# APPLE-CKD

## APolipoprotein L1 in People of african ancestry Living in the UK: Exploration of genetic and environmental factors associated with Chronic Kidney Disease (APPLE-CKD study)



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| **Funder:** | Astra Zeneca – Investigator Led study |
| **IRAS Reference** | 292365 |

# Protocol Version 1.0, 18/1/22

## KEY ROLES AND RESPONSIBILITIES

**SPONSOR:** The sponsor is responsible for ensuring before a study begins that arrangements are in place for the research team to access resources and support to deliver the research as proposed and allocate responsibilities for the management, monitoring and reporting of the research. The Sponsor also has to be satisfied there is agreement on appropriate arrangements to record, report and review significant developments as the research proceeds, and approve any modifications to the design.

**FUNDER:** The funder is the entity that will provide the funds (financial support) for the conduction of the study. Funders are expected to provide assistance to any enquiry, audit or investigation related to the funded work.

**CHIEF INVESTIGATOR (CI):** The person who takes overall responsibility for the design, conduct and reporting of a study. If the study involves researchers at more than once site, the CI takes on the primary responsibility whether or not he/she is an investigator at any particular site.

The CI role is to complete and to ensure that all relevant regulatory approvals are in place before the study begins. Ensure arrangements are in place for good study conduct, robust monitoring and reporting, including prompt reporting of incidents, this includes putting in place adequate training for study staff to conduct the study as per the protocol and relevant standards.

The Chief Investigator is responsible for submission of annual reports as required. The Chief Investigator will notify the R&I Office of the end of the study, including the reasons for the premature termination. Within one year after the end of study, the Chief Investigator will submit a final report with the results, including any publications/abstracts to the REC.

**PRINCIPAL INVESTIGATOR (PI):** Individually or as leader of the researchers at a site; ensuring that the study is conducted as per the approved study protocol, and report/notify the relevant parties – this includes the CI of any breaches or incidents related to the study.

## DECLARATIONS

The undersigned confirm that the following protocol has been agreed and accepted and that the investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the Research Governance Framework 2005 (as amended thereafter), the Trust Data & Information Governance policy, Sponsor and other relevant SOPs and applicable Trust policies and legal frameworks.

I (investigator) agree to ensure that the confidential information contained in this document will not be used for any other purposes other than the evaluation or conduct of this research without the prior written consent of the Sponsor.

I (investigator) also confirm that an honest accurate and transparent account of the study will be given; and that any deviations from the study as planned in this protocol will be explained and reported accordingly.

Chief Investigator:

**Signature**:  Date18/1/22

**Print Name (in full)**: Kate Bramham

**Position**: Clinical Senior Lecturer and Honorary Consultant Nephrologist

## CONTENTS

## KEY WORDS

Pregnancy; Chronic Kidney Disease; Apolipoprotein L1; African Ancestry

## LIST OF ABBREVIATIONS

| | |
|---|---|
| AE | Adverse Event |
| APOL1 | Apolipoprotein |
| BMI | Body Mass Index |
| CKD | Chronic Kidney Disease |
| CI | Chief Investigator |
| CRF | Case Report Form |
| DMC | Data Monitoring Committee |
| GAfREC | Governance Arrangement for NHS Research Ethics |
| GFR | Glomerular filtration rate |
| HRA | Health Research Authority |
| HTA | Human Tissue Authority |
| ICF | Informed Consent Form |
| IPSC | Inducible Pluripotent Stem Cell |
| PCR | Protein Creatinine Ratio |
| PI | Principal Investigator |
| PIS | Participant Information Sheet |
| QA | Quality Assurance |
| QC | Quality Control |
| REC | Research Ethics Committee |
| SAE | Serious Adverse Event |
| SDV | Source Data Verification |
| SOP | Standard Operating Procedure |
| TMF | Trial Master File |

## STUDY SUMMARY

| STUDY OVERVIEW | |
|---|---|
| Full title | **AP**olipoprotein L1 in **P**eople of african ancestry **L**iving in the UK: **E**xploration of genetic and environmental factors associated with Chronic Kidney Disease (APPLE-CKD study) |
| Objectives | Primary:<br>• To establish if epigenetic DNA methylation patterns in the APOL1 gene promoter in renal tissue are comparable to peripheral blood mononuclear cells PBMC (or spleen) and urine in participants with CKD and controls (including deceased kidney donors) with APOL1 high and low-risk genotypes<br>Secondary:<br>• To study the role of APOL1 genetics and epigenetics in the differentiation of inducible Pluripotent Stem Cells into kidney relevant cells<br>• To develop a 'clinical/genetic/epigenetic/inflammatory signature' associated with CKD in people of African ancestry with validation |
| Type of Study | Multicentre observational cohort |
| Health condition(s) or problem(s) studied | Chronic Kidney Disease |
| Target sample size | At least 720 participants with CKD; 220 healthy controls |
| Study design and methods | Multicentre observational cohort with sample collection |
| Study duration per participant: | Up to 2 days |
| Main inclusion/exclusion criteria: | Participants with CKD<br>*Inclusion Criteria*:<br>• Self reported African or Afro-Caribbean ancestry<br>• ≥18 years old<br>• Willing and able to provide written informed consent<br>• Chronic Kidney Disease (KDIGO Criteria)<br>• Group 1: Without diabetic nephropathy or serological or biopsy proven evidence of immune mediated kidney disease<br>• Group 2: With diabetic nephropathy or serological or biopsy proven evidence of immune mediated kidney disease<br><br>*Exclusion criteria*:<br>• Unable to provide informed consent<br>• Pregnancy<br><br>Participant Controls<br>*Inclusion Criteria*:<br>• Self-reported African ancestry<br>• ≥18 years old |

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|---|---|
| | • Willing and able to provide written informed consent<br>• No Chronic Kidney Disease<br>*Exclusion criteria*:<br>• Unable to provide informed consent |
| Statistical methodology and analysis: | Primary Objective - Tissue analysis: APOL1 promoter DNA methylation patterns will be compared between renal tissue and PBMC (spleen) and urine (Cases only) in CKD cases (N=120) and controls (N=120; QUOD; N=40). Methylation patterns in different CKD disease phenotypes will be compared.<br><br>Secondary Objectives<br>i) 'High risk signature' model development: Clinical characteristic, APOL1 genotypes, PMBC or urine DNA methylation patterns at CpG-sites in the APOL1 promoter and inflammatory markers will be compared between Phase 1 cases (N=120) and controls (N=120) in a development cohort, as well as between subjects with and without APOL1 high risk alleles with Bonferroni correction factor for multiple testing. All predictors will initially be prepared for inclusion in the prediction model. Any non-normal variables will be transformed to normal using log or any other appropriate transformation.<br><br>Each predictor will then be tested in univariable logistic regression models with the outcome (CKD), to initially estimate their crude associations. Odds ratios (ORs) with 95% confidence intervals (CIs) will be reported. All predictors will then be included in a multivariable model and, using a backwards approach, subtracted one by one based on p-values. The p-value used will be larger than the standard 0.05 as to not be too stringent. Any pre-specified predictors that are clinically significant will be forced into the model. This process will identify the 3-5 predictors of greatest statistical significance for outcome prediction. The model will be validated in Phase 2 cases (N=600) and controls (N=100). |
| **STUDY TIMELINES** | |
| Study Duration/length | 36 months |
| Expected Start Date | Mar 2022 |
| End of Study definition and anticipated date | End of Study: Analysis of samples from last recruited patient.<br>Anticipated End Date: Nov 2024 |
| Key Study milestones | Months 1 to 24: Recruitment<br>Months 24-36: Data cleaning; analysis; prepare research report |
| **STORAGE of SAMPLES (if applicable)** | |
| Human tissue samples | De-identified plasma, serum, urine and DNA samples will be stored in the HTA approved freezers for up to 10 years after study completion |
| Data collected / Storage | De-identified data will be stored for 10 years after study completion in Iron Mountain secure archiving system. |

# 1 INTRODUCTION

Preventative treatment for kidney disease is urgently needed in order to reduce ethnicity related health inequalities, reduce cost (NHS budget ~3% for renal replacement therapy) and burden of kidney disease to patients, their families and communities. Apolipoprotein L1 (*APOL1*) high-risk genotypes (G1/1; G2/2; G1/2) are associated with increased risk of chronic kidney disease (CKD) in people of African ancestry (1). However, there are wide variations in the association between CKD and high-risk genotypes in different countries, tribes and CKD phenotypes (e.g. strong association with hypertensive sclerosis, focal segmental sclerosis and HIV associated nephropathy (HIVAN)). For example, lower rates of CKD are reported in people with high-risk APOL1 alleles living in Africa, than in the USA and environmental changes are proposed to be contributory. The prevalence of *APO1L1* high-risk genotypes and their association with different CKD phenotypes in a diverse population of people of African ancestry living in the UK has not been explored.

Molecules to moderate the *APOL1* pathway are being developed. However, enhanced risk stratification and improved understanding of pathophysiology of *APOL1* related kidney disease is required to target treatment. We propose a study to explore genetic, epigenetic and inflammatory factors which contribute to development of kidney disease in people with high risk APOL1 genotypes living in the UK.

# 2 BACKGROUND AND RATIONALE

It remains unclear why not all individuals with high-risk *APO1L1* genotypes develop CKD. Additional environmental 'hits' which lead to 'gain-of-function' *APOL1* gene activity appear to be required for disease progression. Epigenetic mechanisms can mediate genetic and environmental crosstalk without changing underlying DNA sequence. Changes include DNA methylation modification which can prevent transcription-factor binding in gene promoters or binding repressor proteins leading to transcriptional silencing (2). Preliminary assessment of the *APOL1* gene, by Dr Jordana Bell (collaborator King's College London), in 27,578 peripheral blood mononuclear cells (PBMCs) from people of European ancestry suggests that there is variability in DNA methylation levels in the promoter region. Furthermore, genetic variants in *APOL1* strongly impact DNA methylation levels at specific CpG-sites in the *APOL1* promoter, which could in turn contribute to differences in gene expression. However, to date *APOL1* DNA methylation patterns have not been explored in subjects with and without CKD.

In addition, it is unknown if PMBC and urine *APOL1* DNA methylation patterns reflect those of renal tissue. We will determine if methylation patterns and protein translation are consistent in different tissues, using blood and urine samples from patients and healthy controls recruited to the study, and from kidney biopsy samples from patients and deceased kidney donors from the Quality in Organ Donation (QUOD) (https://quod.org.uk/) initiative. QUOD is a national consortium to promote research in organ donation and transplantation and have confirmed that 40 tissue samples are available from donors of African ancestry. QUOD control kidney biopsy tissue and splenic mononuclear cells (SMNCs) (as surrogates for PBMCs from deceased donors) will be used to compare epigenetic patterns and renal protein translation with CKD patients.

*APOL1* expression has been demonstrated to be activated by inflammation *in vitro*, but the relationship between inflammation and CKD progression in patients has not been explored. Similarly, additional genetic factors (e.g. Sickle Cell trait) have also been proposed to contribute to development of CKD in people of African ancestry but their interaction with *APOL1* related kidney disease in not understood.

Current models to understand *APOL1* risk variant biology rely on genetically engineered cell lines. Importantly, these genetic modifications are often performed under the reference genome. However, the reference APOL1 haplotype is only a minor haplotype, rarely found in Africans (3). Recent data, utilizing cell lines, has shown that *APOL1* risk variant toxicity is dependent on the APOL1 haplotype background (4). Suggesting that the genetic background might have a strong role in APOL1 risk variant biology in renal relevant cells. To overcome this challenge, and potentially unforeseen ones (like the role of epigenetics), generation of inducible Pluripotent Stem Cells (iPSC) from participants with and without kidney disease, and with and *APOL1* renal risk variants will allow study of the role genetics and epigenetics in the differentiation of iPSCs into kidney relevant cells, including iPSC derived podocytes, endothelial cells or organoids. Development of these highly physiologically relevant cell models *in vitro* will facilitate assessment of *APOL1* high-risk genotype contribution to cell differentiation, cell death, metabolism, inflammation, enable enhanced understanding of the role of *APOL1* high-risk genotypes in the pathophysiology of multiple cells and allow *in vitro* assessment of the causality of potential 'second-hits' observed in the patient cohort (e.g. Inflammatory markers).

We propose that assessment of CpG variability in PBMC and urine and/or renal cells with concurrent inflammatory marker analysis and assessment of *APOL1* high-risk variant role in cell models *in vitro* can a) identify people of African ancestry with APOL1 high-risk genotypes at greatest risk of CKD progression, and b) provide mechanistic insight into underlying pathophysiology.

# 3 OBJECTIVES

## 3.1 Primary Objective

- To establish if epigenetic DNA methylation patterns in the APOL1 gene promoter in renal tissue are comparable to peripheral blood mononuclear cells PBMC (or spleen) and urine in CKD cases and controls with APOL1 high and low-risk genotypes

## 3.2 Secondary Objectives

- To study the role of APOL1 genetics and epigenetics in the differentiation of inducible Pluripotent Stem Cells (iPSC) into kidney relevant cells
- To develop a 'clinical/genetic/epigenetic/inflammatory signature' associated with CKD in people of African ancestry for future validation

# 4 STUDY SCHEDULE

*Enrolment process*: Eligible participants will be identified from GP referral letters, inpatient or outpatient clinical areas.

Healthy Controls will be recruited from other King's College Hospital NHS Trust clinics (e.g. dental clinics), hospital and King's College London university staff websites.

Clinical staff will be given a laminated card with the study eligibility criteria and research team contact details. Participants will be identified by clinical and research staff and provided with a patient information sheet (PIS). Details will be stored in a screening log. Participants who meet the inclusion and exclusion criteria for the study will not require any further screening prior to involvement in the study. Participant will provide written consent and samples taken for analysis.

Patients with CKD and controls will be recruited to the iPSC sub-study until at least 3 samples are available from each genotype where possible (G0/G0, G1/G1 and G2/G2) – 18 in total.

Blood and urine tests will be taken at the same time as tests required as part of routine clinical care where possible. Blood and urine samples will be taken by a research nurse, processed immediately according to Standard Operating procedures including for iPSC sub-study and then stored at -80°C for future measurements.

Renal biopsy samples for patients who have previously undergone a biopsy and Quod samples will be retrieved by relevant pathology services and sent by courier to Renal Sciences, King's College London. Samples will be subsequently sent to:

i) Bart's and the London Genome Centre, Barts and the London, School of Medicine and Dentistry, Blizard Institute, 4 Newark St, Whitechapel, London, E1 2AT for DNA extraction and DNA methylation analysis,

ii) AstraZeneca laboratory Cardiovascular, Renal and Metabolism (Early CVRM), R&D Biopharmaceuticals, AstraZeneca, Gothenburg, Sweden

The following tests will be carried out at Local Laboratories as part of routine clinical care and recorded in the study database.

| Laboratory test | Parameters |
|---|---|
| BLOOD | |
| Haematology | Haemoglobin |
| Serum chemistry | Creatinine (Enzymatic IDMS traceable), urea, sodium, potassium, bicarbonate, calcium, phosphate, albumin, AST or ALT |
| Urine | Albumin Creatinine Ratio (preferred), or Protein Creatinine Ratio, or 24 hour urine protein |

Demographic data including creatinine, ACR, PCR or 24 hour urine protein, use of antihypertensive medications, Angiotensin Converting Enzyme Inhibitor (ACEI) or Angiotensin Receptor Blocker (ARB) use, deprivation score (http://dclgapps.communities.gov.uk/imd/idmap.html), age, ethnicity, smoking, booking visit height and weight, booking and current blood pressure (second of two

readings), presence of diabetes and most recent HbA1c, BMI, other diseases and medication will be collected. Country of origin will be collected for participants, their parents and grandparents.

If control participants are identified to have new diagnosis of hypertension routine information and support to seek further investigation and treatment from their GP will be provided. If control participants are identified to have kidney abnormalities, permission will be requested for referral to their GP or nephrology services, depending on severity.

All data will be handled in accordance with the UK Data Protection Act 2018.

Data will be downloaded for each site from the study database, and de-identified by each site and each participant will be given a trial identification number, for identification and this will be clearly explained to the participant in the Participant information sheet. Data from healthy controls will be recorded in a separate database and similarly de-identified. Participant consent for this will be sought.

It is the responsibility of the investigator to ensure the accuracy of all data entered in the database. The delegation log will identify all those personnel with responsibilities for data collection and handling, including those who have access to the study database.

All data entry must be completed and signed by staff that are listed on the delegation log and authorized by the CI/ PI to perform this duty. The CI/PI is responsible for the accuracy of all data reported in the study database. Data will be available to the coordinating site on the database. The study visit must be entered within 24 hours of recruitment. Source data verification should be completed and all data queries answered prior to submission where possible.

Participants will also be asked if they would be willing for their contact details to be kept for up to 5 years, so that we may contact them to invite them to participate in future studies and to ask about their health in the future. Additional ethics approval would be sought for further work.

*Follow up*: Participants will have routine clinical care and no further study related follow-up.

*Participant withdrawal criteria and procedures*: Participants have the right to withdraw from the study at any time for any reason. It is understood by all concerned that an excessive rate of withdrawals can render the study uninterpretable; therefore, unnecessary withdrawal of patients should be avoided. Withdrawn patients will not be replaced.

Should a patient decide to withdraw consent, all efforts will be made to report the reason for withdrawal in as much detail as possible. After consent withdrawal, participant's information will be destroyed and samples discarded, unless anonymised i.e. in the iPSC study.

*End of the study*: The end of the study will occur after the final patient is recruited and sample analysis has been undertaken.

**Table 1: Schedule of Assessments**

| | Study Visit |
|---|---|
| Informed Consent | |
| Medical History / Medication Review | X |
| Eligibility confirmation | X |
| Blood Pressure | X |
| Clinical blood and urine tests | X |
| Blood and urine samples for research | X |

**Recruitment Phases**
Recruitment will be conducted in two phases with the following anticipated overlapping timelines:

***Phase 1***: Cases (Group 1 CKD) and Controls for Epigenetic and iPSC analysis:
Sites - King's College Hospital NHS Foundation Trust
Anticipated timeline: Mar 22-Dec 22

***Phase 2***: Cases (Groups 1 and 2) and Controls for high-risk signature analysis:
Site - King's College Hospital NHS Foundation Trust; Guy's and St Thomas' NHS Foundation Trust; Barts and the Royal London
Anticipated timeline: Jun 22- Nov 24

## 5 CONSENT

The participant must personally sign and date the latest approved version of the Informed Consent form before any of the study specific procedures are performed.

Written versions of the Participant Information and Informed Consent will be presented to the participants detailing the exact nature of the study, what it will involve for the participant, the implications and constraints of the protocol and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

If he/she wishes to consider the information, the participant will be allowed as much time as he/she needs. He/she will be offered sufficient opportunity to question the Investigator, GP or other independent parties to decide whether she will participate in the study. Participants will not be rushed into making a decision. However, the convenience of the participant will also be considered including the right to immediate consent.

Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the Informed Consent. A member of the research team will go through the written information with the potential participant giving the opportunity for

questions to be asked. Only researchers experienced in consent procedure and trained in Good Clinical Practice will take consent from participants.

In gaining consent from the participant we will ensure that the participant is able to:
1. Understand the purpose and nature of the research
2. Understand what the research involves including potential benefits and burden
3. Understand that the alternative to taking part is for them to receive standard medical care and that this is not affected by participation
4. Retain information long enough to make an effective decision
5. Make a free choice

If there is any further safety information that may result in significant changes in the risk/benefit analysis, the PIS and Informed Consent Form (ICF) will be reviewed and updated accordingly. All participants that are actively enrolled on the study will be informed of the updated information and given a revised copy of the PIS/ICF in order to confirm their wish to continue on the study.

Non-English speakers will be offered the opportunity to participate with a translator if available.

# 6 ELIGIBILITY CRITERIA

## 6.1 Inclusion Criteria – CKD Cases

- Self-reported African ancestry
- ≥18 years old
- Willing and able to provide written informed consent
- Chronic Kidney Disease (KDIGO Criteria)
- Group 1: Without diabetic nephropathy or serological or biopsy proven evidence of immune mediated kidney disease
- Group 2: With diabetic nephropathy or serological or biopsy proven evidence of immune mediated kidney disease
-

## 6.2 Inclusion Criteria – Controls

- Self-reported African ancestry
- ≥18 years old
- Willing and able to provide written informed consent
- No Chronic Kidney Disease (estimated GFR > 60mls/min/1.73m$^2$ and urinary protein: creatinine < 15mg /mol or albumin: creatinine ratio <3mg/mmol or negative urine dip for protein).

## 6.3 Exclusion Criteria – Cases and Controls

- Unable to provide informed consent

- Pregnancy

## 6.4 Additional Inclusion Criteria – CKD Cases for iPSC sub-study

- Hypertension
- Age <50 years
- Estimated GFR – <30mls/min/1.73m$^2$ with or without renal biopsy

## 6.5 Exclusion Criteria – CKD Cases for iPSC sub-study

- Unable to provide informed consent
- Diabetes
- Serological or biopsy evidence of immunologically-mediated renal disease
- Known blood borne viruses (HIV, Hepatitis B and C)

## 6.6 Additional Inclusion Criteria – Healthy Controls for iPSC sub-study

- Age <50 years

## 6.7 Exclusion Criteria – Healthy Controls for iPSC sub study

- Unable to provide informed consent
- Diabetes
- Serological or biopsy evidence of immunologically-mediated renal disease
- Known blood borne viruses (HIV, Hepatitis B and C)

## 6.8 Inclusion Criteria – QUOD Deceased Controls

- QUOD reported African ancestry
- ≥18 years old
- Informed consent for research
- No known Chronic Kidney Disease (estimated GFR > 60mls/min/1.73m$^2$) prior to death
- Renal and spleen tissue sample available

## 6.9 Exclusion Criteria – QUOD Deceased Controls

- No consent for research
- Chronic Kidney Disease (estimated GFR < 60mls/min/1.73m$^2$) prior to death
- Renal and spleen tissue sample unavailable

# 7 RECRUITMENT

Participant recruitment at a site will only commence when the study has:

1. Been confirmed by the Sponsor (or its delegated representative)
2. Received HRA Approval , and
3. Has confirmed Capacity and Capability

Potential participants will be identified through the hospital database and approached in renal outpatients by PI or direct clinical care team and provided with a patient information sheet (PIS). PI or member of the direct care team will ask for the individual's agreement for their details to be made available to the research team so that they can contacted should they wish to participate in the study. Healthy controls will be approached through NHS trust and King's College London networks.

The person taking consent will be suitably qualified and experienced, and will have been delegated this duty by the CI/ PI on the Delegation Log. They will explain the aims, methods, anticipated benefits and potential hazards of the intervention.

It will be recorded in the medical notes (including version and date of the PIS) when the participant information sheet (PIS) has been given to the participant; The Investigator will explain that participants are under no obligation to enter the study and that they can withdraw at any time during the study, without having to give a reason.

A copy of the signed Informed Consent form will be given to the participant. The original signed form will be retained in the study file at site and a copy placed in the medical notes.

No study procedures will be conducted prior to the participant giving consent by signing the Consent form.

If a participant decline to take part, he/she will be asked of their reason not to participate which will then be recorded in the screening log.

## 7.1 Recruitment Summary

| | **Total Number** | **Sample Type** | | | | |
|---|---|---|---|---|---|---|
| *Phase 1* | | *iPSC* | *PMBC* | *Renal Biopsy* | *Urine* | *Plasma /Serum* |
| **CKD Cases** | 120 | 18 | 120 | 120 | 120 | 120 |
| **Healthy Controls** | 120 | 18 | 120 | - | 120 | 120 |
| **QUOD Controls** | 40 | - | 40 (Spleen) | 40 | - | - |
| *Phase 2* | | | | | | |
| **CKD Cases** | >600 | - | >600 | - | >600 | >600 |
| **Healthy Controls** | >100 | - | >100 | - | >100 | >100 |

## 8 STATISTICAL METHODS

**Sample size**: This is an exploratory analysis; therefore, a formal sample size calculation cannot be undertaken. However, the sample numbers proposed are comparable or higher than similar APOL1 related prevalence studies from the USA and epigenetic exploratory studies in promotor regions. Three iPSC lines from each risk genotype will ensure that at least one cell line from each genotype is viable and may allow for biological similarities and variation to be compared

**Statistical analysis plan**:
Primary Objective - Tissue analysis: APOL1 promoter DNA methylation patterns will be compared between renal tissue and PBMC (spleen) and urine (Cases only) in CKD cases (N=120) and controls (N=120; QUOD; N=40). Methylation patterns in different CKD disease phenotypes will be compared.

Secondary Objectives
ii) 'High risk signature' model development: Clinical characteristic, APOL1 genotypes, PMBC or urine DNA methylation patterns at CpG-sites in the APOL1 promoter and inflammatory markers will be compared between Phase 1 cases (N=120) and controls (N=120) in a development cohort, as well as between subjects with and without APOL1 high risk alleles with Bonferroni correction factor for multiple testing. All predictors will initially be prepared for inclusion in the prediction model. Any non-normal variables will be transformed to normal using log or any other appropriate transformation.

Each predictor will then be tested in univariable logistic regression models with the outcome (CKD), to initially estimate their crude associations. Odds ratios (ORs) with 95% confidence intervals (CIs) will be reported. All predictors will then be included in a multivariable model and, using a backwards approach, subtracted one by one based on p-values. The p-value used will be larger than the standard 0.05 as to not be too stringent. Any pre-specified predictors that are clinically significant will be forced into the model. This process will identify the 3-5 predictors of greatest statistical significance for outcome prediction. The model will be validated in Phase 2 cases (N=500) and controls (N=100).

All analyses will be completed in Stata MP 14.2 or later and R 3.5.1 or later.

## 9 PATIENT AND PUBLIC INVOLVEMENT (PPI)

The involvement of patients and participants has been integral to shaping this proposal. We have worked with Africa Advocacy Foundation to develop this proposal. They have also reviewed all patient material facing material. They are highly supportive of work focusing on disease areas that people of African ancestry. Africa Advocacy Foundation will continue to support the study and advise on data interpretation where appropriate and dissemination material.

## 10 FUNDING AND SUPPLY OF EQUIPMENT

The study funding has been reviewed by the KCH R&I Office, and deemed sufficient to cover the requirements of the study.

The research costs for the study have been supported by Astra Zeneca (£428,150.64). Sites will be funded for all consumables, nursing time and £300 for archiving.

## 11 DATA HANDLING AND MANAGEMENT

**Confidentiality**

All participants will give their consent for access to their data. This consent can be revoked at any time and data related to that participant will then be deposed of securely. Research data will be stored in a password-protected database held on a secure encrypted server accessible only to designated research staff. All demographic and clinical data will be stored in de-identified form using hospital number and a study specific number. No patient identifiable data will be shared with collaborators. The CI will act as custodian of the data. Results reported in papers, reports and newsletters will not include personally identifiable information. Reports will only deal with aggregated data. Data will be managed in accordance with the GDPR, NHS Caldecott Principles, Research Governance Framework for Health and Social Care 2005 and the conditions of the Research Ethics Committee approval.

**Case Report Form**

The Case Report Form will include correspondence, protocol and amendments, ethics committee approval, R&D, study site staff records, participant information and consent, inclusion and exclusion checklists, participant information, data management, withdrawal from study, statistics, adverse events and the clinical study report. The CI will be responsible for the completion of the Case Report Form.

**Record Retention and Archiving**

During the course of the research, all study records will be kept in secure conditions and will remain the responsibility of the CI. When the research study is complete, it is a requirement of the Research Governance Framework and Health Board Policy that the records are kept for a further 5 years. Each site will need to keep this locally for the duration and KCH will archive off site in 'Iron Mountain'.

## 12 MATERIAL/SAMPLE STORAGE

In the study, plasma, serum, urine, pre-existing renal tissue and peripheral blood mononuclear cells (PBMC) will be collected from patients in accordance with the patient consent form and patient information sheet and shall include all tissue samples or other biological materials and any derivatives, portions, progeny or improvements as well as all patient information and documentation supplied in relation to them. Samples will be processed, stored and disposed in accordance with all applicable legal and regulatory requirements, including the Human Tissue Act 2004 and any amendments thereafter.

Site research teams will process, store and dispose of plasma, serum, renal biopsy tissue, urine, urine pellets and PBMC samples in accordance with all applicable legal and regulatory requirements, including the Human Tissue Act 2004 and any amendments thereto.

Samples will be de-identified (study ID number) prior to transfer.

The following samples will be sent to the following laboratories for further processing and analysis:

*Genotyping and epigenetic analysis* (funded by Investigator Led Grant)

PBMC (spleen MCs), renal biopsy tissue, urine pellets cases and controls - Bart's and the London Genome Centre, Barts and the London, School of Medicine and Dentistry, Blizard Institute, 4 Newark St, Whitechapel, London, E1 2AT

*APOL1 and Inflammatory marker analysis*
Plasma, serum, renal biopsy tissue, urine supernatent and urine pellets – will be processed at KCL or sent to other approved laboratories (amendment to protocol will be submitted when confirmed).

*Inducible Pluripotent Stem Cell (iPSC) analysis* (funded directly by AstraZeneca)
Cryopreserved PBMCs - Cardiovascular, Renal and Metabolism (Early CVRM), R&D Biopharmaceuticals, AstraZeneca, Gothenburg, Sweden with preliminary sample processing by Takara Bio Europe: https://www.takarabio.com/ into cell lines following IDEXX: https://www.idexxbioanalytics.com/h-impact-human-pathogen-testing-profiles for confirmation of blood borne virus status. Cell lines will be cultured by AstraZeneca collaborators and transformed into renal specific cells +/- organoids (e.g. podocytes, endothelial cells). *APOL1* pathway related DNA/mRNA / protein expression will be assessed, including in response to relevant 'stressor' stimuli e.g. interferons.

The plasma, urine, serum, renal biopsy tissue and PBMC samples will not be processed and/or transferred to any party not identified in this protocol and are not to be processed and/or transferred other than in accordance with the participants' consent. After ethics approval for the study has expired, the plasma, urine, serum and PBMC will be disposed of in accordance with the Human Tissue Act 2004 and any amendments thereto, or transferred to a licensed tissue bank.

## 13 PEER AND REGULATORY REVIEW

The study has been peer reviewed in accordance with the requirements outlined by KCH R&I.

- The Sponsor considers the procedure for obtaining funding from Astra Zeneca to be of sufficient rigour and independence to be considered an adequate peer review.

The study was deemed to require regulatory approval from the following bodies (list). Each approval will be obtained before the study commences.

- HRA
- REC

## 14 ADVERSE EVENTS AND INCIDENT REPORTING

No serious adverse events are anticipated in this observational study. Participants will be managed according to usual care during the course of this study with the addition of extra blood and urine, samples taken at the same time as routine needle and urine tests whenever possible.

Any serious adverse event that is related to the study procedure will be reported immediately upon knowledge of the event to R&D and always within 24 hours. All adverse events will be reported to the CI and the study sponsor.

## 14.1 Definitions of Adverse Events

| Term | Definition |
|---|---|
| Adverse Event (AE) | Any untoward medical occurrence in a patient or study participant, which does not necessarily have a causal relationship with the intervention/treatment/procedure involved. |
| Serious Adverse Event (SAE). | Any adverse event that:<br>• results in death,<br>• is life-threatening*<br>• requires hospitalisation or prolongation of existing hospitalisation**<br>• results in persistent or significant disability or incapacity |
| *A life- threatening event, this refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.<br>** Hospitalisation is defined as an in-patient admission, regardless of length of stay. Hospitalisation for pre-existing conditions, including elective procedures do not constitute an SAE. | |

## 14.2 Assessments of Adverse Events

Each adverse event will be assessed for severity, causality, seriousness and expectedness as described below.

### Severity

| Category | Definition |
|---|---|
| Mild | The adverse event does not interfere with the participant's daily routine, and does not require further procedure; it causes slight discomfort |
| Moderate | The adverse event interferes with some aspects of the participant's routine, or requires further procedure, but is not damaging to health; it causes moderate discomfort |
| Severe | The adverse event results in alteration, discomfort or disability which is clearly damaging to health |

## 14.3 Procedures for recording adverse events

All adverse events will be recorded in the medical records in the first instance.

All adverse events will be recorded in the CRF until the participant completes the study

## 14.4 Procedures for recording and reporting Serious Adverse Events

All serious adverse events will be recorded in the medical records and the CRF.

All SAEs (except those specified in section 13.5 as not requiring reporting to the Sponsor) must be recorded on a serious adverse event (SAE) form. The PI or designated individual will complete an SAE form and the form will be preferably emailed to the Chief Investigator/Trial Manager/Research Nurse within 1 working day of becoming aware of the event. The Chief Investigator will submit all SAE reports to the R&I Office (kch-tr.research@nhs.net).

## 14.5 Serious Adverse Events that do not require reporting

There are no serious adverse events that do not require reporting but this is a low risk observational study with sample collection only therefore it is not anticipated that there will be any serious adverse events related to study procedures.

## 14.6 Reporting Urgent Safety Measures

If any urgent safety measures are taken the CI/ PI shall immediately and in any event no later than 3 days from the date the measures are taken, give written notice to the relevant REC, Health Research Authority and R&I office of the measures taken and the circumstances giving rise to those measures.

## 14.7 Protocol deviations and notification of protocol violations

A deviation is usually an unintended departure from the expected conduct of the study protocol/SOPs, which does not need to be reported to the sponsor. The CI will monitor protocol deviations.

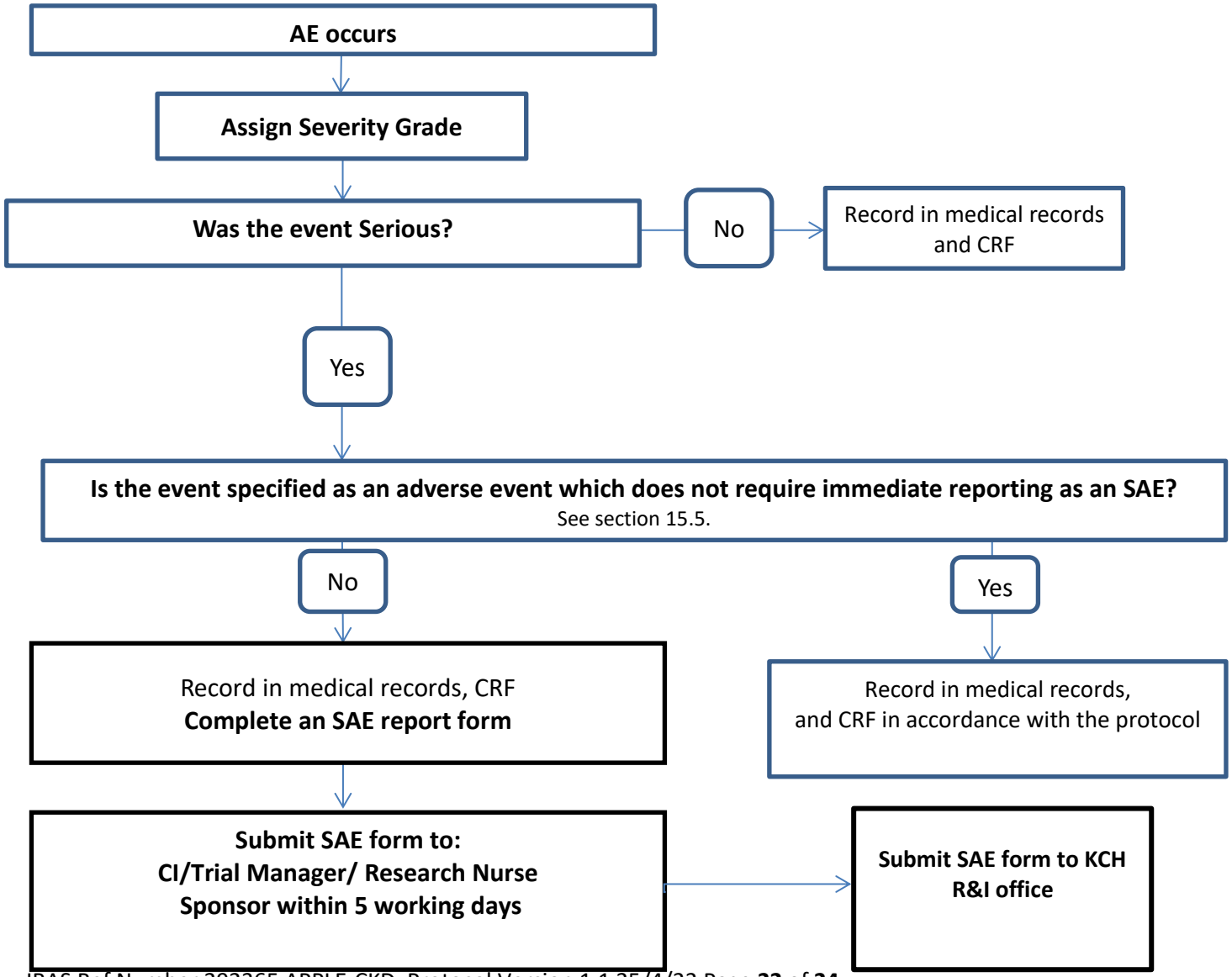
A protocol violation is a breach which is likely to effect to a significant degree –

(a) the safety or physical or mental integrity of the participants of the study; or

(b) the scientific value of the study.

The CI and R&I Office should be notified immediately of any case where the above definition applies during the study conduct phase.

## Flow Chart for SAE reporting



### 14.8 Trust incidents and near misses

An incident or near miss is any unintended or unexpected event that could have or did lead to harm, loss or damage that contains one or more of the following components:

a. It is an accident or other incident which results in injury or ill health.
b. It is contrary to specified or expected standard of patient care or service.
c. It places patients, staff members, visitors, contractors or members of the public at unnecessary risk.
d. It puts the Trust in an adverse position with potential loss of reputation.
e. It puts Trust property or assets in an adverse position or at risk.

Incidents and near misses must be reported to the Trust through DATIX as soon as the individual becomes aware of them.
A reportable incident is any unintended or unexpected event that could have or did lead to harm, loss or damage that contains one or more of the following components:

a) It is an accident or other incident which results in injury or ill health.
b) It is contrary to specified or expected standard of patient care or service.
c) It places patients, staff members, visitors, contractors or members of the public at unnecessary risk.
d) It puts the Trust in an adverse position with potential loss of reputation.
e) It puts Trust property or assets in an adverse position or at risk of loss or damage.

## 15 MONITORING AND AUDITING

The Chief Investigator will ensure there are adequate quality and number of monitoring activities conducted by the study team. This will include adherence to the protocol, procedures for consenting and ensure adequate data quality.

The Chief Investigator will inform the sponsor should he/she have concerns which have arisen from monitoring activities, and/or if there are problems with oversight/monitoring procedures.

## 16 TRAINING

The Chief Investigator will review and provide assurances of the training and experience of all staff working on this study. Appropriate training records will be maintained in the study files.

## 17 INDEMNITY ARRANGEMENTS

KCH will provide NHS indemnity cover for negligent harm, as appropriate and is not in the position to indemnify for non-negligent harm. NHS indemnity arrangements do not extend to non-negligent harm and NHS bodies cannot purchase commercial insurance for this purpose; it cannot give advance undertaking to pay compensation when there is no negligence attributable to their vicarious liability. The Trust will only extend NHS indemnity cover for negligent harm to its employees, both substantive and honorary, conducting research studies that have been approved by the R&D Department. The

Trust cannot accept liability for any activity that has not been properly registered and Trust approved. Potential claims should be reported immediately to the R&I Office

## 18 ARCHIVING

During the study, all data will be kept securely and confidentially at local sites. After the study has ended, paper data recording sheets, the Study Master file and patient consent forms will be archived at a long term storage facility (Iron Mountain) for 10 years. Data spreadsheets will be encrypted, name and contact details removed, and stored on a private Renal research team folder with limited access.

## 19 PUBLICATION AND DISSEMINATION POLICY

The research team plans to disseminate the study research findings in the following settings:

- Conference presentation of study process and results at UK Kidney Week, American Society of Nephrology Conference or the European Renal Association conference and International Society of Nephrology.
- Publication of results in a renal recognised high impact journal.

## 20 REFERENCES


1. Freedman BI, Limou S, Ma L, Kopp JB. APOL1-Associated Nephropathy: A Key Contributor to Racial Disparities in CKD. Am J Kidney Dis. 2018;72:S8-S16.
2. Wanner N, Bechtel-Walz W Epigenetics of kidney disease. Cell Tissue Res.2017; 369:75–92
3. Thomson R, Genovese G, Canon C et al. Evolution of the primate trypanolytic factor APOL1 PNAS, 2014: 111 E2130-E2139
4. Lannon H, Shah SS, Dias L et al. Apolipoprotein L1 (APOL1) risk variant toxicity depends on the haplotype background Kidney Int, 2019;96:1303-1307.


## 21 APPENDICES

## Appendix 1: PROTOCOL VERSIONS

| Versions No | Version Date | Status |
|---|---|---|
| 1.0 | 18/1/22 | |
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