynamics including historic migrations which have led to the admixture of the Bantu populations (Choudhury, *et al*., 2021). This could also be due to the small sample volume (which could have been isolated) compared to the other studies. The 1000 genomes project involved 2504 individuals from 26 populations (Peng, *et al*., 2017). Wudil, *et al*., 2021 studied 2458 individuals from five ethnic groups in Northern Nigeria. The 1104 participants reported on by Hung, *et al*., 2022 were from East, South, Central, and West Africa. When comparing the present study to another South African research, a similarity emerges in both studies reporting *APOL1* G2 as more prevalent than *APOL1* G1. It is notable that both studies had relatively small sample sizes, with n=220 in the present study and n=228 in the study conducted by Kasembeli, *et al*. (2015). Additionally, the South African studies did not provide

information about the ethnicities included in their investigations. However, the primary explanation is that *APOL1* gene is not presenting a selective advantage imposed by *Trypanosoma brucei gambiense* within the South African population.

Table 4.6 Genotype frequencies for *APOL1* $G1^{G+}$ (rs73885319), $G1^{+M}$ (rs60910145) and G2 (rs71785313).

SNPs	**Frequency**	**Percentage**	**Expected frequencies**	**1000 genomes (Global)**	**1000 genomes (Africa)**
$G1^{G+}$ (rs73885319)					
A/A	0.8272(n=182)	82.73	0.8306	0.882 (n=2208)	0.561 (n=371)
A/G	0.1681 (n=37)	16.82	0.1616	0.097 (n=243)	0.359 (n=237)
G/G	0.0045 (n=1)	0.45	0.0079	0.021 (n=53)	0.080 (n=53)
$G1^{+M}$ (rs60910145)					
T/T	0.8318 (n=183)	83.18	0.8347	0.882 (n=2209)	0.563 (n=372)
T/G	0.1636 (n=36)	16.36	0.1578	0.097 (n=242)	0.357 (n=236)
G/G	0.0045 (n=1)	0.45	0.0075	0.021 (n=53)	0.080 (n=53)
G2 (rs71785313)					
I/I	0.7591 (n=167)	75.90	0.7341	0.934 (n=2339)	0.759 (n=502)
I/D	0.1955 (n=43)	19.55	0.2454	0.062 (n=154)	0.224 (n=148)
D/D	0.0455 (n=10)	4.55	0.0205	0.004 (n=11)	0.017 (n=11)

A-adenine; C-cytosine; G-guanine; T-thymine; I-insertion; D-deletion; MAF- Minor allele frequency; HWE- Hardy Weinberg Equilibrium

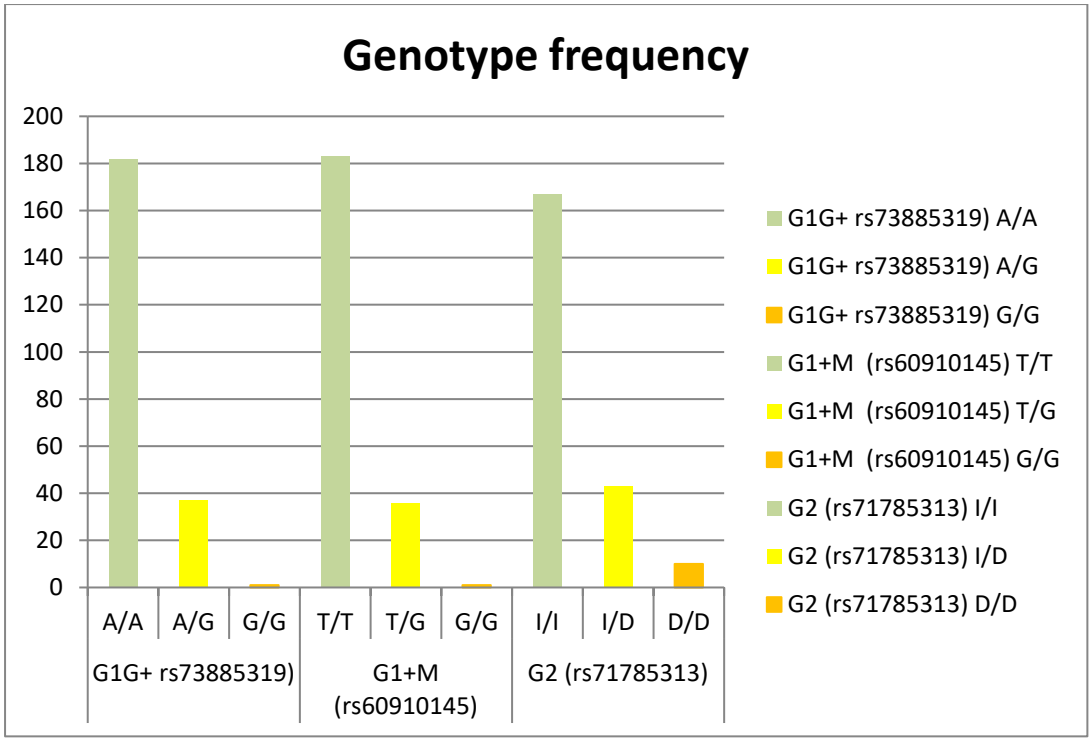

Figure 4.5 Genotype frequencies for $G1^{G+}$ (rs73885319), $G1^{+M}$ (rs60910145) and G2 (rs71785313). (The actual numbers were used to plot values)

4.5. Clinical CKD risk associated *APOL1* genotypes per study group

The genotypes associated with clinical CKD risk, as documented in literature (Friedman and Pollak, 2021; Daneshpajouhnejad, *et al*., 2022), observed within this study population were tabled according to the phenotypic study groups. The data provided in the table represents the distribution of genotypes in four different groups based on the presence or absence of HIV and CKD (Table 4.7, Figure 4.6); each category is represented by 55 individuals. The pair of *APOL1* G1 SNPs ($G1^{G+}$ rs73885319 and $G1^{+M}$ rs60910145) display an almost complete linkage disequilibrium, separated by a span of 128 base pairs, and typically have the same genotype, whether in homozygous or heterozygous form. From this point forward, they will be collectively referred to as *APOL1* G1. The specific alleles are classified as follows: for $G1^{G+}$ (rs73885319), A/A = G0/G0, A/G = G0/G1 and G/G = G1/G1. For $G1^{+M}$ (rs60910145), T/T = G0/G0, T/G = G0/G1 and G/G = G1/G1. For G2 (rs71785313), I/I = G0/G0, I/D = G0/G2 and D/D = G2/G2. In this study population and across all phenotype groups, the most common genotype observed is *APOL1* G0/G0, with a frequency of 60% followed by G0/G1 and G0/G2 with frequencies of

15% and 18.6%, respectively. All of the above-mentioned genotypes represent the no risk (G0/G0) and the low to no risk (G0/G1 and G0/G2). The high-risk genotypes had the lowest frequencies with rarest genotype being *APOL1* G1/G1, with a frequency of 0.5%, followed by the compound heterozygous genotype at 1.4% and G2/G2 having the highest frequency of 4.5% in the high-risk category (Table 4.7, Figure 4.7).

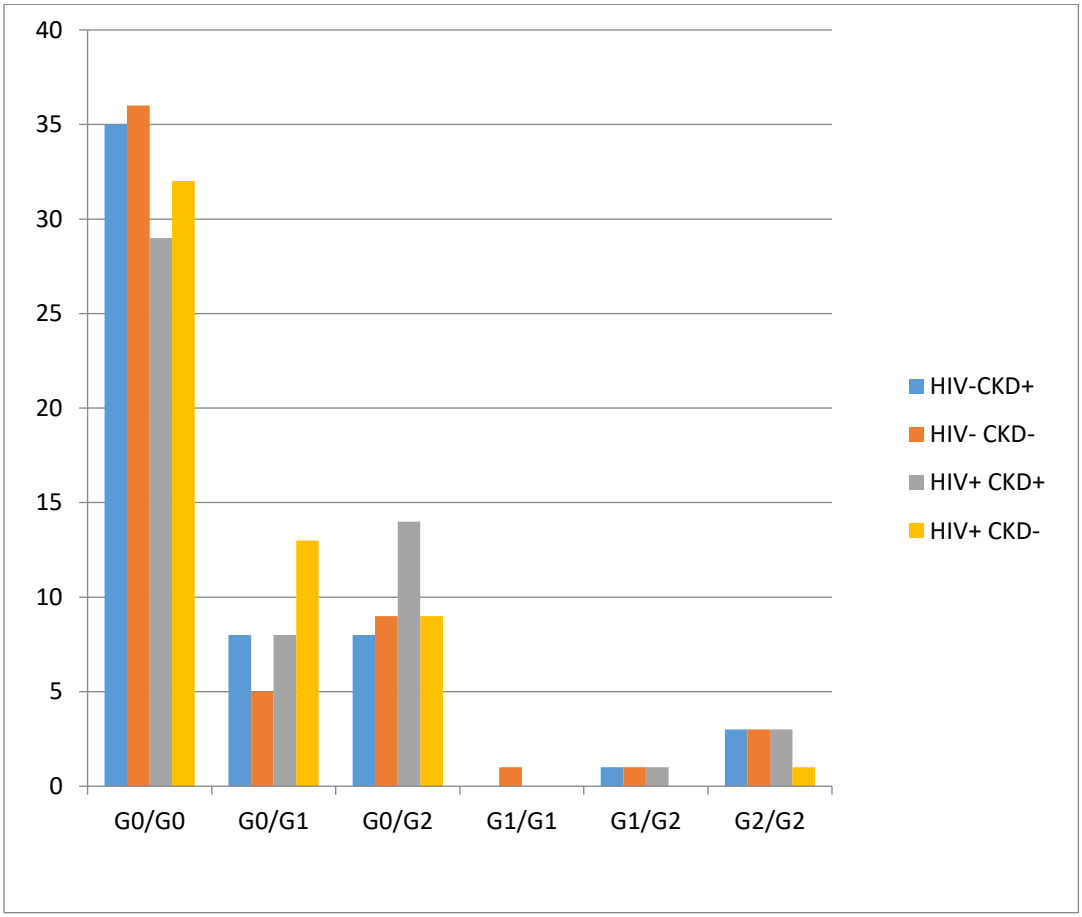

Figure 4.6 *APOL1* Genotypes per clinical CKD risk phenotype: HIV- CKD+, HIV- CKD-, HIV+ CKD+, and HIV+ CKD-.

While *APOL1* G0/G0 is the most common genotype, there are notable differences in the distribution of other genotypes across the different phenotypic groups. The low-risk genotype *APOL1* G0/G2 is more common in the HIV+ CKD+ phenotypic group with the frequency of 6.36, while the other three groups had the same frequency of 4.09 each. Among the low-risk genotypes, *APOL1* G0/G2 had the highest frequency.

The other low risk genotype, *APOL1* G0/G1 was found to be more common in the HIV+ CKD- category, with a frequency of 5.45. This group carried the second highest low risk genotype in the study. The rest of the phenotype groups; HIV+ CKD+, HIV- CKD+ and HIV- CKD had the frequencies of 3.64, 3.64 and 2.27, respectively. When comparing the four phenotype groups, HIV- CKD- had the lowest low risk for both *APOL1* G1 (2.27) and *APOL1* G2 (4.09). The groups with CKD+ status, with and without HIV had the same frequency for the G0/G1 genotype (3.64) but differed significantly on the G0/G2 genotype with HIV- having 4.09 and HIV+ (6.36). The HIV positive groups, both with CKD and without had the highest combined frequencies. G0/G1 for HIV+ CKD+ was 3.64 and G0/G2 was 6.36, with the combined frequency for low-risk genotypes of 10.0, G0/G1 for HIV+ CKD- was 5.45 and G0/G2 was 4.09 with the combined frequency of 9.54. This suggests potential associations between low risk (G0/G1 and G0/G2) genotypes and CKD in HIV positive individuals. The association of just one risk variant and CKD has not been a subject of investigation.

Table 4.7 displays genotype percentages categorized by the level of risk for chronic kidney disease, ranging from no risk to high risk.

Genotype	**HIV-CKD+**	**HIV- CKD-**	**HIV+ CKD+**	**HIV+ CKD-**	**Total risk (%)**
G0/G0	15.91 (n=35)	16.36 (n=36)	13.18 (n=29)	14.55 (n=32)	60 (n=132)
G0/G1	3.64 (n=8)	2.27 (n=5)	3.64 (n=8)	5.45 (n=12)	33.6 (n=74)
G0/G2	4.09 (n=9)	4.09 (n=9)	6.36 (n=14)	4.09 (n=9)	
G1/G2	0 (n=0)	0.45 (n=1)	0.45 (n=1)	0.45 (n=1)	6.4 (n=14)
G1/G1	0 (n=0)	0.45 (n=1)	0 (n=0)	0 (n=0)	
G2/G2	1.36 (n=3)	1.36 (n=3)	1.36 (n=3)	0.45 (n=1)	

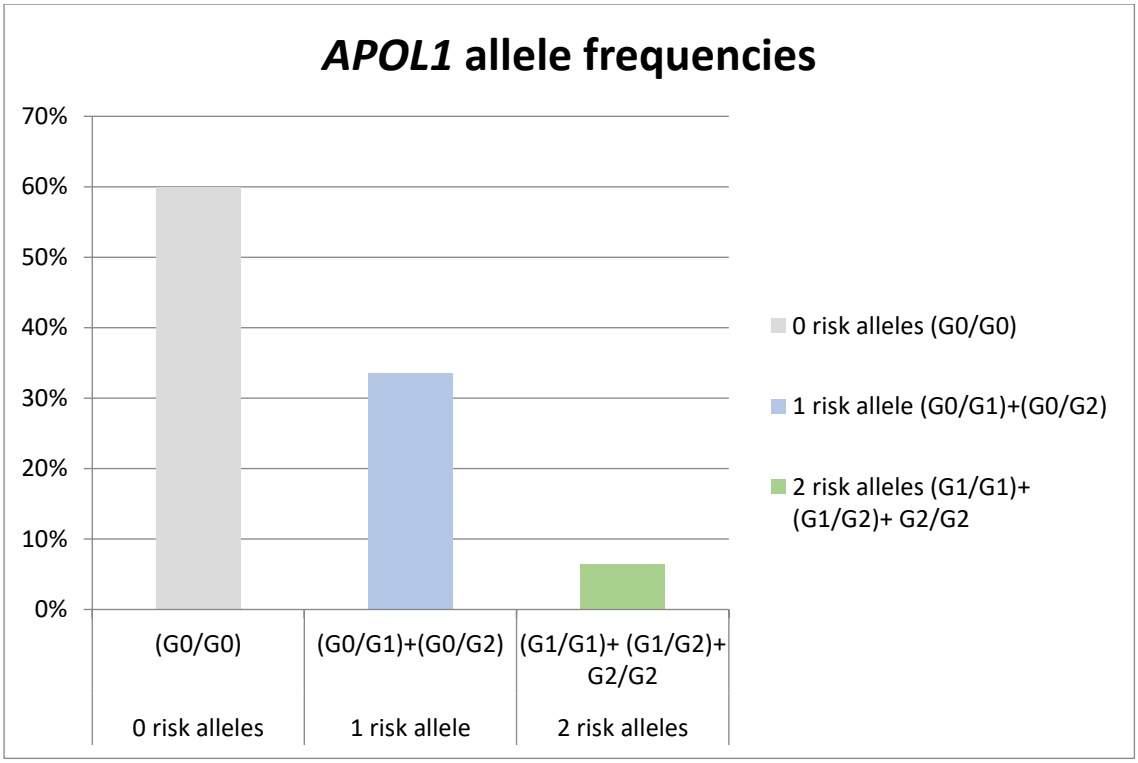

Figure 4.7 The distribution of *APOL1* risk alleles.

No risk: 60% - grey; Low risk: 33.6% - blue; High risk: 6.4% - green.

The genotypes associated with no risk to develop CKD, G0/G0, included all the wild type homozygous *APOL1* genotypes namely: $G1^{G+}$ (rs73885319) = A/A, $G1^{+M}$ (rs60910145) = T/T and for G2 (rs71785313) – I/I (no deletion in either allele) (Figure 4.7). This is to be expected since the wild type alleles are also the major alleles for all the *APOL1* variants studied. The *APOL1* genotypes clinically associated with a low risk to develop CKD contained one variant or mutant allele in the heterozygous form and include: $G1^{G+}$ (rs73885319) = A/G, $G1^{+M}$ (rs60910145) = T/G and for G2 (rs71785313) – I/D (one deletion). The genotypes clinically associated with a high risk to develop CKD contained two variant or mutant allele in the homozygous form and include: $G1^{G+}$ (rs73885319) = G/G, $G1^{+M}$ (rs60910145) = G/G and for G2 (rs71785313) – D/D (deletion on both alleles). The high-risk alleles were less prevalent since they correspond to the minor alleles, as evidenced in population allele databases. Genotypes carrying variant or mutant allele in $G1^{G+}$ (rs73885319) or $G1^{+M}$ (rs60910145), along with one in G2 (rs71785313), in homozygous form totalling four variant or mutant alleles (G1/G1 and G2/G2), also categorized as high-risk genotypes is G1/G2 in a heterozygous form. Only one participant sample

(HAART026) tested fell into the category of G1/G1 and the genotype was as follows: $G1^{+M}$ (rs60910145) = $G1^{G+}/G1^{+M}$ and for in G2 (rs71785313) = G2/G2.

A contingency table was utilized to examine the relationship between the clinical CKD risk genotypes and phenotypic groups. Table 4.8 displays the count of individuals affected and unaffected within each phenotype group. Additionally, it presents the risk levels ranging from no risk to high risk. The number of individuals with no risk (n=61) genotypes was lower in the HIV positive phenotype group with the combined number of low risk (n=43) and high risk (n=6) genotypes being the highest amongst the affected and unaffected categories. Although the number of individuals in the HIV negative category was the highest (n=71), the number of individuals with the high risk (n=9) genotype is also the highest in this study; this deviates from the hypothesis that the high risk variants are higher in HIV-positive individuals than in HIV-negative individuals, but the small population size contribute to this and thus the results should be interpreted with caution as they are not a representation of a larger population size. Additionally, the count of individuals exhibiting the low-risk genotype (30) was lower among the HIV-negative status group compared to the HIV-positive group. This disparity could be due to the presence of risk alleles in a heterozygous form. The data for the CKD affected and unaffected phenotype correlated with the hypothesis that CKD-positive individuals will have a higher frequency of high risk (n=8) compared to CKD-negative individuals (n=7). For comparison between combined phenotype groups, such as HIV positive individuals with CKD, HIV positive individuals without CKD etc., 2x2 contingency tables for each SNP were done. The contingency table for G1 and G2 low and high-risk genotypes is depicted by table 4.9. Based on the data presented in this table, there are no statistically significant associations found between the genotypes categorized by risk and either HIV or CKD.

Table 4.8 The contingency table for examining the association between genotype (with categories: [G0/G0], [G0/G1, G0/G2] and [G1/G1, G1/G2, G2/G2]) and phenotype (with categories: HIV+, HIV-, CKD+, CKD-).

	HIV+	**HIV-**	**OR (95% CI)**	**p-value**	**CKD+**	**CKD-**	**OR (95% CI)**	**p-value**
No risk genotypes (G0/G0)	61	71	1.462 (0.850-2.515)	0.215	64	68	1.164 (0.678-1.997)	0.680
Low risk (G0/G1, G0/G2)	43	30	0.544 (0.331-1.031)	0.085	38	35	0.884 (0.504-1.551)	0.775
High risk (G1/G1, G1/G2, G2/G2)	6	9	1.545 (0.531-4.497)	0.594	8	7	0.867 (0.303-2.478)	1.000

OR – Odds Ratio; CI – Confidence Interval

Table 4.9 The statistical 2x2 contingency table for high low and high genotypes for *APOL1* G1 and G2 (G0/G1 and G1/G1) and (G0/G2 and G2/G2).

***APOL1*G1**	**CKD+**	**CKD-**	**OR (95% CI)**	**p-value**
HIV+	9	13	0.680 (0.161-2.275)	0.516
HIV-	8	7	1.651 (0.440-6.201)	
***APOL1* G2**	**CKD+**	**CKD-**	**OR (95% CI)**	**p-value**
HIV+	18	11	1.909 (0.651-5.598)	0.285
HIV-	12	14	1.524 (0.179-1.536)	

OR – Odds Ratio; CI – Confidence Interval

According to the provided contingency tables, which outline the tallies of individuals categorized by various *APOL1* G1 and G2 genotypes in relation to their HIV and CKD status, no distinct pattern of correlation between genotypes and the

combination of HIV and CKD is evident. The counts are distributed across the categories without a distinct trend or imbalance. *APOL1* G1 had a p-value of 0.516 with OR of 0.680 (0.161-2.275) in the HIV-positive group and OR of 1.651 (0.440-6.201) in the HIV-negative group. For *APOL1* G2, the p-value was 0.285 and OR of 1.909 (0.651-5.598) for HIV-positive group and OR 1.524 (0.179-1.536) in the HIV-negative group. The Odds Ratio of *APOL1* G2 was higher in HIV-positive individuals than the G1 variant. However, due to the lack of statistical significance, OR should not be considered. Based on the absence of strong trends or significant differences in frequencies and p-values across the categories, it can be concluded that there is no apparent association between the genotypes and the combined HIV and CKD status based on the provided data.

Further exploration into the association between the risk alleles and the phenotypic groups involved a detailed breakdown focusing on the specific allele associations, irrespective of CKD risk, with phenotypes. Table 4.10 represent the allele frequencies per phenotype group. The genotype frequencies for each phenotype group are depicted in table 4.11.

Table 4.10 Allele frequencies of each variant per phenotype group.

SNP	Alleles		HIV-CKD+	HIV- CKD-	HIV+ CKD+	HIV+ CKD-
$G1^{G+}$ (rs73885319)	**Major allele**	**A**	0.92 (n=101)	0.93 (n=102)	0.92 (n=101)	0.88 (n=97)
	Minor allele	**G**	0.08 (n=9)	0.07 (n=8)	0.08 (n=9)	0.12 (n=13)
$G1^{+M}$ (rs60910145)	**Major allele**	**T**	0.93 (n=102)	0.93 (n=102)	0.92 (n=101)	0.88 (n=97)
	Minor allele	**G**	0.07 (n=8)	0.07 (n=8)	0.08 (n=9)	0.012 (n=13)
G2 (rs71785313)	**Major allele**	**I**	0.86 (n=95)	0.85 (n=94)	0.81 (n=89)	0.90 (n=99)
	Minor allele	**D**	0.14 (n=15)	0.15 (n=16)	0.19 (n=21)	0.10 (n=11)

Table 4.11 Genotype frequencies per phenotype group

SNP	Genotype	HIV-CKD+	HIV-CKD-	HIV+CKD+	HIV+CKD-	95% CI	OR	P value
$G1^{G+}$ (rs73885319)	**A/A**	47 (0.85)	48 (0.87)	46 (0.83)	42 (0.76)	(0.308-1,294)	0.632	0.279
	A/G	8 (0.15)	6 (0.11)	9 (0.16)	13 (0.24)	(0.826-3.557)	1.714	0.202
	G/G	0 (0)	1(0.02)	0 (0)	0 (0)	(0-NAN)	0	NAN
$G1^{+M}$ (rs60910145)	**T/T**	48 (0.87)	48 (0.87)	46 (0.83)	42 (0.76	(0.281-1.210	0.583	0.202
	T/G	7 (0.13)	6 (0.11)	9 (0.16)	13 (0.24)	0.887-3.925)	1.865	0.140
	G/G	0 (0)	1 (0.02)	0 (0)	0 (0)	(0-NAN)	0	NAN
G2 (rs71785313)	**I/I**	43 (0.78)	42 (0.76)	37 (0.62)	44 (0.80)	(0.444-1,520)	0.822	0.639
	I/D	9 (0.16)	10 (0.18)	15 (0.27)	10 (0.18)	(0.724-2.741)	1.409	0.399
	D/D	3 (0.05)	3 (0.05)	3 (0.05)	1 (0.02)	(0.179-2.285)	0.654	0.752

NAN: No real number; OR: odds ratio; CI: Confidence interval

The expected count for G/G is zero, making the chi-square statistic undefined for that cell. In practical terms, this means that the G/G genotype is very rare in this dataset. Hence, the division by zero matter in the $G1^{G+}$ (rs73885319) - G/G Genotype was corrected by excluding that term from the calculation. The p-value for both $G1^{G+}$(rs73885319) and $G1^{+M}$(rs60910145) suggest that, for those specified genotypes, there is no significant association between the genotype and disease status. The OR for $G1^{G+}$ (rs73885319) and $G1^{+M}$ (rs60910145) are similar for the homozygous normal genotype (A/A) (OR=0.632, 95% CI: 0.308-1.294 and p=0.279) and (OR=0.583, 95% CI: 0.281-1.210 and p=0.202), respectively, and they are higher than those of getting a homozygous mutant genotype (G/G) with the OR of 0. Therefore, the odds of getting *APOL1* G1 in a homozygous state in this population (using this dataset) are nonexistent; however, there was one individual with that genotype. The odds of getting *APOL1* G2 (rs71785313) in a homozygous mutant

state are present with the p-value of 0.752, (OR=0.654, 95% CI: 0.179-2-2285). There is an increase in heterozygous genotypes for all variants tested for, with G2 having a larger number of heterozygous participants than G1. Once more, after conducting the statistical association analysis, no statistically significant associations were found between the SNP alleles and either HIV or CKD. Furthermore, there were no significant associations detected between the SNP genotypes (regardless of CKD risk category) and either HIV or CKD.

4.6. Genotype confirmation

Sanger sequencing was applied to validate the genotypes of positive controls before setting up the qPCR reactions, thereby ensuring the confirmation of their genotypes. In addition, 45% of the study population's samples, including samples reflecting a variety of results that had initially undergone genotyping via qPCR were also Sanger sequence confirmed. All the sequence data has been compiled in Appendices D-G. Figures 4.10 to 4.14 are examples of electropherogram data plots indicating the DNA sequence of base pairs against florescence intensity, for the different genotypes. One hundred samples were sequenced, and a consensus sequence between the forward and reverse direction of sequencing was determined. Participant samples that indicated a positive, negative, or unclear genotyping result from the qPCR platform were selected. Some results were unclear due to the instrument no calling them. Participant DNA samples with heterozygous *APOL1* genotypes (G0/G1 and G0/G2), homozygous genotypes (G0/G0) representing a wild type genotype, positive homozygous genotypes (G1/G1 and G2/G2) for *APOL1* G1 and *APOL1* G2, respectively. A sequence for a compound heterozygous genotype (G1/G2) where one G1 allele and one G2 allele were found was also performed. All sequences conducted correlated 100% with the qPCR genotyping results.

4.6.1. *APOL1* G1 sequence results

APOL1 G1 has been described in literature to consist of two variants, the variants are rs73885319 and rs60910145 resulting in Ser342Gly and Ile384Met amino acid substitutions, respectively. Because these variants are in almost complete linkage

disequilibrium and lack ambiguity, often times a genotype of only rs73885319 are sufficient. However, for quality control purposes G1 SNPs were performed. These SNPs are $G1^{G+}$ (rs73885319) and $G1^{+M}$ (rs60910145). Figure 4.8.a. represents $G1^{G+}$ (rs73885319) and is shown by a green highlight and a green arrow where the A allele was substituted by G allele (single nucleotide substitution). This SNP was present in HAART 026 which was the only sample in the study with this SNP in a homozygous form and was thus also served as the positive control for the variant form of the SNP rs73885319. The *APOL1* G1 ($G1^{+M}$ rs60910145) is depicted by Figure 4.8.b. shown by a red highlight and a red arrow where nucleotide T is substituted by G, in homozygous form.

a) **A – missense mutation G1 rs73885319 (A > G)**

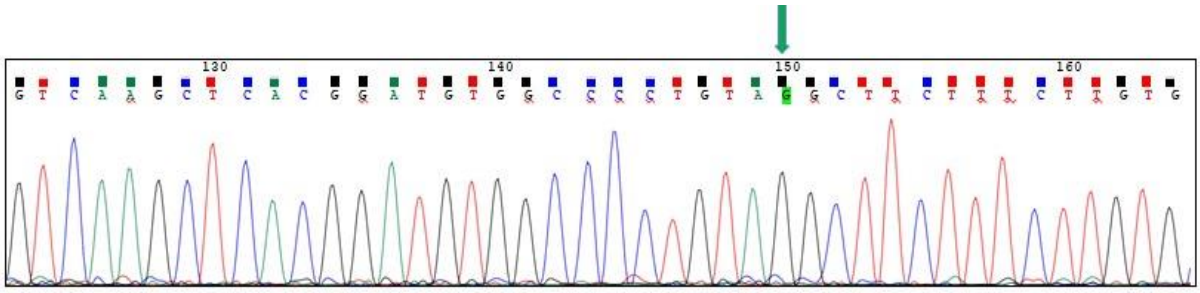

Figure 4.8.a HAART 026: *APOL1* G1/G1

b) **T – missense mutation G1 rs60910145 (T > G)**

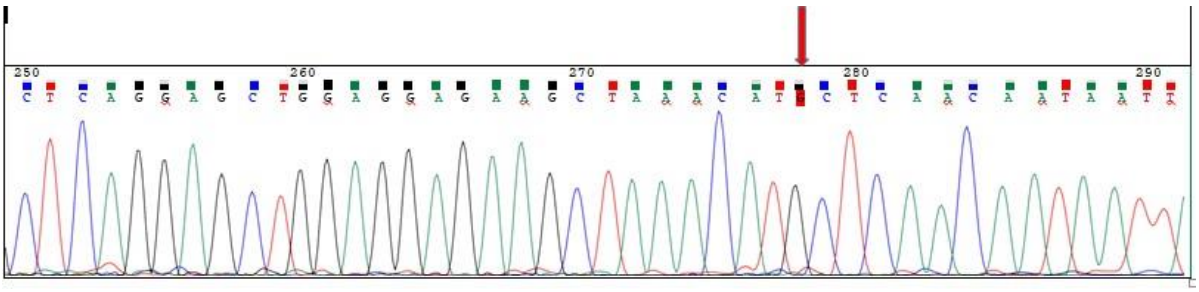

Figure 4.8.b HAART 026: *APOL1* G1/G1

The two SNPs for *APOL1* G1 are in close proximity on genome sequence, only 128 bp apart, thus it is expected that if the variant allele is present on for rs73885319 then it will also be present for rs60910145. However, one sample HAART 030 was heterozygous for only rs73885319 and not rs60910145. This sample was sequenced, and the sequencing results confirmed the findings. The heterozygous SNP $G1^{G+}$ (rs73885319) is represented by a green arrow in figure 4.9.a. Sample

HAART 030 also had a heterozygous genotype for another *APOL1* SNPs, rs16996616 (Asp337Asn), not associated with CKD, shown with a blue arrow. This sample belonged to an individual who was HIV negative but had kidney disease markers. Sumaili, *et al.*, 2018 detected rs16996616 at a higher allele frequency of 20% in the general population in in Kinshasa in a cohort of ESKD cases and controls while the frequency for other regions in Africa was 8%. This variant evolved from the ancestral haplotype (G0). A second SNP variant not associated with CKD is rs373463596 represented by a yellow arrow in figure 4.9.b., present in the DNA sample of patient number HAART 030. This variant is present in population databases at 0.03% frequency but has not been reported on 1000 genomes or any other literature reporting individuals affected with *APOL1*-related disorders.

a)

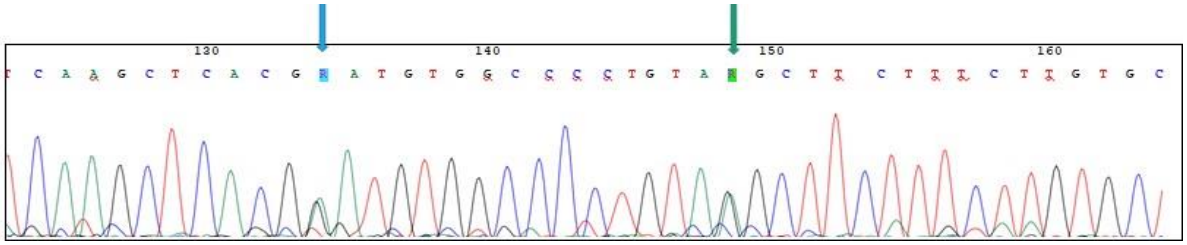

Figure 4.9.a HAART 030: *APOL1* G0/G1, Blue arrow= rs16996616, Green arrow=_rs73885319.

b)

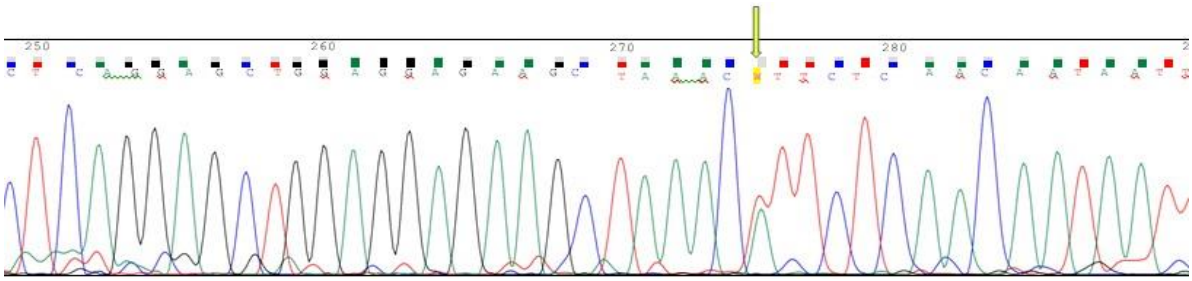

Figure 4.9.b HAART 030: *APOL1* G0/G1 yellow arrow rs373463596.

4.6.2. *APOL1* G2 results

The *APOL1* G2 variant, rs71785313, involves a 6-base pair in-frame deletion, alternatively recognized as rs143830837. This rs143830837 alteration is a 2-base pair micro-deletion causing a distinct frameshift, resulting in an identical DNA

sequence and amino acid sequence as rs71785313 (David, *et al*., 2018; Zhang, *et al*., 2017). *APOL1* genotypes with this deletion result in a shorter protein sequence. Figure 4.10 depicts a homozygous deletion (G2/G2) of the bases TTATAA; this was detected in HAART 509. For heterozygous genotypes (G0/G2), both the wild type and mutant alleles are shown superimposed on one other, this is observed in Figure 4.11 representing HAART 508.

TTATAA – deletion 6 bp G2 rs71785313

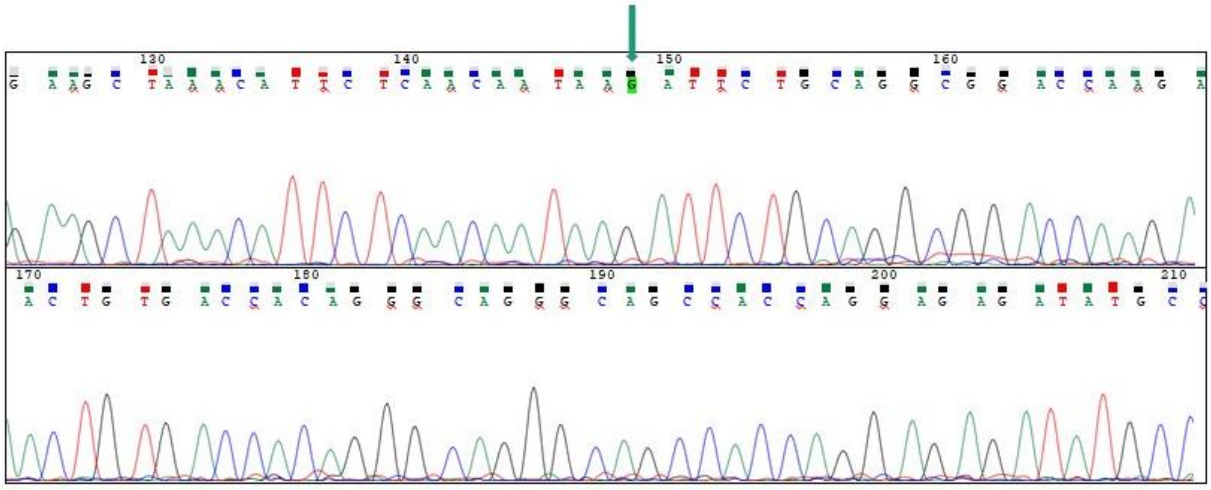

Figure 4.10 HAART 509: *APOL1* G2/G2

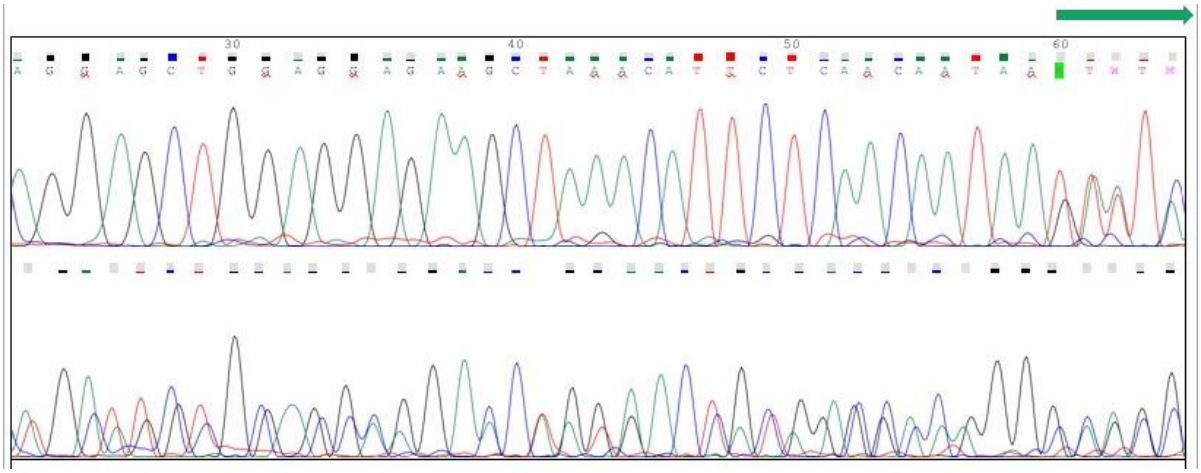

Figure 4.11 HAART 508: *APOL1* G0/G2

HAART 403 (Figure 4.12) represents a compound heterozygous genotype with both the *APOL1* G1 and G2 mutant alleles present (G1/G2). The *A POL1*G1 allele is shown by the blue arrow while the green arrow shows the G2 allele. Figure 4.13

(HAART 176) is a representation of a normal genotype (G0/G0), this represents the reference sequence.

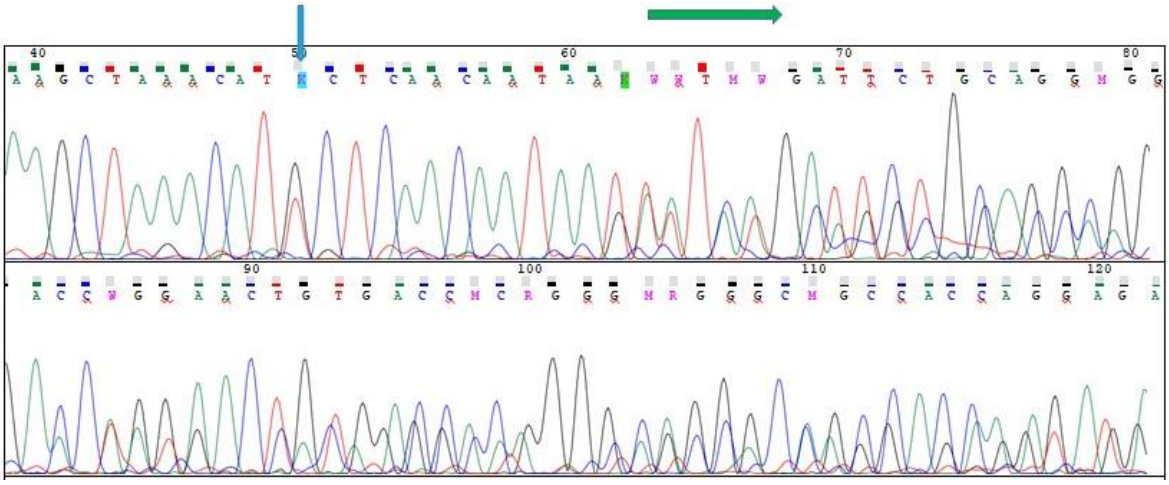

Figure 4.12 HAART 403: *APOL1* G1/G2

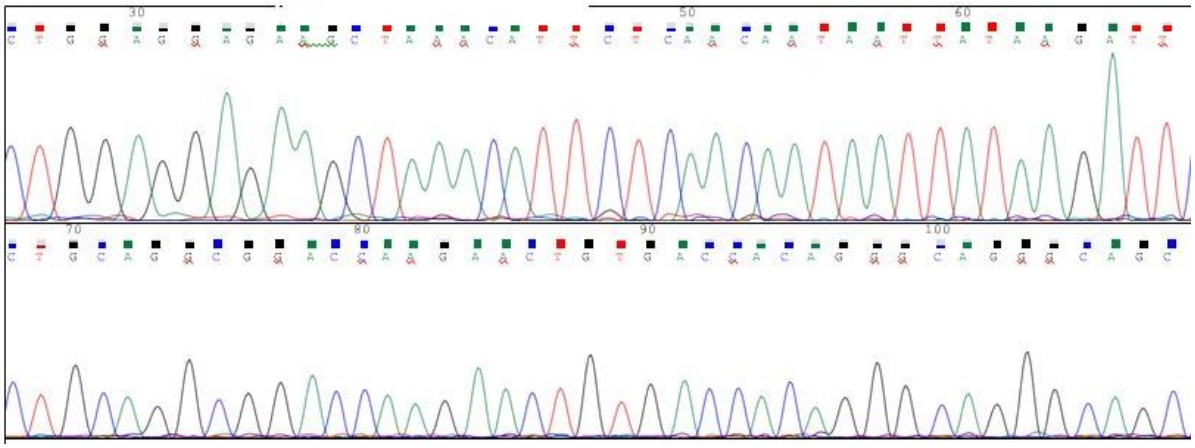

Figure 4.13 HAART 176: *APOL1* G0/G0

4.7. Conclusion

This study sought to correlate *APOL1* genotypic information with kidney disease in HIV positive individuals. It was conducted in the Free State, the province with the second highest HIV infection rate in South Africa. The *APOL1* G1/G1 was the lowest detected genotype and was detected in the population without CKD markers and no HIV infection (HIV- CKD-). The observations regarding the frequencies of CKD high-risk or two-risk *APOL1* allele genotypes (G1/G1, G1/G2, and G2/G2) were notably

lower in this study (6.4%) than in African American and African European populations (12-14%) (1000 genomes database Ensembl). However, when comparing the results to the study conducted on the South African population (2-4%) by Kasembeli, *et al,* 2015, the frequency reported in this study was higher. Interestingly, the one study by Kasembeli in the South African population correlates with our findings in that the G2 variant is more prevalent than the G1 variant. In contrast with one of the largest studies carried out in Nigeria, the high-risk genotype obtained in their research (6.2%) (Wudil, *et al.,* 2021) aligns closely with the findings of this study (6.4%).

This study found the *APOL1* G1 allele to have a frequency of 16%, while the G2 allele had a frequency of 24%, resulting in a combined frequency of 40%. Upon comparing the general population with the study group, it became evident that the *APOL1* G2 variant is more prevalent.

The low values of the high-risk genotypes compared to Igbo Nigerians (38%) (Wudil, *et al.,* 2021) and African Americans (14%) (Limou*, et al.,* 2014) are attributed to the fact that South Africa is outside the *Trypanosomiasis* belt. The African Americans shown to have high risk genotypes were mainly reported to have migrated from West Africa. Individuals with two risk variants are protected against Human African Trypanosomiasis, however this protection comes at a risk of developing kidney disease. Individuals from areas with *Trypanosoma brucei gambiense* have a selective advantage for *APOL1* risk variants, South Africa is not one of them hence the low frequencies of the risk variants in our population. It must be noted that not all HIV-positive individuals of African descent with two high risk variants will develop kidney disease; they do however put an individual at a greater risk of developing kidney disease. The mechanisms behind this are however still a subject of research.

The homozygous genotype for *APOL1* G1 was almost absent in this population with only one individual with this genotype. The high-risk variant with the highest frequency was *APOL1* G2, followed by the compound heterozygote genotype which was present in three individuals. The high-risk frequencies are higher in HIV+ CKD+ individuals than in HIV- CKD+ individuals. This suggests potential associations between certain genotypes and these conditions. However, there was no statistically significant correlation found between the high-risk genotypes and the presence of

chronic kidney disease (CKD) and/or HIV status. Additionally, none of the *APOL1* alleles exhibited a notable association with either CKD or HIV, the two examined phenotypes.

Chapter 5

Conclusion

The *APOL1* gene, which is located on chromosome 22 (Chaudhary, *et al*., 2022), has achieved substantial attention in recent years because to its association with kidney disease, particularly in individuals with African ancestry. The *APOL1* gene plays a role in innate immunity and is up regulated by interferons and other inflammatory cytokines (An *et al*., 2019). When secreted, it protects humans against being infected by the African Parasite *T. brucei.*

The *APOL1* C-terminal variants G1 and G2 trigger overexpression of the gene (gain of function mutation) (Bruggeman, *et al.,* 2019) which is toxic to the cells and can kill *T. rhodesiense*, but these variants also induce kidney podocyte dysfunctions, leading to chronic kidney disease. Two common variants, *APOL1* G1 and G2, are known to have an increased risk of developing various types of kidney disease, including FSGS, HIVAN and hypertension-attributed nephropathy (Friedman and Pollak, 2021; Pollak and Friedman, 2023). Moreover, the interaction between *APOL1* variants and HIV has emerged as a crucial factor influencing kidney disease development.

South Africa carries an enormous burden of HIV and has one of the highest prevalence globally (Zuma, *et al*., 2022). The HIV epidemic does not only impact the healthcare system, but it also adds a layer of complexity to the comprehension of kidney disease. HIVAN has become a daunting challenge, excessively affecting individuals with recent African ancestry (Husain, *et al.,* 2018). According to several research publications, the cooperative relationship between HIV infection and *APOL1* variants magnifies the risk of developing kidney disease (An, *et al.,* 2019; Heron, *et al*., 2020; Goyal and Singhal, 2021). This further emphasizes the need for comprehensive strategies in addressing both the viral infection and genetic predisposition.

A number of studies have found *APOL1* to confer a genetic predisposing to CKD in HIV positive individuals of African descent (Kasembeli, *et al.,* 2015; Hung, *et al*., 2022; Pollak and Friedman, 2023; Abdu, *et al*., 2022). An individual is classified as possessing a high-risk *APOL1* genotype if they carry two CKD risk variants within the *APOL1* gene, specifically identified as G1/G1, G1/G2, or G2/G2 (An, *et al.,* 2019). Studies performed on participants with African ancestry in America and in Europe

reported on a frequency 12-14% in high-risk genotypes (Hung, *et al.,* 2022). In a Nigerian population the frequency for high-risk variants was 6.4% (Wudil, *et al*., 2021). The frequency was even lower in the South African population at 2-4% (Kasembeli, *et al.,* 2015). In this study, we explored the prevalence of *APOL1* gene variants in both HIV-positive and HIV-negative individuals with evidence of kidney disease, shedding light on the intricate relationship between genetics, viral infection, and renal health. This was a descriptive case-control study on a cohort of 220 South African participants. The participants were divided into four phenotype groups, 55 HIV positive participants with CKD, 55 HIV positive participants without CKD, 55 HIV negative participants with CKD and 55 HIV negative individuals without CKD. To determine if the risk genotypes were at higher frequency in HIV-positive individuals with CKD markers and if significant associations could be found between the risk alleles and patients suffering from HIV and CKD.

Genotyping of the *APOL1* gene on these particular SNP variants: $G1^{G+}$ (rs73885319), $G1^{+M}$ (rs60910145) and the G2 haplotype consisting of a 6–base pair in-frame deletion rs71785313 were performed, as they have been highly associated with CKD in literature (Friedman and Pollak, 2021; Daneshpajouhnejad, *et al*., 2022). Allele and genotype frequencies were calculated using Chi-squared and Hardy Weinberg equilibriums, logistic regression analysis and 2x2 contingency tables were used to compare the frequencies between the two phenotypes (HIV and CKD) and *APOL1*. A $p<0.05$ indicated statistical significance.

In this study the frequency of *APOL1* G2 (rs71785313) low risk and high-risk variants was more prevalent that *APOL1* G1 (both $G1^{G+}$ rs73885319 and $G1^{+M}$ rs60910145) low and high-risk variants. In its homozygous state the G1 risk variant was only present in one individual. The prevalence of high-risk genotypes was the same in CKD+ and CKD- individuals and was lower in HIV+ individuals than in HIV- individuals, however when combining the two phenotypes HIV+ and CKD+, the frequency for high-risk variants was 1.81% and for HIV-CKD+ was 1.36%. This meant that HIV+ individuals with CKD had a higher frequency of high-risk genotypes than HIV- individuals without CKD. The results obtained correlated with what was found in the North Nigeria as well as in the South African population residing in Johannesburg.

Following the statistical association analysis, no statistically significant connections were identified between the *APOL1* SNP variant alleles and either HIV or CKD. This remained consistent when analysing the association between CKD risk category *APOL1* genotypes and both HIV status and CKD. Likewise, no notable association was observed between the *APOL1* variant alleles and genotypes and their correlation with HIV status and CKD. Similar outcomes were observed when evaluating the *APOL1* variant genotypes per SNP and their relation to both HIV status and CKD in our South African population. Consequently, the results from this study do not suggest an association between *APOL1* gene variants and the presence of CKD and HIV as a second hit. Subsequently, based on the findings obtained in this study, it appears that the susceptibility associated with *APOL1* gene variants in HIV infection does not play a role in the development of CKD in our South African population. The incorporation of genetic screening for *APOL1* variants, particularly in HIV-positive individuals into routine clinical practice might be advantageous for personalized medicine advancements. This can promote early detection and management of kidney disease, ultimately lessening the burden on the healthcare system and improving the quality of life of affected patients. The study results suggest that altering and implementing treatment regimens universally for CKD and HIV positive patients should not be considered without prior determination of genotypes. This caution is prompted by the insufficient frequency of the *APOL1* CKD risk allele among South African residents, indicating a lack of necessity for immediate modifications in treatments based solely on this genetic factor.

5.2. Future prospects

Future research endeavours should focus on improving our understanding of the prevalence of *APOL1* variants in diverse subpopulations within South Africa. Considerations for geographical, ethnic, and socioeconomic factors should be taken into account. As we try to understand the aetiology of kidney disease in South African individuals of African descent, the connections between genetic research, clinical practice, and public health initiatives are vital. This holistic approach will provide healthcare providers, researchers, and policymakers the knowledge to

address the unique challenges presented by the prevalence of *APOL1* gene variants and the burden of HIV on kidney disease development.

5.3. Study limitations

Urine dipstick testing is commonly used in clinical settings to screen for proteinuria, a hallmark of kidney disease. While urine dipstick analysis can provide a quick and convenient method for detecting the presence of proteinuria, it has limitations, particularly in quantifying the amount of protein present. Inclusion of patients with trace proteinuria based solely on dipstick testing without further confirmation through laboratory tests could introduce a potential bias in the study findings. Patients with trace proteinuria may have been classified as having kidney disease, leading to misclassification and potentially diluting the observed associations between kidney disease and HIV rapid antigen testing accuracy.

Although other CKD markers were measured, only proteinuria was considered for the determination of CKD. This limitation could indeed impact the interpretation of the study results.For future studies other markers should be considered. Patients with established and advanced kidney disease should be recruited from nephrology clinics or dialysis centers compared to control individuals without kidney disease from primary care clinics or community health centers. Inclusion criteria could include a confirmed diagnosis of CKD stage 3 or higher, as determined by eGFR measurements, and the presence of hypertension as a common comorbidity.

For this study, CKD was not classified into different groups. As kidney diseases are diverse, this study did not include individuals with various renal conditions. This should be considered for future studies as the specific type and severity of kidney disease could impact the association between *APOL1* variants and kidney outcomes. The diagnostic criteria for kidney disease should also be considered e.g. HIVAN, FSGS. There were no kidney biopsies taken in this study to determine the type of kidney disease the participants had.

APOL1 risk variants can vary among different populations (irrespective of them being of African origin), therefore there should be a wider representation of ethnic and regional groups in our South African population. HIV itself has various subtypes that

might interact differently with genetic factors. The study did not differentiate among different strains of HIV and their potential interactions with *APOL1*. The impact of CD4 counts was also not captured.

Another limitation was the small sample size that may have limited the statistical power of the study. Disease progression was not tracked in this study making it impossible to detect the effects of *APOL1* high risk variants on CKD. Longitudinal studies tracking disease progression are vital for such conclusions.

Addressing these limitations often involves a multi-faceted approach, combining large sample sizes, diverse cohorts (ethnic), robust statistical methods, careful phenotyping (classification of CKD, diagnostic criteria of CKD using biopsies and HIV strains), and controlling for confounding variables. Collaborative efforts across research groups and regions can strengthen the reliability and applicability of findings, ultimately advancing our understanding of the intricate relationship between *APOL1* gene variants, HIV, and kidney disease, especially in our South African population.

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Appendices

Appendix A. Ethics approval letters for study UFS-HSD2023/0249/2305 from the Health Sciences Research Ethics Committee of the Free State

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

Health Sciences Research Ethics Committee

15-May-2023

Dear **Gerda Botha**

Ethics Clearance: **Prevalence of APOL1 gene risk variants in HIV-positive compared to HIV-negative individuals with evidence of kidney disease.**
Principal Investigator: **Ms Madingaka Notani**
Department: **Genetics Department (Bloemfontein Campus)**
Submission Page

APPLICATION APPROVED

Please ensure that you read the whole document

With reference to your application for ethical clearance with the Faculty of Health Sciences, I am pleased to inform you on behalf of the Health Sciences Research Ethics Committee that you have been granted ethical clearance for your project.
Your ethical clearance number, to be used in all correspondence is:**UFS-HSD2023/0249/2305**

The ethical clearance number is valid for research conducted for one year from issuance. Should you require more time to complete this research, please apply for an extension.

We request that any changes that may take place during the course of your research project be submitted to the HSREC for approval to ensure we are kept up to date with your progress and any ethical implications that may arise. This includes any serious adverse events and/or termination of the study.

A progress report should be submitted within one year of approval, and annually for long term studies. A final report should be submitted at the completion of the study.

Research conducted in any Department of Health facility: Researchers are required to sign and return the HSREC approval letters to the provincial Department of Health where they applied. It is also a requirement for researchers to submit electronic copies of their final research findings, and/or make a presentation of their findings and recommendations at departmental research days when and where indicated.

The HSREC functions in compliance with, but not limited to, the following documents and guidelines: The SA National Health Act. No. 61 of 2003; Ethics in Health Research: Principles, Structures and Processes (2015); SA GCP(2020); Declaration of Helsinki; The Belmont Report; The US Office of Human Research Protections 45 CFR 461 (for non-exempt research with human participants conducted or supported by the US Department of Health and Human Services- (HHS), 21 CFR 50, 21 CFR 56; CIOMS; ICH-GCP-E6 Sections 1-4; International Council for Harmonisation (ICH) Harmonised Guideline, Integrated Addendum to ICH E6(R1), Guideline for Good Clinical Practice (GCP) E6(R2), 2016, SAHPRA Guidelines as well as Laws and Regulations with regard to the Control of Medicines, Constitution of the HSREC of the Faculty of Health Sciences.

The Principal Investigator (PI) bears final responsibility for the RIMS application. In the event of any misconduct or improper activities perpetuated by a third party, the PI will be held vicariously liable. The HSREC will bear no responsibility or liability for any actions of a PI and/or third party or breach of confidentiality caused by the PI and/or third party.

For any questions or concerns, please feel free to contact HSREC Administration: 051-4017794/5 or email EthicsFHS@ufs.ac.za.

Thank you for submitting this proposal for ethical clearance and we wish you every success with your research.

Yours Sincerely

Prof. A. Sherriff
Chairperson: Health Sciences Research Ethics Committee

Health Sciences Research Ethics Committee
Office of the Dean: Health Sciences
T: +27 (0)51 401 7795/7794 | E: ethicsfhs@ufs.ac.za
IRB 00011992; REC 230408-011; IORG 0010096; FWA 00027947
Block D, Dean's Division, Room D104 | P.O. Box/Posbus 339 (Internal Post Box G40) | Bloemfontein 9300 | South Africa
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Appendix B. Permission from the principal investigator of the HAART to HEART study

UFS-UV

19 February 2023

Prof A Sherriff
The Chairperson,
Ethics Committee,
Faculty of Health Sciences
University of the Free State

Dear Prof Sherriff

Project title: Prevalence of *APOL1* gene risk variants in HIV-positive compared to HIV-negative individuals with evidence of kidney disease.

I hereby grant Ms MD Notani permission to conduct the abovementioned research project. The research will be completed in accordance with myself as Head of Department of Internal Medicine and Dr. Gerda Marx as supervisor of this study.

Kind regards

.....

Adjunct Prof TRP MOFOKENG(HOD)
Dept Internal Medicine

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Appendix C. Characteristics of all the participant samples tested, including the HIV status, sex, age, protein mg/dl (indication of CKD) and BMI

Group	Study no	Sex	BMI kg/m2	Age					
					Protein mg/dl	Creatinine and estimated GFR			
						Creatinine (umol/L)	Urine creatinine (mmol/L)	Urine albumin (mg/L)	Urine albumin : creat ratio
HIV+	HAART 0003	M	35,5	49	Negative	77	10,2	13	1,3
HIV+	HAART 0005	F	38,8	40	Negative	52	4,3	2,2	0,5
HIV+	HAART 0010	F	31,6	30	30+	54	6,1	22	3,6
HIV+	HAART 0011	M	24,7	39	Trace	74	8,3	5,5	0,7
HIV-	HAART0012	M	30,6	37	Negative	75	10,5	16	1,6
HIV-	HAART0013	M	21,4	30	Trace	63	14,9	30	2
HIV-	HAART0014	F	28,7	42	Negative	58	9,5	26	2,7
HIV+	HAART0015	F	16,7	30	≥2000++++	88	11,4		
HIV+	HAART0016	F	22,3	45	Trace	53	5,3	4,6	0,9
HIV-	HAART0022	F	27,5	47	Negative	70	22,4	58	2,6
HIV+	HAART0023	F	33,2	47	Negative	71	10,4	5,6	0,5
HIV+	HAART0024	M	23,3	42	Trace	68	18,2	62	3,4
HIV+	HAART0025	F	31,1	32	Trace	58	6,1	0,1	0
HIV-	HAART0026	M	20,8	35	Negative	102	3,7		
HIV-	HAART0028	F	32	31	Negative				
HIV-	HAART0030	M	20,5	42	Trace	65	13,4	25	1,8
HIV-	HAART0032	F	27	58	Negative	71	12,8	4,8	0,4
HIV-	HAART0033	M	18,9	33	Trace	73	3,5	<3.00	
HIV-	HAART0034	M	25,4	35	Negative	77	7,3	4,1	0,6
HIV+	HAART0035	M	18,7	35	Negative	45	16,3	251	15,4

HIV+	HAART0036	M		39	Trace	91	10,6	30	2,8
HIV-	HAART0037	F	27,7	41	Trace	50	8,4	<3	unable to calculate
HIV-	HAART0038	F	20,5	46	Trace	56	9,4	<3.00	unable to calculate
HIV-	HAART0039	F	34,3	46	Trace	58	13,6	6,8	0,5
HIV+	HAART0041	M	18,7	47	Trace	72	7,1	<3.00	unable to calculate
HIV-	HAART0043	F	42	57	Negative	65	15,9	12	0,8
HIV-	HAART0045	M	28,8	56		59	10,7	67	6,3
HIV+	HAART0046	F	35,1	35	Trace	52	14,8	30	2
HIV+	HAART0047	M	22,3	42	Negative	104	7,9	proteinuria	unable to calculate
HIV+	HAART0051	M	19,3	42	Trace	66	2,9	<3,00	unable to detect
HIV+	HAART0053	F	29,8	30	Negative	78	4	13	3,3
HIV-	HAART0054	M	23,2	65	30+	60	17,6	4,4	0,3
HIV-	HAART0055	M	18,1	51	Trace	81	7,9		
HIV-	HAART0056	F	22,8	41	30+	45	11,2	5,1	0,5
HIV-	HAART0057	M	19,8	35	Negative	70	3,8	<3,00	unable to calculate
HIV+	HAART0058	M	19	33	Trace	81	5,5	<3,00	unable to calculate
HIV+	HAART0061	F		55	300+++	53	17,1	unable to calculate	unable to calculate
HIV-	HAART0063	F	33,7	39	Negative	91	20,5	4,6	0,2
HIV-	HAART0065	F	29,4	42	Negative	63	7,2	<3,00	unable to calculate
HIV+	HAART0068	F	36,1	39	Negative	63	7,5	33	4,4
HIV+	HAART0069	M	19,3	37	30+	79	10,1	80	7,9
HIV+	HAART0071	M	21,9	56	30+	55	9,9	29	2,9
HIV-	HAART0073	M	26,1	40	Negative	76	4,4	<3,00	unable to calculate
HIV+	HAART0074	F	22,1	45	Negative	58	11,6	2,8	0,2
HIV+	HAART0075	M	21,3	47	Trace	74	8,4	138	16,4
HIV+	HAART0077	F		30	Trace	69	9,2	<3,00	unable to calculate
HIV-	HAART0080	F	23,1	35	Trace		11,5	<3,00	unable to calculate
HIV+	HAART0084	M	18,4	33	Trace	55	6,3	3	0,5
HIV+	HAART0085	F	24,3	46	30+	75	10,1	28	2,8
HIV-	HAART0088	F	31,6	49	Negative	84	14,7	4,5	0,3

HIV+	HAART0091	M	18,5	36	30+	71	21	16	0,8
HIV-	HAART0093	F	33,6	46	Trace	74	13,5	3,8	0,3
HIV-	HAART0094	F	38,7	60	Negative	80	13,4	<3,00	unable to calculate
HIV-	HAART0095	F	20	49	Trace	63	14,4	3,2	0,2
HIV+	HAART0096	F	15,3	40	Trace	54	6,3	3,5	0,6
HIV+	HAART0097	M	22,8	47	30+	108	22,8	17	0,7
HIV-	HAART0098	M	20,8	28	Trace	72	4,5	<3,00	UNABLE TO CALCULATE RESULT
HIV+	HAART0101	M	20,1	42	30+	81	24,2	10	0,4
HIV+	HAART0102	F	30,1	48	Trace	55	3,2	<3,00	UNABLE TO CALCULATE RESULT
HIV-	HAART0104	F	35,8	38	Negative	64	9,6		
HIV-	HAART0105	M	27,6	38	Negative	85	6,8		
HIV-	HAART0108	F	30,3	29	Negative	61	21,3		
HIV+	HAART0109	F	25,2	42	Trace	90	UNSUITABLE FOR TEST-URINE SPECIMEN REQUIRED		
HIV+	HAART0110	M	14,5	36	30+	55	8,7	20	2,3
HIV-	HAART0111	M	20	58	Negative	83	7,4	<3,0	UNABLE TO CALCULATE RESULT
HIV-	HAART0112	F	37,5	33	Trace	59	21,4	12	0,6
HIV-	HAART0113	M	19,6	32	Trace		12,7	<3,00	UNABLE TO CALCULATE RESULT
HIV-	HAART0114	M	36,2	56	Negative	95	6,4	<3,00	UNABLE TO CALCULATE RESULT
HIV-	HAART0115	M	20	53	Negative	80	9,8	12	1,2
HIV+	HAART0116	F	38,3	51	Negative	79	11,5	118	10,3
HIV+	HAART0117	F	18,6	38	30+	58	17,1	31	1,8
HIV-	HAART0119	M	16,9	31	Trace	93	14,2	11	0,8
HIV-	HAART0120	M	16,8	47	Trace	53	13,6	4,9	0,4
HIV-	HAART0122	F	31,8	33	30+	62	17,4	17	1
HIV+	HAART0125	F	26	34	Trace	66	17,7	34	1,9
HIV-	HAART0128	M	18,9	54	Trace	77	9,2	<3,00	UNABLE TO CALCULATE RESULT

HIV-	HAART0129	F	41,6	31	Negative	70	19,9	7,8	0,4
HIV-	HAART0130	M	21,3	39	300+++	68	34,9	10	0,3
HIV+	HAART0131	F	27,2	43	Negative	52	8,3	6,4	0,8
HIV-	HAART0134	F	20,2	31	300+++	135	10,7		
HIV-	HAART0135	F	29	40	Negative	67	17,5	12	0,7
HIV-	HAART0136	F	24,5	31	Negative	68	9,3	9,8	1,1
HIV-	HAART0139	F	27,9	58	Trace	71	16,6	13	0,8
HIV-	HAART0140	F	36,4	46	30+	80	18,5	9,4	0,5
HIV-	HAART0141	F	33	44	Trace	78	6,7	<3,00	UNABLE TO CALCULATE RESULT
HIV-	HAART0143	M	16,7	33	300+++	96	16,2	7,2	0,4
HIV-	HAART0144	M	28,3	52	Negative	118	3,1	<3,00	UNABLE TO CALCULATE RESULT
HIV-	HAART0146	F	17,6	53	Trace	60	8,9	19	2,1
HIV-	HAART0148	F	36	29	Trace	56	6,6	4,3	0,7
HIV-	HAART0150	M	19,4	28	30+	63	4	6,4	1,6
HIV-	HAART0151	M	28,2	40	Trace	80	16,4	10	0,6
HIV-	HAART0152	F	30,5	38	30+	65	12,7	21	1,6
HIV+	HAART0153	F	18,1	40	30+	44	5	3,1	0,6
HIV+	HAART0154	M	19,7	38	Trace	65	6,1	<3,0	UNABLE TO CALCULATE RESULT
HIV+	HAART0155	F	19,4	31	30+	52	20	28	1,4
HIV+	HAART0156	M	24,9	40	Trace	66	2,3	<3,00	UNABLE TO CALCULATE RESULT
HIV+	HAART0157	F	39,5	40	Trace	50	14,1	13	0,9
HIV+	HAART0158	M	21,9	36	Trace	66	4,6	<3,00	UNABLE TO CALCULATE RESULT
HIV+	HAART0160	F	27,2	31	100++	45	13,8	212	15,3
HIV+	HAART0161	F	21,3	32	30+	75	5,5	42	7,6
HIV-	HAART0162	F	29	30	Trace	70	11,5	<3,00	UNABLE TO CALCULATE RESULT
HIV-	HAART0163	F	33,3	37	Trace	49	4,8	<3,00	UNABLE TO CALCULATE RESULT
HIV-	HAART0165	F	29,8	36	Negative	56	16,9	17	1

HIV-	HAART0166	F	33,7	36	Negative	52	7,2	<3,00	UNABLE TO CALCULATE RESULT
HIV+	HAART0167	F	21,8	30	Negative	56	9,6	4,5	0,5
HIV-	HAART0172	F	28,4	32	Trace	78	REJECTED		
HIV-	HAART0173	M	ERROR	30	Trace	51	REJECTED		
HIV+	HAART0175	F	22,5	41	Negative	8,4	NOT RECEIVED		
HIV+	HAART0176	M	23,6	34	Negative	67	7,3	5,3	0,7
HIV-	HAART0178	F	26,1	32	Negative	59	7,3	<3,00	UNABLE TO CALCULATE RESULT
HIV-	HAART0179	F	40,9	30	Trace	39	9,7	6,5	0,7
HIV-	HAART0180	F	23,4	45	Trace	70	9	12	1,4
HIV-	HAART0181	F	31,4	48	30+	56	26,3	19	0,7
HIV+	HAART0185	F	30,1	37	30+	51	11,2	8,9	0,8
HIV-	HAART0186	M	33,3	35	Trace	75	10,1	<3,00	UNABLE TO CALCULATE RESULT
HIV-	HAART0187	M	21,3	31	Trace	104	8,1	<3,00	UNABLE TO CALCULATE RESULT
HIV-	HAART0189	F	28,8	77	Negative	94	8	4,6	0,6
HIV+	HAART0192	M	16,7	44	30+	74	9,5	18	1,9
HIV-	HAART0193	M	20,9	48	Trace	76	9,4	<3,00	UNABLE TO CALCULATE RESULT
HIV-	HAART0194	F	44,4	57	Trace	77	8	6,4	0,8
HIV-	HAART0197	M	29,4	38	Trace	83	22,5	11	0,5
HIV-	HAART0198	F	41,3	30	Trace	46	11,1	7,7	0,7
HIV+	HAART0200	M	ERROR	53	30+	119	15,3	65	4,3
HIV+	HAART0201	F	18,1	45	Trace	62	4,7	29	6,2
HIV+	HAART0203	F	38,1	46	100++	75	9,9	450	45,5
HIV+	HAART0205	M	18,4	39	30+	75	13	5,6	0,4
HIV-	HAART0207	M	18	29	Trace	105	21,5	<3,00	UNABLE TO CALCULATE RESULT
HIV-	HAART0208	M		30	Negative	98	10,4	<3,00	UNABLE TO CALCULATE RESULT
HIV-	HAART0211	F	31,8	29	Negative	72	10	<3,00	UNABLE TO CALCULATE RESULT

HIV+	HAART0212	M	20	44	Trace	69	2,8	<3,00	UNABLE TO CALCULATE RESULT
HIV+	HAART0213	F	33,4	38	30+	80	5,7	165	29
HIV+	HAART0214	M	21,8	32	300+++	61	22,1	108	4,9
HIV-	HAART0219	F	32,6	43	Trace	76	25,8	23	0,9
HIV-	HAART0220	F	36,5	44	Trace	63	20	25	1,2
HIV+	HAART0223	F	29,6	35	Trace	56	12,4	PROTEIN+, PROTEINURIA PRESENT	
HIV-	HAART0224	F	36,4	41	Trace	40	4,5	4,5	1
HIV-	HAART0233	F	24,7	73	Trace	62	6,9/4	26,44/11,45	3,8/2,9
HIV-	HAART0234	M	20,6	42	Trace	82	7,9	<3,00	UNABLE TO CALCULATE RESULT
HIV+	HAART0235	F	19	41	Negative	49	2,2	<3,00	UNABLE TO CALCULATE RESULT
HIV+	HAART0236	F	22,4	39	30+	64	9,6	14	1,5
HIV+	HAART0237	F	19,3	48	100+	61	23,3	25	1,1
HIV-	HAART0238	M	18,5	28	30+	76	8	<3,00	UNABLE TO CALCULATE RESULT
HIV-	HAART0240	M	UNABLE TO MEASURE	72	Trace	84	3,5	<3,00	UNABLE TO CALCULATE RESULT
HIV+	HAART0243	F	17,5	52	Negative	63	13,8	3,6	0,3
HIV-	HAART0244	M	20,1	37	Negative	88	4,3	4,3	1
HIV+	HAART0248	M	16,9	42	Negative	58	6,9	3,4	0,5
HIV+	HAART0256	M	17,6	42	Negative	59	9,4	20	2,1
HIV+	HAART0258	M	18,4	30	Negative	86	3,8	<3,00	UNABLE TO CALCULATE RESULT
HIV+	HAART0260	F	26.4	33	Trace	56	21,7	27	1,3
HIV-	HAART0251	M	19,4	53	Negative	63	7,9	5,2	0,7
HIV-	HAART0254	F	29	32	Negative	85	23	47	2,1
HIV-	HAART0265	F	17,5	44	30+	61	14,9	483	32,4
HIV-	HAART0266	M	24	37	Negative	106	5,5	68	12,4
HIV-	HAART0273	M	20,9	55	Negative	77	9,1	14	1,5
HIV-	HAART0276	F	38,1	37	Negative	64	4,1	<3,00	UNABLE TO CALCULATE

									RESULT
HIV-	HAART0277	M	34,8	47	Negative	111	32,2	9	0,3
HIV+	HAART0279	M	20,5	40	Trace	71	11,5	8,6	0,7
HIV+	HAART0282	M	24,4	45	Negative	82	12,7	<3,00	UNABLE TO CALCULATE RESULT
HIV+	HAART0284	F	18,3	53	Negative	59	5,3	85	16
HIV+	HAART0291	F	20,1	50	Negative	57	21	14	0,7
HIV+	HAART0295	M	22,2	47	Trace	77	32,2	10	0,3
HIV+	HAART0296	M	20,7	32	Negative	175	6,6	163	24,7
HIV-	HAART0298	M	18,5	34	Trace	72	24	23	1
HIV-	HAART0300	M	20	31	Negative	76	4,9	<3,00	UNABLE TO CALCULATE RESULT
HIV+	HAART0305	M	19,9	54	Negative	109	28,7	100	3,5
HIV-	HAART0306	F	31,5	45	Negative	53	10,8	87	8
HIV+	HAART0308	F	26,8	37	100++	67	15,1	REJECTED	
HIV+	HAART0330	M	32,5	41	Negative	67	10	<3,00	UNABLE TO CALCULATE RESULT
HIV+	HAART0336	F	23,1	46	Negative	51	10,9	131	12
HIV+	HAART0337	F	26,5	43	Negative	60	3,2	69	21,5
HIV+	HAART0343	F	19,9	56	Negative	64	22	28	13,8
HIV-	HAART0345	F	25,6	37	Negative	59	18	5	0,3
HIV+	HAART0350	M	27,9	54	Negative	118	22,2	UNABLE TO CALCULATE	
HIV+	HAART0351	M	18,6	55	Negative	59	4	52	12,9
HIV-	HAART0361	F	40,1	42	Negative	44	7,9	<3,00	UNABLE TO CALCULATE RESULT
HIV+	HAART0364	F	22,6	35	Negative	47	8,8	<3,00	UNABLE TO CALCULATE RESULT
HIV+	HAART0365	F	18,8	35	Negative	65	10,5	<3,00	UNABLE TO CALCULATE RESULT
HIV+	HAART0372	M	21,9	43	Negative	76	25,1	187	7,4
HIV+	HAART0373	M	17,8	40	Negative	85	17,4	10	0,6
HIV+	HAART0376	M	17,9	38	Negative	89	2,7	3,4	1,3
HIV-	HAART0377	M	34,0	48	Negative	121	37,5	6,9	0,2
HIV+	HAART0381	F	25,6	37	Negative	60	17,2	34	1,9
HIV+	HAART0382	F	17,7	64	Negative	77	10,8	33	3,1
HIV+	HAART0383	M	18,5	46	Negative	125	11,2	282	25,2
HIV+	HAART0389	M	17,2	42	Trace	73	18,5	76	4,1
HIV+	HAART0390	M	22,9	30	Negative	119	19,3	15	0,8

HIV-	HAART0397	M	19,4	60	Negative	115	11,2	5,1	0,5
HIV-	HAART0398	M	19,8	31	Negative	78	8	291	36,4
HIV-	HAART0399	M	25,3	60	Negative	87	11,9	14	1,1
HIV+	HAART0403	F	32,9	31	Negative	77	17	9,3	0,5
HIV-	HAART0406	F	21,5	51	Negative	56	4,6	<3,00	UNABLE TO CALCULATE RESULT
HIV-	HAART0407	F	21,5	48	Negative	57	9,7	5,7	0,6
HIV+	HAART0410	M	18,5	32	Negative	87	12,5	3,1	0,2
HIV+	HAART0412	M	24,5	38	Negative	87	18,4	14	0,8
HIV+	HAART0421	F	20,3	63	Negative	115	20,7	18	0,9
HIV+	HAART0422	F	24,7	55	Negative	63	5	307	61,4
HIV+	HAART0423	M	Under weight	30	300+++	137	34,7	>711	UNABLE TO CALCULATE RESULT
HIV+	HAART0428	F	41,8	39	Negative	56	11,1	4,7	0,4
HIV+	HAART0431	F	26,8	35	Negative	49	15,5	22	1,4
HIV-	HAART0436	F	37,5	43		90	22,4	85	3,8
HIV-	HAART0437	M	21,1	36	30+	77	12,3	138	11,2
HIV+	HAART0441	F	31,3	38	Trace	57	5,3	72	13,5
HIV-	HAART0452	F	22,4	61	Negative	77	3,4	7,5	2,2
HIV+	HAART0468	M	21,1	43	Negative	99	20,2	183	9
HIV+	HAART0469	M	15,1	43	Negative	107	10,7	46	4,3
HIV-	HAART0470	M	16,5	48	Negative	73	16,3	39	2,4
HIV+	HAART0484	M	17,9	32	Negative	105	19,9	3,8	0,2
HIV+	HAART0485	F	21,4	31		50	22,2	12	0,5
HIV+	HAART0486	M	17,6	54		73	17,4	7,7	0,4
HIV-	HAART0489	M	19,1	29	Trace	74	14,1	6,3	0,4
HIV-	HAART0491	F	41,8	29	Negative	52	12,4	6,8	0,6
HIV+	HAART0493	M	17,8	35	Negative	85	11,5	13	1,1
HIV-	HAART0497	M	32,8	59	Negative	94	11	5,7	0,5
HIV+	HAART0499	F	15,8	48	Negative	68	15,9	29	1,8
HIV+	HAART0502	F	40,8	48	Negative	37	12	31	2,6
HIV+	HAART0503	F	16,8	57	Negative	55	6,6	9	1,4
HIV+	HAART0504	F	24,1	43	100++	66	38,4	UNABLE TO CALCULATE	0,109
HIV+	HAART0506	F	31,9	36	Negative	66	10,2	0,9	0,1
HIV-	HAART0508	F	34,4	33	Negative	58	19,9	1,3	0,1
HIV-	HAART0509	M	21,8	39	Negative	89	18,7	0,5	0,1

The data provided in APPENDIX D indicate the genotypes for HIV+ with and without CKD. Appendix E represent genotypes for HIV- participants with and without CKD. The table shows all the participants that were genotyped in this study and their genotype results.

Appendix D. Genotyping results for *APOL1* G1 ($G1G^+$ $G1^+M$) and G2 for HIV positive samples, with and without CKD.

HIV+ CKD+	**$G1G^+$** rs73885319	**$G1^+M$** rs60910145	**G2** rs71785313	**HIV+ CKD-**	**$G1G^+$** rs73885319	**$G1^+M$** rs60910145	**G2** rs71785313
Haart 010	G0/G0	G0/G0	G0/G2	**Haart 003**	G0/G0	G0/G0	G0/G0
Haart 015	G0/G1	G0/G1	G0/G0	**Haart 005**	G0/G1	G0/G1	G0/G0
Haart 016	G0/G0	G0/G0	G0/G0	**Haart 023**	G0/G1	G0/G1	G0/G0
Haart 024	G0/G0	G0/G0	G2/G2	**Haart 047**	G0/G1	G0/G1	G0/G0
Haart 025	G0/G0	G0/G0	G0/G2	**Haart 053**	G0/G0	G0/G0	G0/G0
Haart 036	G0/G1	G0/G1	G0/G0	**Haart 068**	G0/G1	G0/G1	G0/G0
Haart 041	G0/G0	G0/G0	G0/G0	**Haart 074**	G0/G0	G0/G0	G0/G0
Haart 046	G0/G0	G0/G0	G0/G0	**Haart 116**	G0/G0	G0/G0	G0/G0
Haart 058	G0/G0	G0/G0	G0/G0	**Haart 131**	G0/G0	G0/G0	G0/G0
Haart 061	G0/G0	G0/G0	G0/G0	**Haart 167**	G0/G0	G0/G0	G0/G0
Haart 069	G0/G0	G0/G0	G0/G0	**Haart 176**	G0/G0	G0/G0	G0/G2
Haart 071	G0/G1	G0/G1	G0/G0	**Haart 235**	G0/G0	G0/G0	G0/G2
Haart 075	G0/G0	G0/G0	G0/G0	**Haart 243**	G0/G1	G0/G1	G0/G0
Haart 077	G0/G0	G0/G0	G0/G0	**Haart 248**	G0/G0	G0/G0	G0/G2
Haart 084	G0/G0	G0/G0	G0/G0	**Haart 256**	G0/G0	G0/G0	G0/G0
Haart 085	G0/G0	G0/G0	G0/G2	**Haart 258**	G0/G0	G0/G0	G0/G0
Haart 091	G0/G0	G0/G0	G0/G0	**Haart 282**	G0/G0	G0/G0	G0/G0
Haart 096	G0/G0	G0/G0	G0/G0	**Haart 291**	G0/G0	G0/G0	G0/G0
Haart 097	G0/G1	G0/G1	G0/G0	**Haart 305**	G0/G1	G0/G1	G0/G0
Haart 101	G0/G0	G0/G0	G0/G2	**Haart 330**	G0/G0	G0/G0	G0/G0
Haart 110	G0/G1	G0/G1	G0/G0	**Haart 336**	G0/G0	G0/G0	G0/G0
Haart 117	G0/G1	G0/G1	G0/G0	**Haart 351**	G0/G1	G0/G1	G0/G0
Haart 125	G0/G0	G0/G0	G0/G0	**Haart 364**	G0/G0	G0/G0	G0/G0
Haart 011	G0/G0	G0/G0	G0/G0	**Haart 365**	G0/G0	G0/G0	G0/G0
Haart 051	G0/G0	G0/G0	G0/G0	**Haart 372**	G0/G0	G0/G0	G0/G2
Haart 102	G0/G0	G0/G0	G2/G2	**Haart 373**	G0/G1	G0/G1	G0/G0

Haart 109	G0/G0	G0/G0	G2/G2	**Haart 376**	G0/G0	G0/G0	G0/G2
Haart 153	G0/G0	G0/G0	G0/G0	**Haart 383**	G0/G0	G0/G0	G0/G0
Haart 154	G0/G0	G0/G0	G0/G0	**Haart 390**	G0/G0	G0/G0	G0/G0
Haart 155	G0/G0	G0/G0	G0/G2	**Haart 403**	G0/G1	G0/G1	G0/G2
Haart 156	G0/G0	G0/G0	G0/G2	**Haart 410**	G0/G0	G0/G0	G0/G0
Haart 157	G0/G0	G0/G0	G0/G2	**Haart 412**	G0/G0	G0/G0	G0/G0
Haart 158	G0/G0	G0/G0	G0/G0	**Haart 421**	G0/G0	G0/G0	G0/G0
Haart 160	G0/G0	G0/G0	G0/G0	**Haart 422**	G0/G0	G0/G0	G0/G0
Haart 161	G0/G0	G0/G0	G0/G2	**Haart 428**	G0/G0	G0/G0	G0/G0
Haart 185	G0/G0	G0/G0	G0/G0	**Haart 431**	G0/G0	G0/G0	G0/G0
Haart 192	G0/G1	G0/G1	G0/G2	**Haart 468**	G0/G1	G0/G1	G0/G0
Haart 200	G0/G1	G0/G1	G0/G0	**Haart 484**	G0/G0	G0/G0	G0/G0
Haart 201	G0/G0	G0/G0	G0/G2	**Haart 485**	G0/G1	G0/G1	G0/G0
Haart 203	G0/G0	G0/G0	G0/G0	**Haart 493**	G0/G0	G0/G0	G0/G0
Haart 205	G0/G0	G0/G0	G0/G0	**Haart 499**	G0/G0	G0/G0	G0/G2
Haart 212	G0/G0	G0/G0	G0/G0	**Haart 502**	G0/G0	G0/G0	G0/G0
Haart 213	G0/G0	G0/G0	G0/G2	**Haart 503**	G0/G0	G0/G0	G0/G2
Haart 214	G0/G0	G0/G0	G0/G2	**Haart 506**	G0/G0	G0/G0	G0/G0
Haart 223	G0/G0	G0/G0	G0/G0	**Haart 469**	G0/G0	G0/G0	G2/G2
Haart 236	G0/G0	G0/G0	G0/G0	**Haart 337**	G0/G0	G0/G0	G0/G2
Haart 237	G0/G0	G0/G0	G0/G0	**Haart 350**	G0/G1	G0/G1	G0/G0
Haart 260	G0/G0	G0/G0	G0/G0	**Haart 296**	G0/G0	G0/G0	G0/G0
Haart 279	G0/G0	G0/G0	G0/G0	**Haart 035**	G0/G1	G0/G1	G0/G0
Haart 504	G0/G0	G0/G0	G0/G2	**Haart 343**	G0/G0	G0/G0	G0/G0
Haart 295	G0/G0	G0/G0	G0/G0	**Haart 284**	G0/G0	G0/G0	G0/G0
Haart 308	G0/G0	G0/G0	G0/G2	**Haart 382**	G0/G0	G0/G0	G0/G0
Haart 389	G0/G0	G0/G0	G0/G2	**Haart 175**	G0/G0	G0/G0	G0/G0
Haart 423	G0/G1	G0/G1	G0/G0	**Haart 486**	G0/G0	G0/G0	G0/G0
Haart 441	G0/G0	G0/G0	G0/G0	**Haart 381**	G0/G0	G0/G0	G0/G0

WT (G0 homozygous) – black text; Heterozygote – blue; Homozygote variant - red

Appendix E. Genotyping results for *APOL1* G1 ($G1G^+$ $G1^+M$) and G2 for HIV negative samples

HIV- CKD+	**$G1G^+$** rs73885319	**$G1^+M$** rs60910145	**G2** rs71785313	**HIV- CKD-**	**$G1G^+$** rs73885319	**$G1^+M$** rs60910145	**G2** rs71785313
Haart 013	G0/G0	G0/G0	G0/G0	**Haart 012**	G0/G1	G0/G1	G0/G0
Haart 030	G0/G1	G0/G0	G0/G0	**Haart 014**	G0/G0	G0/G0	G0/G2
Haart 033	G0/G0	G0/G0	G0/G0	**Haart 022**	G0/G1	G0/G1	G0/G0
Haart 037	G0/G1	G0/G1	G0/G0	**Haart 026**	G1/G1	G1/G1	G0/G0
Haart 038	G0/G0	G0/G0	G0/G0	**Haart 028**	G0/G0	G0/G0	G0/G2
Haart 039	G0/G1	G0/G1	G0/G0	**Haart 032**	G0/G0	G0/G0	G0/G0
Haart 054	G0/G0	G0/G0	G0/G0	**Haart 034**	G0/G0	G0/G0	G0/G0
Haart 055	G0/G0	G0/G0	G0/G2	**Haart 043**	G0/G0	G0/G0	G0/G0
Haart 056	G0/G0	G0/G0	G0/G0	**Haart 045**	G0/G0	G0/G0	G0/G0
Haart 098	G0/G0	G0/G0	G2/G2	**Haart 057**	G0/G0	G0/G0	G0/G0
Haart 112	G0/G0	G0/G0	G0/G2	**Haart 063**	G0/G0	G0/G0	G0/G2
Haart 113	G0/G0	G0/G0	G0/G0	**Haart 065**	G0/G0	G0/G0	G0/G0
Haart 119	G0/G0	G0/G0	G2/G2	**Haart 073**	G0/G0	G0/G0	G2/G2
Haart 122	G0/G0	G0/G0	G0/G2	**Haart 211**	G0/G0	G0/G0	G0/G0
Haart 134	G0/G0	G0/G0	G0/G0	**Haart 088**	G0/G0	G0/G0	G0/G0
Haart 140	G0/G1	G0/G1	G0/G0	**Haart 094**	G0/G0	G0/G0	G0/G0
Haart 143	G0/G0	G0/G0	G0/G0	**Haart 104**	G0/G0	G0/G0	G0/G0
Haart 146	G0/G0	G0/G0	G0/G0	**Haart 108**	G0/G0	G0/G0	G0/G0
Haart 150	G0/G0	G0/G0	G0/G0	**Haart 111**	G0/G0	G0/G0	G2/G2
Haart 151	G0/G1	G0/G1	G0/G0	**Haart 115**	G0/G0	G0/G0	G0/G0
Haart 152	G0/G0	G0/G0	G0/G0	**Haart 470**	G0/G0	G0/G0	G0/G0
Haart 162	G0/G0	G0/G0	G0/G0	**Haart 129**	G0/G0	G0/G0	G0/G2
Haart 163	G0/G0	G0/G0	G0/G0	**Haart 136**	G0/G0	G0/G0	G0/G0
Haart 172	G0/G0	G0/G0	G0/G0	**Haart 165**	G0/G0	G0/G0	G0/G0
Haart 173	G0/G0	G0/G0	G2/G2	**Haart 166**	G0/G0	G0/G0	G0/G0
Haart 181	G0/G0	G0/G0	G0/G0	**Haart 178**	G0/G0	G0/G0	G0/G0
Haart 186	G0/G0	G0/G0	G0/G0	**Haart 208**	G0/G0	G0/G0	G0/G2
Haart 187	G0/G0	G0/G0	G0/G0	**Haart 244**	G0/G0	G0/G0	G0/G0
Haart 219	G0/G0	G0/G0	G0/G0	**Haart 251**	G0/G1	G0/G1	G0/G2
Haart 220	G0/G0	G0/G0	G0/G0	**Haart 254**	G0/G0	G0/G0	G0/G0
H0aart 224	G0/G1	G0/G1	G0/G0	**Haart 266**	G0/G0	G0/G0	G0/G0
Haart 233	G0/G0	G0/G0	G0/G0	**Haart 273**	G0/G0	G0/G0	G0/G0
Haart 234	G0/G0	G0/G0	G0/G0	**Haart 276**	G0/G0	G0/G0	G0/G0
Haart 265	G0/G0	G0/G0	G0/G0	**Haart 277**	G0/G0	G0/G0	G0/G2

Haart 298	G0/G0	G0/G0	G0/G0	**Haart 300**	G0/G0	G0/G0	G0/G0
Haart 437	G0/G0	G0/G0	G0/G2	**Haart 306**	G0/G0	G0/G0	G0/G0
Haart 141	G0/G0	G0/G0	G0/G0	**Haart 345**	G0/G0	G0/G0	G0/G0
Haart 148	G0/G0	G0/G0	G0/G0	**Haart 361**	G0/G1	G0/G1	G0/G0
Haart 193	G0/G0	G0/G0	G0/G0	**Haart 377**	G0/G0	G0/G0	G0/G0
Haart 194	G0/G1	G0/G1	G0/G0	**Haart 397**	G0/G0	G0/G0	G0/G0
Haart 197	G0/G0	G0/G0	G0/G2	**Haart 398**	G0/G1	G0/G1	G0/G0
Haart 198	G0/G0	G0/G0	G0/G0	**Haart 399**	G0/G0	G0/G0	G0/G0
Haart 238	G0/G0	G0/G0	G0/G0	**Haart 406**	G0/G0	G0/G0	G0/G0
Haart 240	G0/G0	G0/G0	G0/G2	**Haart 407**	G0/G0	G0/G0	G0/G0
Haart 207	G0/G0	G0/G0	G0/G2	**Haart 436**	G0/G0	G0/G0	G0/G2
Haart 080	G0/G0	G0/G0	G0/G0	**Haart 452**	G0/G0	G0/G0	G0/G0
Haart 093	G0/G0	G0/G0	G0/G2	**Haart 491**	G0/G0	G0/G0	G0/G0
Haart 095	G0/G1	G0/G1	G0/G0	**Haart 497**	G0/G0	G0/G0	G0/G0
Haart 120	G0/G0	G0/G0	G0/G0	**Haart 105**	G0/G0	G0/G0	G0/G0
Haart 128	G0/G0	G0/G0	G0/G0	**Haart 189**	G0/G0	G0/G0	G0/G2
Haart 130	G0/G0	G0/G0	G0/G2	**Haart 135**	G0/G1	G0/G1	G0/G0
Haart 139	G0/G0	G0/G0	G0/G0	**Haart 508**	G0/G0	G0/G0	G0/G2
Haart 179	G0/G0	G0/G0	G0/G0	**Haart 509**	G0/G0	G0/G0	G2/G2
Haart 180	G0/G0	G0/G0	G0/G0	**Haart 196**	G0/G0	G0/G0	G0/G0
Haart 489	G0/G0	G0/G0	G0/G0	**Haart 144**	G0/G0	G0/G0	G0/G0

WT (G0 homozygous) – black text; Heterozygote – blue; Homozygote variant – red

Appendix F. Sequencing results for HIV+ and CKD+ samples

HIV+ CKD+	G1G+ (rs73885319)	G1+M (rs60910145).	G2 (rs71785313)	Genotype
Haart 010	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G0/G2
Haart 015	cDNA.1253A>G	cDNA.1381T>G	5'- ATAATTATAA-3'	G0/G1
Haart 024	5'CCTGTA**A**GCTTC3	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G2/G2
Haart 025	5'CCTGTA**A**GCTTC3	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G0/G2
Haart 061	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 069	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 075	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 085	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G0/G2
Haart 097	cDNA.1253A>G	cDNA.1381T>G	5'- ATAATTATAA-3'	G0/G1
Haart 109	5'-CCTGTAAGCTTC-3'	v5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G2/G2
Haart 117	cDNA.1253A>G	cDNA.1381T>G	5'- ATAATTATAA-3'	G0/G1
Haart 155	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G0/G2
Haart 160	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 161	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G2
Haart 182	---------	---------	---------	---------
Haart 200	cDNA.1253A>G	cDNA.1381T>G	5'- ATAATTATAA-3'	G0/G1
Haart 203	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 213	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G0/G2

Haart 214	5'CCTGTA**A**GCTTC3	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G0/G2
Haart 223	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 260	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 308	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G0/G2
Haart 423	cDNA.1253A>G	cDNA.1381T>G	5'- ATAATTATAA-3'	G0/G1
Haart 441	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 504	cDNA.1253A>G	cDNA.1381T>G	5'- ATAATTATAA-3'	G0/G1

Highlighted in green: G2/G2 haplotype

Highlighted in blue: G0/G2 haplotype

Highlighted in red: G0/G1 haplotype

No highlight: G0/G0 haplotype

Appendix G. Sequencing results for HIV+ and CKD- samples

HIV+ CKD-	G1G+ (rs73885319)	G1+M (rs60910145).	G2 (rs71785313)	Genotype
Haart 035	cDNA.1253A>G	cDNA.1381T>G	5'- ATAATTATAA-3'	G0/G1
Haart 047	cDNA.1253A>G	cDNA.1381T>G	5'- ATAATTATAA-3'	G0/G1
Haart 048	---------	---------	---------	---------
Haart 053	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 068	cDNA.1253A>G	cDNA.1381T>G	v5'- ATAATTATAA-3'	G0/G1
Haart 176	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G0/G2
Haart 284	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 296	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 305	cDNA.1253A>G	cDNA.1381T>G	5'- ATAATTATAA-3'	G0/G1
Haart 336	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 337	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G0/G2
Haart 343	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 350	cDNA.1253A>G	cDNA.1381T>G	5'- ATAATTATAA-3'	G0/G1
Haart 351	cDNA.1253A>G	cDNA.1381T>G	5'- ATAATTATAA-3'	G0/G1
Haart 372	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G0/G2
Haart 373	cDNA.1253A>G	cDNA.1381T>G	5'- ATAATTATAA-3'	G0/G1
Haart 376	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G0/G2
Haart 382	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0

Haart 383	5'CC'TGTA**A**GCTTC3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 390	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 403	cDNA.1253A>G	cDNA.1381T>G	cDNA.1393_1398delTTATAA	G1/G2
Haart 421	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 422	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 468	cDNA.1253A>G	cDNA.1381T>G	5'- ATAATTATAA-3'	G0/G1
Haart 469	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G2/G2
Haart 483	---------	---------	---------	---------
Haart 499	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G0/G2

Highlighted in green: G2/G2 haplotype

Highlighted in blue: G0/G2 haplotype

Highlighted in yellow: G1/G2 haplotype

Highlighted in red: G0/G1 haplotype

No highlight: G0/G0 haplotype

Appendix H. Sequencing results for HIV- and CKD+ samples

HIV-CKD+	G1G+ (rs73885319)	G1+M (rs60910145).	G2 (rs71785313)	Genotype
Haart 013	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 030	cDNA.1253A>G	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G1
Haart 080	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 093	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G0/G2
Haart 095	cDNA.1253A>G	cDNA.1381T>G	5'- ATAATTATAA-3'	G0/G1
Haart 113	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 120	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 128	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 130	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G0/G2
Haart 134	cDNA.1253A>G	cDNA.1381T>G	5'- ATAATTATAA-3'	G0/G1
Haart 139	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 152	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 179	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 180	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 187	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 207	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G0/G2
Haart 265	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 437	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G0/G2

Haart 489	5’-CCTGTAAGCTTC-3’	5’-AAACATTCTCAAC-3’	5’- ATAATTATAA-3’	G0/G0

Highlighted in blue: G0/G2 haplotype

Highlighted in red: G0/G1 haplotype

No highlight: G0/G0 haplotype

Appendix I. Sequencing results for HIV- and CKD- samples

HIV-CKD-	G1G+ (rs73885319)	G1+M (rs60910145).	G2 (rs71785313)	Genotype
Haart 026	cDNA.1253A>G	cDNA.1381T>G	5'- ATAATTATAA-3'	G1/G1
Haart 028	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G0/G2
Haart 045	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 063	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G0/G2
Haart 105	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 111	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G2/G2
Haart 144	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 189	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G0/G2
Haart 196	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 211	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 251	cDNA.1253A>G	cDNA.1381T>G	cDNA.1393_1398delTTATAA	G1/G2
Haart 254	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'5'- ATAATTATAA-3'	G0/G0
Haart 266	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 277	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	cDNA.1393_1398delTTATAA	G0/G2
Haart 306	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 377	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 397	5'-CCTGTAAGCTTC-3'	5'-AAACATTCTCAAC-3'	5'- ATAATTATAA-3'	G0/G0
Haart 398	cDNA.1253A>G	cDNA.1381T>G	5'- ATAATTATAA-3'	G0/G1

Haart 406	5’-CCTGTAAGCTTC-3’	5’-AAACATTCTCAAC-3’	5’- ATAATTATAA-3’	G0/G0
Haart 414	---------	---------	---------	---------
Haart 436	5’-CCTGTAAGCTTC-3’	5’-AAACATTCTCAAC-3’	cDNA.1393_1398delTTATAA	G0/G2
Haart 452	5’-CCTGTAAGCTTC-3’	5’-AAACATTCTCAAC-3’	5’- ATAATTATAA-3’	G0/G0
Haart 470	5’-CCTGTAAGCTTC-3’	5’-AAACATTCTCAAC-3’	5’- ATAATTATAA-3’	G0/G0
Haart 482	---------	---------	---------	---------
Haart 483	---------	---------	---------	---------
Haart 508	5’-CCTGTAAGCTTC-3’	5’-AAACATTCTCAAC-3’	cDNA.1393_1398delTTATAA	G0/G2
Haart 509	5’-CCTGTAAGCTTC-3’	5’-AAACATTCTCAAC-3’	cDNA.1393_1398delTTATAA	G2/G2
Haart 510	---------	---------	---------	---------

Highlighted in green: G2/G2 haplotype

Highlighted in blue: G0/G2 haplotype

Highlighted in red: G0/G1 haplotype

Highlighted in pink: G1/G1 haplotype

No highlight: G0/G0 haplotype

Appendix J *APOL1* G2 forward primer

INTEGRATED DNA TECHNOLOGIES

SPECIFICATION SHEET WWW.IDTDNA.COM

01-Jun-2023

Order No. **3881070**

Ref. No. **235754464**

Sequence - APOL1 G2 F — 25 nmole DNA Oligo, 20 bases

5'- AAG CAC TTA CAT GAG GGG GC -3'

Properties

Tm (50mM NaCl): 57.5 °C*
GC Content: 55.0%
Molecular Weight: 6,191.1
nmoles/OD260: 5.0
ug/OD260: 30.9
Ext. Coefficient: 200,400 L/(mole·cm)

Amount Of Oligo

6.3 = OD_{260} 31.2 nmoles = 0.19 mg

For 100 µM: add 312 µL

Shipped To

GERDA MARX
UNIVERSITY OF THE ORANGE FREE STAT
GENETICS
BLOEMFONTEIN, 9301
SOUTH AFRICA
0827869684
Customer No. 4233223 *PO No. 124-0000830805*

Secondary Structure Calculations

Lowest folding free energy (kcal/mole): 0.26 at 25 °C
Strongest Folding Tm: 20.7 °C

Oligo Base Types	Quantity
DNA bases	20
Modifications and Services	**Quantity**
Standard Desalting	1

Disclaimer

See on reverse page notes (I) (II) & (III) for usage, label license, and product warranties

Mfg. ID 379890827

Labels - Peel here

235754464 IDT
G.MARX
APOL1 G2 F

235754464 IDT
G.MARX
APOL1 G2 F

INSTRUCTIONS

*Lyophilized contents may appear as either a translucent film or a white powder. This variance does not affect the quality of the oligo.

*Please centrifuge tubes prior to opening. Some of the product may have been dislodged during shipping.

*The Tm shown takes no account of Mg^{2+} and dNTP concentrations. Use the OligoAnalyzer® Program at www.idtdna.com/schools to calculate accurate Tm for your reaction conditions.

M

Appendix K *APOL1* G2 reverse primer

INTEGRATED DNA TECHNOLOGIES

SPECIFICATION SHEET WWW.IDTDNA.COM

01-Jun-2023

Order No. **3881070**

Ref. No. **235754465**

Sequence - APOL1 G2 R

25 nmole DNA Oligo, 20 bases

5'- GGC ATA TCT CTC CTG GTG GC -3'

Properties

Tm (50mM NaCl):* 57.6 °C
GC Content: 60.0%
Molecular Weight: 6,100
nmoles/OD260: 5.6
ug/OD260: 34.0
Ext. Coefficient: 179,500 L/(mole·cm)

Amount Of Oligo

6.6 = 36.6 = 0.22
OD_{260} nmoles mg

For 100 µM: add 366 µL

Shipped To

GERDA MARX
UNIVERSITY OF THE ORANGE FREE STAT
GENETICS
BLOEMFONTEIN, 9301
SOUTH AFRICA
0827869684
Customer No. 4233223 PO No. 124-0000830805

Secondary Structure Calculations

Lowest folding free energy (kcal/mole): -0.07 at 25 °C
Strongest Folding Tm: 26.2 °C

Oligo Base Types	Quantity
DNA bases	20
Modifications and Services	Quantity
Standard Desalting	1

Disclaimer

See on reverse page notes (I) (II) & (III) for usage, label license, and product warranties

Mfg. ID379890828

Labels - Peel here

235754465 IDT G.MARX 379890828 01-Jun-2023 APOL1 G2 R

235754465 IDT G.MARX 379890828 01-Jun-2023 APOL1 G2 R

I N S T R U C T I O N S

•Lyophilized contents may appear as either a translucent film or a white powder. This variance does not affect the quality of the oligo.

•Please centrifuge tubes prior to opening. Some of the product may have been dislodged during shipping.

*The Tm shown takes no account of Mg^{2+} and dNTP concentrations. Use the OligoAnalyzer® Program at www.idtdna.com/scitools to calculate accurate Tm for your reaction conditions.

M

Appendix L *APOL1* G1 forward primer

INTEGRATED DNA TECHNOLOGIES

SPECIFICATION SHEET WWW.IDTDNA.COM

29-Sep-2023

Order No. **3948381**

Ref. No. **236896903**

Sequence - APOLG1_F 25 nmole DNA Oligo, 20 bases

5'- CTT CAG TCA GTA CCG CAT GC -3'

Properties

Tm (50mM NaCl):* 56.0 °C
GC Content: 55.0%
Molecular Weight: 6,053
nmoles/OD260: 5.4
ug/OD260: 32.9
Ext. Coefficient: 184,100 L/(mole·cm)

Amount Of Oligo

5.5 = 29.7 = 0.18
OD_{260} nmoles mg

For 100 µM: add 297 µL

Shipped To

GERDA MARX
UNIVERSITY OF THE ORANGE FREE STAT
GENETICS
BLOEMFONTEIN, 9301
SOUTH AFRICA
0827869684
Customer No. 4233223 *PO No. 124-0000849522*

Secondary Structure Calculations

Lowest folding free energy (kcal/mole): 0.63 at 25 °C
Strongest Folding Tm: 14.6 °C

Oligo Base Types	Quantity
DNA bases	20
Modifications and Services	Quantity
Standard Desalting	1

Disclaimer

See on reverse page notes (I) (II) & (III) for usage, label license, and product warranties

Mfg. ID 380674585

Labels - Peel here

236896903 IDT
G.MARX
380674585
APOLG1_F

236896903 IDT
G.MARX
380674585
APOLG1_F

I N S T R U C T I O N S

•Lyophilized contents may appear as either a translucent film or a white powder. This variance does not affect the quality of the oligo.

•Please centrifuge tubes prior to opening. Some of the product may have been dislodged during shipping.

*The Tm shown takes no account of Mg^{2+} and dNTP concentrations. Use the OligoAnalyzer® Program at www.idtdna.com/scitools to calculate accurate Tm for your reaction conditions.

M

Appendix M *APOL1* G1 reverse primer

INTEGRATED DNA TECHNOLOGIES

SPECIFICATION SHEET WWW.IDTDNA.COM

29-Sep-2023

Order No. **3948381**

Ref. No. **236896904**

Sequence - APOLG1_R 25 nmole DNA Oligo, 20 bases

5'- GGC ATA TCT CTC CTG GTG GC -3'

Properties

Tm (50mM NaCl):* 57.6 °C
GC Content: 60.0%
Molecular Weight: 6,100
nmoles/OD260: 5.6
ug/OD260: 34.0
Ext. Coefficient: 179,500 L/(mole·cm)

Amount Of Oligo

3.7 = 20.4 = 0.12
OD_{260} nmoles mg

For 100 µM: add 204 µL

Shipped To

GERDA MARX
UNIVERSITY OF THE ORANGE FREE STAT
GENETICS
BLOEMFONTEIN, 9301
SOUTH AFRICA
0827869684
Customer No. 4233223 PO No. 124-0000849522

Secondary Structure Calculations

Lowest folding free energy (kcal/mole): -0.07 at 25 °C
Strongest Folding Tm: 26.2 °C

Oligo Base Types	Quantity
DNA bases	20
Modifications and Services	**Quantity**
Standard Desalting	1

Disclaimer

See on reverse page notes (I) (II) & (III) for usage, label license, and product warranties

Mfg. ID 380674586

Labels - Peel here

I N S T R U C T I O N S

*Lyophilized contents may appear as either a translucent film or a white powder. This variance does not affect the quality of the oligo.

*Please centrifuge tubes prior to opening. Some of the product may have been dislodged during shipping.

*The Tm shown takes no account of Mg^{2+} and dNTP concentrations. Use the OligoAnalyzer® Program at www.idtdna.com/scitools to calculate accurate Tm for your reaction conditions.

M

Appendix N Certificate of analysis: TaqMan® SNP Genotyping Assays

applied biosystems
by Thermo Fisher Scientific

Certificate of Analysis

TaqMan® SNP Genotyping Assays

Sales Order: 1613481
Delivery Number: 901213291
Ship Date: 17-AUG-2023
Page: 1 of 1

Part Number: 4351379 **Part Description:** TaqMan® SNP Genotyping Assays, Human, SM

Assay ID:	RS Number:	Lot Number:	Expiration Date:
C_89555688_30	rs60910145	P230612-005C06	10-AUG-2028

Vial Type	Quality Control Test	Result
TaqMan (TM)	Fill Volume	PASS
TaqMan (TM)	Mass Spectrometry	PASS
TaqMan (TM)	Genomic Functional Test	NA

Assay ID:	RS Number:	Lot Number:	Expiration Date:
C_96253221_10	rs73885319	P230612-005C05	10-AUG-2028

Vial Type	Quality Control Test	Result
TaqMan (TM)	Fill Volume	PASS
TaqMan (TM)	Mass Spectrometry	PASS
TaqMan (TM)	Genomic Functional Test	NA

Assay ID:	RS Number:	Lot Number:	Expiration Date:
C_102754756_10	rs71785313	P230612-005C07	10-AUG-2028

Vial Type	Quality Control Test	Result
TaqMan (TM)	Fill Volume	PASS
TaqMan (TM)	Mass Spectrometry	PASS
TaqMan (TM)	Genomic Functional Test	NA

Applied Biosystems' oligo manufacturing process requires a fill volume check on each assay lot to verify that the volume is consistent with the required volume printed on the product label. Applied Biosystems' oligo manufacturing process also requires a mass spectrometry test for each component of an assay to verify that the identity of each oligo meets set specifications. All human assays receive a genomic functional test (GFT) on first synthesis. Human assays that pass testing on first synthesis do not require additional genomic functional testing; subsequent syntheses of already tested human assays and non-human assays receive fill volume check and mass spectrometry. NA = Not applicable and/or not available. A "+" sign appears for Assay IDs and RS Numbers greater than 20 characters. Full entry recorded in Assay Information File (AIF).

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

ISO 9001
ISO 13485
REGISTERED

Pleasanton,CA

Electronically generated, no signature required.

Doc p/n: 4425294 rev.D

Appendix O TaqMan® SNP Genotyping Assays Data Sheet

applied biosystems
by Thermo Fisher Scientific

TaqMan™ SNP Genotyping Assays Data Sheet

Sales Order:	1613481	**Order Date:**	10-Aug-2023
Rack ID:	8152492_1	**Customer P.O.:**	124-0000840524 / 183714 S
Delivery No:	901213291	**Ship-To Country:**	ZA
Customer:	LTC Tech South Africa (Pty) Ltd	**Contact:**	Zaahidah Wessels
Contact Email:	zaahidah.wessels@thermofisher.com		
Contact Phone:	123		
Number of Assays in this Rack:	3	**Number of Assays in this Order Made to Order:**	3

Assays in this Shipment

Tube Position	Assay ID*	RS Number*	Part Number
A01	C__98253221_10	rs73885319	4351379
B01	C__89555688_30	rs60910145	4351379
C01	C_102754756_10	rs71785313	4351379

** "+" sign appears for entries greater than 20 characters. Full entry recorded in Assay Information File (AIF).*

applied biosystems
by Thermo Fisher Scientific

Other Details

Assay Details

Part Number	Product	Reporter / Quencher	Volume (µL)	Formulation	No. of Reactions (5µL Reaction Size)
4351379	TaqMan™ SNP Genotyping Assays, Human, SM	Allele 1: VIC/MGB-NFQ Allele 2: FAM/MGB-NFQ	188	40x	1,500
4351376	TaqMan™ SNP Genotyping Assays, Human, MED	Allele 1: VIC/MGB-NFQ Allele 2: FAM/MGB-NFQ	625	40x	5,000
4351374	TaqMan™ SNP Genotyping Assays, Human, LG	Allele 1: VIC/MGB-NFQ Allele 2: FAM/MGB-NFQ	750	80x	12,000
4351384	TaqMan™ SNP Genotyping Assays, Non-human, SM	Allele 1: VIC/MGB-NFQ Allele 2: FAM/MGB-NFQ	188	40x	1,500
4351382	TaqMan™ SNP Genotyping Assays, Non-human, MED	Allele 1: VIC/MGB-NFQ Allele 2: FAM/MGB-NFQ	625	40x	5,000
4351380	TaqMan™ SNP Genotyping Assays, Non-human, LG	Allele 1: VIC/MGB-NFQ Allele 2: FAM/MGB-NFQ	750	80x	12,000

Probe and primer concentrations available on Assay Information File (AIF).

applied biosystems
by Thermo Fisher Scientific

Storage Conditions

−25°C to −15°C

Supporting Documentation

Supporting documentation and files associated with this product are available for download at thermofisher.com/tagmanfiles:

- Ordering Protocol: Procedures for using online search
- User Guide: "How to" guide for performing the application
- Assay Information File (AIF): Technical details for all assays in the shipment
- Certificate of Analysis: Validation results for your assays
- Data Sheet: Electronic version of this document
- Safety Data Sheet (SDS): Safety guidelines
- Understanding Your Shipment: Directory for understanding the contents of your shipment

Additional Quality Information

A standard certificate of analysis is included in the supporting documentation for this product. For additional quality information, visit thermofisher.com/support.

Technical Support

Visit www.thermofisher.com/contactus

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.thermofisher.com/us/en/home/global/terms-and-conditions. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.

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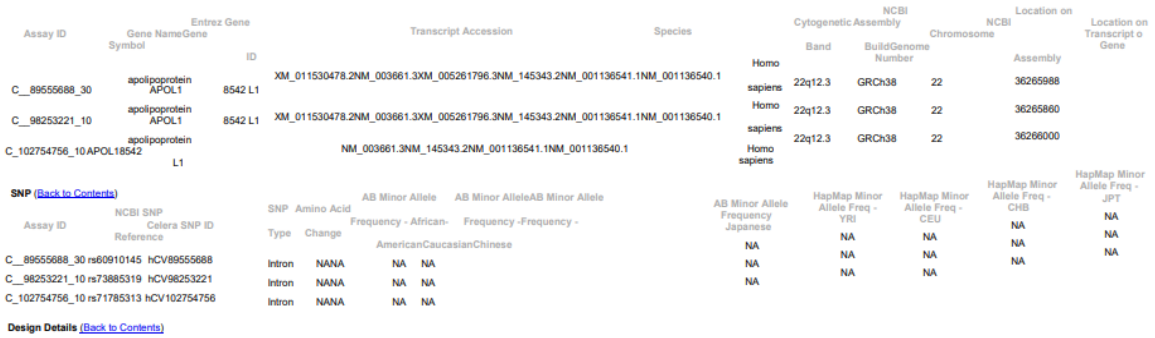
Trademarks

Pub. no. 4425293 rev.B

Appendix P Assay Information: TaqMan® SNP Genotyping Assays



Assay ID	Gene Name	Gene Symbol	Entrez Gene ID	Transcript Accession	Species	Cytogenetic Band	NCBI Assembly BuildGenome Number	Chromosome	Location on NCBI Assembly	Location on Transcript o Gene
C__89555688_30	apolipoprotein APOL1	APOL1	8542 L1	XM_011530478.2NM_003661.3XM_005261796.3NM_145343.2NM_001136541.1NM_001136540.1	Homo sapiens	22q12.3	GRCh38	22	36265988	
C__98253221_10	apolipoprotein APOL1	APOL1	8542 L1	XM_011530478.2NM_003661.3XM_005261796.3NM_145343.2NM_001136541.1NM_001136540.1	Homo sapiens	22q12.3	GRCh38	22	36265860	
C_102754756_10	apolipoprotein APOL1	APOL1	8542 L1	NM_003661.3NM_145343.2NM_001136541.1NM_001136540.1	Homo sapiens	22q12.3	GRCh38	22	36266000	

SNP (Back to Contents)

Assay ID	NCBI SNP Reference	Celera SNP ID	SNP Type	Amino Acid Change	AB Minor Allele Frequency - African-American	AB Minor Allele Frequency - Caucasian	AB Minor Allele Frequency - Chinese	AB Minor Allele Frequency Japanese	HapMap Minor Allele Freq - YRI	HapMap Minor Allele Freq - CEU	HapMap Minor Allele Freq - CHB	HapMap Minor Allele Freq - JPT
C__89555688_30	rs60910145	hCV89555688	Intron	NANA	NA	NA		NA	NA	NA	NA	NA
C__98253221_10	rs73885319	hCV98253221	Intron	NANA	NA	NA		NA	NA	NA	NA	NA
C_102754756_10	rs71785313	hCV102754756	Intron	NANA	NA	NA		NA	NA	NA	NA	NA

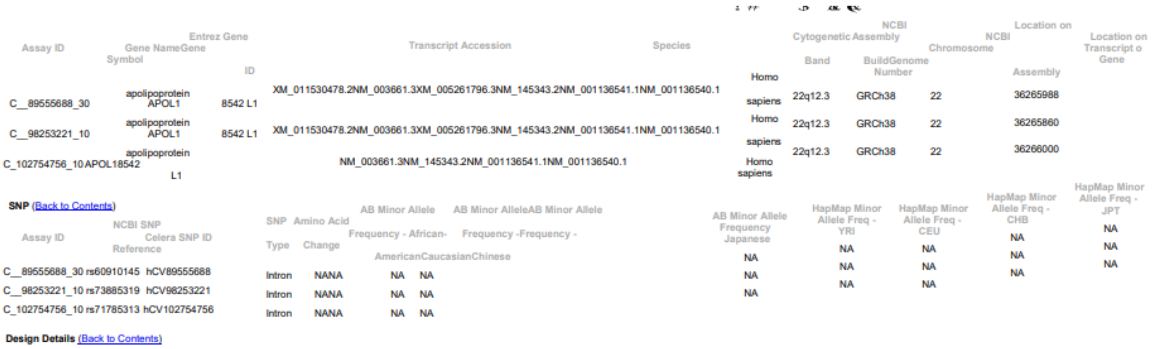
Design Details (Back to Contents)

10/25/23, 12:22 PMAssay_Info_TaqMan_SNP_SalesOrder_1613481_RackID_8152492_1.mhtml

Forward Reverse

Assay ID	Assay Mix Concentration	Reporter 1 Dye	Reporter 1 Concentration (µM)	Reporter 1 Quencher	Reporter 2 Dye	Reporter 2 Concentration (µM)	Reporter 2 Quencher	Primer Concentration (µM)	Primer Concentration (µM)	Context Sequence	Amplicon Size
C__89555688_30	40x	VIC	8	NFQ	FAM	8	NFQ	36	36	AGGAGCTGGAGGAGAAGCTAAACAT[G/T]CTCAACAATAATTATAAGATTCTGC	NA
C__98253221_10	40x	VIC	8	NFQ	FAM	8	NFQ	36	36	CAAGCTCACGGATGTGGCCCCTGTA[A/G]GCTTCTTTCTTGTGCTGGATGTAGT	NA
C_102754756_10	40x	VIC	8	NFQ	FAM	8	NFQ	36	36	AGCTAAACATTCTCAACAATAA[TTATAA/-]GATTCTGCAGGCGGACCAAGAAC	NA

NA = Not applicable or not available.



Assay ID	Gene Name	Gene Symbol	Entrez Gene ID	Transcript Accession	Species	Cytogenetic Band	NCBI Assembly BuildGenome Number	Chromosome	Location on NCBI Assembly	Location on Transcript o Gene
C__89555688_30	apolipoprotein APOL1	APOL1	8542 L1	XM_011530478.2NM_003661.3XM_005261796.3NM_145343.2NM_001136541.1NM_001136540.1	Homo sapiens	22q12.3	GRCh38	22	36265988	
C__98253221_10	apolipoprotein APOL1	APOL1	8542 L1	XM_011530478.2NM_003661.3XM_005261796.3NM_145343.2NM_001136541.1NM_001136540.1	Homo sapiens	22q12.3	GRCh38	22	36265860	
C_102754756_10	apolipoprotein APOL1	APOL1	8542 L1	NM_003661.3NM_145343.2NM_001136541.1NM_001136540.1	Homo sapiens	22q12.3	GRCh38	22	36266000	

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C__89555688_30	rs60910145	hCV89555688	Intron	NANA	NA	NA		NA	NA	NA	NA	NA
C__98253221_10	rs73885319	hCV98253221	Intron	NANA	NA	NA		NA	NA	NA	NA	NA
C_102754756_10	rs71785313	hCV102754756	Intron	NANA	NA	NA		NA	NA	NA	NA	NA

Design Details (Back to Contents)

10/25/23, 12:22 PMAssay_Info_TaqMan_SNP_SalesOrder_1613481_RackID_8152492_1.mhtml

Forward Reverse

Assay ID	Assay Mix Concentration	Reporter 1 Dye	Reporter 1 Concentration (µM)	Reporter 1 Quencher	Reporter 2 Dye	Reporter 2 Concentration (µM)	Reporter 2 Quencher	Primer Concentration (µM)	Primer Concentration (µM)	Context Sequence	Amplicon Size
C__89555688_30	40x	VIC	8	NFQ	FAM	8	NFQ	36	36	AGGAGCTGGAGGAGAAGCTAAACAT[G/T]CTCAACAATAATTATAAGATTCTGC	NA
C__98253221_10	40x	VIC	8	NFQ	FAM	8	NFQ	36	36	CAAGCTCACGGATGTGGCCCCTGTA[A/G]GCTTCTTTCTTGTGCTGGATGTAGT	NA
C_102754756_10	40x	VIC	8	NFQ	FAM	8	NFQ	36	36	AGCTAAACATTCTCAACAATAA[TTATAA/-]GATTCTGCAGGCGGACCAAGAAC	NA

NA = Not applicable or not available.