CHRONIC RENAL INSUFFICIENCY COHORT (CRIC) STUDY

Appendix to Protocol Amendment #3
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APPENDIX to PROTOCOL AMENDMENT #3

Introduction

This appendix accompanies the Chronic Renal Insufficiency Cohort (CRIC) Study Protocol Amendment #3. It provides the full protocol and informed consent form for the CRIC Plus study which will be incorporated into the core CRIC protocol, as well as the four ancillary studies described in the Amendment #3, Section D.

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A.1. CRIC Plus Protocol

STUDY AIM/PURPOSE

The Chronic Renal Insufficiency Cohort (CRIC) study is an observational cohort study of patients with reduced kidney function who do not yet requiring dialysis or transplantation. The purpose of this "renal Framingham" is to understand risk factors for progressive loss of renal function among these patients and reasons for their increased cardiovascular disease. No interventions are planned.

BACKGROUND/SIGNIFICANCE

It is estimated that 8 million American suffer from chronic renal insufficiency (CRI) with decrease in glomerular filtration rate (GFR) to < 60 ml/min/1.73m^2.[1] Morbidity and mortality associated with CRI derive from progression of CRI to end-stage renal disease (ESRD) and disproportionate risk of cardiovascular disease (CVD) in the setting of CRI. Recognizing major gaps in our knowledge regarding CRI, the NIH-NIDDK issued a RFA which eventually resulted in the funding of the current study.

Improved understanding of the risk factors for progressive loss of renal function and increased cardiovascular disease will lead to improved risk-stratification and targeted interventions in these patients.

METHODS

a. General Study Design

This cohort study will recruit patients from 7 clinical centers (University of Pennsylvania Medical Center, Johns Hopkins University/University of Maryland, Case Western Reserve University, University of Michigan at Ann Arbor, University of Illinois at Chicago, Tulane University Health Science Center and Kaiser Permanente of Northern California/University of California at San Francisco). The overall PI for the Kaiser/UCSF clinical site is Dr Alan Go at Kaiser Division of Research. Dr Hsu is the PI of the UCSF subcontract.

Patients will be recruited from both UCSF and Kaiser but study related activities from obtaining consent to performance of procedures will be take place only at Kaiser Division of Research (except electron beam tomography which will take place at Heartscan Institute). Patients will be observed for up to five years during which they will be monitored for development of clinical events (e.g., need for dialysis or stroke) as well as sub-clinical progression of disease (e.g., loss of GFR).

b. Methods of data-analysis

Standard descriptive statistics will be used to describe baseline characteristics and follow-up measures, both overall and within comparison subgroups. Failure-time analyses will be conducted for discreet outcomes such as hospitalization for myocardial infarction. For study outcomes that are repeated measures of continuous or categorical variables, standard mixed effects growth curve models will be used and supplemented with marginal models estimated by generalized estimating equations. Nested analyses will be performed for several important study variables (including iothalamate GFR, electron beam tomography and some chemistry measures) that will be performed only on a subset of study subjects. The actual power to detect specified associations will depend on the particular outcome of interest.

c. Subject selection

1) Who and Why: The estimated GFR to define eligibility will be evaluated using the simplified MDRD estimating equation[2,3]: GFR (ml/min/1.73m^2) = 186 x [serum Cr (mg/dL)] $^{-1.154}$ x [age] $^{-0.203}$ x [0.742 if female] x [1.212 if African-American/Black]. Potential subjects for the CRIC study must fulfill the following age-based estimated GFR inclusion criteria.

Age Stratum	Eligible Estimated GFR Range (ml/min/1.73 m^2)
21-44 years	20-70
45-64 years	20-60
65-74 years	20-50

In order to establish a diverse cohort of participants the following target ranges below have been established to include approximately equal numbers of men and women, diabetic patients and racial/ethnic groups that reflect the population of the participating clinical center areas.

Variable	Value	Percent of Population
Age (years)	21-44	20-30
	45-64	40-60
	65-74	20-30
Diabetes	No	40-60
	Yes	40-60
GFR	Lower half of range*	40-60
	Upper half of range	40-60
Race/Ethnicity	White	40
	African-American/Black	40
	Other [Latino/Hispanic]	15
	Asian/Pacific Islander/Other	5

* for ages 21-44: <45, for ages 45-64, <40; for ages 65+, <35

2) Total Number: The CRIC Study will enroll 3000 individuals. Each of the seven clinical centers plan to enroll approximately 430-500 patients using a recruit with replacement approach, to establish the baseline cohort of 3000 CRIC participants. The estimated rate of dropout during the first year is estimated at 3 – 5 %. We anticipate that 100-150 participants will be recruited from UCSF, the rest from Kaiser.

For largely budgetary reasons, only subcohort of 1000 participants will be selected to undergo iothalamate GFR measurement and electron bean CT. We will seek consent for subcohort participation from the entire cohort, then randomly select participants from those who consent.

3) Exclusion criteria:

General Exclusion Criteria	
Institutionalized (e.g., prisoner, nursing home resident, skilled nursing facility resident)	Previously received dialysis (peritoneal and/or hemodialysis) lasting more than one month based on patient self-report
Unable or unwilling to provide informed consent	Prior organ or bone marrow transplant; prior renal transplant based on patient self-report
Life expectancy less than three years as judged by the site investigator's assessment	Received immunosuppressive or other immunotherapy for primary renal disease or systemic vasculitits that affects the kidneys (i.e., anti-GBM, ANCA, SLE, IgA nephropathy, cryoglobulin, etc.) within the past six months before enrollment based on patient self-report. This does not include, for example, use of prednisone for the treatment of reactive airways disease.
NYHA Class III or IV heart failure at baseline	Received chemotherapy or alkylating agents for systemic cancer other than non-melanoma skin cancer within two years prior to enrollment based on patient self-report

General Exclusion Criteria	
Known cirrhosis based on patient self-report	Previous diagnosis of multiple myeloma or renal carcinoma based on patient self-report
Known HIV infection and/or AIDS based on patient self-report	Previously diagnosed polycystic kidney disease based on patient self-report
Present participation in the AASK Cohort Study	Currently participating in an interventional clinical trial (i.e., primarily trials of therapeutic agents that may have an effect on renal or cardiovascular outcomes as assessed by a Central Adjudication Committee)
Additional Exclusion Criteria for Participants Undergoing ^{125}I-Iothalamate GFR Testing	
Known iodine allergy	Currently breast feeding, or pregnant based on urine HCG test
Impaired urinary voiding	Radiation exposure to γ-emitting isotope other than technetium

d. Subject recruitment

1) Sources: At UCSF, we have identified four sources of patient recruitment. One, CRI patients who are attending the UCSF Nephrology/ Hypertension Faculty Practices (including Parnassus, Mt. Zion, Lakeside and office of Dr. Alan Coleman, Clinical Professor at UCSF [non-salaried]). Two, patients who have participated in Pilot Study of Chronic Renal Insufficiency (CHR approval #H10536-21053-01A) and who have given consent for us to contact them regarding this study. Three, subjects identified to have CRI using the laboratory and demographic information contained in STOR (Summary Time Oriented Record) at UCSF Medical Center. This third category of subjects is already captured in our pilot study database. Four, study recruitment information is posted on UCSF public search web site.

2) Initial Contact Method: This will vary depending on source of patients. One, for CRI patients seen in clinic, they will be approached by Drs Hsu, Chertow or their representative during the regularly scheduled clinic visit. To augment recruitment, Dr. Coleman will inform his patients about CRIC and invite them to participate in study informational sessions. These sessions will be conducted by study personnel and will provide additional information regarding CRIC. Two, for CRI subjects who have gone through the Pilot Study and have agreed to be contacted, they will receive a phone call. (See appendix 1 for draft of CRIC study telephone pre-screening script). In all instances, we will ask all subjects to sign an authorization form before releasing their contact information to our colleagues at Kaiser (appendix 2).

Three, for patients already identified as having CRI in STOR but not yet contacted as part of pilot study, initial contact will be in the form of a letter signed by Dr Hsu and the patient's UCSF provider identified through STOR (appendix 3 is example of initial contact letter). Before this happens, we will first e-mail each provider a list of patient names that we intend to contact (appendix 4 shows e-mail text to provider). We will send letters only to patients approved for contact by their UCSF provider. The provider will also have an opportunity to direct us to another provider that they deem more appropriate (for example, in cases where the primary care provider is actually a medical house officer that we had not identified in STOR). We will then approach each provider and ask him or her to sign one copy of the letter which is complete except for a blank space for the patient name field. We will then make copies of this provider-signed letter and fill in each patient's name as appropriate. The letter will be accompanied by a postcard with 3 choices: essentially "yes", "no" and "tell me more" (appendix 5 is example used in Pilot Study). Follow-up phone calls will be made only if we receive a postcard indicating "yes" or "tell me more." These are very similar to the steps approved by the UCSF CHR for our pilot study (H10536-21053-01A). (In other words, no new STOR searches are planned.)

An information sheet regarding the study will be provided to all interested health personnel.

e. Consent Process and Documentation

Written consent will be obtained during the face-to-face meeting with the patient at Kaiser Division of Research. No patients will be screened or enrolled without first obtaining written consent. Please see attached copy of Consent Form. Note that we are requesting that the UCSF CHR defer to the Kaiser IRB for approval of this consent form since consent and study will take place at Kaiser.

f. Procedures

1) Study procedures: CRIC study participants will be followed for up to five years, depending on the date of enrollment.

If subjects express interest, participants will be scheduled for a *screening visit* at Kaiser Division of Research during which the following will occur: informed consent obtained; eligibility assessment questionnaire completed; contact and demographic information recorded; non-fasting blood drawn (10 cc) for serum creatinine (to calculate GFR and determine eligibility), cystatin C and glucose; urine tested for presence of glucose, protein, and hematuria by dipstick.

This visit will take approximately 1.5 to 2 hours. Instructions and supplies will be provided for the collection of a 24 hour urine sample for use in the event the GFR estimation identifies the potential participant as eligible. If eligible, participants will be instructed to complete a food frequency questionnaire that asks detailed information about the food they eat, and return the completed questionnaire at the baseline visit. Participants selected for the subcohort studies will be informed of this shortly after this visit to prepare for the baseline visit procedures.

If a person is eligible according to the information collected during the screening visit, she/he will be scheduled within 30 to 45 days for a *baseline visit*. This visit is considered study enrollment during which the following will occur: eligibility assessment confirmed; detailed medical history obtained; fasting blood drawn (130 cc) (for complete blood count; metabolic panel; lipid profile; cystatin C, HbA1C, homocysteine, troponin I, iPTH, fibrinogen and uric acid and DNA *if* consent given for genetic studies); urine assayed for creatinine, protein, albumin, urea nitrogen; concomitant medication information obtained. The following measurements will also be performed: electrocardiogram (ECG), ankle-brachial index, anthropometric measures (height, weight, mid-abdominal circumference, hip circumference, and possibly additional measures); bioelectrical impedance assessment (BIA); questionnaires of dietary intake, physical activity, quality of life, depression, cognitive function and health resource utilization; finger nail clippings for future study of heavy metal exposure and finally iothalamate - GFR test (if selected for this subcohort).

This visit will take approximately three to four hours. Participants who are selected for the GFR testing component of the study will spend an additional three to four hours at this visit. The GFR test will be repeated two and four years after enrollment.

One year and four years after enrollment, all participants will also be scheduled for an echocardiogram test. One year and four years after enrollment, the same subjects who undergo GFR testing will also be scheduled for an electron beam tomography (EBT) test.

During the *follow-up* phase, participants will be contacted by *telephone* six months after the baseline and annual clinic visits to update contact information, to ascertain interim medical history and potential outcome events, and to assess health resource utilization (six months follow-up materials are attached to this protocol). Participants may also be contacted at other times during the year to answer additional questions or to join in a new part of this study.

Annual clinic visits will be scheduled to occur within a range of one month before to one month after the anniversary of the baseline enrollment date. Participants will experience many of the same procedures as those which occurred at the baseline visit.

A summary of the visit schedule and current plan of testing and data collection is shown below:

CRIC Visit Schedule	Pre-Screen Contact	Screening Visit	Base Line Visit	6 Mos. Phone Contact	Y 1	6 Mos. Phone Contact	Y 2	6 Mos. Phone Contact	Y 3	6 Mos. Phone Contact	Y 4	6 Mos. Phone Contact	Y 5
Eligibility Assessment	X	X											
Informed Consent (s)		X											
Medical Record Consent		X			X		X		X		X		X
Contact Information		X		X	X	X	X	X	X	X	X	X	X
Labs: Serum creatinine, cystatin C, glucose		X											
Demographic Information		X											
Eligibility Confirmation			X										
Medical History [CV, renal, health behaviors]			X		X		X		X		X		X
Genetic Sample			X		X		X		X		X		X
Labs: [CBC, metabolic panel, cystatin C, HbA1C, homocysteine, troponin I, iPTH, fibrinogen]			X		X		X		X		X		X
Urinary Assay: 24 Hour Urine [creatinine, protein, albumin, urea nitrogen]		X	X		X		X		X		X		X
ABI/Anthropometry/Nail clipping			X		X		X		X		X		X
Bioelectrical impedance analysis (BIA)			X				X				X		
ECG			X		X		X		X		X		X
Echocardiogram					X						X		
EBCT (1/3 Participants)					X						X		
I-GFR (1/3 Participants)			X				X				X		
Physical Activity			X				X				X		
Concomitant Medications			X		X		X		X		X		X
Questionnaires			X		X		X		X		X		X
Recent Medical History & Outcome Event Data				X	X	X	X	X	X	X	X	X	X

The protocol changes introduced as a result of additional funding for "CRIC-Plus" is as follows. For subjects who develop more advanced renal insufficiency (defined as an estimated GFR <20 ml/min/1.73m^2), an additional echocardiogram will be scheduled if the subject is not due to get an echocardiogram anyway (i.e., if their GFR does not fall below 20 ml/min/1.73m^2 at years 1 or 4). In additional, 24-hour urine collections for patients with advanced chronic renal insufficiency will be performed every 6-months rather than every year. Patients will have to come in person to turn in the 24-hour urine collection and undergo a blood draw to determine concurrent serum creatinine and blood urea nitrogen in order to calculate renal clearance. During this 6-month in-person visit, for efficiency, individual clinical centers and patients may wish to complete items scheduled for the 6-month telephone contact. Subjects with GFR <20 ml/min/1.73m^2 will undergo yearly (rather than every other year) bioelectrical impedance analysis (BIA).

For subjects who progress to develop end-stage renal disease requiring maintenance dialysis therapy or kidney transplantation, an additional echocardiogram will be scheduled within 6 months of this event. We will also obtain information regarding details of the dialysis procedure (such as dose of dialysis) from each subject's outpatient dialysis unit chart. Consent for this release of medical record will be obtained along with consent for release of other medical records in CRIC overall.

In summary, there are no additional procedures introduced as a result of "CRIC-Plus," only more frequent performance of procedures among those with advanced chronic renal insufficiency, including transition into end-stage renal disease.

2) Time: The investigators are sensitive to the issue of study burden on the enrollees and have striven to balance that with maximizing the scientific benefits of the study. Food will be provided as soon as the fasting blood draws are completed. We estimate that the screening visit will take approximately 1.5 to 2 hours. The baseline visit will take approximately three to four hours. Participants who are selected for the GFR testing component of the study will spend an additional three to four hours at the baseline visit visit. The six-month telephone contacts will take less than 30 mins. The annuals clinic visit should take approximately the same amount of time as the baseline visit.

3) Study Site: No research related activity except recruitment and six-month follow-up phone contacts will take place at UCSF. All visits and studies (except the EBT) will take place at the Kaiser Permanente Division of Research clinic (3505 Broadway St., 9th Floor, Oakland, CA, 94611). Patients will undergo EBT at the Heartscan Institute– Walnut Creek (2161 Ygnacio Valley Road, Walnut Creek, CA 94598)(there is also a backup Heartscan site in South San Francisco).

g. Risks/Discomforts

The main potential risks to the study, other than patient confidentiality (which is addressed separately under l. confidentiality of records) involve the physical examination and diagnostic testing those participants will undergo as part of the study, which are described below:

Anthropometry and Body Composition. Height, weight, waist measurement, and non-invasive bioelectrical impedance assays will be performed at the baseline and five annual study visits. There are no known risks associated with these measurements.

Blood pressure and Ankle-Brachial Index. Systolic and diastolic blood pressures will be taken at the screening, baseline, and five annual study visits using a standard manual aneroid sphygmomanometer. The potential risk is temporary arm discomfort with inflation of the blood pressure cuff. The ankle-brachial index requires additional measurement of blood pressure at the ankle through use of a standard manual aneroid sphygmomanometer applied at the thigh. The potential risk with this procedure is temporary leg discomfort with inflation of the blood pressure cuff. Standard methods applied by trained, certified study staff will be used to reduce the amount of time necessary to obtain an accurate reading.

Fingernail Clippings. At the baseline and five annual study visits, samples of fingernail clippings will be obtained using a research quality nail clipper. The potential risks include mild discomfort in manipulating the finger, temporary pain with clipping the nail, minor bleeding if the nail bed is cut, and in diabetic patients, an uncommon but serious risk of infection. To reduce these risks, study staff will be trained to obtain nail clippings safely, with particular care being given to diabetic patients, which will constitute ~50% of the study sample at our site.

Phlebotomy: All participants will have annual blood collection of ~100-130 cc total. There is minimal risk of temporary mild pain, discoloration, bruising or very rarely, infection, at the place where the needle was inserted. The risks also include possible fainting. These risks will be minimized by the use of certified phlebotomists and the proper physical setting that currently exists at the 3505 Broadway Kaiser Division of Research facilities.

Urine tests: There are no known risks to providing random spot urine samples or 24-hour urine collections, which will be done in all subjects annually.

Electrocardiogram. Standard methods will be used to obtain 12-lead surface electrocardiograms in all subjects at the Baseline, Year 2, and Year 4 study visits. There are no known risks from electrocardiography.

Transthoracic echocardiography. Non-invasive surface transthoracic echocardiography will be performed in all subjects at Year 1 and Year 4 study visits using standard methods. There are no known physical risks from echocardiography and there is no radiation exposure from this test.

^{125}I-iothalmate clearance test for GFR (Glomerular Filtration Rate): In one third of participants (~150 at our clinical center), a more detailed measurement of GFR will be done using the ^{125}I-iothalamate clearance test at the baseline, Year 2 and Year 4 visits. We will inject 35 Ci of ^{125}I-iothalamate under the skin. The tracer provides a way of measuring the amount of dye that the kidney is able to remove from the blood over about a four-hour period. Participants will be asked to drink about four tablespoons (60cc) of water containing potassium iodide to protect their thyroid gland. A small intravenous line will be inserted into the arm and about one teaspoon of blood taken from the line every hour. During the test, participants will be asked to drink about 1 quart of water and to empty their bladder about every half hour. This test will take about four hours total. The main risk of this test is exposure to low-level radiation from the ^{125}I-iothalmate. The amount of radiation is approximately the same as for a standard chest x-ray, with radiation dose estimates for organs summarized below at 4.8 hours following iothalamate injection:

	Estimated Radiation Dose 4.8 hours	
Organ	mCy MBq	Rad mCi
Bladder	0.16	0.058
Kidneys	0.016	0.059
Liver	0.0040	0.015
Ovaries	0.0026	0.0095
Bone marrow	0.0017	0.0063
Testes	0.0057	0.021
Total Body	0.0021	0.0077

Since there is a small amount of iodine in the ^{125}I-iothalamate, patients with known iodine allergy will be excluded from the study. Of note, pregnant and breast-feeding women will be excluded from the overall study to avoid unnecessary radiation exposure, and all women of child-bearing age who are not known to be pregnant will undergo urine pregnancy screening prior to enrollment in the study and prior to each ^{125}I-iothalamate clearance test.

EBT (Electron Beam Tomography): In the same subcohort that receives ^{125}I-iothalamate GFR testing, electron beam tomography to measure coronary calcification will be performed at the Year 1 and Year 4 study visits. The main risk associated with EBT is exposure to a small amount of radiation that is slightly less than a set of dental x-rays, with total body exposure of ~2-rem. This is approximately 7 times the average annual exposure a person in the United States receives from natural background radiation. As noted above, all pregnant and breast-feeding women will be excluded from the main study. All women of child-bearing age who are not known to be pregnant will undergo urine pregnancy screening prior to enrollment in the study and prior to each EBT. The other potential risk for participants is the identification of non-cardiac abnormalities. Consistent with the policy used by a similar cardiovascular cohort study sponsored by the National Heart, Lung, and Blood Institute (NHLBI) at the NIH, all non-cardiac fields on the EBT (i.e., primarily lungs and liver) will be blacked out and not be read by the EBT Central Reading Center. This is justified since the purpose of the test is to evaluate coronary calcification and not other non-cardiac findings. There is no existing evidence that provides sound guidance on clinical follow-up and management even if those parts of the EBT were read.

h. Treatment and Compensation for Injury

The consent form contains the standard UCSF wording regarding treatment and compensation for injury. All study related adverse events will be tracked in CRIC using a standardized adverse event report form.

i. Alternatives

The alternative to not participating in this observation only study is to receive usual clinical care by the subject's health care providers.

j. Costs to Subjects

All tests associated with the study will be provided to the subjects free of charge.

k. Reimbursement of Subjects

Subjects will be reimbursed $20 after completing the Screening Visit and $25 after the Baseline Visit and each subsequent annual study visit. For those who are part of the substudy that involves GFR testing and EBT, each subject will receive an additional $25 after the visits that include these tests. In addition, parking at Kaiser will be validated and subjects will be reimbursed for their travel expenses calculated at $0.35 per mile traveled from residence.

l. Confidentiality of Records

Extensive efforts will be made to ensure that participants' confidentiality is maintained. Each participant will be assigned a unique study identification number, and participants will never be tracked through the study by name, social security number, medical record number, or any other personal identifier (unless permission is given by patient to use social security number to cross-link with external sources of medical information such as Medicare). A paper log of the participant names, participant ID numbers, and pertinent registration information (e.g. home address, telephone number, and emergency contact information) is maintained in a locked area at the Kaiser Division of Research with restricted access to the PI and only necessary research personnel, all of whom will have signed a confidentiality agreement. An electronic version of this log will be kept as a password-protected file with only access to the PI, Project Manager, and other necessary research personnel.

To enhance subject retention in the study, the UCSF recruiter and study coordinator Ms. Irina Gorodetskaya will have access to the log of UCSF-recruited patients in order to contact them for follow-up and feedback. Any information on UCSF subjects that is removed from Kaiser will be stored in locked file cabinets in a locked area in UCSF Laurel Heights.

The staff at the CRIC Data Coordinating Center at the University of Pennsylvania does not have access to this log. Only the encrypted participant ID number is given to the CRIC Data Coordinating Center staff and entered into the study database by each of the seven participating Clinical Centers. Any communication between the Data Coordinating Center and Kaiser regarding participant data occurs using the participant ID number. Any forms or documents sent to the CRIC Data Coordinating Center, IRBs, or Regulatory Authorities will have all personal identifiers removed. Authorized representatives of the Sponsor, the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK), National Institute of Health (NIH), as well as the IRB have access to and may copy both medical records and records from participation in this study consistent with the policy of the NIH Certificate of Confidentiality. Such access is necessary to insure the accuracy of the findings and the safety and welfare of participants. All research reports, articles, and presentations will report only aggregate findings, and participants will never be identified by name or any other personal identifier.

All blood, urine, and fingernail samples will be labeled only with the unique, encrypted study identifier that will be used to track the samples throughout the processing and storage procedures. No personal identifiers will be attached to the blood, urine, or nail samples.

Each participant will be asked as part of the informed consent procedure to approve use of their DNA for genetic testing to study aspects of kidney disease, cardiovascular disease, and related conditions. Participants will not receive any results of genetic testing. No results from these tests will be included in the participant's medical records nor will their medical providers be given the results.

At the end of the study, any unused samples will remain at the NIDDK-contracted sample repository for up to 50 years; unless additional studies are performed or the participant requests the samples to be destroyed. Participants will be asked as part of the informed consent process to provide the limits for use of their blood, urine, and nail samples. To participate in this study, they must agree to allow testing of their blood, urine, and nail samples for the core list of laboratory measures. In addition, as part of the initial informed consent process, they will be asked to allow or not allow use of their samples for future use in (1) other kidney- or cardiovascular-related studies, and/or (2) studies unrelated to kidney or cardiovascular disease.

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A.2. CRIC PLUS Informed Consent Form

INTRODUCTION:

The Chronic Renal Insufficiency Cohort (CRIC) study has been established by the U. S. Department of Health and Human Services (DHHS), the National Institutes of Health (NIH), and National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). The purpose of the CRIC study is to identify and follow the progress of people with chronic renal insufficiency (CRI), otherwise known as chronic kidney disease. If you agree to be part of this study, you may help doctors gain a better understanding of chronic renal insufficiency and its effects on your health.

STUDY PURPOSE:

You are being asked to join this research study because you may have chronic renal insufficiency. You may be eligible for the Chronic Renal Insufficiency Cohort (CRIC) study. Chronic renal insufficiency is a condition that limits your kidneys' ability to function. Over time, this condition may become worse and may also affect your heart. The goal of this study is to learn what causes this to happen. This is an observational study, which means that we will ask questions and gather information about your health for research purposes. We will not medically treat you or change any treatments you may be receiving. If you are eligible and want to join this study, you will remain under the care of your usual doctor(s). With your permission, results of study tests and procedures will be shared with your doctor. It will be up to your doctor to act on these study results.

We hope that this study will lead to improvement in the treatment of chronic renal insufficiency. We plan to have 3000 patients included in this study at seven medical centers across the country.

You will be asked to be in the study for a total of up to five (5) years, depending on when you start. After the first two visits (Screening and Baseline, which are described below), you will be asked to come to the clinical center at the Division of Research, 3505 Broadway, Oakland, once a year. You will also have telephone interviews between clinic visits.

VISITS:

The table on the last page of this form lists the schedule of study visits and tests. As part of the CRIC study, you will be asked to do the following:

a. Screening Visit

The Screening Visit will be a short visit to the clinical center. It will last about two hours. We will describe the study and get your informed consent. We will ask you questions to find out if you qualify for the study and will record your sex, race/ethnicity, medical information, and contact information. We will take a non-fasting blood sample (10 cc, or about two teaspoons) to test your serum creatinine level (a kidney function test) and your glucose level (blood sugar). We will check your resting blood pressure and do a test to check for glucose, protein and blood in your urine. You will receive a Food Frequency Questionnaire, which asks questions about the food you eat, and instructions for filling it out. We will also give you directions for collecting a 24-hour urine sample. Soon after this visit, we will call you to let you know if you qualify for this study.

b. Baseline Visit

If you qualify for the study and are interested after the Screening Visit, you will be scheduled for the Baseline Visit. The Baseline Visit will take about four hours. During this visit, we will review and confirm that you qualify for the study. You will be asked to bring your 24-hour urine sample so we can measure how well your kidneys are working.

You will also be asked to bring your completed Food Frequency Questionnaire. At this visit we will:

- Perform an Electrocardiogram (ECG) to record your heart rhythm. (All of the procedures are described later).
- Check your resting blood pressure and heart rate.
- Measure the difference between your ankle and arm blood pressure, called the Ankle
- Brachial Index (ABI) to find any circulation problems you may have.
- Draw 130 cc (about ½ cup) fasting blood sample for a complete blood count and tests of your metabolism.
- Perform several other heart and kidney tests.
- Record your weight, height and waist size.
- Measure your body water and fat content by Bioelectrical Impedance Analysis (BIA).
- Ask you about your medical and family history.
- Ask you about medicines you have used recently.
- Take a sample of clippings of your nails to measure heavy metal (like mercury or lead) exposure.
- Ask you to complete a series of questionnaires about your quality of life, diet, mood and physical activity.

c. Annual Clinical Center Visits

During each of your yearly visits to our clinical center, we will perform the following procedures: update your medical history; take a blood sample of up to 130 cc (about ½ cup) for laboratory tests; collect a urine sample; record your weight; and measure your blood pressure, heart rate, and ABI. We will also ask you about medications you are taking, your quality of life, diet, mood and physical activity. We may perform additional tests during annual visits, and if so, you will be informed about these tests and why they are being requested at that time. The table on the last page of this form describes the testing performed at each visit. These visits will take up to about four hours. Telephone Contacts Between your yearly visits, you will a get telephone call from us to check your contact information and ask about your recent medical history, health behaviors and use of medical services. These calls will each take about 15-20 minutes. Study personnel may call you at other times during the year to ask you some additional questions or to invite you to join in a new part of this study. You may also receive forms in the mail to complete and return.

PROCEDURES AND RISKS:

Blood draw. At the Baseline Visit and each annual visit, we will be taking a blood sample of up to 130 cc (about ½ cup) for various tests. The blood will be drawn from a vein in your arm. There is a small risk of temporary mild pain, discoloration, bruising or infection at the place where the needle was inserted. The risks also include possible fainting. These risks are very small because the tests will all be done by people who are specially trained in drawing blood.

Ankle-Brachial Index (ABI) is a test used to find circulation problems in the blood vessels of your legs. We will compare the blood pressure in your arm to the blood pressure in your leg. A Doppler, which is a pencil-shaped, sound-sensitive instrument, is used to measure the blood pressure in your leg. We will also use the regular blood pressure cuff that is used to measure blood pressure in the arm. This index will be measured at the baseline visit and every year. The only risk is temporary discomfort when the blood pressure cuff is inflated.

Bioelectrical Impedance Analysis (BIA) is a test used to measure your body's fat, water, and muscle content. This test involves lying on a bed for about two minutes while two electrodes (sticky pads with a wire attached to them) are attached to your foot and hand. A tiny current is

passed through your body (less electricity than that of a "AA" battery) and a reading is taken which is stored in a computer, along with your height and weight, to calculate your body fat, water, and muscle mass content. This procedure is completely painless. The sticky pads may leave a small residue which is easily washed off with water. We will do this measurement at the baseline visit and after two and four years. Individuals with implanted defibrillators may not take part in BIA testing.

Electrocardiogram (ECG) is a test that measures the electrical activity of the heart. The electrical signals from your heartbeat can be picked up on the surface of the skin using sticky pads or “electrodes.” The ECG can tell us about the condition of your heart and will detect an abnormal heartbeat or rhythm. We will perform an ECG at the baseline visit and at each annual visit. There are no known risks associated with having an ECG. This test is painless.

Echocardiogram is a test that uses sound waves to form a moving picture of the heart. A microphone is moved over the chest wall above the area of the heart while the echocardiography machine sends sound waves to the microphone. The sound waves are sent back by the heart walls and heart valves and changed into a picture that tells us how well the heart is functioning. We will perform this test at one year and four years after the baseline visit. There are no known risks associated with having an echocardiogram. It is painless.

Nail clippings. Using a standard nail-clipper, we will take a sample of your fingernails that will be stored for future measurement of heavy metal exposure (e.g., mercury or lead). The potential risks include mild discomfort in manipulating the fingers, temporary pain with clipping the nail, minor bleeding if the nail bed is cut, and in diabetic participants, an uncommon but serious risk of infection. We will minimize this risk by using staff trained in collecting nail clippings safely.

PROCEDURES FOR SUBSTUDY PARTICIPANTS:

You have a one in three chance of being selected to take part in a substudy to measure more information about your kidney function. If selected and you agree, you will have both GFR (Glomerular Filtration Rate) and EBT (Electron Beam Tomography) testing performed. If you are chosen for this part of the study, we will let you know soon after the screening visit.

GFR (Glomerular Filtration Rate): The purpose of the GFR test is to more accurately measure your kidney function. A small intravenous (IV) line is put in a vein in your arm so that we don’t have to keep sticking you with a needle to take the blood samples. Next, you will be asked to drink about four tablespoons (60cc) of water containing a bitter substance called SSKI (potassium iodide). A nuclear medicine technician at the Kaiser Oakland Medical Center (280 W. MacArthur Blvd.) will inject a small amount of dye (a “tracer” called 125I-Iothalamate) for the GFR test. The tracer provides a way of measuring the amount of dye that the kidney is able to remove from the blood over a four-hour period. During the test, you will be asked to drink about one quart of water and to empty your bladder often (about every half-hour). This is to make sure that you have enough urine flow. About one teaspoon of blood will be taken from the IV line about every half-hour. A small dose of heparin, a blood thinner, will be sent through the IV line after each blood draw to keep the IV line open. This test will take about four hours. We will do GFR testing at the baseline visit and repeat it two years and four years after the baseline visit for participants who were selected for this substudy.

Risks associated with GFR: For substudy participants who have GFR testing, using 125I-Iothalamate involves minimal exposure to radiation. This is about the same amount as having a chest x-ray. Pregnant and breast-feeding women cannot take part in GFR testing. Women of childbearing age will be given a urine pregnancy test before the exam. Those who may be pregnant, based on the urine pregnancy test, may not take part in GFR testing. There is a tiny amount of iodine in the dye, so if you are allergic to iodine or shellfish, you should **not** take this

test. Patients who have received other types of radiation within the 30 days before this test will also be excluded. Please be aware that this radiation exposure is necessary for this study, but is not necessary for your medical care. The use of radiation in this research study has been approved as being within the Kaiser Permanente of Northern California guidelines for research participants. Heparin will be used to flush the IV line to keep it open. If you have an allergy to heparin, or a history of heparin-induced thrombocytopenia (HIT, a condition where blood clots form as a result of an allergic reaction to heparin), we will use normal saline instead. There is a very small risk of blood clots. There is also a small risk of bruising or infection at the site of the needle stick to put the IV line in. Very skilled people will do this test so the risks are very small.

EBT (**Electron Beam Tomography):** At the Year 1 and Year 4 Visits, we will ask those people who have been selected for the substudy to have EBT testing performed by the HeartScan Institute in Walnut Creek. EBT measures the amount of calcium in the blood vessels in the heart. This is a fairly new test in which a very fast x-ray scanner is used to create a series of pictures of the heart. The EBT may show early signs of heart disease. This test takes about 30 minutes and is painless.

Risks associated with EBT: Participants are exposed to small amounts of radiation from the EBT exam. Each EBT examination adds the risk of radiation exposure (~2 rem) that is slightly less than a set of dental x-rays, or about 7 times the amount of radiation that a typical person in the United States is exposed to from background radiation in one year. Pregnant and breast-feeding women may not take part in EBT testing. Women of childbearing age will be given a urine pregnancy test before the exam. Those who may be pregnant, based on the urine pregnancy test, may not take part in EBT testing.

PROCEDURES FOR PARTICIPANTS WHOSE ESTIMATED GLOMERULAR FILTRATION RATES IS BELOW 20 ml/min/1.73m^2

If your estimated or measured GFR is found to be below 20 ml/min/1.73m^2 at an annual study visit, you will be informed by the research team. An echocardiogram will be performed within 6 months of this visit to examine the function and structure of your heart. You will also be asked to collect an additional 24-hour urine sample every six months instead of every year to monitor more closely your kidney function. An additional non-fasting blood sample of 10 cc (or about two teaspoons) will be drawn when you come in person to turn in the extra 24-hour urine collection in between the regular yearly visits to measure your serum creatinine and blood urea nitrogen. Bioelectrical Impedance Analysis (BIA) will be done every year instead of every other year as a measure of your body composition.

Should you develop the need for dialysis or kidney transplantation by the time of your next annual study visit, an echocardiogram will be performed within 6 months of that visit. You also give us authorization to collect information about the dialysis therapy or kidney transplant.

STORED SPECIMENS: Your blood, urine and nail samples will be stored at a central laboratory (at the University of Pennsylvania) or an NIDDK storage facility for future studies of kidney and heart disease. This sample will be connected to your study results only by a unique study number, which you will be given at the time of your consent. Your name and any other personal information will not be linked to your blood, urine, or nail samples. The samples will be stored through the end of the study. If you approve, it can be used at any time during that period for more studies of kidney and heart disease. It is possible that new tests might be available in the future, which could be useful in understanding kidney and heart disease. You may or may not be able to find out the results of these future tests depending on the specific study. These studies will not be about your genetic makeup or in any way describe your genes or DNA. Researchers who plan to use your sample(s) for future scientific study will be required to request and receive all of the necessary approvals or waivers from the NIDDK and CRIC study

investigators before using your sample. Samples will only be released to scientists who are qualified and prepared to conduct a research study.

POSSIBLE BENEFITS:

You may not benefit personally from being in this study. It is possible that you may benefit from early detection of health problems, if present, and from the free diagnostic tests. You will also have the satisfaction of being in a research study that may help others and lead to a better understanding of kidney and heart disease.

COSTS and PAYMENTS:

All of the tests in this study will be done at no cost to you. You will not be paid for taking part in the study, but we will give you a small thank you for your time and effort. After completing the Screening Visit, you will receive $20. After the Baseline Visit and each subsequent annual study visit, you will receive $25. If you are part of the substudy that involves GFR testing and EBT, you will receive an additional $25 after the visits that include these tests. In addition, we will reimburse you for any travel-related costs to attend study visits. You will receive a check following each completed visit after a delay of up to 6 to 8 weeks.

ALTERNATIVES:

This study will examine the effects of CRI on your health and lifestyle but does not provide any treatments. Your alternative is not to participate in this research study. Your regular doctor will be able to give you information about standard treatments for CRI.

VOLUNTARY PARTICIPATION:

Participation in this study is completely voluntary. You are free to refuse to participate in any part of the study or to refuse to answer any questions. If you decide to participate, you are free to change your mind and stop at any time. If you choose not to enter the study or if you enter the study and later decide that you want to stop, this will not affect your medical care or eligibility for future care or membership in Kaiser Foundation Health Plan (KFHP) or care at the University of California, San Francisco. The Principal Investigator may also decide to stop your participation in the study at any time. If new information becomes available about any of the tests that were done before you left the study, we will notify you in a timely manner.

RESEARCH-RELATED INJURY:

In the event of a research-related injury, please contact the study Principal Investigator at Kaiser Permanente, Alan S. Go, MD, at 510-891-3553, the study Co-Principal Investigator at UCSF, Chi-yuan Hsu, MD, at 415-353-2379, and/or the Research Project Manager at Kaiser Permanente, Nancy Jensvold, at 510-891-3544. Any injury or condition experienced by a member of KFHP, as a result of being in this study, will be treated according to the member's Health Plan coverage, as described in the Service Agreement.

If you are injured as a result of being in this study, treatment will be available. The costs of such treatment may be covered by the University of California depending on a number of factors. The University does not normally provide any other form of compensation for injury. For further information about this, you may call the office of The Committee on Human Research at (415) 476-1814, or write: Committee on Human Research, Box 0962, UCSF, San Francisco, CA 94143.

CONFIDENTIALITY:

Every effort will be made to maintain your privacy. You will be given a unique study identification number. This number will be used to record your study information. You will never be tracked through the study by name, medical record number or other personal information unless you

give us permission to use your social security number, which would only be unscrambled to connect to other sources of medical information such as Medicare. A list of participant names, participant identification numbers, and personal information (such as home address, telephone number, and emergency contact information) will be maintained in a locked area at the Kaiser Permanente clinical site only.

The University of Pennsylvania is the Data Coordinating Center for this study. The study information from all research centers, with all personal identifiers removed, will be stored in secure electronic files at the University of Pennsylvania. All study data (except personal information such as home address, telephone number, and emergency contact information) will be sent to the Data Coordinating Center. Only authorized research staff will have permission to see this data.

We will ask you to tell us your social security number. This number will be recorded but it will not be open to anyone outside of the research study. It will be stored in a scrambled fashion so that the numbers cannot be easily figured out. When used to track your health status, it will be unscrambled to connect to other sources of medical information, such as Medicare. Only authorized study personnel will be permitted to view your social security number and then it will be scrambled again after the necessary medical information is obtained.

Please respond to the following statement and **CIRCLE** either "**YES**" or "**NO**" and write your initials and today's date:

_____ _____

Initials **Date**

I will provide my Social Security Number. **YES** **NO**

As stated in the Introduction, we will send your lab and health information to the health care provider that you choose.

Please answer the following statement and **CIRCLE** either **"YES"** or **"NO"** and write your initials and today's date:

_____ _____

Initials **Date**

I permit my test results to be sent to my doctor/health care provider. **YES** **NO**

Authorized representatives of the Sponsor, the US Department of Health and Human Services (DHHS), National Institutes of Health (NIH), the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), and the Institutional Review Boards (IRBs) of Kaiser Permanente and the University of California, San Francisco, may have access to and copy medical records and records from this study as permitted by law. This is necessary to insure the accuracy of the findings and the safety and welfare of participants.

To help us protect your privacy, we have obtained a Certificate of Confidentiality from the National Institutes of Health. With this Certificate, the researchers cannot be forced to disclose information that may identify you, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify you, except as explained below.

The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of Federally funded projects or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA).

A Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about yourself or your involvement in this research. If an insurer, employer, or other person obtains your written consent to receive research information, then the researchers may not use the Certificate to withhold that information. No voluntary disclosures will be made, without your consent, of information that would identify you as a participant in the research project. If publications or presentations result from this research, you will not be identified by name or any other personal information.

SUBJECTS' RIGHTS/QUESTIONS:

If you think of more questions about the research at a later time, you may contact the Principal Investigator, Alan S. Go, MD at 510-891-3553, the study Co-Principal Investigator, Chi-yuan Hsu, MD, at 415-353-2379, or the Research Project Manager, Nancy Jensvold, at 510-891-3544. Kaiser participants with questions about their rights as a study participant, comments or complaints about the study may contact the Institutional Review Board for the Protection of Human Subjects, Kaiser Foundation Research Institute, 1800 Harrison Street, Oakland, CA 94612-3433, telephone toll-free 1-866-241-0690. Participants from the University of California at San Francisco may contact the office of the Committee on Human Research at 415-476-1814 or write: Committee on Human Research, Box 0962, UCSF, San Francisco, CA 94143.

SIGNATURES: I have had this research study explained to me. I have read and understand the informed consent document. I have had the chance to ask questions. All of my questions about the study have been answered to my satisfaction. I understand that my signature below means that I voluntarily agree to participate in the research study. I will be given a copy of this signed form, the Authorization to Use and Disclose Protected Health Information, and a copy of the "Research Participants' Bill of Rights" for my records.

Date	Signature of Participant (Or Legally Authorized Representative – note relationship to participant)	Printed Name

Date	Signature of Witness (Only required if the participant cannot read this consent)	Printed Name

I have discussed this clinical study with the participant and/or his/her authorized representative, using language that is understandable and appropriate. I believe that I have fully informed the participant of the nature of this study and its possible benefits and risks.

Date	Signature of Person Obtaining Consent	Printed Name

SEPARATE SIGNATURE REQUIREMENTS FOR OPTIONAL GENETIC SAMPLE

PROCEDURE FOR GENETIC/DNA SAMPLE: We will ask you to allow genetic testing on a blood sample that will be collected and stored as part of the CRIC study. This test does not require a separate blood draw.

DNA is the part of your blood sample that holds genetic information. Your DNA sample will be stored at a central laboratory under a unique study identification number. All results of genetic testing will be stored under this study identification number without personal information (such as name or medical record number) in a secure, password-protected database. You will not be informed of any of the results of the genetic testing on your DNA. The results will not be placed in your medical record. Your blood sample will be used to prepare DNA. DNA from the blood will be used to study cardiovascular disease, kidney disease and related conditions. DNA can be removed from blood samples and stored separately for future genetic analyses. Or, to provide a larger amount of DNA from your blood for analysis in the future, we can store your blood in a way that allows blood cells to live and grow indefinitely. This is called creating a cell line from your blood cells. The DNA samples will be kept through the end of the study.

RISKS: The kind of genetic information being analyzed in the CRIC study is not likely to have any direct effect on your health. There is the unlikely risk that if people other than the researchers got your genetic information they could misuse it. The chance of this ever happening to you is very small. The risks of the blood draw are the same as listed in the consent for participation in the study.

BENEFITS: There is no direct benefit to you for participating in the genetic part of the CRIC study. You will not be informed of any results of the genetic testing on your DNA. However, the medical knowledge gained may improve the health of patients with kidney disease in the future.

CONFIDENTIALITY: Results of DNA testing will be kept confidential and protected. Your DNA will be kept at a central storage facility under the direction of the National Institutes of Health and National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) for future studies. These future studies may include medical research projects for medical conditions other than kidney disease or cardiovascular disease. The NIDDK storage facility was established to store samples from many research projects around the country in order to conduct large research studies. Your name and other personal information that could identify you will not appear on the DNA samples or results. Only approved study investigators who are working directly with the genetic samples will have the master code that links your name with the study identification number. This master code will be kept in a secure location.

To help us ensure your privacy, we have obtained a Certificate of Confidentiality from the National Institutes of Health. With this Certificate, the researchers cannot be forced to disclose information that may identify you, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify you, except as explained below.

The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of Federally funded projects or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA).

A Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about yourself or your involvement in this research. If an insurer, employer, or other person obtains your written consent to receive research information, then the

researchers may not use the Certificate to withhold that information. No voluntary disclosures will be made, without your consent, of conformation that would identify you as a participant in the research project.

Researchers who plan to use your sample for future scientific study will have to request and receive all of the necessary approvals from the NIDDK and CRIC study investigators before using your sample. Samples will only be released to scientists who are qualified and prepared to conduct a research study.

ALTERNATIVES: Your alternative is not to participate in the genetic testing part of the study. If you choose not to participate in this part of the study, it will have no effect on your current or future medical care or on your ability to take part in the main CRIC study.

VOLUNTARY CONSENT: Your participation in the genetic testing part of this research study is completely voluntary. You may choose not to join in this part of the study even if you decide to participate in the CRIC study previously described in this informed consent form.

If you do decide to participate in this genetic testing, but later change your mind and wish to withdraw from this part of the study, you must notify the Principal Investigator of this study in writing so that no additional genetic testing will be performed on your samples. You may also decide to participate in some level of genetic testing but not another, as described in the choices below.

CONSENT FOR GENETIC TESTING

Instructions: For each question, please **CIRCLE "YES"** or **"NO"** and write your initials and today's date in each row where indicated. Please circle either **"YES"** or **"NO"** for each of the following four questions.

Initials	**Date**		
▪ I give my permission to prepare DNA from my blood samples		**YES**	**NO**
▪ I give my permission to create a cell line from my blood cells.		**YES**	**NO**
▪ I give my permission to test my DNA for genes related to the main goals of this study: learning the causes and effects of diseases of the kidney, heart and blood vessels.		**YES**	**NO**
▪ I give permission to test my DNA for genes related to other health conditions.		**YES**	**NO**

SIGNATURES:

I have had the optional genetic testing section of this research study explained to me. I have read and understand the informed consent document. I have had the chance to ask questions. All of my questions have been answered to my satisfaction. My signature below means that I voluntarily agree to participate in the genetic testing part of the research study. I have chosen the types of testing I have agreed to. I will be given a copy of this form for my records.

Date	**Signature of Participant (Or Legally Authorized Representative – note relationship to participant)**	**Printed Name**
Date	**Signature of Witness (Only required if the participant cannot read this consent)**	**Printed Name**

I have discussed the optional genetic testing part of the research study with the participant or his/her authorized representative, using language that is understandable and appropriate. I believe that I have fully informed the participant of the nature of this study and its possible benefits and risks.

Date	Signature of Person Obtaining Consent	Printed Name

APPENDIX B. CRIC ANCILLARY STUDY DOCUMENTS

RETINOPATHY (RCRIC) PROTOCOL AND CONSENT

Protocol Title: Retinopathy in Chronic Renal Insufficiency (RCRIC)

1. Purpose:

To gain understanding of the relationship between progressive renal disease and cardiovascular illness, the National Institute of Diabetes, Digestive, and Kidney Diseases (NIDDK) established the Chronic Renal Insufficiency Cohort (CRIC) Study. This is an eight year long multicenter study that will include about 3000 adults with varying severity of chronic renal insufficiency (CRI). The principal goals of the CRIC Study are to examine risk factors for CRI and cardiovascular disease (CVD) events among participants with varying severity of CRI, and develop predictive models that will identify high-risk subgroups with CRI.

The CRIC Study did not include an eye examination as part of the overall assessment of the clinical condition of the participants. Because many of the CRIC Study participants are at high risk of developing significant retinopathy due to diabetes mellitus, systemic hypertension and other vascular diseases, it is important to assess the ocular condition. In addition, because both diabetic and hypertensive retinopathy have been shown to be associated with CRI and CVD, identification of these abnormalities in CRIC participants may improve predictive models aimed at identifying high risk subgroups with CRI.

The CRIC Study provides a unique opportunity to investigate the relationship between retinopathy and chronic renal insufficiency. To achieve this goal, we are proposing a study entitled “Retinopathy in CRI” (RCRIC), in which we will perform one set of fundus photographs on CRIC participants. We propose to merge the ocular data obtained through this grant with the vast amount of data collected by the CRIC Study to address the following Specific Aims:

Specific Aim 1:

Hypothesis 1. Diabetic and hypertensive retinopathy are non-traditional risk factors that are associated with both progression of CRI and development of end-stage renal disease. (Non-traditional risk factors indicate risk factors that have not yet been well studied in renal disease in contrast to well-studied factors such as blood pressure and proteinuria). We will examine the relationships between retinopathy and CRI and subsequent development of end-stage renal disease.

Specific Aim 2:

Hypothesis 2. Diabetic and hypertensive retinopathy are non-traditional risk factors that are associated with CVD events and measures of CVD progression in the setting of CRI. We will examine the relationships between retinopathy and subsequent CVD events and measures of CVD progression.

The data from the RCRIC ocular study will be merged with the extensive primary data collected within the CRIC Study. The statistical methods that will be used will follow the broader analysis plan already developed for the CRIC Study. We will perform analyses designed for prediction of outcomes, as well as those designed to elucidate mechanisms of disease progression. We also will use analyses relating baseline characteristics of the cohort to clinical outcomes.

2. Duration:

The duration of the entire RCRIC study will be five years. If follow-up of CRIC patients is extended beyond 2008, the period of data analysis may be extended. However, data collection specific to RCRIC will be completed during a 1-year time span between Spring 2006 and Spring 2007. .

3. Subject Recruitment and Selection

Subjects included in this project will be participants of the Chronic Renal Insufficiency Cohort (CRIC) study, an 8-year multicenter, collaborative project sponsored by the NIDDK. The CRIC Study Coordinating Center is located at the University of Pennsylvania and the protocol has been approved by the IRB of the University of Pennsylvania and the IRB of each participating center. The RCRIC research project will be an ancillary study to the CRIC study in which we will obtain a single set of fundus photographs on CRIC participants either their baseline visit or an annual visit marking 1, 2, or 3 years of participation in the CRIC Study. This spread over the years of participation in the CRIC study is due to the staggered recruitment of the CRIC study.

The CRIC is a multi-center study drawing a clinical population from six institutions across the United States. Based on the estimated distributions of race/ethnicity in the available populations at the CRIC Clinical Centers, we estimate the race/ethnic composition of participants in our RCRIC study to be approximately 40% White/Caucasian, 40% African American, 15% Latino/Hispanic, and 5% Asian/Pacific Islander and Other. We plan to enroll approximately equal numbers of men and women.

Our RCRIC project will recruit 2200 volunteers from the 3000 subjects participating in the CRIC Study across the United States. Recruitment is planned to start on March 1, 2006 and end on February 28, 2007. The University of Pennsylvania will be one of the 6 Clinical Centers participating in this project. We will recruit in our RCRIC study at the University of Pennsylvania about 480 patients.

Ages of the participants will be between 21 and 76 years at the time of enrollment. The lower age limit was chosen since the focus of the CRIC Study is on adults, which is defined by the NIH as 21 years or older for research purposes. The upper age limit of 74 years was chosen to address the role of renal dysfunction in older participants and to increase power for cardiovascular analyses without a significant impact of competing risks and censoring due to death or dropout. All participants will have some degree of renal insufficiency, half of the participants will have Diabetes Mellitus, and about 75% will have Systemic Hypertension.

4. Location

The RCRIC is a multicenter study that will be carried out in six Clinical Centers: 1) University of Pennsylvania Medical Center, Philadelphia, PA. 2) University of Maryland, Baltimore, MD. 3) Case Western Reserve University, Cleveland, OH. 4) University of Michigan, Ann Arbor, MI. 5) University of Illinois, Chicago, IL. 6) Kaiser Permanente of California, Oakland, CA. It will be the responsibility of each clinical center to conduct the study according to the study protocol and applicable regulatory guidelines which will include the approval of this project by the IRB of each of the institutions involved.

The Coordinating Center and the Fundus reading Center of the RCRIC study will be located in the Department of Ophthalmology at the University of Pennsylvania.

5. Background

Diabetes mellitus and systemic hypertension are diseases that are often associated with CRI. About 50% percent of the participants included in the CRIC Study will have diabetes mellitus and about 80% of the participants will have systemic hypertension. We estimate that a large percent of CRIC Study participants will have significant ocular morbidity from these diseases, and therefore, it is important to assess the ocular condition of the CRIC participants. In addition, both of these diseases have been shown to be associated with increased risk of morbidity and mortality in patients with CRI.

Therefore, we are proposing the performance of an ancillary study to the CRIC in which we will obtain baseline fundus photographs in CRIC participants. Data obtained through this ancillary study will assess the relationship between diabetic and hypertensive retinopathy and the progression of CRI and CVD within the cohort of CRIC participants.

6. Research Design

We are planning to include in our ancillary Retinopathy in CRI Study all 2145 CRIC participants to be recruited in six of the seven CRIC clinical centers. At each of the six centers, an informed consent specific for our retinopathy ancillary study will be obtained using a consent form approved by each local IRB. Each participant will also sign a consent form including the HIPAA regulations of April 2003.

Because of the staggered recruitment of CRIC participants (which is being carried out during 33 months) CRIC participants will be enrolled in our study at baseline or the annual visit at year 1 2, or 3 of the CRIC Study for the patient. All patients will have one set of fundus photographs. \

In all CRIC Study participants who agree to take part in our Retinopathy in CRI ancillary study, one set of fundus photography will be performed in both eyes with a non-mydriatic fundus camera that does not require pupillary dilatation.

A CRIC coordinator will present the purposes and detailed protocol of our ancillary study to all CRIC participants. The presentation of our RCRIC study by a CRIC coordinator familiar to the participant will improve the chances that CRIC participants will agree to participate in our study. Following the signature of our consent form, the CRIC coordinator will obtain fundus photographs of the disk and macula of both eyes. No dilatation of the pupils will be necessary because we will be using a non-mydriatic camera that does not require dilatation. Fundus photography will be obtained in a darkened room. To induce a natural dilatation of the pupil, a period of dark adaptation of about 5 minutes will precede photography. Time required to complete the photography will be about ten minutes.

A letter with information about the findings observed in the fundus photographs will be sent to the patients. Patients that have fundus findings that require treatment will be advised to seek a complete eye examination by their Ophthalmologist of choice.

7. Potential Risks

Our ancillary study will pose minimal risks to participants. We will be performing a single non-dilated fundus photography session during the study in all participants.

8. Consent Procedures

CRIC participants will be approached by the CRIC Study coordinator and will be asked to participate in our Retinopathy in CRI (RCRIC) ancillary study. Following signature of the appropriate consent forms fundus photography will be obtained by the CRIC coordinator at the time of the regular CRIC visit.

Each clinical center will prepare an informed consent form following the guidelines of their local Institutional Review Board (IRB) and applicable regulations for Informed Consent. This consent will be separate from the main consent form of the CRIC Study. The informed consent form must be signed and dated by the participant prior to initiation of any study related activity and at a minimum, contain a description of the potential risks, benefits, expense to the participant, and alternative treatment.

The consent process may differ somewhat by clinical center according to local IRB guidelines. This form will cover all aspects of the study protocol. The Health Insurance Portability and

Accountability Act (HIPAA) became effective on April 14, 2003 and affects the CRIC Study and our Retinopathy in CRI ancillary study with regard to the use or disclosure of protected health information. Each institution may hold different requirements regarding HIPAA. Some institutions may request that this HIPAA language be inserted into the appropriate sections of the informed consent form; other institutions may require a stand alone document. We will be using at the University of Pennsylvania a separate HIPAA consent form in this study.

9. Protection of subjects:

During the fundus photography subjects will be under direct supervision by CRIC personnel. Extensive efforts will be made to ensure that participants' confidentiality is maintained. Each participant will be assigned a unique study identification number and will never be tracked through the study by name, social security number, medical record number, or other personal identifier. A log of the participant names, participant ID numbers, and pertinent registration information (e.g. home address, telephone number, and emergency contact information) will be maintained in a locked area at each clinical site as part of the main CRIC study procedures. Only the participant ID number will be sent to the RCRIC investigators. Any communication between the RCRIC investigators and clinical sites regarding participant data will occur via the participant ID number. Any forms or documents sent to the CRIC Study Data Coordinating Center, IRB or Regulatory Authorities will have all personal information removed.

If any publication or presentations result from this research, participants will not be identified by name or other personal identifier. All research reports, articles, and presentations will report only aggregate findings. Throughout the study we will adhere to the HIPAA guidelines of April 14, 2003 regarding all issues of participant confidentiality.

10. Potential benefits

Participants will benefit from having an assessment of any diabetic and hypertensive retinopathy from their fundus photographs, an important benefit since many of the participants in the study will have significant eye conditions. Patients will be informed of any information observed in the photographs that may require treatment. While this is a direct benefit to the individual participants of this study, there is also considerable potential benefit to future participants, and to society as a whole if predisposing factors for progression of CRI and development of CVD are identified from this study. Identification of such factors may potentially lead to interventions that may reduce the burden of chronic renal failure.

11. The Risk/Benefit ratio

The risk involved in this project is very small. Knowledge gained will help identify participants at risk for progression of CRI and CVD as well as diabetic and hypertensive retinopathy. This will allow a better treatment and follow up of these conditions. The risks involved in this study are minimal in comparison to the importance of the knowledge that may be gained through this study.

Protocol Title: Retinopathy in Chronic Renal Insufficiency (RCRIC)

Principal Investigator: _____

Emergency Contact: _____

Phone: _____

Introduction

You have been invited to participate in a research study entitled **Retinopathy in Chronic Renal Insufficiency (RCRIC)** that will investigate the effect of chronic kidney disease on the eyes. You are currently participating in the Chronic Renal insufficiency Cohort Study (CRIC) which is looking at the relationship between kidney disease and heart conditions. Because Chronic Renal Insufficiency can also have a significant effect on your eyes, it is important to investigate the relationship between kidney disease and eye disease

The CRIC study, in which you are currently participating, does not include eye photographs. We would like to perform eye photography in CRIC study participants to investigate the impact of kidney disease on the retina. The retina is the film in the back of the eye that enables you to see things.

The Research Team wants you to know that Federal laws require that you be given the information needed for you to make your decision about participating in this study. This means that you know what is involved with this research study, what is expected of you, and what risks and potential benefits you may experience. After reading this consent form, you should ask questions about what you have read. Use this information to decide whether or not you want to participate. By signing this form you document that you have been informed about this study and give your consent to participate.

What is the purpose of this research study?

The purpose of this research is to gather information on the status of the eye by taking one set of eye photographs. The information gathered through these photographs will allow us to determine the relationship between kidney disease and eye conditions.

How long will my participation last? How many people will be in the study?

There will be a total of 2200 volunteers participating in this study. About 480 of these patients will be followed at the University of Pennsylvania. Each subject will have eye photographs taken one time during the study.

What am I being asked to do?

You are currently participating in the CRIC study. In addition to the protocol of that study, you will have eye photography at the CRIC Study Center. The eye photography will be performed on the regular CRIC Study visit. You will be asked to sit for about five minutes in a darkened room in front of an eye camera. A few photographs will be taken of each eye. The procedure will take about 10 minutes.

What are my potential risks or discomforts?

There is no risk involved in the eye photographs that will be taken. You will not have your eyes dilated for these photographs. This study will use a camera that does not require the eye to be dilated for photographs.

What if new or additional information becomes available about the study that can affect me?

You will receive a letter describing the findings detected in the eye photographs. In those cases in which the photos show conditions that should be treated you will be advised to have an eye examination with your eye doctor of your choice. During the course of this study if any new information becomes available that could have an effect on the expected safety of the study, you will be promptly contacted informing you of this information.

What are the possible benefits of the study?

You will benefit from having eye photographs, an important benefit since many of the participants in the CRIC study will have significant eye conditions. The photographs will be carefully analyzed and you will be notified if any eye conditions that require treatment are detected in your photographs. There is considerable potential benefit to future patients with chronic renal insufficiency and to society as a whole if predisposing factors for progression of kidney disease and development of heart disease are identified from this study. Identification of such factors may potentially lead to interventions that may reduce the burden of chronic renal failure.

Will I be paid for this study?

There is no compensation for participation in this study.

What alternatives do I have if I choose not to participate?

You are free to decide whether or not to participate in this study. Your care at this institution will not be affected if you choose not to enroll in this study or if you choose to withdraw from the study after you enroll.

Will I have to pay for anything?

The cost for eye photographs to be performed during this investigation will be covered by the study. Treatment for any conditions discovered in the photographs will not be covered by the study.

What happens if I am injured or hurt?

In the event of any physical injury resulting from research procedures, medical treatment will be provided without cost to you, but financial compensation is not otherwise available from the University of Pennsylvania.

Treatment for any illness or injury you may experience during the course of this research trial not related to your participation in this study will not be reimbursed.

You or your third party payer, if any, may be billed for medical expenses associated with this study if they are deemed medically necessary and such expenses would have been incurred independent of the study.

When is the study over? Can I leave the study before it ends?

This study is expected to end at the same time that the CRIC study will end. If you decide to participate, you are free to leave the study at anytime. Withdrawal will not interfere with your future care.

Who can see or use my information?

Every attempt will be made to keep all information collected in this study strictly confidential. Authorized representatives of the University of Pennsylvania, as well as the Food and Drug Administration (FDA) and Sponsor representatives, may review and copy data collected from

this study and your medical records. If any publications result from this research, you will not be identified by name.

What are my rights?

If you have a medical emergency during the study, or if you feel the medical emergency will affect your ability to participate in the study, you may contact Dr. Juan E. Grunwald, MD at (215)- 662 8039. You may contact (215)- 662 8100 for any emergencies with the 24-hour and ask for the resident on call.

If you have any questions as a research subject or if you have questions regarding your participation in this research study, you may contact the Director of the University of Pennsylvania Office of Regulatory Affairs at (215) 898-2614.

I have had the chance to have my questions answered. A copy of this form has been given to me. I agree to participate in this research study under the direction of the principal investigator listed above

A copy of this consent form will be given to you.

Date	Signature of Patient	Printed Name
Date	Signature of Person Obtaining Consent	Printed Name

COGNITIVE FUNCTION STUDY PROTOCOL AND CONSENT

Cognitive Function in Chronic Renal Insufficiency: An Ancillary Study to CRIC

July 28, 2005

Identifying Information

Investigators:

Kristine Yaffe, MD (Principal Investigator, UCSF)
Manju Kurella, MD (Co-Investigator, UCSF)
Glenn M. Chertow, MD, MPH (Co-Investigator, UCSF)
Alan S. Go, MD (Co-Investigator, KPNC/UCSF)
Harold L. Feldman, MD (Co-Investigator, University of Pennsylvania)
Deborah Cohen, MD (Co-Investigator, University of Pennsylvania)
Ashwini Sehgal, MD (Co-Investigator, Case Western Reserve University)
James P. Lash, MD (Co-Investigator, University of Illinois at Chicago)

CRIC cognitive function study sites and anticipated enrollment number:

Kaiser Permanente of Northern California/UCSF: 180 subjects
University of Illinois at Chicago: 180 subjects
University of Pennsylvania: 180 subjects
Case Western Reserve University: 180 subjects

Background

Cognitive impairment is common in patients with end-stage renal disease (ESRD) although the etiology of cognitive impairment in ESRD remains unclear. Both dialysis and renal transplantation appear to reverse these deficits (1,2). Correction of anemia with erythropoietin has been shown to improve cognitive function in anemic persons with ESRD (3). However, these interventions only partially correct the cognitive deficits associated with ESRD. Furthermore, the role of comorbid conditions associated with both cognitive impairment and renal failure has not been well studied. For example, type II diabetes mellitus has been associated with cognitive decline (4,5) and vascular dementia (6) in several large prospective studies in the general population, but its effect on cognitive function in persons with kidney disease has not been well characterized. Other metabolic derangements associated with chronic renal insufficiency (CRI) and ESRD, such as secondary hyperparathyroidism, chronic inflammation, and accelerated atherosclerosis may also be potential mediators of cognitive impairment in renal disease. Thus, whether the burden of cognitive impairment in persons with ESRD is due to a higher prevalence of predisposing comorbid conditions, or is secondary to non-traditional, “CRI-specific” risk factors is unclear.

Most previous studies of cognitive function and kidney disease have been limited to persons with ESRD. In one large single center study, cognitive impairment was present in 30% of prevalent hemodialysis patients (7). Relatively little is known about the scope and progression of cognitive impairment in persons starting with mild-to-moderate CRI. Furthermore, there have been no systematic studies of risk factors for and potential mediators of cognitive impairment in this growing population.

Study Rationale

The design of the Chronic Renal Insufficiency Cohort (CRIC) Study provides the ideal framework to study the prevalence, progression, and potential mediators of cognitive impairment in persons with CRI; and to examine the relationship between progression of CRI and changes in cognitive function over time. Stratification of the CRIC enrollment by age and by

diabetes status among a racially and ethnically diverse population will allow us to examine the relative importance of traditional risk factors for cognitive impairment (e.g. age, education, diabetes, race/ethnicity), from non-traditional or "CRI specific" risk factors for cognitive impairment. While CRIC currently is obtaining cognitive function on the entire cohort with the MMSE, a more complete battery will supplement this measure. In addition, the MMSE is a notoriously insensitive test and does not provide information on different cognitive domains. Thus, by adding a more comprehensive battery to the CRIC study, we will be able to more sensitively detect cognitive impairment and to determine if this impairment is more common is specific domains such as executive function.

Specific Aims:

1. To determine the association between severity of chronic renal insufficiency (CRI) and cognitive function and 3-4 year cognitive decline in CRIC participants over age 55 years.

   Hypotheses:

   a) Participants with lower estimated glomerular filtration rate (GFR) will have worse baseline cognitive function and more pronounced cognitive decline over 3-4 years of follow up.

   b) The association between CRI and cognitive function and decline will be of greater magnitude for specific cognitive domains, such as executive or frontal lobe function.

2. To evaluate whether the association between CRI and cognitive function is consistent among different patient subgroups such as those of different races and ethnicities, and those with and without diabetes mellitus.

   Hypothesis:

   a) *Among elders with diabetes and CRI, baseline cognitive function will be worse* and 3-4 year decline will be more pronounced over time than those without diabetes.

   b) The association between CRI and cognitive function and decline can be identified within major ethnic groups, and will be independent of education, socioeconomic status and other confounders.

3. To elucidate several mechanisms that may mediate the association between CRI and cognitive decline among older CRIC participants, including potential modifiable risk factors such as cardiovascular disease (CVD) risk factors, inflammation and vascular calcification.

   Hypotheses:

   a) The association between CRI and cognitive function is mediated, in part, by cerebrovascular disease. Participants with CRI and cognitive impairment will have a higher prevalence of traditional and nontraditional CVD risk factor and greater burden of clinical CVD such as stroke and myocardial infarction.

   b) *Participants with more severe degrees of CRI will have higher levels of chronic inflammatory markers that will be inversely related to cognitive function and 3-4 year cognitive decline.*

Preliminary Data

We analyzed cross-sectional data collected on 1015 women with known coronary heart disease enrolled in the Heart Estrogen/progestin Study (HERS) who underwent a battery of cognitive function testing. Compared to women with normal renal function (defined as an estimated GFR

$\geq$ 60 mL/min/1.73 m^2), women with CRI had deficits (defined by score >1.5 sd from the mean) in global cognitive function, executive function, language, and memory. There was a 27% increase in the risk of global cognitive impairment per each 10 mL/min/1.73 m^2 decrement in estimated GFR, and women with severe CRI (defined as an estimated GFR < 30 mL/min/1.73 m^2) were 5-fold more likely to have low 3MS scores than those with normal or near normal GFR. We also recently completed a cross-sectional study of cognitive function in 80 persons with CRI (mean estimated GFR 25.2 $\pm$ 10.9 ml/min/m^2) at UCSF, confirming the independent association between estimated GFR and performance on several cognitive function measures. Together, these results suggest that CRI is likely an important risk factor for cognitive impairment.

Methods

We propose to administer a battery of cognitive tests to participants enrolled in 4 of the 7 CRIC sites. This battery would be administered at the first possible annual CRIC clinic visit, or if necessary, at a separate study visit conducted close to the annual CRIC visit, and repeated at each annual visit thereafter. Given that age is the biggest risk factor for both cognitive impairment and for many comorbidities, we propose to obtain cognitive testing only on CRIC participants over age 55 years.

Proposed Cognitive Battery:

The choice of cognitive tests is driven by scientific goals, feasibility, ease of training and administration, prior use in CRI patients, and minimizing the burden to CRIC participants and CRIC staff. The current proposed battery of 5 tests would take approximately 40 minutes to administer.

- Trails A and B (5 minutes): measure visuospatial scanning, sequential processing, motor speed, executive function, and attention.
- Category Fluency (2 minutes): measures verbal production, semantic memory, and language with higher scores indicating better performance.
- Modified Mini Mental Status Exam (3MS) (10-15 minutes): a brief, general cognitive battery with components for orientation, concentration, language, praxis, and immediate and delayed memory with a maximum (best) score of 100. Of note, this is not the same as the MMSE but provides complementary information and is more sensitive than the traditional MMSE. The 3MS is increasingly becoming a widely used cognitive test in epidemiological studies of aging. Using the 3MS in addition to the already collected MMSE is slightly redundant but provides expanded cognitive domains and would allow for inter-conversion with the shorter MMSE (for example is needed for missing data).
- Buschke Selective Reminding Test (10-15 minutes): The Buschke is a well-established validated test of verbal memory with immediate and delayed components.
- Boston Naming (5 minutes): a brief test of naming and language.

Statistical Analysis and Sample Size Estimates

The primary analysis will be comparing baseline and 3-4 year change in cognitive scores as a function of renal disease severity using random effects models. In addition, we will determine the relative risk of developing cognitive impairment of the test scores (defined as <80 on the 3MS and as >1.5 sd of mean change score on the other cognitive tests) among CRIC participants with varying CRI severity (eg., estimated GFR 20-30 vs. 30-40 vs. >40 ml/min/1.73 m^2). Participants will then be stratified by presence of diabetes, and the association between CRI and cognition will be analyzed separately for those with and without diabetes. Multivariable models will be performed including the residual effects of age and race, and other using

possible confounders (those associated with cognition and CRI at a P value <0.1 on bivariate analyses).

Review of our preliminary data suggests that cognitive impairment associated with CRI is uncommon in persons ≤ 55 years of age. Therefore we propose restricting cognitive function testing to persons > 55 years of age, or roughly half of the CRIC cohort. Power calculations were performed using estimates from the HERS data (assuming a normal distribution of scores). Substitution using the data gathered at UCSF did not appreciably change these estimates.

Based on a sample size of 720 (180 from each site), we will have excellent power to detect relatively low effect sizes on all cognitive tests for participants at approximately 4 sites, even after stratifying by presence of diabetes. We will have good to excellent power to detect low effect sizes among the largest ethnic minority in CRIC, African-American participants. The power will remain robust even if some individuals decline participation in these studies, or are otherwise unable to complete some or all of them (e.g., blindness). While the sample size estimates were based on cross-sectional data, we estimate the sample size needed for longitudinal effects would be similar or possibly smaller.

Anticipated Burden to Sites and Participants

Additional time for cognitive testing would be the main additional burden to both Clinical Center staff and their participants. However, we have found from other studies, that participants often enjoy cognitive testing and are curious about their performance. We have purposely chosen a cognitive battery that is as time limited as possible while still providing valuable information. We will try to integrate the cognitive testing into the annual visit; ideally taking advantage of "down-time" during the GFR assessment or in no GFR years, taking advantage of a slightly shorter visit with fewer demands.

There would be no additional risk added to the participants from this ancillary study. Clinical Center personnel would receive appropriate training and financial support to conduct the proposed study.

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7. Sehgal AR, Grey SF, DeOreo PB, Whitehouse PJ: Prevalence, recognition, and implications of mental impairment among hemodialysis patients. *Am J Kidney Dis* 30:41-49., 1997

DRAFT of INFORMED CONSENT for CRIC COGNITIVE STUDY

1. Addendum to the Informed Consent Form

This addendum specifically addresses the use of questionnaires to measure cognitive function. The addendum to the Informed consent form will read as follows:

[Site letterhead]

Addendum to the Informed Consent Form

For measurement of cognitive function for the participant in the Chronic Renal Insufficiency Cohort

Investigator: _____ **Telephone #:** _____

Study location: _____
(Name of Institution, City, State and Country) if letterhead is not used.

Purpose of the investigation _____

Some changes in memory and other cognitive function are thought to occur more frequently in patients with chronic kidney disease compared to those without kidney disease. However, how commonly cognitive problems occur and what the mechanism is linking kidney disease to cognition is unknown. It may be due to kidney disease itself, or it may be due to other medical conditions that sometimes accompany kidney disease, such as high blood pressure or diabetes mellitus. The goal of this cognitive function study is to understand the relationship and mechanisms between chronic kidney disease and cognitive function in people older than 55 years.

You are being invited to take part in this cognitive function study because you are a participant in the Chronic Renal Insufficiency Cohort and are over 55 years of age.

Description of the Investigation

The cognitive function study involves interviews and questionnaires in addition to the ones already in the main CRIC study. If you agree to be in this additional study, you will take part in five (5) standard cognitive function tests at each yearly CRIC visit. These five (5) tests will add about forty (40) minutes to the time required for your usual visit and include the following:

- Trails A and B. This test measures attention, visual scanning and mental flexibility.
- Category Fluency. This is a test of word memory and language production and it takes about 2 minutes to complete.
- Modified Mini Mental Status Exam (3MS). This is a series of 24 questions that measure concentration, language, and memory and takes 10-15 minutes to complete.
- Buschke Selective Reminding Test. This is a test of ability to remember new information and a test of delayed memory.
- Boston Naming. This is a brief test of naming and language.

Participation and termination

Your participation in the cognitive function testing is voluntary. If you decide not to take part in these assessments it will not affect your overall participation in the CRIC study or your future medical care. If at any time you decide to stop participating in the CRIC study you will not be

able to continue with this cognitive function study. In general the assessments will take part during your scheduled CRIC study visits, however you may be asked to come for another visit.

Risks and benefits

Participation in this testing does not give you any additional benefits. However, the information that is obtained from this study might help to discover the relationship between chronic kidney disease and cognitive function. We do not believe that there is any risk for you as a consequence of participating in this investigation.

Contact Information

If you need additional information about any of the procedures or tests that compose this investigation or if at any moment you feel you may have been injured during any of the procedures, you may contact Dr. *[Principal Investigator]_*, Phone: *[PI's phone number].*

This cognitive function study and Informed Consent Form has been reviewed and approved by the IRB of *[Name of the IRB & Institute]*, if you have any question or need additional information about the investigation in general or about your rights as a participant you may contact *[Name of IRB contact person]* at *[IRB phone number]*.

Informed Consent Form

..... have read the above text and fully understand the nature and purpose of the testing in which I have been asked to take part.

I have had the opportunity to ask questions concerning this additional study and have been given satisfactory answers.

I am free to withdraw my consent for testing at any time during the study, without the need to justify my decision and without affecting my taking part in the CRIC study or future medical care.

I know that the data collected about me in this study, as in the CRIC study, will be kept confidential. Data will be identified only by a code number and kept in a secure place. No individual will be identified in any research report that may result from this study.

I understand that the goal of the cognitive function testing is to evaluate the population in the CRIC study, and that, therefore; only group results will be reported. We do not believe, at this time, that the reporting of such group results should cause any unforeseeable risks to the patient group taking part in this study, including, for example, groups of subjects of different racial or ethnic backgrounds, or with specific medical conditions. However, if the battery of test shows that I have impaired cognitive function for my age, I will be referred to my doctor for further evaluation.

I voluntarily do* / do not* consent to take part in cognitive function assessment.

* delete as appropriate

Subject's signature	**Printed Name**	**Date**
Signature of Clinical Investigator	**Printed Name**	**Date**
Signature of Witness	**Printed Name**	**Date**

SLEEP STUDY PROTOCOL AND CONSENT

Sleep Disturbance as a Nontraditional Risk Factor in CKD - Wrist Actigraphy Protocol
DETAILED PROTOCOL

Background

Sleep in ESRD and in CKD

A number of studies have reported a high prevalence of sleep disturbances and poor quality sleep in patients with ESRD [1-4]. Sleep complaints are estimated to occur in 45–80% of ESRD patients and are associated with considerable morbidity and reduced quality of life [5-8]. Among patients with chronic renal failure, the prevalence of SDB is estimated to be between 30-80%, which is much higher than the prevalence in the general population [4, 8-12]. Additionally, approximately 80% of dialysis patients experience symptoms of restless legs syndrome [4]. In a study of 694 hemodialysis patients, insomnia was present in 45% of subjects [7]. Thus, ESRD patients may be at a greater risk of developing co-morbidities, such as cardiovascular disease and stroke that are known to be associated with impaired sleep. Furthermore, higher quality of life was significantly associated with lower daytime sleepiness in patients on hemodialysis [4, 13]. The cause of the high prevalence of sleep disorders in CRI is unclear but sympathetic overactivity and the fact that a majority of patients are overweight or obese are likely to play a role. Nocturnal hemodialysis improves sleep apnea, and this could be due to decreased sympathetic tone [14] [15].

In contrast to ESRD, very little is known about sleep disturbances in patients with CKD who do not have ESRD. Not a single study has examined objective measures of sleep duration and quality. One study published in 2004 [2] examined subjective sleep quality in 120 patients with CKD who had creatinine clearances between 7-61 ml/min using a well-validated questionnaire, the Pittsburgh Sleep Quality Index. Fifty-three percent of patients had poor sleep, defined as a Pittsburgh Sleep Quality Index (PSQI) > 5 [2]. There was no relation between creatinine clearance and global PSQI score, although there were significant correlations between serum urea nitrogen and creatinine concentration, and sleep efficiency. Our study (summarized below) is the only other report on subjective sleep quality in CKD. The Kidney Disease Quality of Life Sleep Scale (KDQOL; a scale that includes four items addressing perceived sleep disturbance, adequacy, daytime sleepiness and global sleep quality) was administered to 78 patients with CKD. Forty-one percent of CKD patients reported sleep maintenance disturbances. Importantly, in non-African American patients, there was a significant direct association between estimated GFR and scores on the sleep scale of the KDQOL.

Despite the fact that a majority of CKD patients are overweight or obese, a major risk factor for SDB, not a single study has assessed the incidence of SDB in CKD.

Sleep loss and sleep disturbances as risk factors for hypertension, CVD and diabetes

There is increasing evidence from both epidemiologic and laboratory studies for an association between sleep duration and/or quality and the prevalence and/or severity of major chronic diseases, including diabetes, hypertension, cardiovascular disease, and stroke.

By self-report, sleep duration in America had decreased by one and a half to two hours from the 1960's to today [16, 17]. The rate of ESRD has increased steadily over the same time period. Daily sleep duration appears to have significant health implications [18]. Four recent epidemiological studies have found an association between self-reported sleep duration and body mass index [19-22]. Women who report short sleep duration (<7 hours) have an increased risk of symptomatic diabetes and coronary heart disease [23-25]. In middle-aged men, self-

reported difficulties of falling asleep or the regular use of hypnotics were shown to be associated with an increased risk of diabetes after full adjustments were made for age, biological risk factors, lifestyle, family history and social class [26]In a Japanese study [27], insufficient sleep was associated with a two- to three-fold increased risk of non-fatal acute myocardial infarction. In a recent survey study conducted by our group, short self-reported sleep duration in African-American patients with type 2 diabetes was negatively associated with glycemic control, as assessed by hemoglobin A1C levels (see section C-3).

Laboratory studies of experimental sleep restriction in healthy volunteers have indicated that sleep loss is associated with reduced glucose tolerance [28], increased plasma and urinary catecholamine levels [29, 30], decreased heart rate variability (reflecting a shift of the cardiac sympatho-vagal balance towards increased sympathetic and decreased parasympathetic activity) [28, 31], elevated blood pressure [32, 33], elevated levels of TNF-α [34], elevated levels of CRP [35], and elevated levels of IL-6 [34, 36].

Sleep disorders generally involve a reduction in total sleep time and are therefore pathological conditions of sleep loss. The intrinsic effects of the sleep disruption are enhanced by the associated sleep loss. Furthermore, behavioral sleep restriction worsens sleep disorders such as SDB [37].Sleep disturbances are highly prevalent in ESRD and include SDB, restless leg syndrome and insomnia [38]. The prevalence of these disorders in CKD is unknown. The sympathetic hyperactivity that is characteristic of CKD [14]is likely to promote sleep disturbances. Moreover, since a majority of CKD patients are overweighed or obese, the prevalence of SDB may be particularly high.

SDB has been associated with the development of hypertension and increased cardiovascular morbidity [39-43]. Over 50% of patients with SDB have hypertension [44], and it is estimated that 30% of patients with hypertension manifest SDB [45]. In patients with angiographically documented coronary artery disease, prevalence of SDB has been estimated to be between 30% to 39% [41, 46, 47] and 40 to 50% of patients with congestive heart failure have been shown to manifest SDB [48, 49]. SDB has also been shown to correlate with the risk of cerebrovascular accidents, one third of which occur during sleep [40, 41, 43]. Growing evidence strongly suggests that SDB is an independent risk factor for the development of hypertension [50-75] and cardiovascular morbidity and mortality [39-43, 76].

SDB with its nocturnal hypoxemia and sleep fragmentation leads to sympathetic stimulation [77-85], attenuation of the sleep-induced decrease in blood pressure [85, 86], and blunted heart rate variability [87]. SDB is also associated with increased levels of pro-inflammatory cytokines such as TNF-α, IL-6 and C-reactive protein [88-93], increased levels of reactive oxygen species and increased adhesion molecules [94-98], decreased nitrous oxide levels with blunted endothelial responsiveness, increased levels of endothelin-1 and endothelial growth factor [80, 83, 99-106], increased platelet activation and aggregation [107-109], and increased homocysteine and leptin levels [109-116]. These associations make SDB a potential risk factor for the progression of chronic renal insufficiency (CRI) as well as for the development and progression of cardiovascular disease (CVD). The fact that successful treatment of SDB leads to the resolution of most of these pro-inflammatory and pro-thrombotic abnormalities provides further support for this hypothesis [98, 99, 101, 103].

In recent years, SDB has been associated with significant neuroendocrine abnormalities[117, 118] and is now recognized as a risk factor for increased insulin resistance, independently of the degree of obesity [28, 65, 119-128]. Because insulin resistance is the major pathway to type 2 diabetes, SDB increases diabetes risk.

Periodic limb movement (PLM) disorder and restless legs syndrome (RLS) are two sleep disorders involving abnormal leg movements during sleep and reduced sleep quality. The

prevalence of RLS has been estimated at 5.5 % in the general population [129] and at 21.5% in ESRD [130]. The prevalence of PLM in sleep in essential hypertension is over 18% [131] as compared to 3.9% in the general population [129]. PLM in sleep are associated with cardiac acceleration, even in the absence of arousal [132, 133] and the heart rate response to PLM is stronger and more acute than during wakefulness [134].

Finally, insomnia and the disruptions of sleep continuity are associated with elevations of norepinephrine levels and hyper activity of the hypothalamo-pituitary-adrenal axis [135].

The Sympathetic Nervous System as a Mediator of Kidney Disease Progression

The studies reviewed in section B-2 indicate that increased sympathetic activation is a consistent correlate of sleep loss and sleep disturbances. Recovery from sleep loss or effective treatment of the sleep disorder are capable of correcting sympathetic overactivation. In patients with obstructive sleep apnea and normal renal function, muscle sympathetic nerve activity (MSNA) during waking was found to be nearly two-fold higher than in control subjects [85, 86]. CKD in and of itself is also associated with increased MSNA. Thus, while sleep disturbances could partly be caused by sympathetic overactivity in CKD, they are also likely to exacerbate it. It has been suggested that sympathetic activation via release of catecholamines promotes the progression of kidney damage [14] and therefore, sleep loss and its associated increased SNA and catecholamine release could accelerate the progression of CKD towards ESRD. We review here the evidence for a role of increased sympathetic nervous activation (SNA) in the progression of CKD.

Overactivity of the sympathetic nervous system is indeed often observed in patients in chronic kidney disease (CKD) and it has been speculated that this may a factor that contributes to the progression of CKD [136]. The kidneys have both afferent and efferent sympathetic innervation and therefore may act as both a source and target of sympathetic activation. Supporting the kidney as the origin of increased SNA, muscle SNA is increased in patients on dialysis but not in dialysis patients who have had bilateral nephrectomy [137]. The literature also suggests that earlier stages kidney disease due to different etiologies can be associated with increased SNA [138, 139]. Evidence from clinical human studies is also suggestive that sympathetic over activity may be an important factor in progression of CKD. In a small series of patients with diabetic nephropathy, parasympathetic dysfunction (permitting unopposed sympathetic tone) was associated with greater progression of renal disease over 12 months [140]. However, significant questions remain regarding the role of sympathetic nerve activity in the progression of CKD. In another study, ACE-inhibitor therapy was found to control hypertension and decrease sympathetic hyperactivity, whereas therapy with a calcium channel blocker increased hyperactivity [141]. The fact that inhibitors of the angiotensin-converting enzyme and of the angiotensin receptor blockers have become well-accepted therapeutic interventions for slowing renal disease progression further supports a role for sympathetic overactivity in the progression of CKD [142].

Sleep, the Renin-Angiotensin System and Kidney Disease Progression

The hormones of the renin-angiotensin-aldosterone system exhibit large diurnal variations that are dependent on sleep [143]. Both plasma renin activity (PRA) and aldosterone levels are markedly elevated during sleep [144, 145] and exhibit large ultradian oscillations that are synchronous with the REM-nonREM cycle. Studies that examined the patterns of plasma renin activity and plasma aldosterone levels in subjects submitted to an abrupt shift of the sleep-wake cycle showed that these nocturnal elevations are largely controlled by the timing of sleep rather than by circadian factors [146]. Figure B-1 illustrates these remarkable sleep-dependent patterns.

Interestingly, during sleep, PRA is higher during deep non-REM sleep, when delta wave activity is present in the EEG. This is may appear paradoxical since cardiac sympatho-vagal balance, as assessed by measures of heart rate variability, is lowest during deep non-REM sleep. Thus, while high SNA is normally associated with high PRA during wake, the reverse is true during sleep when PRA peaks when SNA is minimal. A well-documented study has examined in detail the relationship of the ultradian renin oscillations to sleep-stage related changes in arterial blood pressure and EEG activity in the delta range [147]. The sequence of changes during a normal non-REM/REM is first a decrease in sympathetic tone concomitant with a decrease in mean arterial blood pressure, and this is followed within 10-20 min with an elevation of EEG delta activity and a rise in PRA. These observations suggest that blood pressure-dependent mechanisms are involved in the generation of nocturnal oscillations in PRA.

The nocturnal increase in PRA and aldosterone levels is markedly blunted by acute total sleep deprivation [144, 148]and this alteration is associated with increased natriuresis. One study has shown that the nocturnal elevations of PRA and aldosterone are also blunted in SDB [146]. However, the ultradian oscillations of PRA and aldosterone retained their relationship to the REM-nonREM cycle. Treatment with continuous positive airway pressure (CPAP) restored a normal nighttime pattern. Daytime levels were not measured and therefore, it is not known whether the increased daytime SNA that is characteristic of SDB and sleep loss in general is associated with increased levels of PRA and aldosterone. Such a chronobiological alteration in the activity of the renin-angiotensin-aldosterone system could play a role in the pathophysiology of chronic kidney disease. Daytime levels of PRA and aldosterone are indeed elevated in patients with CKD as compared to controls [149] [150].We are not aware of a report on nighttime levels of PRA and aldosterone in CKD patients.

The importance of the renin angiotensin system in mediating kidney damage is supported by both experimental and clinical studies. In experimental models of renal disease, it was found that glomerular capillary hypertension, maintained largely by angiotensin-dependent mechanisms, ultimately leads to glomerular scarring and nephron dropout [151]. Furthermore, therapeutic measures that reduced glomerular capillary pressure (such as protein restriction and angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) resulted in the slowing of the progression of experimental renal disease [152-154]. As a result of these types of investigations, it became apparent that angiotensin II is a central mediator of the glomerular hemodynamic changes associated with progressive renal injury. More recently, it has become evident that the nonhemodynamic effects of angiotensin II are also important in the progression of renal disease. Some of the wide range nonhemodynamic effects studied include aldosterone-induced fibrosis, nuclear factor-kappa B–induced up-regulation of cytokines, chemokines, transforming growth factor-ß (TGF- ß), connective tissue growth factor (CTGF), and chemotactic and cell adhesion molecule expression [142]. These factors are though to be important in leading to progressive glomerulosclerosis and tubulointerstitial fibrosis, resulting in the final common pathway of progressive kidney damage. Further evidence of the importance of the renin angiotensin system is provided by a number of clinical studies in both diabetic and nondiabetic renal disease which have demonstrated that reduction of proteinuria using an angiotensin converting enzyme inhibitors (ACE-I) or an angiotensin receptor blocker (ARB) results in slowing of the rate of loss of renal function which is independent of the effect on systemic blood pressure [155-158].

Subjective sleep quality in CKD patients enrolled in the CRIC study

Subjective sleep quality in CKD has been examined in one study, currently in press [159], and our group has obtained preliminary data from the UIC Chronic Renal Insufficiency Cohort (CRIC). So far, not a single study has examined objective sleep quality (polysomnography) in CKD prior to ESRD.

Investigators from the CRIC study, including members of the present team, administered to 156 subjects the Kidney Disease Quality of Life (KDQOL) four-item sleep subscale, which consists of four items corresponding to sleep maintenance disturbance, sleep adequacy, daytime somnolence, and global sleep quality [159]. Half of the patients had CKD and the other half had ESRD. The first three questions of the KDQOL are: 'Over the past four weeks how often do you (1) awaken during the night and have trouble falling asleep again?, (2) get the amount of sleep you need?, (3) have trouble staying awake during the day'?. Each of these questions is answered on a qualitative 6-point scale ranging from 'none of the time' to 'all of the time.' The fourth question asks respondents to rate their overall quality of sleep on a 10-point scale. All four items are weighted equally, and scores reported on a scale of 0-100, with higher scores indicating higher self-reported quality of sleep.

Median scores on the KDQOL sleep scale were 59 in ESRD subjects and 69 in CKD subjects (p=0.04). Total KDQOL sleep subscale scores were positively correlated with age (r=0.26, p=0.001) and inversely correlated with serum creatinine (r=-0.25, p=0.002), potassium (r=-0.18, p=0.03), phosphorus (r=-0.17, P=0.05), and systolic (r=-0.17, p=0.03) and diastolic (r=-0.20, p=0.013) blood pressure. Individual questions were examined for ESRD subjects and for CKD subjects divided into two groups based on median GFR (advanced CKD:GFR<25 mL/min/1.73m^2; mild-moderate CKD: GFR>25 mL/min/1.73m^2). Thirty-four percent of subjects with ESRD, 27% of subjects with advanced CKD, and 14% of subjects with mild to moderate CKD had sleep maintenance disturbances ("trouble falling asleep after awakening") (p=0.05). Thirteen percent of subjects with ESRD, 11% of subjects with advanced CKD, and no subjects with mild-moderate CKD had complaints of daytime somnolence (p=0.03).

When the analysis was restricted to subjects with CKD, subjects with lower estimated GFR had lower KDQOL sleep scores (p=0.01) when adjusted for the significant residual effect of African American race and the race x GFR interaction. A 10-point difference in KDQOL sleep score translated into a 15 mL/min/1.73 m^2 difference in estimated GFR among non-African American subjects with CKD and no relation between estimated GFR and KDQOL sleep score in African Americans.

Preliminary data were also obtained by Dr. Lash who distributed a short sleep questionnaire to 83 CKD patients recruited at the University of Illinois at Chicago. The mean self-reported sleep duration was 7.3 (± 1.8 SD) hours and the mean self-reported sleep quality on a scale of 1 (very bad) to 10 (very good) was 7.5 (± 2.1). Of those who responded, 32.5% reported trouble staying awake during the day. Another 36.1% reported snoring heavily and 24.1% reported having restless legs that disturbed sleep. Finally, 14.5% reported having trouble falling asleep. In comparison, the National Sleep Foundation (NSF) poll from 2001 surveyed 1004 Americans over the age of 18 from the general population. The self-reported sleep duration was 7.0 hours on weekdays and 7.8 hours on weekends, which would average to 7.2 hours per night. Only 7% reported daytime sleepiness that interferes with daily activities every day or almost every day. Twenty-five percent of the NSF sample reported snoring every night or almost every night, and 7% experienced symptoms of restless legs syndrome every night or almost every night. Finally, 15% of the NSF respondents reported trouble falling asleep every night or almost every night. These preliminary data and their comparison with the annual NSF poll thus suggest that CKD patients are more likely to experience daytime sleepiness and to suffer from restless legs syndrome than the general population.

Significance

There is substantial evidence to suggest that sleep loss and sleep disturbances are likely to be highly prevalent in CKD and could exacerbate the severity of the disease and accelerate the progression to ESRD. The proposed studies are designed to test these hypotheses and to

explore the pathophysiological mechanisms linking sleep loss and the regulation of renal function. The identification of sleep loss as a novel non-traditional risk factor for CKD and CVD in CKD would be of particular public health interest because behavioral and pharmacologic strategies to improve sleep quality and duration are readily available.

Hypotheses

The present protocol seeks to capitalize on the ongoing CRIC study to explore the role of decreased sleep duration and/or quality as a non-traditional risk factor for the progression of CKD and for the development of cardiovascular disease in CKD. The specific aim of this specific protocol is:

to test the hypothesis that habitual at-home sleep duration (assessed by wrist activity monitoring and sleep logs) and subjective sleep quality (assessed by wrist and leg activity monitoring and validated questionnaires) will each predict changes in renal function (as assessed by the estimated GFR) and cardiovascular risk (as assessed by plasma C-reactive protein levels) over study period. Secondary end points will include a measure of insulin resistance (the HOMA index), left ventricular mass, blood pressure, heart rate and ankle brachial index (ABI). *All CRIC participants at the University of Illinois at Chicago (UIC) and at Case Western University will be invited to participate to obtain a sample size of 800 patients.*

The results of the proposed studies are expected to determine to role of sleep disturbances as a previously unrecognized risk factor for the progression of CKD to ESRD and for the development of CVD in CKD. They will also explore possible physiopathological mechanisms linking sleep and kidney function. Most importantly, the identification of sleep disturbances as a risk factor for the progression of CKD to ESRD and for the development of CVD in CKD would be of major benefit to the patients and of particular public health significance because sleep disturbances are amenable to treatment.

Methodology

This protocol will involve the distribution by mail of a well-validated wrist activity monitor (Actiwatch, Mini-Mitter Co. Inc. Bend, OR), and of validated questionnaires assessing subjective sleep duration, subjective sleep quality, subjective daytime sleepiness and depression (Karolinska Sleep Log, Pittsburgh Sleep Quality Index, Epworth Sleepiness Scale, Berlin Questionnaire and Center for Epidemiologic Studies Depression questionnaire) to CRIC participants. We will invite all UIC participants and participants from one additional site to participate. Our goal is a total sample size of 800. Patients will wear an Actiwatch on their wrist for 5 days (Tuesday through Sunday, i.e. three week day nights and two week-end nights) continuously to record sleep and complete the Karolinska sleep log daily. They will wear a second Actiwatch on one leg at night only to screen for periodic leg movements and obtain a measure of frequency and amplitude of leg movements. They will complete the four questionnaires, and mail all instruments back to investigators. Two separate sleep assessments will be performed in each patient two years apart. Additionally we will request that insulin and C-reactive protein (CRP) be measured on a small aliquot (2 ml) of existing blood from the blood draw performed upon enrollment as well as on the blood drawn during the last year of the CRIC study period. This specific protocol does not involve any blood collection.

We will use the daily Karolinska sleep logs combined with the wrist activity monitoring to estimate nocturnal sleep duration on each day of recording and calculate the mean nocturnal sleep duration for the 5 nights of recording (using appropriate weights for weekday nights and week-end nights). The mean of the two separate assessments will be used in the final analysis for sleep duration. For subjective sleep quality, we will use the mean of the two Pittsburgh Sleep Quality Indices (PSQI) as the primary variable. The responses on the Berlin

Questionnaire and on the Epworth Sleepiness Scale will be used to verify the consistency of related items on the PSQI. Our primary outcome measures will be GFR (as marker of the progression of renal insufficiency) and plasma CRP levels (as a marker of cardiovascular risk). Secondary outcome measures obtained from CRIC exams will be the homeostatic model assessment (HOMA) index (a marker of insulin resistance, a risk factor for diabetes and hypertension), left ventricular mass, systolic blood pressure, heart rate and ankle brachial index (ABI).

We will test the following hypotheses:

1. sleep duration will be a significant predictor of the changes in estimated GFR over the study period
2. sleep duration will be a significant predictor of the changes in plasma CREP over the study period
3. subjective sleep quality will be a significant predictor of the changes in estimated GFR over the study period
4. subjective sleep quality will be a significant predictor of the changes in plasma CREP over the study period

Similar hypotheses will be tested for each of the secondary outcome variables, i.e. HOMA index, left ventricular mass, systolic blood pressure, heart rate and ABI.

Additionally, we will compare the changes in estimated GFR in two subgroups of the subjects who participated in this study: those with short sleep duration and poor sleep quality (mean sleep duration < 6 hours and PSQI >10) and those with normal sleep duration and good subjective sleep quality (mean sleep duration ≥ 7 hours but <8.5 hours and PSQI<5). We will test the hypothesis that short/poor sleepers have greater decrements in GFR than normal/good sleepers after controlling for age, gender, BMI, ethnicity and use of anti-hypertensive medications affecting the renin-angiotensin-aldosterone system (ACE inhibitors, ARBs, beta blockers). Calcium channel blockers and alpha-adrenergic receptor blockers do not affect the renin-angiotensin-aldosterone system in most cases [160].

Duration of Protocol.

Subjects are asked to wear the wrist actigraphy monitor for 5 days. This 5-day measurement will be repeated in each subject approximately 2 years later.

Location of Research

Subjects will be going about their usual activities throughout the study period.

Special Precautions

Subjects will have the telephone number of study investigators for any

Experimental Controls.

No controls are included.

Experimental Subjects.

We plan to recruit and enroll 400 subjects from each of the two CRIC sites, University of Illinois, Chicago and Case-Western Reserve University, for a total of 800 subjects. Only subjects who have already been enrolled in CRIC will be recruited. For this protocol, there are no additional exclusion criteria beyond the exclusion criteria for enrollment into CRIC. CRIC excludes patients outside the following ranges of estimated GFR (ml/min/1.73m2) are excluded: 20-70 for 21-44 year olds; 20-60 for 45-64 year olds; 20-50 for 65-74 year olds. Exclusion criteria also include: inability to provide consent, NYHA Class III or IV heart failure at baseline,

cirrhosis/chronic active hepatitis, known HIV infection/AIDS, current participation in a clinical trial, prior dialysis lasting >1 month, organ/bon marrow transplant, immunosuppressive for renal disease or vasculitis within 6 months, chemotherapy for malignancy within 2 years, and polycystic kidney disease.

Statistical Analysis.

The primary outcome measures in this study aim are the changes in GFR and CRP over the 5-year CRIC study period, with larger decrease in GFR indicating worse progression of CKD and larger increase in CRP greater risk for CVD. Secondary outcome measures include HOMA index, left ventricular mass (LV), systolic blood pressure (BP), ABI, and heart rate. Risk factors are sleep duration obtained by Actiwatch and subjective sleep quality measured by PSQI. Four hypotheses will be tested (see protocol of specific Aim 1). We now describe some of the statistical procedures that will be used to answer the questions of this study aim.

First, we will carry out a correlation and regression analysis. We will compute correlation of sleep duration or sleep quality with change in GFR or change in CRP, and test its significance. We will also fit a simple linear regression model with the change in GFR or the change in CRP as dependent variable, and sleep duration or sleep quality as explanatory (i.e., independent) variable. We will extend the model to a multiple linear regression model to control for age, gender, ethnicity, BMI and use of antihypertensive medications affecting the RAA system as covariates. We will test the significance of regression coefficients in both models. All hypotheses will be tested using 5% significance level unless otherwise specified. For a large sample size as large as 800 patients, we would expect any test to have a good power even the effect size is small. For significant test of correlation, we have 99% power to detect a significant correlation even the expected correlation is only 0.15. For testing the effect of a risk factor after controlling for confounders in multiple linear regression, even a sample size of 100 will achieve a 94% power to detect a significant effect of the risk factor, given that 10% of the variance of the dependent variable is explained by the controlled variables and additional 10% is explained by the risk factor.

Second, we will repeat the above correlation and regression analyses for each of the two subgroups of patients: poor sleep group (sleep duration<6 hours and PSQI>10); and normal sleep group (those with sleep duration≥ 7 hours but<8.5 hours and PSQI<5), and compare their correlations and regression coefficients. We expect there will be about 160 (20% of 800) patients in each group. Table D1 shows that a sample size of 160 will have sufficient power (>94% and >89% for one and two sided tests, respectively) to detect a significant correlation when the true correlation is 0.25 or higher. For comparison of two independent correlations, Table D2 shows that a sample size of 160 for each group will have sufficient power (>89-96% and >82-93% for one and two sided tests, respectively) to detect a significant difference when the expected difference is 0.3 or higher.

Third, we will apply a linear random effect model for longitudinal data to estimate and compare the rate of decrease per year in GFR between the poor sleep and the normal sleep subgroups. We will test the hypothesis that the rate (GFR slope) in the poor sleep group is significantly different (or greater for a one-sided test) than that of the normal sleep group. We will use GFR values at baseline, and two and four years after enrollment to estimate the rate of decrease for each patient. Using the AASK data from Dr. Lash, we obtain an estimate of the standard deviation of GFR of 12.9 ml/min/1.73 m^2, and an estimate of correlation between repeated GRF measurements to be 0.732. Similar to the AASK study we will assume 4% loss to follow-up per year in CRIC. However, since our sleep study will be performed around the third year, we will apply a 4% loss to follow-up only to the period between the second and third GFR measurements. Based on this information, the sample size required to detect a significant

difference in the rate of change per year is given in Table 1. It can be seen that our sample size of 160 per group is sufficient to detect a significant difference when the true rate of change difference is about 0.80 (ml/min/1.73 m^2/year) or higher. The statistical procedures described above for GFR and CRP will be used to analyze other outcome measures. In the comparison of good and poor sleepers groups, we have omitted the middle group of 480 patients in order to gain a bigger effect size with respect to the outcome measures of interest. We may combine this middle group with the good or the poor sleep group in the final data analysis.

Table 1. Sample size required to detect with 90% power a significant difference in rate of change: Sample sizes $n_1=n_2=160$; Time (years)=(0, 2, 4)

Difference in Rate of change	Sample Size One-sided test	Sample Size two-sided test
0.70	199	244
0.75	173	213
0.80	152	187
0.85	135	166
0.90	120	148
0.95	108	133
1.00	98	120

Risks and Benefits.

There are no known risks to wearing an Actiwatch. There is the risk of breach of confidentiality, but as described above measures are taken to prevent accessing of data by non-investigators. There are no direct benefits of participation to the subject. If this study finds our hypothesis to be correct, we will have identified an altogether unrecognized and modifiable behavioral factor that is contributing to poor prognosis of chronic kidney disease.

Subject Payment.

Subjects receive $50 for each 3-5 day sleep assessment. If subjects wear the wrist actigraphy monitor for less than 3 days and do not complete the logs or questionnaires, they will not be paid because the actigraphy data cannot be used. If they complete only the questionnaires and daily sleep logs, they will be paid $10

Informed Consent.

Informed consent will be obtained by CRIC investigators at each of two sites: University of Illinois, Chicago and Case Western Reserve University. Copies of all signed consent forms will be kept in a locked file at the University of Chicago.

Confidentiality

Study records will be kept confidential. All of the information collected for this research will be stored in a locked file in the offices of the research team and will only be accessible to members of the research team working on this study. The data will be entered into a computer database by a member of the research lab. This data base will be accessible only to a member of the research lab. All computers require a password to access them.

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Recruiting Methods.

CRIC investigators will recruit current participants in the CRIC study. CRIC participants will be told about they study and if they are interested, the study will be explained in greater detail and informed consent will be obtained. Copies of the consent forms are then sent to University of Chicago investigators who will then contact the subject.

Primary Physician.

University of Chicago investigators will not contact primary physicians because this information will not be available to us. CRIC investigators will be notified, and they can contact primary physicians.

Coordination between Faculty.

Three institutions are involved in this protocol. Kristen Knutson, PhD, will serve as the liaison and coordinator between sites. Conference calls will be scheduled as necessary to discuss study issues between investigators.

Pregnancy Test.

When initially enrolled into the parent study, CRIC, pregnant women are excluded. However, for this specific protocol, women who may have become pregnant since enrollment into CRIC are not excluded because the methodology is not dangerous for pregnant women.

Exclusion of Children.

The proposed age distribution of CRIC participants reflects a balance between the competing goals of ensuring an adequate number of cardiovascular outcome events (benefited by an older age structure) and keeping deaths due to non-renal/CVD etiologies and other non-informative censoring events to an acceptable minimum. Thus the ages range from 21 to 74 years and no children are included.

Drug Infusion.
No drugs are used in this protocol.

GENETICS OF ATHEROSCLEROSIS IN CHRONIC KIDNEY DISEASE (CKD)

Purpose:

To gain understanding of the relationship between progressive renal disease and cardiovascular illness, the National Institute of Diabetes, Digestive, and Kidney Diseases (NIDDK) established the Chronic Renal Insufficiency Cohort (CRIC) Study. This is an eight year long multicenter study that will include about 3000 adults with varying severity of chronic renal insufficiency (CRI). The principal goals of the CRIC Study are to examine risk factors for CRI and cardiovascular disease (CVD) events among participants with varying severity of CRI, and develop predictive models that will identify high-risk subgroups with CRI.

Atherosclerotic cardiovascular disease (CVD) is an inflammatory disorder with a complex genetic basis. The prevalence of chronic kidney disease (CKD), a major risk factor for CVD, is increasing rapidly in the U.S. The mechanistic basis for accelerated atherosclerosis in CKD remains largely undetermined. Recent studies suggest that insulin resistance, kidney dysfunction and CVD may share, in part, a common genetic basis. Innate immunity and insulin resistance converge in atherosclerosis and are prominent features of CKD. We hypothesize that, in this setting, genetic variation in innate immune and insulin resistance pathways will promote atherosclerotic CVD. As an ancillary to the Chronic Renal Insufficiency Cohort (CRIC), an NIH sponsored study (N=3,000) of renal and CVD complications of CKD, our proposal will examine multiple CVD traits that provide mechanistic insight into our primary analyses of clinical CVD. Thus, we will determine the relationship of tagSNPs and estimated haplotypes in innate immune and insulin resistance genes to (a) inflammatory and insulin resistance biomarkers, (b) coronary artery calcification (CAC), a direct measure of atherosclerosis, and (c) the risk of CVD. Further, we utilize re-sequencing data, generated by NIH-sponsored programs, to select tagSNPs in candidate genes for genotyping in the full cohort.

Genes identified to date account for a small proportion of the population-attributable fraction of CVD. Our unifying hypothesis is that multi-locus candidate pathway gene effects will contribute, in a clinically relevant manner, to the attributable risk for CVD in the CKD population. This is not purely a theoretical concern, but rather a public health consideration, given the largely unexplained heritability of atherosclerotic CVD. In order to maximize the likelihood of detecting biologically and clinically meaningful multi-locus effects, we have focused on appropriate candidate pathways that converge in promotion of CVD in CKD and we will examine genes with key functions that interact either physically or regulate each others expression.

Specific Aim 1: To test the hypothesis that common variation in candidate innate immune genes are associated with inflammatory and insulin resistance biomarkers and the burden of sub-clinical atherosclerosis, and predict CVD events in CKD. Innate immune receptors including toll-like receptors (TLRs) provide a critical link between pathogens and the initiation of host innate immune defenses. TLR activation leads to a rapid elaboration of inflammatory cytokines, including tumor necrosis factor alpha (TNF, interleukin-1β (IL1B) and IL6. Circulating levels of oxidized lipoproteins, fatty acids and C-reactive protein (CRP), endogenous TLR ligands, are elevated in CKD and promote chronic inflammation, insulin resistance and atherosclerosis. We will examine a panel of innate immune signaling genes with physical or regulatory interactions that are also strong candidates for insulin resistance and atherosclerosis.

Specific Aim 2: To test the hypothesis that common genetic variation in insulin resistance pathway genes are associated with inflammatory and insulin resistance biomarkers and the burden of sub-clinical atherosclerosis and, predict CVD events in CKD. Adipose secreted factors play a central role in modulating insulin signaling and inflammatory responses. Plasma levels of insulin and adipokines are altered in CKD and have been linked to vascular insulin

resistance, inflammation and atherosclerosis. Little is known of the contribution of genetic variation in this pathway to atherosclerotic CVD. Thus, insulin resistance and adipokine pathway genes with strong links to vascular inflammation and atherosclerosis will be examined.

Specific Aim 3: To test the hypothesis that candidate gene variants modify the effects of other pathway gene variants in predicting inflammatory and insulin resistance biomarkers, the burden of sub-clinical atherosclerosis and the risk of CVD events in CKD. Innate immune and insulin resistance pathways converge at multiple points in promoting atherosclerosis (Figure 1). Hence, there are likely to be complex multi-locus interactions of genes within and across these pathways in the development of CVD that will not be addressed by analyses of main effects in Specific Aims 1 and 2. Application and extension of methods developed by Dr Foulkes and others will permit estimation of multi-locus effects on the variability in CVD traits and the attributable risk for CVD in the CKD population.

Duration

The duration of the entire EBTCRIC study will be five years. If follow-up of CRIC patients is extended beyond 2008, the period of data analysis may be extended. However, data collection specific to EBTCRIC will be completed during a 3-year time span between Spring 2006 and Spring 2009.

Subject recruitment and selection

Subjects included in this project will be participants of the Chronic Renal Insufficiency Cohort (CRIC) study, an 8-year multicenter, collaborative project sponsored by the NIDDK. The CRIC Study Coordinating Center is located at the University of Pennsylvania and the protocol has been approved by the IRB of the University of Pennsylvania and the IRB of each participating center. The EBTCRIC research project will be an ancillary study to the CRIC study in which we will obtain an EBT scan in all appropriate subjects at the following clinical centers: University of Pennsylvania, University of Michigan, Tulane University and Kaiser Permanente of California. We will include in the analysis of EBT the data from the subjects in the sub-cohort from the other participating centers. In addition, all subjects in the CRIC study who are followed for events will be included in the event analysis.

The CRIC is a multi-center study drawing a clinical population from six institutions across the United States. Based on the estimated distributions of race/ethnicity in the available populations at the CRIC Clinical Centers, we estimate the race/ethnic composition of participants in our RCRIC study to be approximately 40% White/Caucasian, 40% African American, 15% Latino/Hispanic, and 5% Asian/Pacific Islander and Other. We plan to enroll approximately equal numbers of men and women.

Ages of the participants will be between 21 and 76 years at the time of enrollment. The lower age limit was chosen since the focus of the CRIC Study is on adults, which is defined by the NIH as 21 years or older for research purposes. The upper age limit of 74 years was chosen to address the role of renal dysfunction in older participants and to increase power for cardiovascular analyses without a significant impact of competing risks and censoring due to death or dropout. All participants will have some degree of renal insufficiency, half of the participants will have Diabetes Mellitus, and about 75% will have Systemic Hypertension.

Location

The EBT-CRIC is a multicenter study that will be carried out in four Clinical Centers: 1) University of Pennsylvania Medical Center, Philadelphia, PA. 2) University of Michigan, Ann Arbor, MI. 3) Tulane University, New Orleans, LA. 6) Kaiser Permanente of California, Oakland, CA. It will be the responsibility of each clinical center to conduct the study according to the study

protocol and applicable regulatory guidelines which will include the approval of this project by the IRB of each of the institutions involved.

Background

Atherosclerosis is a complex disease with multiple environmental and genetic influences. Novel risk factors, including common single nucleotide polymorphisms (SNPs) in atherosclerotic pathway genes, are likely to be associated with modest increases in atherosclerotic CVD risk or only increase risk in the presence of one or many other environmental or genetic risk factors. These modest main effects as well as epistatic effects will be missed in smaller studies that are underpowered. The large multi-center CRIC study offers a unique opportunity to study the complex genetic basis of atherosclerotic CVD in CRI as it relates to the candidate MetSyn and innate inflammatory genes as well as novel loci, of specific relevance to CRI, derived from family-based linkage analysis studies. In this ancillary RO1, we will examine the association of novel and candidate genomic regions, and gene-gene interactions, with (1) coronary artery calcification (CAC) at electron beam tomography (EBT), a quantitative measure of sub-clinical atherosclerosis, and (2) cardiovascular outcomes in the CRIC study. Specific genes/genomic regions, including inflammatory and metabolic genes, will be selected because of their potential role in promoting atherosclerosis in CRI. Examination of a measure of atherosclerosis (CAC) as well as clinical outcomes will provide evidence for a direct link between candidate gene regions and atherosclerosis in the promotion of clinical atherosclerotic CVD in CRI.

Research Design

All CRIC participants who have consented to genetic testing will be used in the genetic analyses (for analysis of CVD outcomes). In addition, we plan perform additional EBT scans on subjects at four participating centers (one scan in as many subjects as possible). At each of the centers, an informed consent specific including our ancillary study will be obtained using a consent form approved by each local IRB. Each participant will also sign a consent form including the HIPAA regulations of April 2003.

Because of the staggered recruitment of CRIC participants (which is being carried out during 33 months) CRIC participants will be enrolled in our study at baseline or the annual visit at year 1 2, 3 or 4 of the CRIC Study for the patient. The goal is to make this additional visit to be as minimal of a burden to the CRIC participants. In all CRIC Study participants who agree to undergo an EBT scan as part of our CRI ancillary study, an EBT will be performed at the local scanning center (used for all subjects in the sub-cohort). Scans will be read at the central reading center and reports made available to the participant and their physician.

Potential Risks

The additional procedures involved in this ancillary study are the additional EBT scan at participating centers. Risks to participants are the exposure to small amounts of radiation from the EBT exam. Each EBT examination adds the risk of radiation exposure that is slightly less than a set of dental x-rays. All pregnant and breast feeding women are excluded from EBT testing.

Consent Procedures

CRIC participants at participating centers who are not part of the sub-cohort will be approached by the CRIC Study coordinator and will be invited to undergo an EBT scan as part of this ancillary study. Following signature of the appropriate consent forms the EBT scan will be scheduled by the CRIC coordinator at a time convenient to the participant.

Each clinical center will prepare an informed consent form following the guidelines of their local Institutional Review Board (IRB) and applicable regulations for Informed Consent. This consent

will be separate from the main consent form of the CRIC Study. The informed consent form must be signed and dated by the participant prior to initiation of any study related activity and at a minimum, contain a description of the potential risks, benefits, expense to the participant, and alternative treatment.

The consent process may differ somewhat by clinical center according to local IRB guidelines. This form will cover all aspects of the study protocol. The Health Insurance Portability and Accountability Act (HIPAA) became effective on April 14, 2003 and affects the CRIC Study and our Retinopathy in CRI ancillary study with regard to the use or disclosure of protected health information. Each institution may hold different requirements regarding HIPAA. Some institutions may request that this HIPAA language be inserted into the appropriate sections of the informed consent form; other institutions may require a stand alone document. We will be using at the University of Pennsylvania a separate HIPAA consent form in this study.

Protection of subjects

Extensive efforts will be made to ensure that participants' confidentiality is maintained. Each participant will be assigned a unique study identification number and will never be tracked through the study by name, social security number, medical record number, or other personal identifier. A log of the participant names, participant ID numbers, and pertinent registration information (e.g. home address, telephone number, and emergency contact information) will be maintained in a locked area at each clinical site as part of the main CRIC study procedures. Only the participant ID number will be sent to the RCRIC investigators. Any communication between the CRIC investigators and clinical sites regarding participant data will occur via the participant ID number. Any forms or documents sent to the CRIC Study Data Coordinating Center, IRB or Regulatory Authorities will have all personal information removed.

If any publication or presentations result from this research, participants will not be identified by name or other personal identifier. All research reports, articles, and presentations will report only aggregate findings. Throughout the study we will adhere to the HIPAA guidelines of April 14, 2003 regarding all issues of participant confidentiality.

Potential benefits

Participants who undergo an EBT scan may gain important information about their risk for coronary disease. While immediate direct benefit cannot be guaranteed to the individual participants of this study, there is considerable potential benefit to future patients and to society as a whole if predisposing factors for progression of CRI and development of CVD are identified from this study. Identification of such factors would potentially lead to interventions that may reduce the burden of chronic renal failure. Finally, laboratory and radiographic data obtained as part of this study is made available to participants' treating physicians. Conceivably, this information, which is additional to that obtained in the course of usual care, may provide diagnostic insights to treating physicians that would benefit study participants directly (e.g. treatment of elevated cholesterol).

The Risk/Benefit Ratio

The risk involved in this project is very small. Knowledge gained will help identify participants at risk for progression of CRI and CVD. This will allow a better treatment and follow up of these conditions. The risks involved in this study are minimal in comparison to the importance of the knowledge that may be gained through this study.

INFORMED CONSENT

To be inserted into the informed consent for each of the participating sites:

Most participants at the University of Pennsylvania (or other participating sites) will be invited to have an electron beam CT scan to measure the amount of calcium in the arteries of your heart at one of your visits. You will not be invited to have this test if you weigh more than 300 lbs or if you have previously had a stent placed in your coronary artery or if you have had coronary artery bypass surgery. The EBT may show early signs of heart disease. However, this test is fairly new and its role in predicting heart disease is still not completely understood. This test takes about 30 minutes. This test will either be done as part of the main CRIC study or as part of an additional study which is being performed at the University of Pennsylvania and a few other (University of Michigan, Tulane University, Kaiser/Permanente Medical Center).

Risks associated with EBT: Participants are exposed to small amounts of radiation from the EBT exam. Each EBT examination adds the risk of radiation exposure that is slightly less than a set of dental x-rays. All pregnant and breast feeding women are excluded from EBT testing.