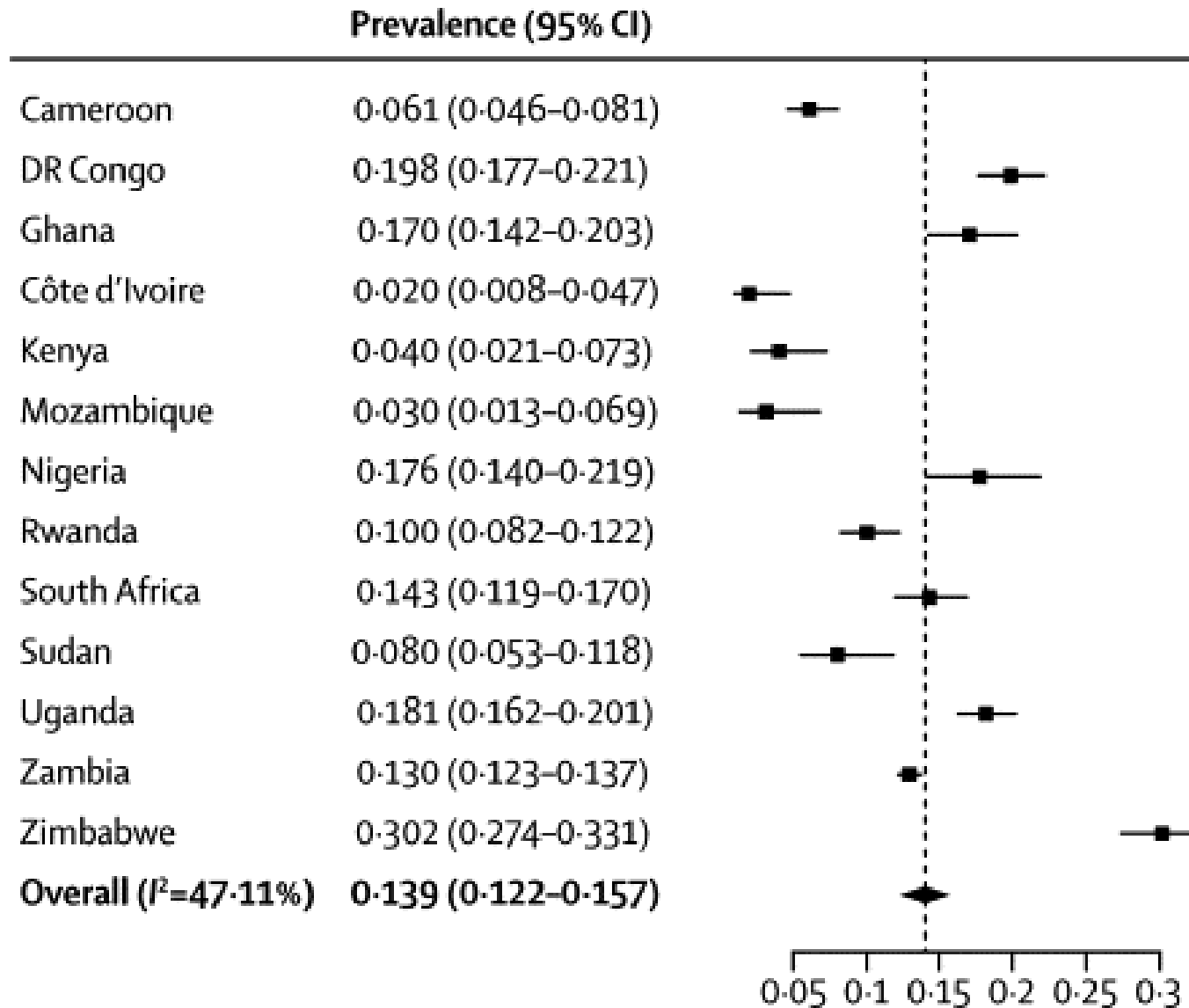# H3Africa Kidney Disease Research Network

C. Osafo, D. Adu, S. Ajayi, F. Arogundade, T. Olarenwaju, S. Mc'Ligeyo, M. Manmak, J. Plange-Rhule, I. Ulasi, B. Salako, Y. Tadasse, N. Tiffin, B. Tayo, D. Burke, A. Adeyemo, R. Parekh, Y. Raji, A. Ghansah, A. Ojo, R. Gbadegesin, M. Pollak, F. Hildebrandt, J. Kopp, C. Winkler, R. Cooper, M. Kretzler, M. Boehnke, J. Moran, A. Nyarko.

Burden of Kidney Disease in Africa

Prevalence (95% CI)

	Prevalence (95% CI)
Cameroon	0·061 (0·046–0·081)
DR Congo	0·198 (0·177–0·221)
Ghana	0·170 (0·142–0·203)
Côte d'Ivoire	0·020 (0·008–0·047)
Kenya	0·040 (0·021–0·073)
Mozambique	0·030 (0·013–0·069)
Nigeria	0·176 (0·140–0·219)
Rwanda	0·100 (0·082–0·122)
South Africa	0·143 (0·119–0·170)
Sudan	0·080 (0·053–0·118)
Uganda	0·181 (0·162–0·201)
Zambia	0·130 (0·123–0·137)
Zimbabwe	0·302 (0·274–0·331)
Overall (I^2=47·11%)	0·139 (0·122–0·157)

Breakdown of CKD Subtypes in Kidney Disease Research Network

Diagnosis-specific eligibility	Average Age (years)	Rate of progression to ESRD
Glomerular Disease **Steroid Resistant Nephrotic Syndrome**	10-15	Years
Biopsy Proven Glomerular Disease (focal segmental glomerulosclerosis, minimal change disease, or membranous nephropathy)	10-30	Months to years
HIV Chronic Kidney Disease	Adult	Variable
Sickle Cell Chronic Kidney Disease	Adult	Variable
Hypertensive Non Diabetic Chronic Kidney Disease	Adult	Few years if uncontrolled
Diabetic Chronic Kidney Disease	Adult	Few years if uncontrolled
Chronic Kidney Disease of Unknown Etiology	Adult	Unknown

# Risk of Chronic Kidney Disease In 1.2 Million Persons
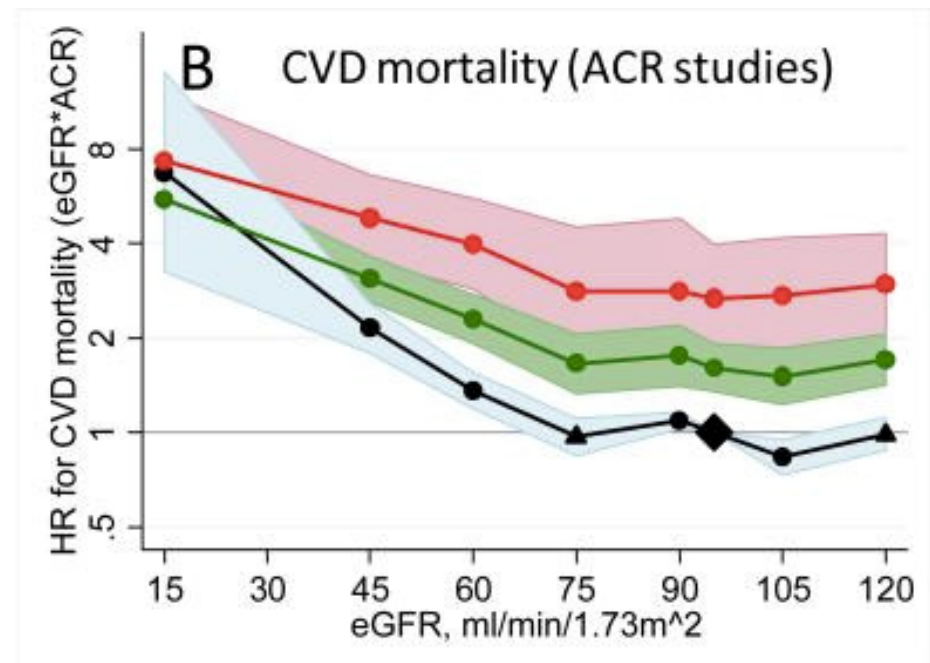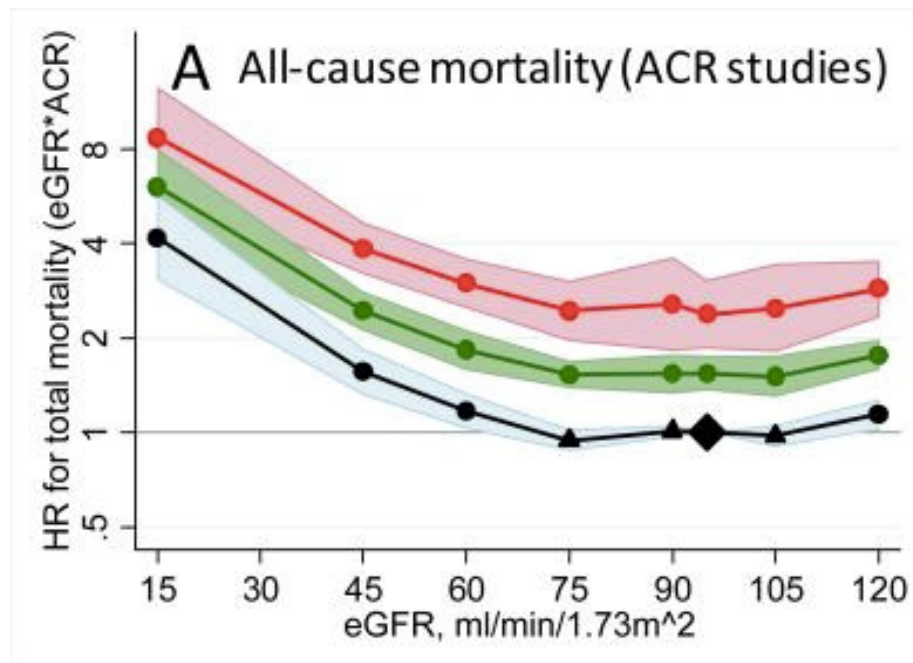
H3Africa Kidney Disease Research Network:
Objectives

1. Comprehensive phenotyping of 4,000 cases and 4000 controls in four African countries

2. Conduct genetic and translational research projects in adult and pediatric glomerular disease and CKD

3. Training of clinical research personnel and genomics investigators

4. Two genomic research laboratories in West Africa using sustainable, low capital-intensity laboratory technology platform

H3A Kidney Disease Research Network:
Research Projects

H3Africa Initiative
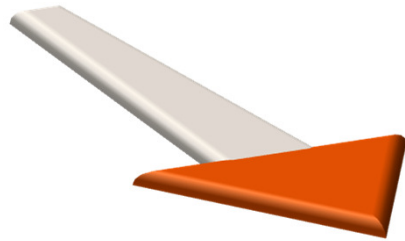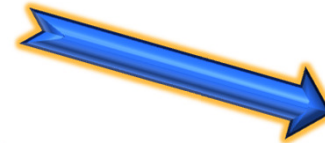
H3Africa Kidney Disease Research Network

HIV Nephropathy Study
(N=1000)

Renal Candidate Genes
(APOL1, etc)
Studies
(N=8000)

GWAS
(N=2000)

Familial Nephrotic
Syndrome
(N=50 families)

Proposed study subcohorts

Sample Sizes of the Participants and Controls			
Diagnosis-specific eligibility	Age	Cases	Controls
Steroid resistant nephrotic syndrome[1]	<18	200	-
Biopsy Proven Glomerular Disease (FSGS/MCD & MN)	≤74	200	-
HIV Chronic Kidney Disease	≤74	500	500[2]
Sickle Cell Chronic Kidney Disease	≤74	500	500[3]
Hypertensive Non-diabetic Chronic Kidney Disease	≤74	800	800
Diabetic Chronic Kidney Disease	≤74	800	800[4]
Chronic Kidney Disease of Unknown Aetiology	≤74	1,000	-
General Population Controls			1400
Total		4,000	4,000

[1]Includes 50 families with index cases and affected family members
[2]Patients with HIV and no CKD
[3]Patients with sickle cell disease and no CKD
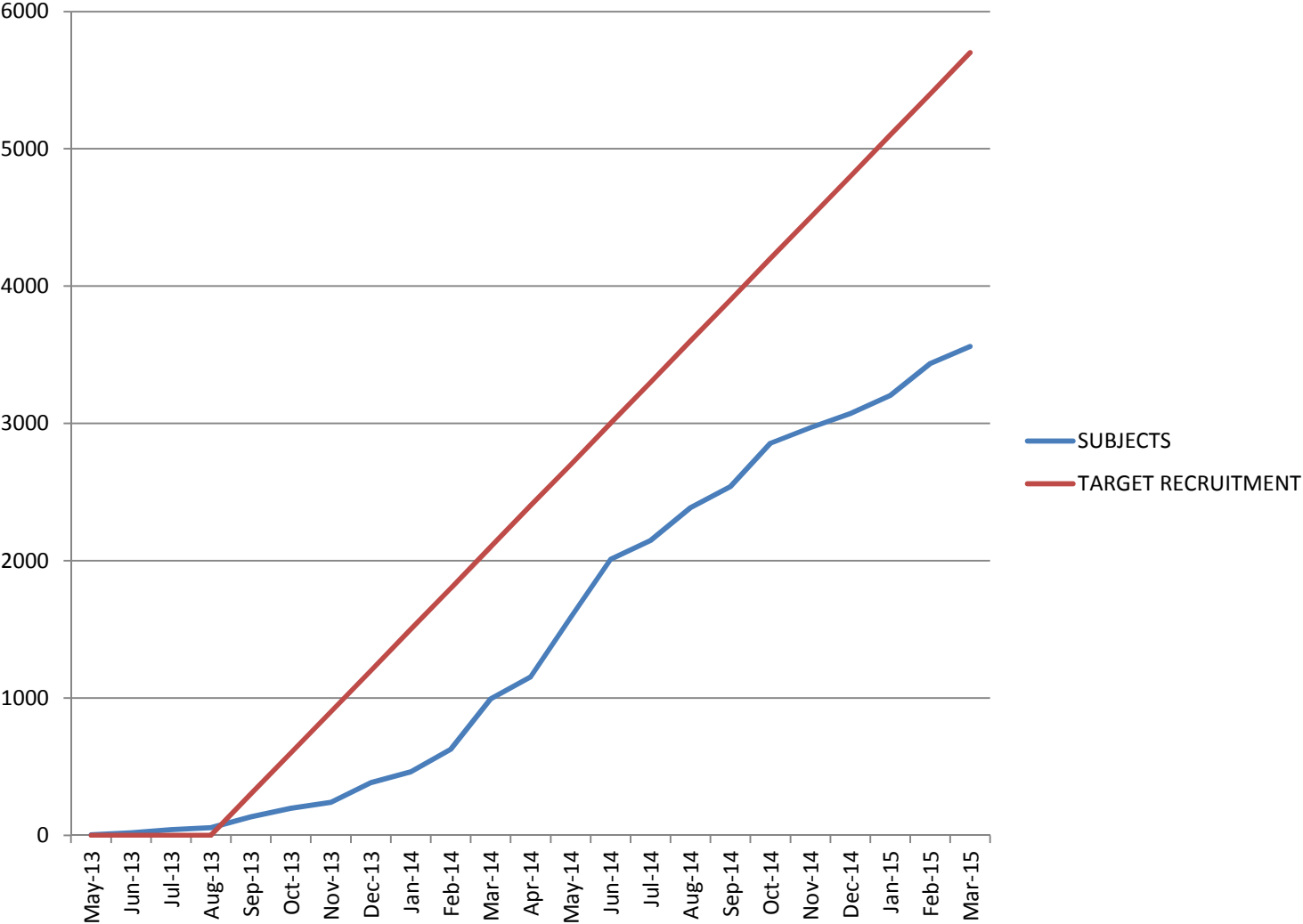[4]Patient with diabetes mellitus and no
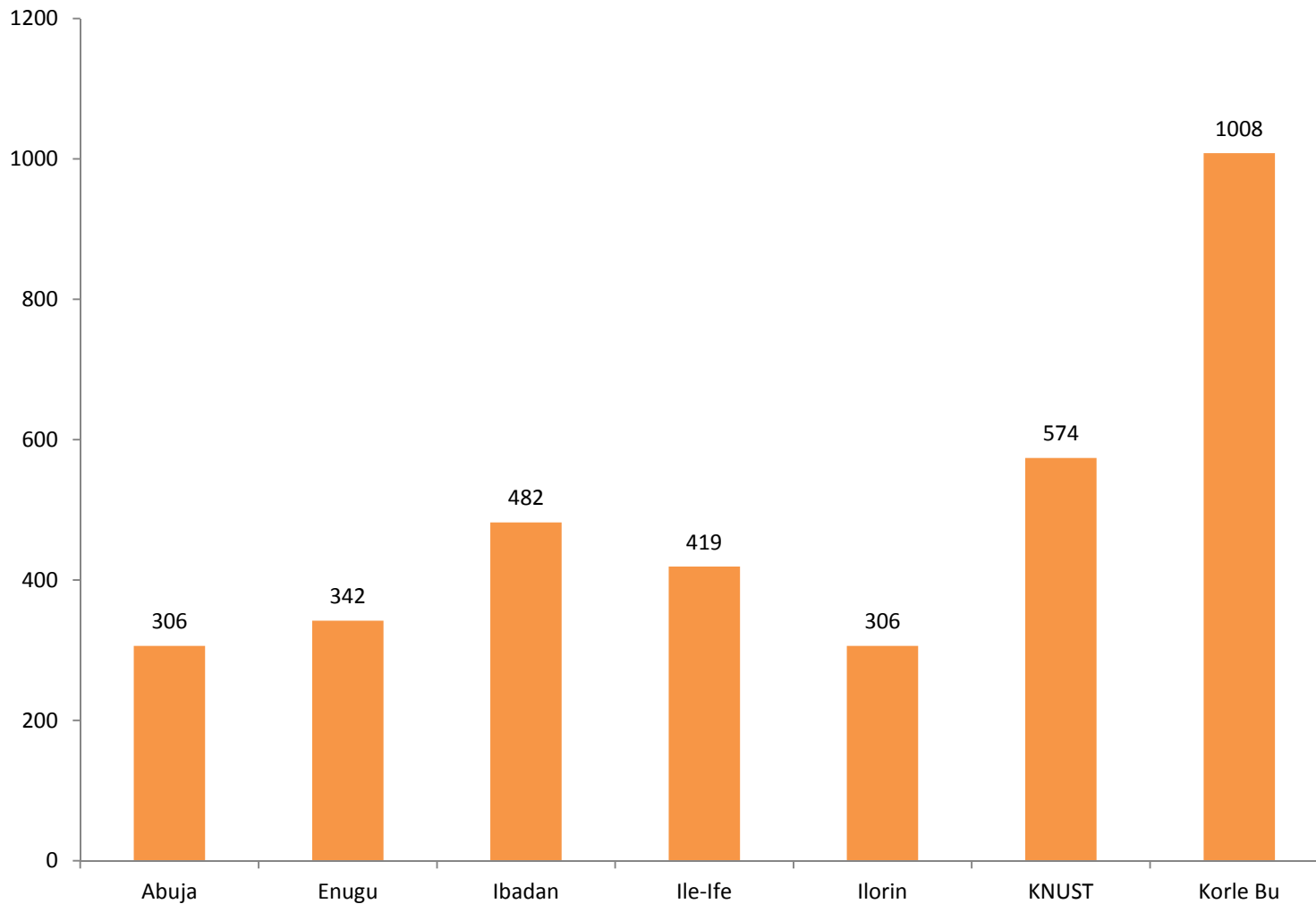
H3Africa Kidney Disease Research Network: IRB

- Eight out of nine centers have IRB approval
- By end of September 2013, 3 out of 9 centers were recruiting
- By end of October 2013 seven out of nine centers were recruiting
- Kenya started recruitment in May 2015
- Awaiting IRB approval for Ethiopia
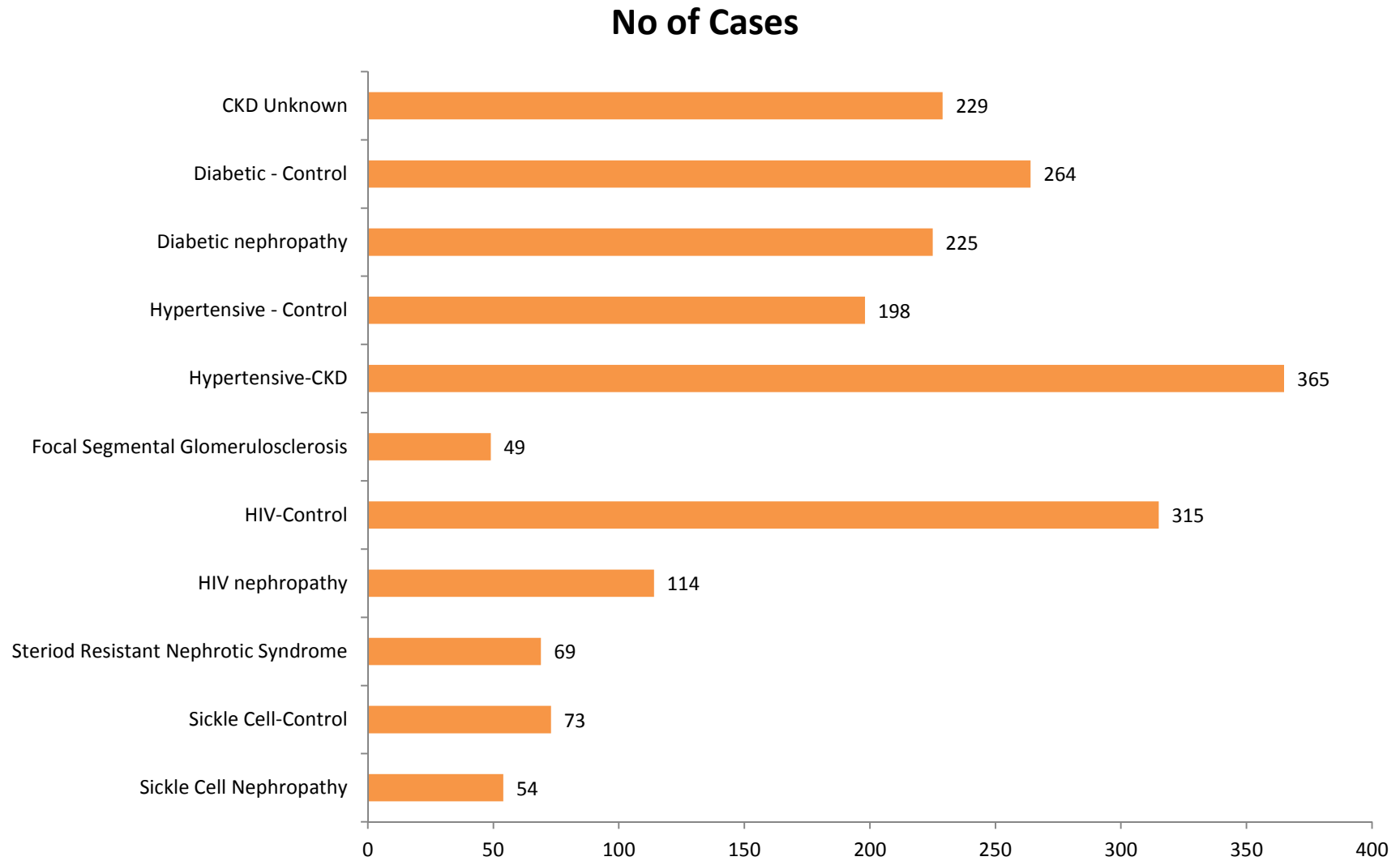- Genetic studies of this population would make our study more comprehensive

Cumulative Enrolment from May 2013 to March 2015

Enrolment by Clinical Center February 2015

Kidney Disease in Study Participants

No of Cases

Category	No of Cases
CKD Unknown	229
Diabetic - Control	264
Diabetic nephropathy	225
Hypertensive - Control	198
Hypertensive-CKD	365
Focal Segmental Glomerulosclerosis	49
HIV-Control	315
HIV nephropathy	114
Steriod Resistant Nephrotic Syndrome	69
Sickle Cell-Control	73
Sickle Cell Nephropathy	54

Baseline characteristics of participants in the H3Africa Kidney Disease Study (n=1897)

Variable	Cases (n=600)	Controls (n=1297)
Female % (n)	35.17 (211)	57.21 (742)
Age, years	48.49 ± 13.95	43.05 ± 14.66
Weight, kg	70.00 ± 15.71	70.07 ± 17.92
Body mass index, kg/m^2	25.11 ± 5.63	26.28 ± 6.30

Baseline clinical characteristics of participants in the H3Africa Kidney Disease Study (n=1897)

Variable	CASES (n=600)	CONTROLS (n=1297)
Self-reported Diabetes mellitus , % (n)	14.00 (84)	10.25 (133)
Blood pressure, mm Hg		
Systolic, mean (±SD)	141.36 ± 25.82	124.66 ± 21.68
Diastolic, mean (±SD)	83.13 ± 16.76	74.93 ± 13.80

Baseline characteristics of participants in the H3Africa Kidney Disease Study (n=1897)

| Variable	CASES (n=600)	CONTROLS (n=1297)
Estimated glomerular filtration rate mean (±SD) (CKD-EPI)	25.76 ± 22.07	92.27 ± 12.30
eGFR<=15 % (n)	46.50 (279)	0
Serum creatinine, mean (±SD), mg/dL	5.88 ± 5.53	0.85 ± 0.23
Urine Albumin to creatinine ratio (mg/mmol) mean (±SD)	189.47 ± 1471.80	10.20 ± 61.71
Hemoglobin, mean (±SD), g/dL	10.53 ± 6.15	13.39 ± 8.65

Biospecimen Management

- Samples from clinical sites shipped by DHL to MDS-Lancet Laboratories in Ghana

- Samples for DNA (blood, buffy coat and saliva) couriered to Noguchi Memorial Institute for Medical Research at the University of Ghana

- Biochemical assays carried out at MDS-Lancet and aliquots stored at -80^0C

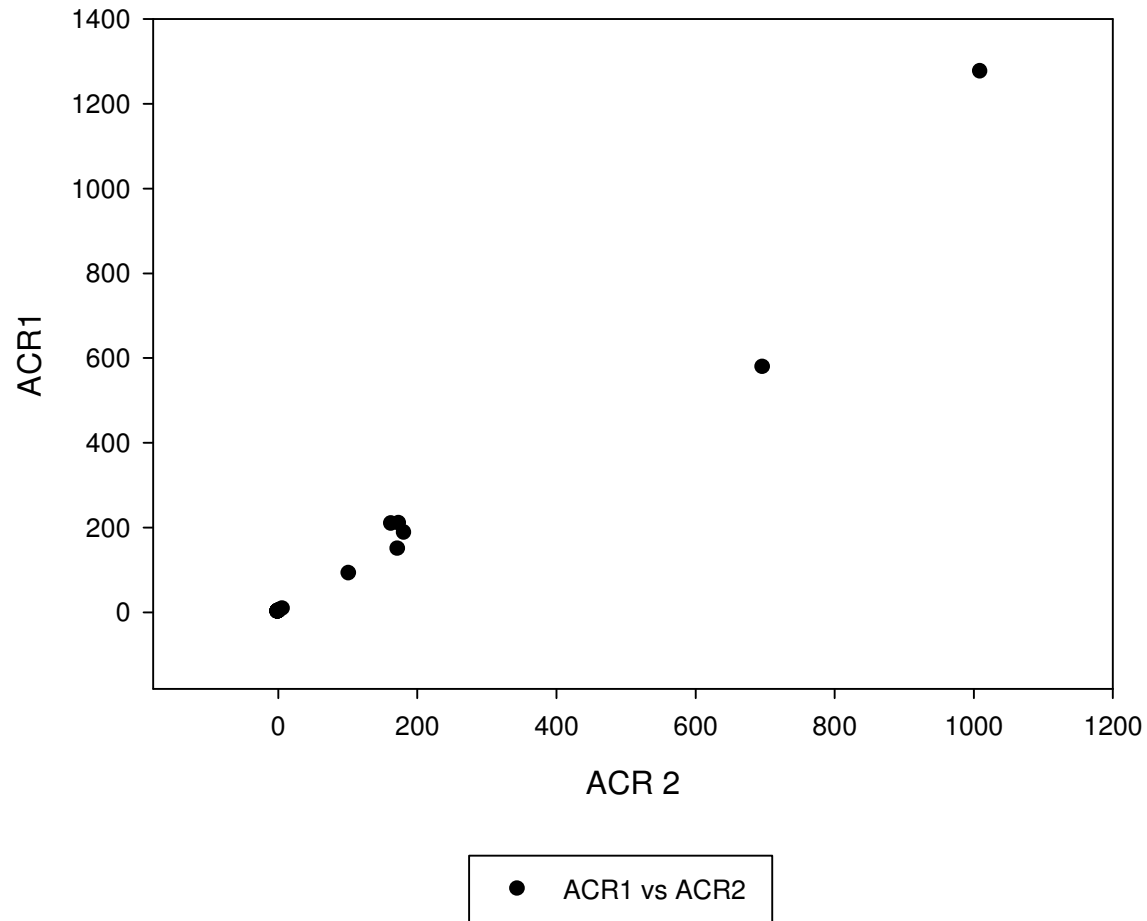- DNA stored at Noguchi Memorial Institute for Medical Research -80^0C

QUALITY CONTROL REPEAT SERUM CREATININE
SCATTER PLOT

PLOT OF REPEAT SERUM CREATININE
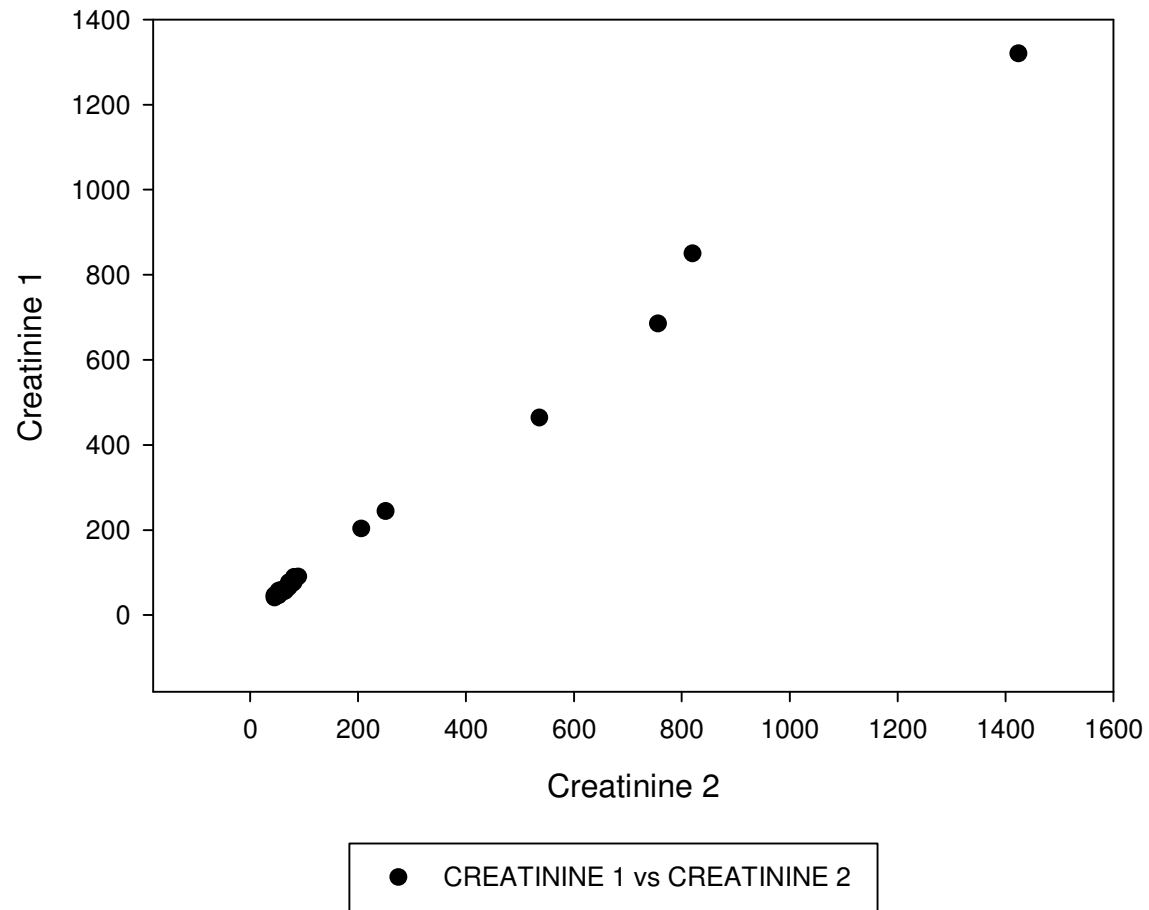
QUALITY CONTROL REPEAT ALBUMIN CREATININE RATIO SCATTER PLOT

Plot of Repeat Urine Albumin Creatine Ratios

DNA isolation and genotyping

- Primary DNA isolated from blood buffy coat using the QIAamp DNA Blood Mini Kit.

- Additional DNA from saliva was isolated by a salting out protocol.

- DNA isolation performed at The Kidney Genomics lab, NMIMR, University of Ghana from 13/12/2013 to date

- 2270 DNA preparations (60%) have been performed to date on blood and 2160 on saliva

- Each isolated DNA preparation was individually assayed by agarose gel electrophoresis.

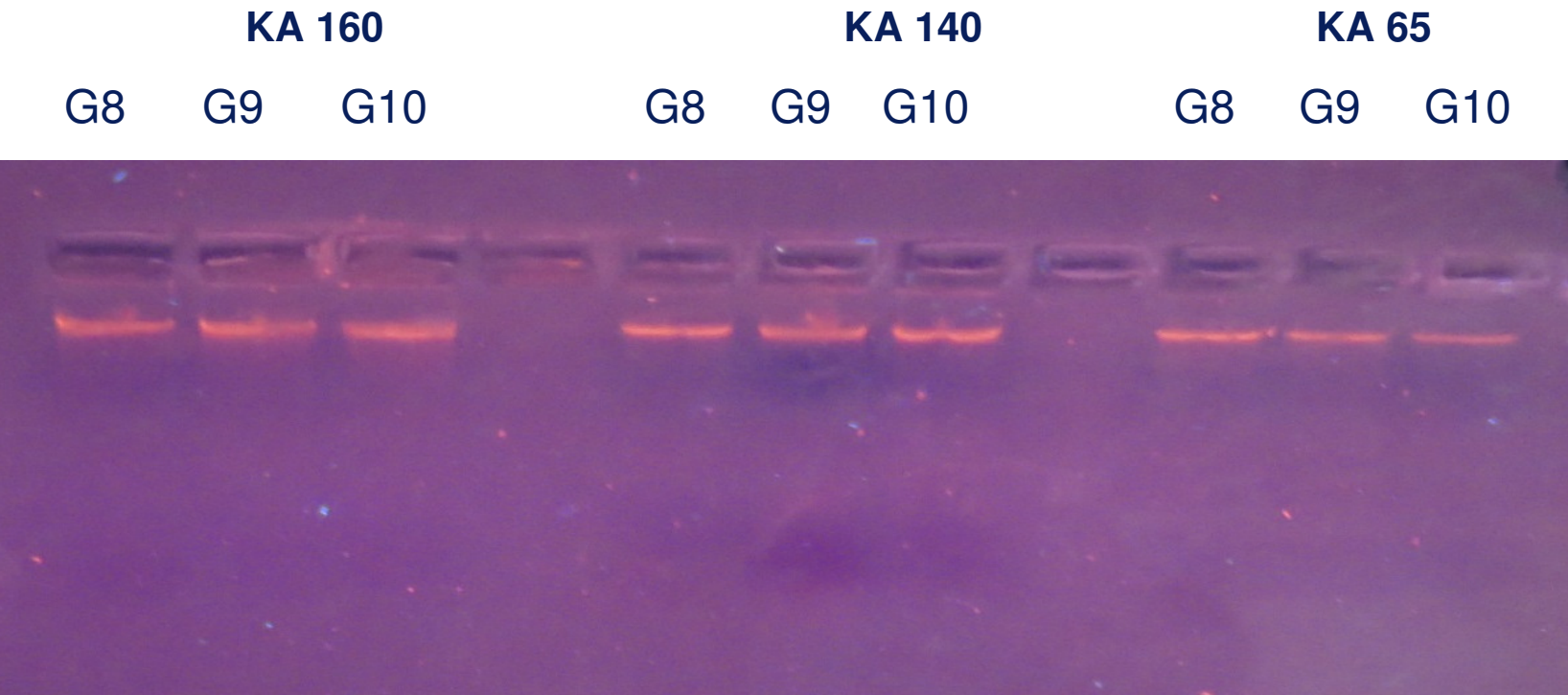# QC documentation: All aliquots of selected samples

Percentage Gel: 1% Gel
Sample type: 5µl Buffy Coat gDNA
Fluorescent dye: Gel Red
Buffer: 1X SB Buffer
Location: University of Ghana, Noguchi Inst.

H3Africa SNP Chip: Baylor Whole Genome Sequencing

- Samples (N= 230) shipped on dry ice from Ghana to USA for QC testing and trans-shipment to Baylor University.
- Initial set of 207 samples from Ghana contained DNA of high molecular weight (gel electrophoresis).
- Initial set of 207 samples supported multiple PCR reactions.
- All 207 samples supported SNP detection by Ligation Detection reaction.
- 50 samples sent for Whole Genome Sequencing at Baylor Human Genome Sequencing Center (>2 µg each).
- Represent 6 West African ethnic/language groups.

SNP genotyping tests

- Ligation detection reaction (LDR), followed by polyacrylamide electrophoresis.
- Male/Female detection by amelogenin sequence
- Hemoglobin C
- Hemoglobin S
- APOL1, rs73885319 (S342G, G1)

  rs60910145 (I384M, G1)

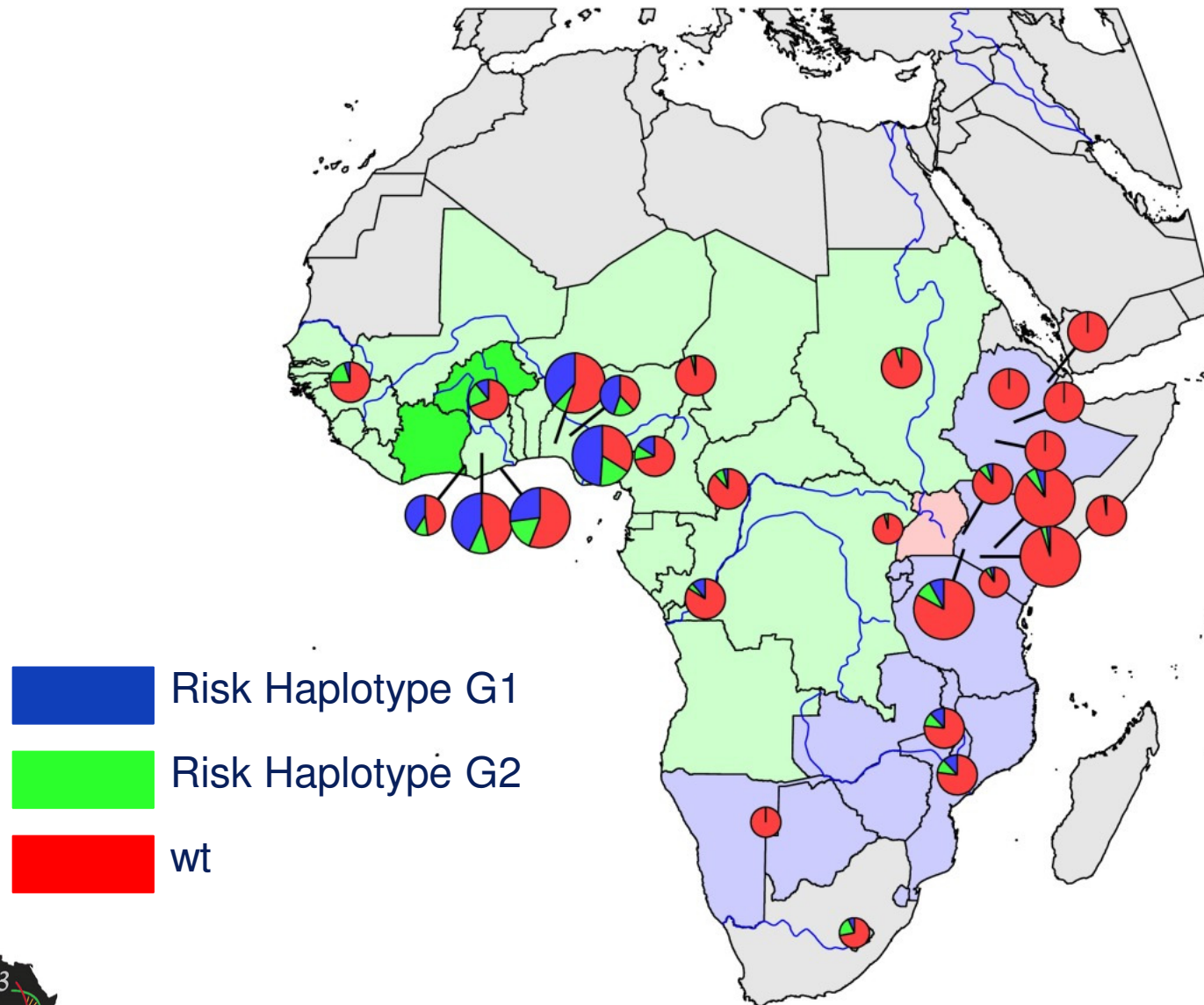 The APOL1 SNP haplotype "G1" is associated with kidney disease in several populations.

SNP genotyping: example results
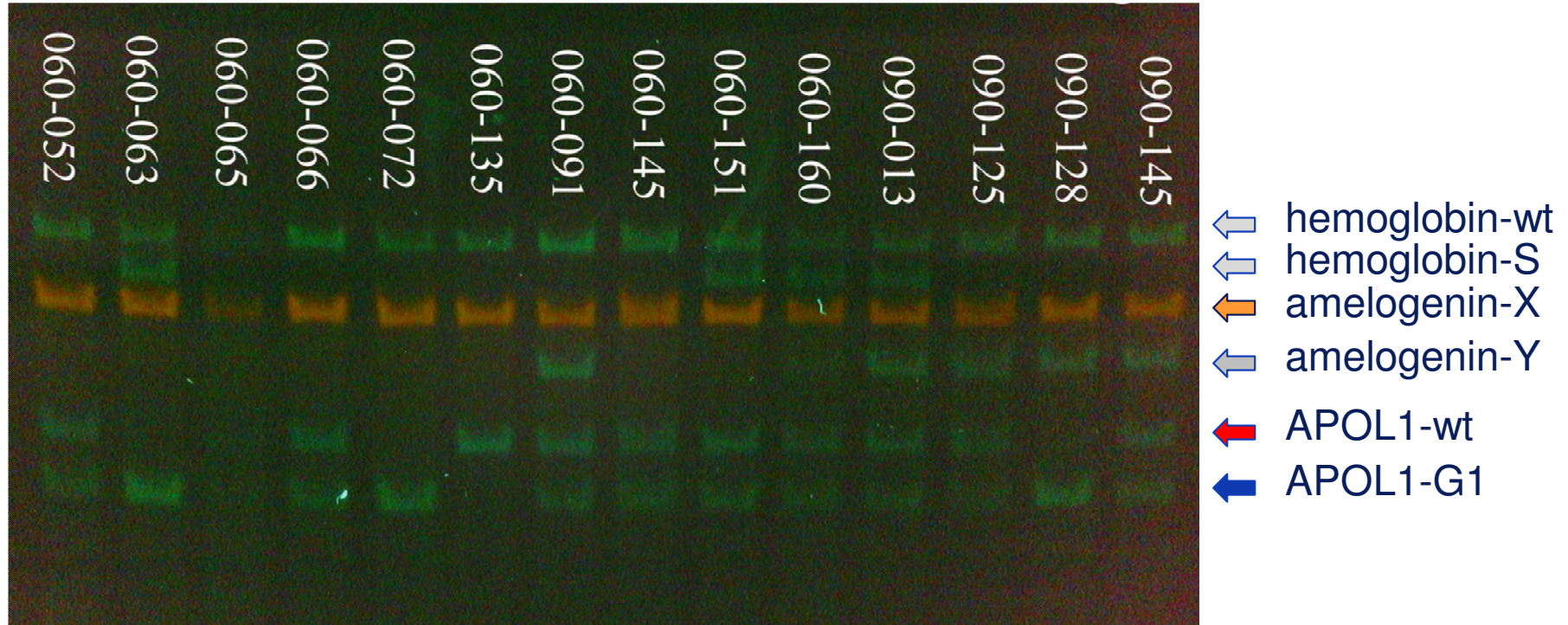
The LDR SNP detection system is now being used at the Noguchi Lab.

Allele distribution of trypanolytic factor APOL1

Risk Haplotype G1

Risk Haplotype G2

wt

Population frequency of APOL1 alleles

Country	Population	N	Latitude	Longitude	% of G1	% of G2	Ref.
Ghana	Akan	171	6.7	−1.6	43	11	New
Ghana	Ga-Adangbe	139	5.6	−0.2	27	17	New
Nigeria	Ibo	190	6.5	7.5	49	17	New
Kenya	Kikuyu	112	−0.4	37	5	6	New
Kenya	Luo	895	−0.5	34.7	8	9	New
Kenya	Masai	102	−1.1	35.9	2	3	New
Somalia	Somali	30	2	45.4	0	2	New
Nigeria	Yoruba	113	7.4	3.9	38	7	Hapmap
Kenya	Luyha	90	0.6	34.6	5	7	Hapmap
Kenya	Bantu_NE	12	−3	37	5	5	HGDP
Ghana	Bulsa	22	10.7	−1.3	11	20	U.C.L.
Ghana	Asante	35	5.8	−2.8		11	U.C.L.
Ghana	Various	207			34	.	H3Kidney

In 207 samples from the KDN study population the frequency of G1 APOL1 is 0.34

H3KIDNEY TRAINING

- 5 doctors have completed a 2-year training program on Clinical Research Methods and Biostatistics

- 5 biomedical scientists training in laboratory genomics

- 1 MPhil student training in biochemistry

- 3 scientists training in bioinformatics

- 2 research administrators training in NIH programme

RESEARCH MANAGEMENT TRAINING IN ACCRA

SUMMARY

- Recruitment is underway at nearly all sites

- Recruitment is nearly on target, by geography and by disease sub types

- Samples are being collected

- Samples are good (excellent!) quality

H3AFRICA KIDNEY INVESTIGATORS-African Investigators

NAMES	AFFILIATIONS
Dwomoa Adu (PI)	University of Ghana
Charlotte Osafo (PI)	University of Ghana
Alexander Nyarko	University of Ghana
Vincent Boima	University of Ghana
Michael Mate-Kole	University of Ghana
Victoria Adabayeri	Korle-Bu Teaching Hospital, Accra, Ghana
Ivy Ekem	University of Ghana
Jacob Plange-Rhule (PI)	Kwame Nkrumah University of Science and Technology
Yewondwossen Mengistu (PI)	Addis Ababa University
S. O. Mc'Ligeyo (PI)	University of Nairobi
Babatunde Salako (PI)	University of Ibadan
Olukemi Amodu	University of Ibadan
Yemi Raheem Raji	University of Ibadan
Adebowale Ademola	University of Ibadan
Chijioke Adindu	University of Ilorin
Timothy Olanrewaju (PI)	University of Ilorin
Clement Bewaji	University of Ilorin
Fatiu Arogundade (PI)	Obafemi Awolowo University
Samuel Ajayi	University of Abuja Teaching Hospital
Manmak Mamven	University of Abuja Teaching Hospital
Ifeoma Ulasi (PI)	University of Nigeria, Enugu
Chuba Ijoma	University of Nigeria, Enugu
Nicki Tiffin (PI)	University of Western Cape
Junaid Gamiedien	University of Western Cape
Darlington Mapiye	University of Western Cape

H3AFRICA KIDNEY INVESTIGATORS-
North American Investigators

NAMES	AFFILIATIONS
Richard Cooper (PI)	Loyola University
Bamidele Tayo	Loyola University
Rasheed Gbadegesin	Duke University
Akinlolu Ojo (PI)	University of Michigan
Matthias Kretzler	University of Michigan
Michael Boehnke	University of Michigan
John Moran	University of Michigan
David Burke	University of Michigan
Robert Lyons	University of Michigan
Frank (Chip) Brosius	University of Michigan
Daniel Clauw	University of Michigan
Friedhelm Hildebrandt	Harvard Medical School
Martin Pollak	Harvard Medical School
Rulan Parekh (PI)	University of Toronto
Jeffrey Kopp	NIDDK
Paul Kimmel	NIDDK
Cheryl Winkler	NIH
Rebekah Rasooly	NIDDK
Marva Moxey-Mims	NIDDK
Adebowale Adeyemo	NIH
Karl Skorecki	Technion - Israel Institute of Technology
Walter G. Wasser	Rambam Health Care Campus Haifa, Israel

Victoria Falls