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The Promise of Metabolomics and Exposomics in CKDu

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Chronic kidney disease of unknown cause (CKDu, also called Mesoamerican nephropathy) is a form of nondiabetic, nonhypertensive CKD most often described in impoverished agricultural communities. The strongest evidence to date indicates that heat stress is an important factor in the pathogenesis of CKDu in many, although not all, affected populations. Other nephrotoxic exposures, including agrochemicals, metals and metalloids, infections, and medications, may also contribute to disease development, either in isolation or in conjunction with heat exposure. The natural history of the disease may include recurrent acute kidney injury (AKI) leading to eventual chronic disease.[1]

In this issue of KI reports, Stem *et al.*[2] apply exposomic and metabolomic analysis to urine samples from sugarcane workers, a group with high rates of CKDu. Their core questions were as follows: (i) what changes in elemental composition and metabolome arise over several months of sugarcane work, and (ii) can these changes inform CKDu pathophysiology and identify potential biomarkers?

They followed 202 Guatemalan sugarcane workers aged >18 years over the November 2017 to April 2018 harvest season. Eighty of 202 workers had morning urine samples suitable for exposomic and metabolomic analysis; of these, 19 workers had a decline in estimated glomerular filtration rate of >9% and were designated the kidney function decline (KFD) group, whereas 16 had an estimated glomerular filtration rate that increased between 0% and 5% and were designated the non-KFD group. Ten participants were randomly selected from each group for analysis of specimens from the beginning and end of the harvest season. Elemental analyses were conducted using inductively coupled plasma mass spectrometry and metabolomic analyses with untargeted liquid chromatography–coupled mass spectrometry.

Elemental analysis showed that silicon and phosphorus increased from November to April in 100% and 85% of KFD and non-KFD samples, respectively, whereas levels of heavy metals (nickel, cadmium, lead, and arsenic) did not increase and were overall generally low. Untargeted metabolomics identified 4799 compounds, 1154 of which were detectable in all samples. Amino acids, botanical compounds, and fatty acids changed the most over the harvest season, as did metabolic pathways related to impaired beta oxidation, mitochondrial function, perturbed energy metabolism, and kidney injury. Among pesticide-related metabolites identified, levels of carbofuran-3-keto, metolachlor, diquat, and paraquat all increased by 1.5-fold or greater over the course of the harvest season. No major differences in either elemental or metabolic features were seen between KFD and non-KFD groups.

Studies such as Stem *et al.*[2] provide a great example of how metabolomic and exposomic tools can be applied to kidney disease.[3] Metabolomics, particularly when applied within a multiomics framework, has contributed toward important advances in our understanding of both AKI and CKD.[4] For example, the identification of nicotinamide adenine dinucleotide metabolic derangement in AKI helped reveal the fundamental role of altered central energy metabolism in the injury state, and also suggested ways these alterations may be targeted to prevent or mitigate injury.[5] Metabolomics and exposomics represent important pillars of investigation as we move toward precision medicine, the understanding of how individual level genetics, exposures, and behaviors

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Figure 1. Application of metabolomics and exposomics to chronic kidney disease of unknown cause research.

unite to drive disease development and therapeutic response. Patients ultimately benefit when these findings can be translated into drug discovery, therapeutic target identification, and preventative measures.

The etiology of CKDu may be complex; silica, heavy metals, heat stress, medications, pesticide exposure have all been studied as potential exposures linked to disease development, and the disease process itself remains poorly understood. Metabolomic and exposomic techniques are therefore particularly promising tools when applied to CKDu because they can help us evaluate both exposures and disease physiology (Figure 1).

The most well-established risk factor for CKDu development is exposure to heat stress.[1] Although metabolomics and exposomics may be used to indirectly assess potential downstream effects of heat stress such as increased gut permeability,[6] they do not directly assess heat stress. However, a number of other risk factors for CKDu development can be readily assessed by metabolomic and exposomic techniques to study them as independent entities and as important cofactors with heat stress. Stem *et al.*[2] have demonstrated the utility of these techniques in evaluating exposure to 3 potentially important CKDu risk factors: metals (including silica), agrochemicals, and nephrotoxic medications.

Metabolomics can provide insight into the physiology of CKDu. Stem *et al.*[2] identify changes arising over the harvest season in a number of metabolites and metabolic pathways known to be associated with kidney injury or CKD progression in other forms of kidney disease. Their coverage of metabolic pathways is at times restricted to only a few metabolites, limiting interpretability, but is nonetheless a valuable proof of concept.

A crucial step in moving our understanding of CKDu forward is exploring the intersection between overlapping exposures. To date, we have very little data on how different risk factors interact to drive CKDu. Stem *et al.*[2] demonstrate one of the most useful elements of metabolomics in CKDu research: the ability to capture concurrent data on multiple exposures and physiologic changes with a single analytic test.

That Stem *et al.*[2] captured a wide variety of agrochemical exposures, but did not capture many of the key metabolites in endogenous metabolic pathways, shows the trade-offs of using untargeted versus targeted metabolomics. Untargeted metabolomics do not prespecify metabolites of interest and are therefore quite valuable for exploratory analyses. However, because there is no prespecification, there is also no guarantee that untargeted metabolomics will capture specific compounds that may be of interest. Missing endogenous metabolites can hinder analyses of metabolic pathway activity, and it can be difficult to determine whether missing exogenous metabolites are truly absent or just not identified using the untargeted technique.

Overall study design is crucial in research applying metabolomics and exposomics to CKDu. Two key components are the timing of specimen collection and the outcome variable selected. CKD development is the most concrete outcome, but the timing of specimen collection for metabolomic analysis is important. Evaluation early in the disease course helps to avoid questions of reverse causality related to the effects of changing kidney function on metabolite levels, and to identify opportunities for prevention before significant disease arises. However, given that the natural history of CKDu may involve recurrent AKI, randomly selecting sampling time points early in the disease course may lead investigators to miss the

active injury process. Evaluating exposures and physiologic processes during AKI events is more likely to capture the processes most relevant to injury, but it remains unclear whether these AKI events truly drive CKDu. Stem *et al*.[2] used decrease in estimated glomerular filtration rate over a sugarcane harvest season (categorized as KFD or non-KFD) as an outcome variable, and measured how features change from the beginning to the end of the season; in doing so, they strike a balance between AKI and CKD as an outcome variable, but also lose some of the concrete clinical relevance of CKD and the close temporal coupling between exposure, physiology, and outcome of AKI.

As Stem *et al*.[2] acknowledge, and as is the case with many studies of CKDu, their research is more hypothesis-generating than hypothesis-confirming. The immediate clinical relevance of the findings of Stem *et al*.[2] related to both exposure and physiology is also tempered by the lack of findings differentiating KFD and non-KFD groups.

The evidence that silica exposure occurs over the course of a harvest season is compelling. Taken in concert with the authors' previous work investigating silica as a driver of CKDu,[7] further exploration is certainly warranted, particularly related to its potential intersection with heat stress. Evidence of the presence of nephrotoxic agrochemical exposure over the harvest season also merits follow-up.

Identifying evidence of minimal exposure to heavy metals exposure is also valuable. However, this finding comes with 2 important caveats. First, urine is not always the optimal specimen for exposure evaluation across all the substances evaluated.[8] Second, the company employing these workers had recently implemented a program to reduce exposure to heavy metal–contaminated water, and it remains unclear whether exposure was greater prior to this intervention.

Endogenous metabolites changing from preharvest to postharvest suggest the emergence of a number of established kidney disease–related processes over this time span, although the lack of difference between KFD and non-KFD groups raises some questions about their applicability to CKDu development. Further targeted studies measuring some of the key metabolites in the pathways discussed, that were not identified with their untargeted methodology, would be useful to more fully understand metabolic changes arising over the course of a sugarcane harvest season.

With any exploratory study on exposures in CKDu, we have to be cautious with how aggressively we act on the findings. The story of the short-lived glyphosate ban in Sri Lanka provides a valuable case study.[9] In 2015, based on emerging evidence linking exposure to glyphosate, a pesticide, with CKDu in regions of Sri Lanka, the government instituted a complete ban. In subsequent years, they relaxed the ban and eliminated it entirely in 2022. The reasons for reversing course were complex and tied to a rapidly changing political situation, but boiled down to the following 2 key factors: (i) banning glyphosate resulted in significant adverse economic consequences, and (ii) subsequent evidence failed to corroborate a substantial causal relationship between glyphosate and CKDu.[9]

Any efforts to address silica and agrochemical exposure in individuals at risk for CKDu in Central America should be considered with the Sri Lanka glyphosate experience in mind. Encouraging the provision and use of adequate personal protective equipment among sugarcane workers exposed to burned sugarcane or pesticides is unlikely to be harmful. However, further evidence substantiating the role of silica and pesticide exposure in CKDu pathophysiology is needed before we consider either exposure as an important target for clinical guidelines or public health policy aimed at preventing CKDu.

DISCLOSURE

All the authors declared no competing interests.

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