

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

1. An integrative study of the **genetic**, social and environmental determinants of chronic kidney disease characterized by tubulointerstitial damages in the North Central Region of **Sri Lanka**. Nanayakkara S, Senevirathna ST, Abeysekera T, Chandrajith R, Ratnatunga N, Gunarathne ED, Yan J, Hitomi T, Muso E, Komiya T, Harada KH, Liu W, Kobayashi H, Okuda H, Sawatari H, Matsuda F, Yamada R, Watanabe T, Miyataka H, Himeno S, Koizumi A. J Occup Health. 2014;56(1):28-38.

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.

2. Whole-exome sequencing reveals **genetic** variants associated with chronic kidney disease characterized by tubulointerstitial damages in North Central Region, **Sri Lanka**. Nanayakkara S, Senevirathna ST, Parahitiyawa NB, Abeysekera T, Chandrajith R, Ratnatunga N, Hitomi T, Kobayashi H, Harada KH, Koizumi A. Environ Health Prev Med. 2015 Sep;20(5):354-9.

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.

3. Association of CYP1A1 gene polymorphism with chronic **kidney** disease: a case control study. Siddarth M, Datta SK, Ahmed RS, Banerjee BD, Kalra OP, Tripathi AK. Environ Toxicol Pharmacol. 2013 Jul;36(1):164-70.

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

4. Increased level of organochlorine pesticides in chronic **kidney** disease patients of unknown etiology: role of GSTM1/GSTT1 polymorphism. Siddarth M, Datta SK, Mustafa M, Ahmed RS, Banerjee BD, Kalra OP, Tripathi AK. Chemosphere. 2014 Feb;96:174-9.

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.

5. CKD of Uncertain Etiology: A Systematic Review. Lunyera J, Mohottige D, Von Isenburg M, Jeuland M, Patel UD, Stanifer JW. Clin J Am Soc Nephrol. 2016 Mar 7;11(3):379-85.

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 heritable component to CKDu.

Agrochemical exposure

1. Exposure to glyphosate-based herbicides and risk for Non-Hodgkin Lymphoma: A Meta-analysis and Supporting Evidence. Zhang L, Rana L, Shaffer R, Taioli E, Sheppard L. Mutation Research. In press, available online 10 February 2019

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.

2. Pesticide exposures and chronic kidney disease of unknown etiology: an epidemiologic review. Valcke M, Levasseur ME, Soares da Silva A, Wesseling C. Environ Health 2017 May 23;16(1):49

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.

3. Simultaneous exposure to multiple heavy metals and glyphosate may contribute to Sri Lankan agricultural nephropathy. Jayasumana C, Gunatilake S, and Siribaddana S. BMC Nephrology 2015;16:103

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.

4. Changes in kidney function among Nicaraguan sugarcane workers. Laws RL, Brooks DR, Amador JJ, Weiner DE, Kaufman JS, Ramírez-Rubio O, et al. Int J Occup Environ Health. 2015;21:241–250.

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.

5. Risk factors for reduced glomerular filtration rate in a Nicaraguan community affected by Mesoamerican nephropathy. Raines N, González M, Wyatt C, Kurzrok M, Pool C, Lemma T, et al. *MEDICC Rev.* 2014;16:16–22

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.

6. Increased level of organochlorine pesticides in chronic kidney disease patients of unknown etiology: role of GSTM1/GSTT1 polymorphism. Siddarth M, Datta SK, Mustafa M, Ahmed RS, Banerjee BD, Kalra OP, et al. *Chemosphere.* 2014;96:174–179.

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

- 1) Nephrotoxic contaminants in drinking water and urine, and chronic kidney disease in rural Sri Lanka. Rango T, Jeuland M, Manthirithilake H, McCornick P. *Sci Total Environ.* 2015;518–519:574–585.

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.

- 2) Environmental exposures of trace elements assessed using keratinized matrices from patients with chronic kidney diseases of uncertain etiology (CKDu) in Sri Lanka. [Diyabalanage S](#), Fonseca S, [Dasanayake DMSNB](#), [Chandrajith R.](#) *J Trace Elem Med Biol.* 2017 Jan;39:62-70.

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

1) Climate Change and the Emergent Epidemic of CKD from Heat Stress in Rural Communities: The Case for Heat Stress Nephropathy Jason Glaser, Jay Lemery, Balaji Rajagopalan, Henry F. Diaz, Ramón García-Trabanino, Gangadhar Taduri, Magdalena Madero, Mala Amarasinghe, Georgi Abraham, Sirirat Anutrakulchai, Vivekanand Jha, Peter Stenvinkel, Carlos Roncal-Jimenez, Miguel A. Lanaspa, Ricardo Correa-Rotter, David Sheikh-Hamad, Emmanuel A. Burdmann, Ana Andres-Hernando, Tamara Milagres, Ilana Weiss, Mehmet Kanbay, Catharina Wesseling, Laura Gabriela Sánchez-Lozada, and Richard J. Johnson: Clin J Am Soc Nephrol. 2016 Aug 8; 11(8): 1472–1483.

2) Kidney Diseases in Agricultural Communities: A Case Against Heat-Stress Nephropathy. Chula Herath, Channa Jayasumana, P. Mangala C.S. De Silva, P.H. Chaminda De Silva, Sisira Siribaddana, and Marc E. De Broe. Kidney Int Rep. 2018 Mar; 3(2): 271–280.

This pair of articles outlines the evidence in support and against heat stress as a major factor in CKDu based in 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.

3) Fructokinase activity mediates dehydration-induced renal injury. Roncal Jimenez CA, Ishimoto T, Lanaspa MA, Rivard CJ, Nakagawa T, Ejaz AA, Cicerchi C, Inaba S, Le M, Miyazaki M, Glaser J, Correa-Rotter R, González MA, Aragón A, Wesseling C, Sánchez-Lozada LG, Johnson RJ. Kidney Int. 2014 Aug;86(2):294-302.

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.

4) Heat stress, dehydration, and kidney function in sugarcane cutters in El Salvador--A cross-shift study of workers at risk of Mesoamerican nephropathy. García-Trabanino R, Jarquín E, Wesseling C, Johnson RJ, González-Quiroz M, Weiss I, Glaser J, José Vindell J, Stockfelt L, Roncal C, Harra T, Barregard L. Environ Res. 2015 Oct;142:746-55.

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.

5) Changes in kidney function among Nicaraguan sugarcane workers. Laws RL, [Brooks](#) DR, Amador JJ, Weiner DE, Kaufman JS, Ramírez-Rubio O, Riefkohl A, Scammell MK, López-Pilarte D, Sánchez JM, Parikh CR, McClean MD. Int J Occup Environ Health. 2015 Jul-Sep;21(3):241-50.

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

1) Clinical markers to predict progression from acute to chronic kidney disease in Mesoamerican nephropathy. Fischer RSB, Vangala C, Mandayam S, Chavarria D, García-Trabanino R, Garcia F, Garcia LL, Murray KO. *Kidney Int.* 2018 Dec;94(6):1205-1216.

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.

2) Leptospira seropositivity as a risk factor for Mesoamerican Nephropathy. Riefkohl A, Ramírez-Rubio O, Laws RL, McClean MD, Weiner DE, Kaufman JS, Galloway RL, Shadomy SV, Guerra M, Amador JJ, Sánchez JM, López-Pilarte D, Parikh CR, Leibler JH, Brooks DR. *Int J Occup Environ Health.* 2017 Jan;23(1):1-10.

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.

3) Chronic kidney disease of uncertain etiology in Sri Lanka: Are leptospirosis and Hantaviral infection likely causes? Gamage CD, Sarathkumara YD. *Med Hypotheses.* 2016 Jun;91:16-19.

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.