Annex S1: Annotated Bibliography, organized by candidate cause(s) of CKDu

We culled key articles testing leading hypotheses, and present these in no particular order.

Genetic Predisposition


This broad study of CKDu in Sri Lanka includes a genome-wide association study that suggests genetic variants near the SLC13A3 gene may predispose to disease. One putative function of the protein product of the SLC13A3 gene is to act as a xenobiotic transporter, which may point toward environmental toxins as an important trigger of disease.


Exome sequencing in a small cohort followed by validation in a larger cohort suggest a possible role for the voltage gated potassium channel KCNA10, the significance of which is not yet clear.


Candidate gene study assessing the xenobiotic transporter CYP1A1, a P450 enzyme, for association with CKDu in India.


Candidate gene study designed around the hypothesis that organochlorine pesticides (OCPs) may contribute to CKDu in India. Investigators focused on genetic variants in the GSTM1 and GSTT1 xenobiotic metabolizing enzymes that have well-described common null alleles. Investigators demonstrated an association between these genetic variants and CKDu, while also demonstrating associations between the alleles and blood levels of various OCPs.

Literature review that demonstrates a consistent association between family history of CKD and CKDu in many studies in Asia and Central America. It suggests that there may be an important hereditable component to CKDu.

**Agrochemical exposure**


   This study serves as an example of evaluation of an agrochemical exposure and health outcomes, with 5 studies meta-analyzed, one of which was a large prospective study with over 10 years of follow up in 54,251 US agricultural workers. Particularly notable is the fact that in all of the included studies, glyphosate use is abstracted via self report (lifetime days), rather than via any measurements.


   A systematic review identified 21 studies assessing association between pesticides and CKDu. Of these, only 3 were prospective. A majority evaluated pesticide exposure using self-report, some very generally, without attempting to ascertain lifetime exposure or intensity of exposure. Three (one from Sri Lanka and two from India) evaluated measured exposures for organochlorine and organophosphates. Investigators did not feel sufficient data exists to support a link between pesticide use and CKDu.


   A case control analysis evaluating urine in 10 participants with clinically diagnosed CKDu, 10 control participants with normal kidney function living in endemic area, and 10 control participants living near Colombo (urban area). Illustrative of types of exposure assessment needed, and types of controls needed. Overall exposure to glyphosate seems to be higher in people living in the endemic agricultural area compared with the urban area, although conclusions in cases versus controls are limited given small sample size.


   A cross-harvest study of 283 to evaluate risk for eGFR decline by job category. Job title of agrochemical applicator (as a proxy of short term agrochemical exposure) was not associated with immediate decline in eGFR, although effects beyond 6 month harvest remain unknown and sample sizes small for each job category.

Case-control questionnaire based analysis to evaluate a range of risk factors for eGFR < 60 ml/min/1.73m² in a CKDu endemic community. Median lifetime days applying pesticides were 0 for both cases and controls, there was no difference in level of personal protective equipment worn, but ‘past inhalation of pesticides’ was associated with eGFR reduction. A questionnaire based assessment that was more granular than previously performed.


Set in a non-endemic (urban) clinic, 270 CKD patients and 270 age-matched controls in whom organochlorine pesticides were measured in the blood, and noted overall to be higher in patients with CKD. The authors then explored whether polymorphisms of xenobiotic metabolizing enzymes were associated with higher organochlorine pesticides.

**Heavy metal exposures**


*Sampled water from 20 communities, geographically diverse in terms of reported prevalence of CKDu. No drinking water samples had high levels of nephrotoxic heavy metals. No evidence for higher mean urinary concentration of these heavy metals in clinically-diagnosed CKDu cases versus controls.*


*Hair and nail samples from 77 participants with biopsy proven CKDu and age matched volunteer participants, with concentrations of trace elements reported using ICPMS, and skin examined via a dermatologist. No differences in cutaneous manifestations, but difficult to interpret any meaningful differences in trace elements concentrations in cases versus controls.*
Heat Stress


This pair of articles outlines the evidence in support and against heat stress as a major factor in CKDu based on large part on epidemiology data. Though there is disagreement about the role of heat stress in general, evidence for heat stress as an important factor in CKDu appears to be stronger in Mesoamerican Nephropathy than in the corresponding disease entity in Sri Lanka, for example.


One of several papers exploring mouse models as a way to test theories about potential causal factors in CKDu as a proof-of-principle experiment. Mice were exposed to cyclic heat and dehydration, which caused renal injury. The fructokinase enzyme and polyol pathway were implicated in heat-associated kidney injury.


Study typifying the cross-shift approach to examining changes in renal indices before and after a workday performing hard manual labor in a hot, humid climate.

Study illustrating the cross-season approach, measuring kidney indices before and after the harvest season among sugar cane workers in Nicaragua. Workers with more demanding jobs had larger decreases in eGFR compared to workers with less exertion in the heat.

Infectious Disease


Recent paper that chronicles an acute syndrome preceding development of CKDu. The acute presentation includes fever, leukocyturia, and leukocytosis, suggesting a possible infectious etiology, though urine cultures in this and other studies were negative.


Testing of a candidate pathogen, leptospirosis, for association with CKDu in Nicaragua. Leptospirosis is a well-known cause of acute kidney injury. The connection between leptospirosis seropositivity and CKDu was inconclusive.


The authors posit that leptospirosis and hantavirus, two pathogens known to cause kidney injury, may contribute to CKDu in Sri Lanka. They propose cross sectional and case-control studies in endemic and non-endemic areas to test this hypothesis.