Profiling Gene Expression Pattern Changes in HIV-1-Associated Kidney Disease

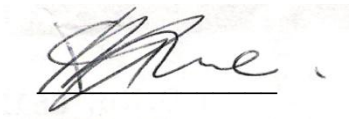
Keleabetswe Lerato Mpye

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy.

Johannesburg, 2015

Declaration

I, Keleabetswe Lerato Mpye, hereby declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted for any other degree or examination at any other University.



Keleabetswe Lerato Mpye

This _**17th** ____ day of ____**June**____, 2015.

Abstract

HIV-1 infected individuals are at risk of developing clinically and morphologically diverse renal complications. Most of the ensuing renal complications are similar to those observed in non-HIV patients while some are limited to HIV-infected patients. Kidney diseases are amongst the top 10 leading causes of death in the general population and affect over 10 % of the adult population. Two to ten percent of HIV-1 infected patients ultimately suffer chronic kidney disease, and specifically HIV-associated nephropathy (HIVAN), characterized clinically by nephrotic range proteinuria and histologically by collapsing focal segmental glomerulosclerosis (FSGS). Genetic mutations and disruption of the podocyte foot process and actin-based cytoskeleton architecture through dysregulation of its principal element, actin, or its associated proteins, lead to proteinuria and nephrotic syndrome. The majority of these proteins are susceptible to proteolysis by a lysosomal cysteine protease, cathepsin-L. The current study set out to profile changes in gene expression in HIVAN relative to HIV negative FSGS, minimal change disease (MCD) and non-proteinuric healthy controls. Probing the presence and localization of cathepsin-L and dynamin in these proteinuric kidney diseases, we showed that cathepsin-L and dynamin expression is enhanced in HIVAN and FSGS, relative to the healthy controls and MCD. We further profiled the expression of the 3 isoforms of cathepsin-L and dynamin using Quantitative reverse transcriptase PCR. *CTSL-3* expression was similar between all disease groups whilst *CSTL-2* was reduced relative to the normal tissue. There was most variation in the expression of *CSTL- 1* between the disease tissues with down-regulation of *CSTL-1* in both HIVAN and FSGS relative to the control and MCD tissue. The expression of dynamin remained largely unchanged between the disease groups with the exception of *DYN-1* which was downregulated in MCD relative to FSGS. Given that dynamin expression was barely altered across the proteinuric states, compared to the healthy non-proteinuric controls, signifies that proteolysis of the actin-associated proteins synaptopodin and/or slit diaphragm-associated CD2AP by the cathepsins-L 2 and 3 was responsible for the proteinuria in HIVAN, FSGS and MCD. Our results show that FSGS manifests through attenuated CD2AP, up-regulated TGF-β1 and cathepsin-L activity and not via dynamin degradation. The attenuated mRNA expression levels of dynamin, CD2AP and synaptopodin in renal tissue of the proteinuric kidneys validate that podocyte injury, in the form of loss of foot process integrity and slit diaphragm permeability, manifests through the over-expressed cathepsin-L enzymatic activity. Many of the genes that were altered in proteinuric kidney diseases, as observed from the pathway analysis of the PCR array data, are

mediated by transcriptional activation of nuclear factor kappa B (NF-KB). Transcriptional mRNA expression levels, validated in real-time quantitative RT-PCR analysis illustrated that HIV-1 infection induces uncontrolled activation and induction of immunity and inflammatory responses, indicated by down-regulation of *SIGIRR*, and modulation of expression patterns of immunity and inflammatory response genes in proteinuric kidney diseases. Other genes that were down-regulated in HIVAN are involved in the MAPK JNK cellular signaling pathway and apoptosis. HIV-1 infection modulates expression patterns of the genes that are involved in immunity and inflammatory responses in proteinuric kidney disease, compromising the structure and function not only of the kidney but of the entire immune system.

This work is dedicated to:

The world's most beautiful and charismatic daughter, Agonnejaalo Mamotlopi, and my family who continuously support and encourage me in all I do.

My grandfather, Abram Ramotlopi Mphye (1928 – 2002), for always believing in me and sowing the seed of success in my life. You must be beaming with pride as you look down on us from the heaven above.

"*Ke setlogolo sa Matebele, kgodumo e e lorole, Mohwaduba, Kgomo.*
Pula!!!"

Acknowledgements

i. My supervisors Dr. Raquel Duarte and Prof. Saraladevi Naicker for their guidance, for taking under their wing and giving me another chance at this degree

ii. Thérése Dix-Peek for always willing to help, discuss and resolve whatever the challenges I encountered. For the immense help with the statistical analysis of the data (even bringing in her personal computer for me to use), primer design and real-time PCR technique. For always having her office open for me to come vent, beg, the small talks and the good laughs

iii. Dr Caroline Dickens for helping with the primer design

iv. Dr Gago Saleshando for helping with the FFPE slides used for immunohistochemistry

v. Alex Kasembeli for helping with FFPE tissue acquisition

vi. Prof. Martin Hale, Dr. Pulane Mosiane and the staff at the Anatomical Pathology Department, National Health Laboratory Services, Charlotte Maxeke Johannesburg Academic Hospital, University of the Witwatersrand, Johannesburg, for providing the FFPE tissue

vii. Staff at the Anatomy Department, University of the Witwatersrand, Johannesburg, for allowing me to use their microscope

viii. Dr Tanya Augustine for the CellProfiler course

ix. Peter at the x-ray unit, area 623, Charlotte Maxeke Johannesburg Academic Hospital for making the developing station available to us at all times

x. Kiashanee Moodley for sharing the strain of printing, collating the thesis and submission, and for always being enthusiastic to lend a helping hand

xi. Members of the "Nephroncology" Research Unit, University of the Witwatersrand, Johannesburg, for the support and being a family to me

xii. The National Research Foundation, Poliomyelitis Research Foundation, the NKFSA Adcock Ingram and David Hepburn Awards for funding

xiii. Mama for the financial and emotional support throughout my studies

xiv. My cousin Kefilwe Balulu for giving up her precious time to take care of 2 months old daughter so that I can go back to the lab to complete this degree

xv. Mmane Anna for being a mother and a best friend every step of the way. You are remarkably splendid

xvi. My family: Sonia, Thapelo, Itu, Ago and daddy for their unwavering support and keeping me going

xvii. Kgothatso Ramaeto, Jabu Maleka and Kwena Nkoana for volunteering their laptops when mine bailed-out on me at the 11th hour. I am blessed

xviii. My friends and everyone else for their contributions

xix. I profusely thank the Lord for His grace, abundant blessings and for giving me a purpose in life. I am highly favoured and eternally grateful

Research outputs

Keleabetswe Lerato Mpye, Therésè Dix-Peek, Caroline Dickens, Jaya George, Saraladevi Naicker and Raquel Duarte. *HIV-1-Associated Kidney Disease: Disease- and Signaling Pathway-Specific Gene Expression Alterations*. World Congress of Nephrology (WCN), March 2015. Cape Town, South Africa. Poster presentation

Keleabetswe Lerato Mpye, Thérèse Dix-Peek, Jaya George, Gago Saleshando, Mpoti Seboka, Saraladevi Naicker and Raquel Duarte. *Profiling Gene Expression Pattern Changes in HIV-1-Associated Kidney Disease.* University of the Witwatersrand Health Sciences Research day, September 2014. Johannesburg, South Africa. Oral presentation

Keleabetswe Lerato Mpye, Thérèse Dix-Peek, Jaya George, Gago Saleshando, Mpoti Seboka, Saraladevi Naicker and Raquel Duarte. *Profiling Gene Expression Pattern Changes in HIV-1-Associated Kidney Disease*. South African Society of Biochemistry and Molecular Biology (SASBMB), July 2014, Cape Winelands, South Africa. Poster presentation

Table of contents

Declaration ii

Abstract iii

Dedications v

Acknowledgements vi

Research outputs viii

Table of contents ix

List of figures xiii

List of tables xv

ist of abbreviations xvii

1. BACKGROUND 1

1.1. Kidneys: An overview 1

1.2. Kidney diseases 6

1.2.1. Chronic kidney disease 8

1.2.2. Focal segmental glomerulosclerosis 8

1.2.3. HIVAN and HIVICK 9

1.3. Human immunodeficiency virus 11

1.4. HIV-1 acquisition in renal cells 13

1.5. HIV-1 genes implicated in HIVAN manifestation 14

1.6. Host genes and gene products implicated in renal disease 15

1.6.1. Dynamin 16

1.6.2. Cathepsin-L 17

1.6.3. Synaptopodin 18

1.6.4. CD2-associated protein 19

1.6.5. NPHS1 *and* NPHS2 20

1.7. HIV renal disease and treatment 21
1.8. Antiretroviral drug-induced nephrotoxicity 21
Study hypothesis 24
Aim 24
Objectives 24
2. MATERIALS AND METHODS 25
2.1. Sample acquisition 25
2.2. Immunohistochemistry 25
2.3. RNA isolation, purification and quantification 28
2.4. Template preparation 29
2.5. Primer design 32
2.6. Real-time quantitative polymerase chain reaction for assessing cathepsin-L and dynamin expression 32
2.7. Determining expression levels of podocyte cytoskeleton proteins 39
2.8. PCR arrays 39
2.9. Validation of PCR arrays 44
2.9.1. Validation of altered HIV-host response genes 44
2.9.2. Validation of altered TLR genes 47
2.10. Protein extraction 49
2.11. Western-blotting 49
2.12. Data analysis 51
2.12.1. Immunohistochemistry quantification 51
2.12.2. Statistical analysis of qPCR data 51
3. RESULTS 52
3.1. Immune-markers, cathepsin-L and dynamin, localization 52

3.2. Cathepsin-L and dynamin expression patterns 62
3.2.1. Transcriptional mRNA expression of cathepsin-L and dynamin 62
3.2.2. Western-blot validation of cathepsin-L and dynamin protein expression 65
3.3. Expression patterns of podocyte cytoskeleton proteins 69
3.4. Polymerase chain reaction arrays 72
3.4.1. HIV host response PCR arrays 73
3.4.1.1. qPCR validation of altered HIV-host response genes 76
3.4.2. Toll-like receptor PCR arrays 81
3.4.2.1. Western-blot validation of altered TLR proteins 81
3.4.2.2. qPCR validation of altered TLR genes 86
4. DISCUSSION 89
Dynamin localization in the kidney 89
Cathepsin-L localization in the kidney 90
Cathepsin-L and dynamin expression levels in renal tissue 91
Cathepsin-L enzymatic activity in the podocyte cytoskeleton 92
Altered host genes and pathways 93
Concluding remarks 98
Study limitations and future work 100
5. REFERENCES 101
Appendix 121
Ethics clearance certificate 121
Copyright certification 122
Immunohistochemistry 127
RNA isolation, purification and quantification 127
Template preparation 128

PCR arrays 129

Sodium dodecyl sulphate polyacrylamide gel electrophoresis 137

Western-blotting 138

List of figures

Figure 1: Kidney cross-section 2
Figure 2: Glomerular filtration barrier 3
Figure 3: Schematic representation of actin-based podocyte cytoskeleton 5
Figure 4: Re-organization of the Podocyte cytoskeleton and proteinuria 7
Figure 5: Genomic organization of HIV-1 12
Figure 6: HIV-1 life-cycle 23
Figure 7: A representative of a light microscopy section of negative control kidney tissue 53
Figure 8: Probing cathepsin-L and dynamin expression and localization in renal tissue 55
Figure 9: Comprehensive analysis of cathepsin-L and dynamin distribution within the kidney .. 56
Figure 10: CellProfiler analyzed images 57
Figure 11: Quantification of immunohistochemical marker localization 58
Figure 12: qPCR renal cathepsin-L expression 64
Figure 13: Box-Whisker plots of qPCR renal dynamin expression 67
Figure 14: Western-blot analysis of cathepsin-L and dynamin expression 68
Figure 15: Expression patterns of podocyte cytoskeleton proteins 70
Figure 16: HIV Host Response Panel arrays gene expression pattern in HIVAN vs HIV negative FSGS 74
Figure 17: HIV Host Response Panel arrays gene expression pattern in HIVAN and HIV negative FSGS compared to the healthy non-proteinuric controls 75
Figure 18: mRNA expression patterns of altered *CCL4*, *CXCL12* and *SLPI* from HIV-1 host response panel of arrays 77
Figure 19: Altered HIV-host response genes in HIVAN and the HIV negative FSGS relative to healthy controls 79
Figure 20: Toll-Like Receptor arrays gene expression pattern of HIVAN vs HIV negative FSGS 82
Figure 21: Toll-Like Receptor arrays gene expression pattern in HIVAN and HIV negative FSGS compared to the healthy non-proteinuric controls 83
Figure 22: MAPK signaling pathway 84
Figure 23: Western-blot analysis of JNK proteins 85

Figure 24: mRNA expression of altered TLR array genes in HIVAN and HIV negative FSGS relative to the healthy control. 87
Figure 25: Gene expression pattern changes in HIV-1-associated kidney disease..... 99

List of tables

Table 1: Preparation of the High Capacity cDNA 2 × reverse transcription master mix 30
Table 2: Thermo-cycling conditions for cDNA synthesis using High Capacity cDNA reverse transcription kit 31
Table 3: Primer sequences of cathepsin-L and dynamin genes 33
Table 4: Primer sequences of reference genes 34
Table 5: Preparation of real time quantitative PCR reaction mix using Brilliant II SYBR 35
Table 6: Thermo-cycling conditions for real time quantitative PCR reaction using Brilliant II SYBR 37
Table 7: Primer sequences used for actin-associated cytoskeleton gene products. 40
Table 8: Real-time PCR cycling conditions for pre-amplification of cDNA target template used in RT^2 PCR array experiments 42
Table 9: Real-time cycling conditions used for RT^2 PCR array experiments 43
Table 10: Primer sequences for up- or down-regulated HIV-host response genes in HIVAN compared to HIV negative FSGS 45
Table 11: Primer sequences for altered HIV-host response genes in HIVAN and HIV negative FSGS compared to the healthy controls 46
Table 12: Primers for altered TLR genes in FSGS and HIVAN compared to healthy controls 48
Table 13: Comparison of glomerular cathepsin-L and dynamin localization intensities between disease states 59
Table 14: Comparison of tubular cathepsin-L and dynamin localization intensities between disease states 60
Table 15: Comparison of qPCR expression levels of cathepsin-L in the kidneys of proteinuric groups and the healthy non-proteinuric controls. 63
Table 16: Comparison of qPCR expression levels of dynamin in the kidneys of proteinuric groups and the healthy non-proteinuric controls. 66
Table 17: Comparison of qPCR expression patterns of actin-associated podocyte cytoskeleton gene products in proteinuric kidney diseases and the healthy controls. 71
Table 18: Comparison of qPCR expression patterns of CCL4, CXCL12 and SLPI genes in proteinuric kidney diseases and the healthy controls. 78

Table 19: Comparison of qPCR expression patterns of HIV-host response genes in proteinuric kidney diseases compared to healthy controls. 80
Table 20: Comparison of qPCR expression patterns of *CCL2* and *REL1* genes was assessed. ... 88

List of abbreviations

°C	Degrees Celsius
Δ*G*	Change in Gibbs free energy
$2^{-\Delta\Delta Cq}$	Fold change
AIDS	Acquired immune deficiency syndrome
APS	Ammonium persulfate
ARV	Antiretroviral
C_t	Cycle threshold
C_q	Quantification cycle
CCL2	Chemokine (C-C Motif) ligand 2 or Monocyte chemoattractant protein 1
CCL3	Chemokine (C-C Motif) ligand 3 or Macrophage inflammatory protein 1-α
CCL4	Chemokine (C-C Motif) ligand 4
CCR5	Chemokine (C-C motif) receptor 5
CDKN1A	Cyclin-dependent kinase inhibitor 1A
CKD	Chronic kidney disease
CXCL12	Chemokine (C-X-C Motif) Ligand 12
CXCR4	Chemokine (C-X-C motif) receptor 4
Da	Daltons
DMEM	Dulbeco's modified eagle medium
DMSO	Dimethylsulphoxide
DTT	Dithiothreitol
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
Env	Envelope glycoprotein
ERK	Extracellular signal-regulated kinase
ESL	Endothelial surface layer
ESRD	End-stage renal disease
FSGS	Focal segmental glomerulosclerosis
GBM	Glomerular basement membrane
H_2O	Water

H_2O_2	Hydrogen peroxide
HAART	Highly active antiretroviral treatment
HCl	Hydrochloric acid
HIV-1	Human immunodeficiency virus type I
HIVAN	Human immunodeficiency virus-associated nephropathy
HIVICK	Human immunodeficiency virus immune complex kidney disease
HMGB1	High Mobility Group Box 1
IFN-γ	Interferon gamma
IHC	Immunohistochemistry
IL-1β	Interleukin-1 beta
IRAK	Interleukin-1 receptor-associated kinases
JNK	c-Jun NH2-terminal kinase
ℓ	Litre
LY96	Lymphocyte antigen 96
M	Molar
MAPK	Mitogen-Activated Protein Kinases
MAP3K1	Mitogen-Activated Protein Kinase Kinase Kinase 1
MAP4K4	Mitogen-Activated Protein Kinase Kinase Kinase Kinase 4
MAPK8	Mitogen-Activated Protein Kinase 8
μℓ	Microlitre
mℓ	Millilitre
NaCl	Sodium chloride
NPHS1	Nephrosis 1, congenital, Finnish type (nephrin)
NPHS2	Nephrosis 2, idiopathic, steroid-resistant (podocin)
NRTI	Nucleoside reverse transcriptase inhibitor
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PELI1	Pellino E3 Ubiquitin Protein Ligase 1
PI	Protease inhibitor
qPCR	Quantitative polymerase chain reaction

RAG	Recombination activating gene
REL	V-rel reticuloendotheliosis viral oncogene homolog (avian)
RNA	Ribonucleic acid
RPL13A	*Homo sapiens* ribosomal protein L13a
rpm	Revolutions per minute
RT	Reverse transcriptase
SDHA	*Homo sapiens* succinate dehydrogenase complex, subunit A, flavoprotein
SDS	Sodium dodecyl sulphate
SERPINA1	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin)
SIGIRR	Single Immunoglobulin and Toll-Interleukin 1 Receptor (TIR) Domain
SLPI	Secretory Leukocyte Peptidase Inhibitor
STAT1	Signal transducer and activator of transcription 1
SYNPO	Synaptopodin
TEMED	N, N, N', N'-Tetramethylethylenediamine
TGF-β1	Transforming growth factors beta 1
TLR	Toll-like receptor
TNFα	Tumor necrosis factor alpha
TNFSF10	Tumor necrosis factor (ligand) superfamily , member 10
TRAF6	Tumor necrosis factor receptor associated factor
µℓ	Micro litre
VDJ	Variable, diversity and joining
x g	Times gravity (relative centrifugal force)
YWHAZ	*Homo sapiens* tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide.

The IUPAC-IUBMB one and three letter notations for the amino acids have been used.