

BIORESOURCE PAPER

An Australian Chronic Kidney Disease Biobank to Support Future Research

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The Chronic Kidney Disease (CKD) Biobank is a repository for plasma, erythrocytes, serum, peripheral blood mononuclear cells (PBMCs), DNA, kidney core biopsies, and clinical data. These materials are collected from CKD patients and healthy controls in Queensland, Australia. Plasma, erythrocytes, serum, urine, and PBMCs are collected annually; DNA and kidney core biopsies (not collected in health controls) are one-off collections; and clinical data is updated annually. This Biobank aims to provide CKD Centre of Research Excellence investigators in addition to domestic and international investigators of CKD with a resource to support future research to improve clinical outcomes of CKD patients.

Keywords: Chronic kidney disease; chronic kidney disease biobank; Australian biobank; clinical biobanking; metropolitan biobanking

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(1) Bioresource Overview

Project description

Chronic Kidney disease (CKD), a clinical syndrome with many adverse sequelae, is stratified by estimated glomerular filtration rate (eGFR) and albuminuria (**Table 1**). It is defined as the occurrence of kidney damage and/or reduced kidney function present for at least 3 months [1]. CKD is a major health burden in Australia with 1 in 10 affected and total annual costs attributed solely to CKD in 2012 estimated to be \$4.1 Billion [2, 3]. It is also a burden internationally with CKD ranked as the 18th most prevalent cause of death [4]. Irrespective of aetiology, many patients will progress to end-stage kidney disease (Stage 5) requiring kidney replacement therapies [5]. In contrast, some patients do not progress but stabilise or recover kidney function [6]. Because there is no cure, clinicians approach the problem with a therapeutic goal of preventing or slowing progression. Understanding the pathophysiological mechanisms of CKD will allow development of much-needed prognostic or diagnostic tests and therapies. To do this, access to bio-specimens and clinical data of CKD patients is necessary. However,

recruiting a statistically relevant sample size of CKD patients and matched healthy controls is difficult and time consuming.

The participant recruitment hurdle to clinical CKD research can be overcome via a clinical biobank. This provides many advantages over traditional participant recruitment because of the ready availability of bio-specimens and clinical data from heterogeneous populations that characterise a complex and dynamic disease. The National Health and Medical Research Council (NHMRC) CKD Centre of Research Excellence (CKD.CRE) has established the CKD Biobank to improve CKD knowledge and management [7]. It aims to provide a resource that advances and coordinates biobanking capabilities for clinical CKD research by national investigators of the CKD.CRE, and international investigators with similar goals. By establishing the CKD Biobank, the CKD.CRE will maintain biomedical research leadership and the delivery of solutions to this emerging and complex health threat.

Classification (1)

Human

Table 1: Stages of chronic kidney disease and goals of management.

CKD stage	eGFR	Albuminuria Stage		
		Normal Male: <2.5 Female: <3.5	Microalbuminuria Male: 2.5–25 Female: 3.5–35	Macroalbuminuria Male: >25 Female: >35
1	≥90			
2	60–89			
3a	45–59			
3b	30–44			
4	15–29			
5	<15 or on dialysis			
Treatment priority	Goals of management			
Low	Not CKD unless haematuria or kidney structural abnormalities present.			
Medium	Investigations to determine underlying cause, reduce progression of kidney disease, assessment of absolute cardiovascular risk, avoidance of nephrotoxic medications or volume depletion.			
High	Early detection and management of complications, adjustment of medication doses to levels appropriate for kidney function, appropriate referral to a Nephrologist when indicated.			
Severe	Prepare for kidney replacement therapy if appropriate, prepare for non-dialysis supportive care if appropriate.			

Chronic kidney disease (CKSD) stages are classified by estimated glomerular filtration rate (eGFR; ml/min/1.73m²) and severity indicated by albuminuria (mg/mmol).

Species

N/A

Classification (2)

Biological samples with associated clinical data.

Context

Spatial coverage

Participants are recruited from South-East Queensland, Australia. CKD patients are recruited from kidney health outpatient services while healthy age-matched controls are recruited from the local community.

Temporal coverage

The CKD Biobank was established in 2016 with participant recruitment and bio-specimen and clinical data collection beginning in 2017. Funding for this project through the NHMRC of Australia is available until 2020 with additional funding for beyond 2020 being sought.

Temporal coverage for accessibility

No date has been set for any destruction of banked bio-specimens and clinical data. Banked material will only be destroyed when there is no scientific value to be gained from stored bio-specimens and clinical data; in the event of the closure of the CKD Biobank and no entity can be found to curate the collection; or upon the request of a donor.

(2) Methods

Steps

1. Recruitment

Potential donors are approached either when attending kidney health service clinics (individuals with CKD) or via community outreach programs (healthy controls).

2. Collection of Bioresources

Blood and urine bio-specimens are collected either by kidney health service clinical staff upon consent or via Pathology Queensland at a future point in time. Buccal swabs for subsequent DNA isolation are collected either by kidney health service clinical staff upon consent or by participants in their home, both using a home collection kit. Kidney core biopsies are collected from only CKD patients when requested by treating clinicians as part of the participant's clinical management; tissue not used in a participant's pathology diagnosis is banked.

3. Transportation of Bioresources

Blood and urine bio-specimens are couriered by Pathology Queensland from collection centres, kidney health service clinics or Pathology Queensland clinics, to the CKD Biobank within a 24-hour period. Buccal swabs are transported to the CKD Biobank via the local postal system within a 1-month period. Kidney core biopsies are couriered from Pathology Queensland laboratories (where pathological

analysis of the tissue takes place) to the CKD Biobank in a 24-hour period.

Stabilization/preservation

A maximum of 20 mL of venous blood is collected in vacuum tubes (BD Bioscience), 10 mL into K2 ethylenediaminetetraacetic acid (K2E) coated tubes and 10 mL into silica coated tubes with gel plugs. A maximum of 20 mL of spot urine is collected into a collection pot with no preservative. Venous blood and urine are kept at 4°C upon collection. Buccal swabs are collected using an Oragene DNA Self-Collection kit that may be kept at ambient temperature. Kidney core biopsies are preserved by either formalin-fixation or fresh-frozen protocol, as specified by the collecting entity conducting the CKD patient's routine pathology testing.

Type of long-term preservation

PBMCs are isolated from K2E preserved whole blood using Histopaque 1077 (Sigma-Aldrich) and are re-suspended in a freeze down medium (Dulbecco's Modified Eagles Medium with 1000 U/mL penicillin, 1000 µg/mL streptomycin, 10% foetal bovine serum, and 10% dimethyl sulphoxide). The remaining K2E preserved blood is centrifuged at 1,200g for 10 minutes at 4°C to separate the plasma and erythrocytes, a portion of this is further preserved with 100 mM butylated hydroxytoluene (BHT). A maximum of 10 mL of urine is centrifuged at 1,200g for 10 minutes at 4°C to pellet cells while an additional maximum of 10 mL is stored unspun. DNA is isolated and preserved from buccal swabs using the QIAmp DNA mini kit (Qiagen). A summary of preservatives used with aliquot volume and number for each type of bio-specimen is presented in **Table 2**.

Storage temperature

Plasma, erythrocytes, BHT-preserved plasma, BHT-preserved erythrocytes, serum, urine and isolated DNA are stored at -80°C. Isolated PBMCs are stored in a Mr. Frosty Freezing Container at -80°C for 24 hours before being transferred to liquid nitrogen vapour phase. Fresh-frozen kidney core biopsies will be stored at -80°C while formalin-fixed tissue will be stored at 4°C.

Shipping temperature from patient/source to preservation or research use

Venous blood and urine are transported from collection clinics to the CKD Biobank on ice (0–4°C). Buccal swabs are transported at ambient temperature from collection centres or a participant's homes to the CKD Biobank. Fresh-frozen kidney core biopsies are transported on dry ice (-80°C) while formalin-fixed tissues are transported on ice (0–4°C) to the CKD Biobank.

Shipping temperature from storage to research use

Plasma, erythrocytes, BHT-preserved plasma, BHT-preserved erythrocytes, serum, urine and isolated DNA will be shipped to approved clinical research participants on dry ice (-80°C). Isolated PBMCs will be transported using a dry shipper (-170°C to -190°C) to participating clinical researchers. Kidney core biopsies will be transported on

ice (0–4°C) if formalin-fixed and on dry ice (-80°C) if fresh frozen.

Quality assurance measures

Bio-specimen quality will be assessed 1, 12, 36, or 60 months after collection using general biomarkers of bio-specimen quality with the aim of assessing 10% of each type of bio-specimen banked (randomly selected) per year. Quality of plasma, serum and urine samples will be assessed by protein yield (measured using a Bicinchoninic acid assay) and proteome integrity (assessed by electrophoresis). Quality of DNA will be assessed by yield and purity (Measured using a Thermo Scientific Nanodrop Lite), and integrity (assessed by electrophoresis). Quality of PBMCs will be assessed by typan blue exclusion test of cell viability and counting the number of viable cells. Freeze-thaw cycles are minimized by preparing multiple aliquots of each bio-specimen, distribution of bio-specimens across multiple freezer boxes, compartmentalization within a freezer to minimize temperature fluctuations when the freezer door is open, onsite backup generators and monitored alarm systems to ensure continued operation of freezers during power outages, distributing aliquoted bio-specimens across multiple sites, and recording freeze-thaw episodes.

Source of associated data

Clinical data associated with CKD Biobank donors is also collected. Integrated Electronic Medical Record (ieMR), a Queensland Health digital resource that encompasses a patient's medical history and links to other Queensland Health Clinical Systems, is used as a source of clinical data for recruited CKD patients. Additional clinical information for CKD patients can be sourced from ieMR at the request of withdrawing investigators if relevant to the proposed research project. A questionnaire taken during recruitment is used as an additional source of clinical data from healthy controls. Clinical information recorded is date of birth, sex, height, weight, ethnicity, country of birth, post-code, smoker status, highest level of education, kidney disease diagnoses and comorbidities (CKD patients only), and previous episodes of disease.

Ethics Statement

The Royal Brisbane & Women's Hospital (RBWH) Human Research Ethics Committee approved Establishment of the CKD Biobank, reference number HREC/15/QRBW/610, on the 29/03/2016. Donor recruitment and collection of bio-specimens and clinical data within the Metro North Hospital and Health Service (HHS) area and Metro South HHS area were approved by the RBWH HREC, Reference Number SSA/17/QRBW/57, on the 14/08/17 and Metro South HHS HREC, Reference Number SSA/17/QPAH/344, on the 14/08/17.

The CKD Biobank utilizes a broad consent model that specifies banked material will be used exclusively in CKD research by bona fide CKD researchers. This consent model includes elements of a tiered consent for whether a donor wishes to be recontacted for annual bio-specimen collection and whether to be notified of incidental findings. This consent model was chosen because

Table 2: Biological matrices collected from each donor.

Bio-specimen		Additive/Isolation	Volume Collected	Maximum Aliquot Volume:number
<i>Blood</i>	Plasma/Erythrocytes	K2E	5 mL	500µL:8
	BHT Preserved Plasma/Erythrocytes	K2E	1 mL	250µL:4
	PBMCs	K2E/Histopaque 1077	3 mL	500µL:4
	Serum	Silica	10 mL	500µL:10
<i>Urine</i>		No	20 mL	500µL:40
<i>Saliva</i>	DNA	Oragene DNA Self-Collection Kit/QIAmp	2 mL	25µL:8
<i>Kidney</i>	Fresh Frozen	N/A	N/A	1 piece
	Formalin Fixed	Formalin	N/A	1 piece

it offers donors a reasonable description of how their donated materials will be used in future unspecified research projects; allows consenting to an additional component of the CKD Biobank; and allows consenting to being informed of incidental findings. Additionally, this consent model reduces the burden on operational staff and the inconvenience to donors that would be experienced under a study-by-study consent model by removing the need to recontact and consent donors to every single research project withdrawing from the CKD Biobank. Because of the “in perpetuity” of the CKD Biobank, the withdrawal of consent process is described. Donors can withdraw consent from the CKD Biobank at any point in time. Withdrawal of consent will trigger the destruction of an individual’s banked material not currently withdrawn. In the instance the CKD.CRE ceases custodianship of the CKD Biobank, custodianship will be transferred to another invested party or endeavours will be made to transfer custodianship to a local academic entity with the capacity to continue operation of the CKD Biobank. If a new custodian cannot be identified all banked material will be destroyed/erased.

Constraints

A substantial constraint to the recruitment of participants and the collection of bio-specimens and clinical data is geography and available infrastructure for urban and rural areas outside of South East Queensland, Australia. Pathology Queensland does not have the infrastructure available to facilitate collection and transportation to the CKD Biobank of venous blood and urine from donors residing within these areas within the required time period. Regardless of a donor’s geographical location, kidney core biopsies are problematic to bank. This is because of the invasive nature and risks associated with the collection of these biopsies, the infrequent nature of this collection, and the potential for it to be entirely used during diagnosis.

(3) Bioresource description

Object name

The CKD Biobank houses multiple “objects”:

- Plasma,
- BHT-preserved plasma
- Erythrocytes
- BHT-preserved erythrocytes
- Serum
- Urine
- DNA
- Kidney core biopsy
- Clinical data

Bioresource name

Chronic Kidney Disease Biobank (CKD Biobank)

Bioresource location

The CKD Biobank is located in the Translational Research Institute (Level 5 West) 37 Kent Street, Woolloongabba, Queensland 4102, Australia.

Bioresource contact

ckd.biobank@uq.edu.au or g.gobe@uq.edu.au

Bioresource URL

<https://cre-ckd.centre.uq.edu.au/project/ckd-biobank>

Identifier used

N/A

Bioresource type

Nephrology

Type of sampling

Participants with CKD and healthy controls will have longitudinal, annual collection of venous blood and urine. Buccal swabs and kidney core biopsies (only for donors with CKD) will be a one-off collection.

Anatomical site

N/A

Disease status of patients/source

The CKD Biobank recruits from two groups of individuals, those who have CKD and those who are healthy controls.

Clinical characteristics of patients/source

All donors will be 18 years or older, either male or female, and capable of providing informed consent at the time of consent to participation in the CKD Biobank. Recruitment will focus on CKD patients with CKD stage 3A, 3B, 4, and 5 (see **Table 1**). Healthy controls will have no major illnesses or evidence of kidney dysfunction.

Size of the bioresource

106 donors have currently been recruited to the CKD Biobank; this includes 96 CKD patients 10 healthy controls. Recruitment and collection of bio-specimens and clinical data are ongoing. An upper estimate of the number of CKD patients that can be recruited to the CKD biobank is almost 7000. This value is based on the number of CKD patients recruited to the CKD Queensland Registry [8], a clinical database of individuals with CKD in Queensland, Australia.

Vital state of patients/source

Alive

Clinical diagnosis of patients/source

Patients recruited will have a clinical diagnosis of CKD.

Pathology diagnosis

CKD diagnosis is by eGFR, an estimate of kidney function, and albuminuria (**Table 1**). Using eGFR, individuals can be stratified into CKD Stage 1 (≥ 90 ml/min/1.73m²), CKD Stage 2 (60–89 ml/min/1.73m²), CKD Stage 3a (45–59 ml/min/1.73m²), CKD Stage 3b (30–44 ml/min/1.73m²), CKD Stage 4 (15–29 ml/min/1.73m²), and CKD Stage 5 (< 15 ml/min/1.73m²). Additional, individuals can be stratified into normal (male < 2.5 mg/mmol, female < 3.5 mg/mmol), microalbuminuria (male 2.5–25 mg/mmol, female 3.5–35 mg/mmol), and macroalbuminuria (male > 25 mg/mmol, female > 35 mg/mmol). Individuals with CKD are required to have evidence of kidney damage and/or reduced kidney function for at least 3 months.

Control samples

Healthy controls will have no clinical history or current diagnosis of CKD and other major illness and no evidence of elevated albuminuria.

Biospecimen type

Several biological matrices are collected from each donor (**Table 2**). These are venous blood (separated into serum, plasma, erythrocytes, and PBMCs) urine, DNA (isolated from buccal swabs), and kidney core biopsies.

Release date

N/A

Access criteria

Bona fide researchers wishing to withdraw material from the CKD Biobank will need to submit: the project title and synopsis; name and credentials of the principal

investigator and research team; justification for the types and number of bio-specimens and clinical data requested; experimental protocol that outlines how withdrawn material will be utilized; and evidence of research project funding and ethics approval for the proposed research. The governance committee of the CKD Biobank will judge applicants on capacity to complete the proposed research project, the contribution the research will make towards CKD knowledge, and the ethical robustness of the research project. Upon approval, applicants will also need to sign a research agreement with the CKD Biobank that sets out the terms and conditions of transfer, storage, use, publication of results and disposal of withdrawn material. If the access criteria are met and the withdrawing researchers have signed the research agreement, the withdrawn bio-specimens and clinical data will be transferred at the expense of the withdrawing researchers.

(4) Reuse potential

CKD poses a major health and economic burden that needs further research into its pathophysiology to develop novel prognostic or diagnostic tests and therapies. The CKD.CRE has established the CKD Biobank to address these issues and empower CKD.CRE, domestic, and international CKD researchers to close the gaps in CKD knowledge and improve clinical outcomes. This biobank will help investigators to elucidate the pathophysiological mechanisms underpinning CKD by using longitudinally collected bio-specimens and clinical data to answer various proposed hypotheses. Moreover, the number of available banked material gives investigators the capacity to increase the statistical power of analyses performed. It also allows research to be conducted at a faster pace by minimising the time and resources required to recruit a statistically significant sized population of CKD patients and healthy controls, especially in longitudinal research projects.

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Competing Interests

The authors have no competing interests to declare.

Author Roles

Owens, Evan P is the Bioresource Manager; Hoy, Wendy E is an Operational Advisor; Cameron, Anne is an Operational Advisor; Coombes, Jeff S is an Operational Advisor; Gobe, Glenda C is the Curator.

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