




EDITORIAL COMMENT

Aguascalientes: one of the hottest chronic kidney disease (CKD) hotspots in Mexico and a CKD of unknown aetiology mystery to be solved

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ABSTRACT

In a recent issue of *Clinical Kidney Journal* (CKJ), Gutierrez-Peña *et al.* reported a high incidence and prevalence of advanced chronic kidney disease (CKD) in Aguascalientes, Mexico. This contradicts Global Burden of Disease estimates, which should be updated. A key component of this high burden of CKD relates to young people ages 20–40 years in whom the cause of CKD was unknown [CKD of unknown aetiology (CKDu)]. The incidence of kidney replacement therapy in this age group in Aguascalientes is among the highest in the world, second only to Taiwan. However, high-altitude Aguascalientes, with a year-round average temperature of 19°C, does not fit the geography of other CKDu hotspots. Furthermore, kidney biopsies in young people showed a high prevalence of focal segmental glomerulosclerosis. Potential causes of CKDu in Aguascalientes include the genetic background (no evidence, although podocytopathy genes should be explored) and environmental factors. The highest prevalence of CKD was found in Calvillo, known for guava farming. Thus guava itself, known to contain bioactive, potentially nephrotoxic molecules and pesticides, should be explored. Additionally, there are reports of water sources in Aguascalientes contaminated with heavy metals and/or pesticides. These include fluoride (increased levels found in Calvillo drinking water) as well as naturally occurring arsenic, among others. Fluoride may accumulate in bone and cause kidney disease years later, and maternal exposure to excess fluoride may cause kidney disease in offspring. We propose a research agenda to clarify the cause of CKDu in Aguascalientes that should involve international funders. The need for urgent action to identify and stem the cause of the high incidence of CKD extends to other CKD hotspots in Mexico, including Tierra Blanca in Veracruz and Poncitlan in Jalisco.

Keywords: burden of disease, CKD hotspot, CKD of uncertain aetiology, fluoride, focal segmental glomerulosclerosis, Mexico

CKD HOTSPOTS AND CKDu

In 2014, a *Clinical Kidney Journal* (CKJ) manuscript first defined chronic kidney disease (CKD) hotspots as countries, regions, communities or ethnicities with an incidence of CKD higher

than average [1]. Since then, the term has been popularized, as it may provide information leading to identification of causative agents that explain the high incidence of CKD in certain communities. Earlier, a novel form of CKD of uncertain

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Conceptual framework for CKDu pathogenesis



FIGURE 1: Conceptual framework for CKDu pathogenesis. Based on available evidence regarding the concentration of CKD cases within certain geographic locations and families, as well on the association with certain environments and occupations, a conceptual framework implying a genetic susceptibility background in association or interacting with environmental exposures is a reasonable starting point to unravel the pathogenesis of CKDu.

aetiology (CKDu) was described in several subtropical regions and first named as such in Sri Lanka, although in Central America it was known as Mesoamerican nephropathy [2, 3]. Although Mesoamerican nephropathy was initially described in sugarcane workers and thought to be an occupational disease, children residing in regions of Nicaragua at high risk for Mesoamerican nephropathy may experience subclinical kidney injury prior to occupational exposures [4]. Indeed, a systematic review and meta-analysis not only showed an association with male gender, water intake and lowland altitude, potentially reflecting higher temperatures and agricultural work as drivers, but was also associated with a family history of CKD, potentially implying a genetic background or common intrafamilial exposure [5, 6] (Figure 1). Moreover, a high water intake may represent a risk if such water is contaminated. However, there were no significant associations with pesticide exposure, non-steroidal anti-inflammatory drugs intake, heat stress or alcohol consumption.

EPIDEMIOLOGY OF CKD IN MEXICO

Mexico is among the countries with the highest incidence and prevalence of CKD. According to the Global Burden of Disease (GBD) database 2019, the incidence of CKD is 457/100 000 and the prevalence is 13 017/100 000. The states with the highest prevalence and incidence in 2019 were Mexico City, Veracruz, Morelos and Tamaulipas [7] (Table 1). The annual increase in mortality rate due to CKD reported by the GBD from 1990 to 2019 was 4.85%/year [7]. Diabetes and hypertension, two of the main diagnosed causes of CKD, are highly prevalent in Mexico. According to data from the 2018 National Health and Nutrition Survey, 10.3% of the population ≥ 20 years of age reported a diagnosis of diabetes and 18.4% of hypertension [8].

Mexico has no centralized national registry for kidney disease. Epidemiological data on patients with CKD receiving medical attention in health systems showed a male:female ratio of 1.2:1.0, with a mean age of 60 years [11]. Jalisco kidney replacement therapy (KRT) data are the most widely known internationally and have long been part of the US Renal Data System (USRDS) international comparisons [12]. The KEEP Mexico study from Mexico City and Jalisco found 7% of patients in CKD category G1, 8 and 16% in G2, 6 and 9% in G3 and 1% in G4–5, respectively [13]. Primary causes were diabetes mellitus (DM; 53%), hypertension (34%), glomerular disease (7%), polycystic kidney disease (2%), inborn disease (2%) and others (2%) [11]. Strikingly, CKDu apparently accounted for <2% of patients with CKD. KRT by dialysis mainly involves peritoneal dialysis (PD; 59%, of which 27% corresponds to ambulatory PD and 32% of continuous ambulatory PD) and

haemodialysis (41%) [14]. The states with the highest rates of transplant were Mexico City, Jalisco, Aguascalientes, San Luis Potosi and Coahuila.

CKDu IN MEXICO

CKDu has been reported in Mexico. In the period 2008–2018, 30 of 57 (53%) patients with end-stage kidney disease (ESKD) referred for renal transplant to the Instituto Nacional de Cardiología met the criteria for CKDu [15]. Biopsies in five of six patients showed findings compatible with Mesoamerican nephropathy. Tierra Blanca in Veracruz and Poncitlan in Jalisco have been identified as CKDu hotspots (Figure 2A). A cross-sectional study with 7717 participants ages 20–60 years from communities in Tierra Blanca reported a prevalence of 25% of probable CKD, and 44% of them could be considered CKDu cases. The CKDu hotspot status of Poncitlan is supported by population screenings for CKD that have been performed in the State of Jalisco since 2007. The CKD prevalence in Poncitlan was 20.1, versus 10.4% in the rest of the state, with KRT prevalence being 2-fold higher than the rest of the state [16]. ESKD cases from Tierra Blanca represent 25% of the state's waiting list for kidney transplantation, although the municipality represents only 1.3% of the state's population.

AGUASCALIENTES: LIKELY THE HOTTEST CKD HOTSPOT IN MEXICO

In a recent issue of *CKJ*, Gutierrez-Peña et al. [18] reported on the Aguascalientes State Registry of CKD and Renal Biopsy, providing data on KRT incidence (2019) and prevalence (April 2020) and kidney biopsy diagnoses. The registry included all patients with KRT in private and public institutions from Aguascalientes State Health Services. Aguascalientes is a small state located at high altitudes in central Mexico, with a dry climate characterized by stable yearlong temperatures (average 19°C) (Figure 2). Data from the Aguascalientes State Registry of CKD were recently incorporated into USRDS international comparisons and 2018 Aguascalientes data are part of the USRDS 2020 report [19]. Aguascalientes has emerged as one of the hottest CKD hotspots in Mexico and among the hottest in the world for 20- to 40-year-olds (Figure 3A).

The 2020 prevalence and 2019 incidence of KRT in Aguascalientes were 1997 per million population (pmp) and 336 pmp, respectively. More strikingly, the average age was 46 years, with peaks at ages 20–40 and 50–70 years. In contrast, KEEP Mexico data from Mexico City and Jalisco showed CKDu was the main cause of KRT, followed by diabetes. CKDu was by far the most common cause (73% of cases) of KRT in patients 20–40 years of age. Patients were classified as CKDu if there was no history of DM, glomerulopathy, long-standing hypertension or any other identifiable cause such urinary tract or polycystic kidney disease. Kidney biopsy results supported the high prevalence of a form of CKDu characterized by focal segmental glomerulosclerosis (FSGS) associated to other features of vascular and tubulointerstitial non-specific CKD. These biopsies were mainly obtained from persons ages 21–30 years.

These results are significant since they point to unidentified pockets of CKD within Mexico and emphasize the need for country-wide statistics. Indeed, the GBD ranked Aguascalientes 27th out of 32 Mexican states in terms CKD prevalence. Based on data from Gutierrez-Peña et al. [18], these estimates may be way off the mark. As an alternative explanation, there might be

Table 1. CKD prevalence, incidence and age standardized mortality rate according to the GBD study (1.2) and kidney transplant status (CENATRA study) in Mexico by state

State	CKD prevalence/ 100 000 [7]	CKD incidence/ 100 000 [7]	Age-standardized CKD mortality rate (95% UI) [8]		Kidney transplants (n in 2019) [9]	Total population 2020 [10]	Donation rate (pmp)
			Males	Females			
Aguascalientes	12 195	426	30.4 (28.3–32.4)	29.7 (28.0–31.7)	110	1 425 607	77.46
Baja California	12 187	428	32.1 (30.2–34.2)	29.2 (27.5–31.1)	62	3 769 020	16.4
South Baja California	11 649	401	26.3 (24.5–28.4)	22.7 (21.1–24.4)	16	798 447	20.25
Campeche	12 266	419	20.4 (19.0–21.8)	25.8 (23.8–27.6)	0	928 363	0
Chiapas	10 434	354	22.0 (20.6–23.6)	25.5 (23.8–27.2)	6	3 146 771	1.93
Chihuahua	12 389	433	31.8 (29.8–33.7)	28.4 (26.7–30.3)	30	731 391	41.09
Coahuila	13 080	476	36.6 (34.3–38.8)	32.9 (31.0–35.0)	136	5 543 828	24.54
Colima	13 226	468	32.6 (30.7–35.2)	30.7 (28.7–32.8)	0	3 741 869	0
Durango	12 453	421	28.7 (26.9–30.7)	27.6 (25.7–29.4)	0	1 832 650	0
Estado De Mexico	12,667	460	32.4 (30.5–34.6)	33.1 (31.2–35.1)	95	16 992 418	5.59
Mexico City	16 000	575	34.2 (32.2–36.3)	26.4 (24.9–28.0)	831	9 209 944	90.32
Guanajuato	12 928	452	38.9 (36.5–41.6)	36.8 (34.5–39.1)	210	6 166 934	34.09
Guerrero	11 771	440	20.7 (19.3–22.1)	18.2 (16.9–19.6)	1	3 540 685	0.28
Hidalgo	13 271	460	26.1 (24.5–28.0)	27.3 (25.6–28.8)	32	3 082 841	10.38
Jalisco	13 075	454	33.1 (31.3–35.0)	33.2 (31.3–35.1)	624	8 348 151	74.82
Michoacan	12 879	431	26.1 (24.5–27.8)	26.2 (24.7–27.9)	46	4 748 846	9.70
Morelos	13 736	488	28.7 (27.0–30.3)	27.9 (26.1–29.8)	22	1 971 520	11.16
Nayarit	12 940	435	24.5 (23.0–26.3)	22.9 (21.3–24.6)	1	1 235 456	0.81
Nuevo Leon	13 350	481	29.7 (28.0–31.6)	29.2 (27.5–30.9)	146	5 784 442	25.25
Oaxaca	13 095	445	29.4 (27.5–31.4)	34.2 (32.1–36.2)	4	4 132 148	0.96
Puebla	12 614	443	36.1 (34.0–38.5)	37.9 (35.8–40.1)	139	6 583 278	21.12
Queretaro	12 241	426	30.8 (28.9–33.1)	31.6 (29.4–33.6)	47	2 368 467	19.91
Quintana Roo	10 367	353	19.3 (17.8–20.8)	20.6 (19.0–22.2)	0	1 857 985	0
San Luis Potosi	12 849	428	22.5 (21.2–24.0)	23.8 (22.2–25.2)	122	2 822 255	43.26
Sinaloa	13 353	459	20.7 (19.4–22.0)	19.4 (18.1–20.7)	13	3 026 943	4.30
Sonora	12 660	438	27.9 (26.0–29.8)	28.2 (26.4–30.0)	51	2 944 840	17.34
Tabasco	12 842	458	26.2 (24.5–28.0)	28.3 (26.4–30.1)	18	2 402 598	7.50
Tamaulipas	13 702	486	28.3 (26.6–30.1)	25.9 (24.4–27.5)	11	3 527 735	3.12
Tlaxcala	13 031	461	38.0 (35.5–40.5)	40.1 (37.5–42.7)	5	1 342 977	3.73
Veracruz	14 637	515	25.2 (23.7–26.8)	23.9 (22.4–25.4)	122	8 062 579	15.13
Yucatan	12 792	430	22.7 (21.3–24.3)	24.8 (23.2–26.2)	49	2 320 898	21.12
Zacatecas	12 935	430	23.7 (22.2–25.4)	23.4 (22.0–25.0)	10	1 662 138	6.02

Aguascalientes is the location with the highest incidence and prevalence of CKD.
UI: uncertainty interval.

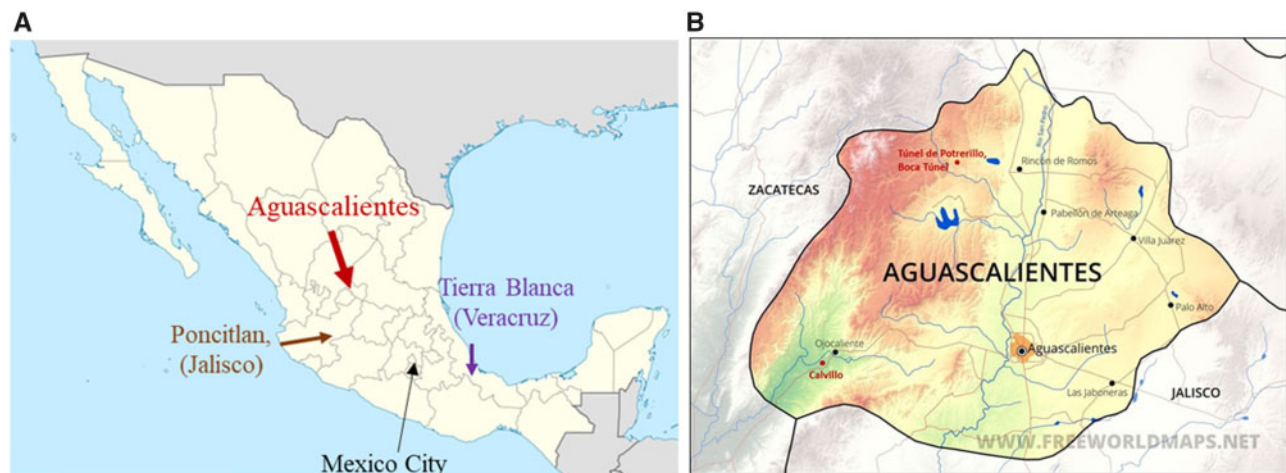


FIGURE 2: Location and geography of Aguascalientes. (A) Location of Aguascalientes within Mexico as well as of the CKD hotspots in Jalisco, Veracruz and Mexico City [17]. (B) Geography of Aguascalientes. The two main river systems (San Pedro and Calvillo Rivers) drawing water from the same mountain range. The locations of Calvillo and Tunel de Potrerillo/Boca Tunel are shown. <https://www.freeworldmaps.net/>.

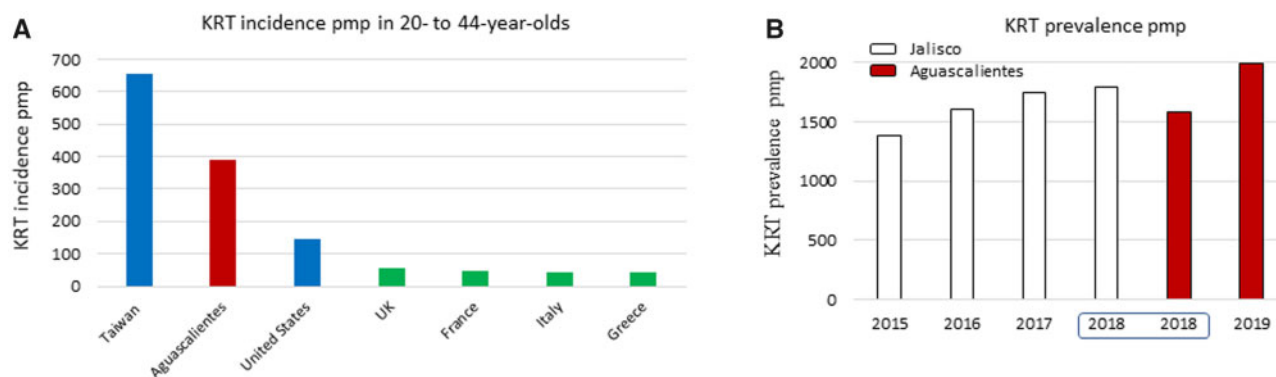


FIGURE 3: Epidemiology of CKD in Aguascalientes, international perspective. (A) KRT incidence pmp in 20- to 44-year-olds. (B) KRT prevalence pmp in Jalisco and Aguascalientes. Up to 2018, source was USRDS [12]; for 2019, source was Gutierrez-Peña et al. [18].

Table 2. Features of Aguascalientes nephropathy, Mesoamerican nephropathy and Sri Lanka CKDu

Feature	Aguascalientes nephropathy	Mesoamerican nephropathy	Sri Lanka CKDu
Age at ESKD (years), range	20–44	3–5th decade [20]	Wide age range [20]
Sex	Males	Males	Male > female for CKD G3/G4 [20]
Climate	Dry, average yearlong temperature 19°C	Humid, average temperature 26°C	Dry [20], average temperature in coast 28°C
Altitude	Highland (Aguascalientes 1888 m; Calvillo 1632 m)	Lowland	Lowland
Occupation	–	Agricultural	Chena farming [20]
Family clustering	–	Yes	–
Childhood evidence of disease	–	Yes	–
Hypertension	–	Rare	–
Proteinuria	–	Minor or non [20]	Minimal [20]
Sediment	–	Sterile pyuria [20]	Without clusters [20]
Urinary biomarkers	–	NGAL [21–23], KIM-1 [4]	NGAL and KIM-1 [24]
Histology	FSGS, interstitial fibrosis, tubular atrophy, arteriolar changes predominantly median hyperplasia	Extensive glomerulosclerosis/signs of chronic glomerular ischaemia, chronic tubulointerstitial nephropathy, mild vascular lesions [3, 25]	Glomerular sclerosis/glomerular collapse, interstitial fibrosis and tubular atrophy, interstitial mononuclear cell infiltration, fibrous intimal thickening and arteriolar hyalinosis [26]

NGAL: neutrophil gelatinase-associated lipocalin; KIM-1: kidney injury molecule-1.

suboptimal provision or registration of KRT in other Mexican states. The annual 2020 USRDS report displays Aguascalientes data for 2018 with more granularity than the data from Jalisco, the traditional source of Mexican information for USRDS international comparisons [19]. One of the most worrisome aspects of the report is the very high incidence of KRT in Aguascalientes among younger people (20–40 years of age), second only to Taiwan, the country with the highest incidence of KRT in the world (Figure 3A). Moreover, the most recent Aguascalientes data, reported by Gutierrez-Peña et al. [18], may position Aguascalientes above Jalisco in terms of KRT prevalence (Figure 3B).

The second issue raised is about the cause of such high KRT incidence and prevalence. Lessons learned in this regard may help other CKD hotspots. One of the most remarkable findings was the high prevalence of renal biopsy registry diagnoses of FSGS, potentially pointing to secondary or genetic causes of FSGS. However, FSGS is a non-specific lesion, indicative of progressive podocyte loss, a feature of any progressive

CKD. The geographical distribution of KRT cases within Aguascalientes may also contribute to pinpoint the cause, as there was a high incidence in certain populations, such as Calvillo (Figure 2B).

WHY IS AGUASCALIENTES DIFFERENT FROM MESOAMERICAN NEPHROPATHY AND SRI LANKA CKDu?

Aguascalientes is a highland, and thus differs from Sri Lanka and classical sites for Mesoamerican nephropathy, which are lowlands and have higher average temperatures (Table 2). Additionally, kidney biopsy reports emphasized the presence of FSGS rather than a predominantly tubulointerstitial and vascular disease. However, the histological descriptions from Aguascalientes, Mesoamerican nephropathy and Sri Lanka CKDu may all be consistent with non-specific CKD findings of glomerular sclerosis, tubular atrophy, interstitial

inflammation and vascular injury that may be found in any form of advanced CKD. Thus, in our view, this may not represent a major difference. Centralized analysis of biopsies from these diverse regions by a single pathologist may be desirable.

WHAT MIGHT CAUSE AGUASCALIENTES NEPHROPATHY?

The yet unsolved mystery of one of the hottest CKD hotspots in Mexico should be addressed from different approaches. What clues on potential genetic and/or environmental drivers of CKD in Aguascalientes are currently available?

The age clue

KRT due to Aguascalientes CKDu peaks between the ages of 20 and 40 years. The occurrence of KRT at such a young age may be consistent with genetic predisposition and/or occupational exposures. However, it cannot be discarded that the very same cause was contributing to kidney disease in older patients diagnosed with diabetic kidney disease (a diagnosis usually made in the absence of a kidney biopsy) or with hypertensive nephropathy. The diagnosis of hypertensive nephropathy is one of exclusion, as is usually made in hypertensive individuals with CKDu [27]. Indeed, in US African Americans, so-called hypertensive nephropathy is now widely acknowledged to represent a genetic predisposition to CKD due to *APOL1* alleles, which most recently have been linked to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-associated kidney disease [28]. In this regard, Gutierrez-Peña et al. [18] report a low prevalence of African ancestry in the region.

The FSGS clue

The most frequent kidney biopsy pattern in Aguascalientes was FSGS, which differs from CKDu descriptions from El Salvador, Nicaragua and Sri Lanka (with mainly interstitial involvement). FSGS is classified as a podocytopathy, which may be primary (usually associated to nephrotic syndrome) secondary to several conditions, including loss of kidney mass (hyperfiltration) from any cause, or genetic, the latter two not associated with nephrotic syndrome [29, 30]. Nephrotic syndrome was present in just one-fourth of patients between 21 and 40 years old, placing the focus on the other causes. Patients with hyperfiltration-mediated podocytopathies frequently have FSGS and glomerulomegaly, the latter a finding seen in 62% of patients in this age range in Aguascalientes [18]. In addition to acquired kidney disease, low birth nephron endowment is a cause of reduced kidney mass. Low birthweight and prematurity are frequent causes of low nephron number that are not frequently recorded for adult patients [31].

Regarding genetic causes, type IV collagen (*COL4A*) gene variants linked to Alport syndrome have recently emerged as the most common (44–56%) cause of genetic FSGS in adults [32]. Variants in other podocyte-expressed genes found in adults with FSGS include *ACTN4*, *TRPC6*, *INF2*, *ANLN* and *NPHS2* [33, 34]. However, there are no data regarding familial history and little is known about the timeline of the high prevalence of CKDu in Aguascalientes over the past decades. Purely genetic causes would not explain a recent surge in CKD cases, unless there was an environment–genetic interaction and the environment changed.

The genetics clue

Beyond the suggestion that FSGS cases may have a genetic component, there are historical records that suggest a high level of inbreeding in Jalisco and Aguascalientes up to the 19th century (83% of all marriages in the 16th century and 75% in the 18th century), including church documents allowing consanguineous marriages, although inbreeding decreased dramatically in the 19th and 20th centuries [35, 36]. This may have facilitated a local prevalence of genetic variants that is higher than expected that either predispose to kidney disease by themselves or predispose to kidney disease under certain environmental conditions. Clustering of cases in small villages that have seen a milder influx of out-of-state persons in recent decades may be a clue in this regard. Gutierrez-Peña et al. [18] report a pilot study that did not find evidence of inbreeding in patients with Aguascalientes CKDu. We are not aware of information on genes predisposing to CKD in the Aguascalientes context. However, we did find an interesting piece of information that merits exploration. A Google search for genetic variants and Aguascalientes identified a methodological manuscript on genetic variants of *ATP6V0A2* [36]. The study was triggered by prior reports of two patients in two Aguascalientes locations who were born to consanguineous parents [37]. *ATP6V0A2* encodes for the lysosomal H⁺-transporting ATPase V0 subunit A2. Pathogenic mutations in this gene have been associated with autosomal recessive cutis laxa disease type II-A (Online Mendelian Inheritance in Man 219200). While the selection criteria for the studied individuals were not detailed and the number was small ($n = 10$), the manuscript states that the incidence of the truncating genetic variant c.187C>T (p.Arg63Ter) was 30%, somehow implying that the sample was random. Participants were from Túnel de Potrerillo (population 154), Boca del Túnel (population 100) and Túneles (Rincón de Romos, Aguascalientes) (Figure 2B). According to the gnomAD version 2.1.1 database, this genetic variant was only found in Latino/admixed populations (1 in 11 500) or African/African American populations (1 in 16 200), but not in Europeans or in Asians [38]. Thus, potentially this genetic variant may be common in Aguascalientes. Interestingly, *ATP6V0A2* messenger RNA (mRNA) is expressed mainly in podocytes and distal tubules according to single-cell transcriptomics studies, as well as in macrophages [39] (Figure 4A). The Protein Atlas confirmed these data at the protein level (Figure 4B). Regarding kidney disease, single-cell transcriptomics identified *ATP6V0A2* mRNA as potentially downregulated in diabetic kidneys in podocytes and increased in infiltrating leucocytes, although the adjusted P-values were not significant [40]. Moreover, in the Nephroseq database of kidney transcriptomics studies, higher expression of *ATP6V0A2* mRNA in kidney, glomeruli or tubulointerstitium was associated with decreased estimated glomerular filtration rate (eGFR) and proteinuria, i.e. *ATP6V0A2* mRNA levels were correlated with the severity of kidney injury in diverse human nephropathies, including FSGS (Table 3). This or other genetic variants not yet associated to kidney disease in the literature may be more prevalent in Aguascalientes than in other regions and underlie the high incidence and prevalence of KTR.

The environmental clue

Environmental factors have been linked to CKDu. Although these have been mainly associated with tubulointerstitial disease, at this stage we cannot yet exclude that the FSGS lesions observed in Aguascalientes are secondary to decreased kidney mass from other causes.

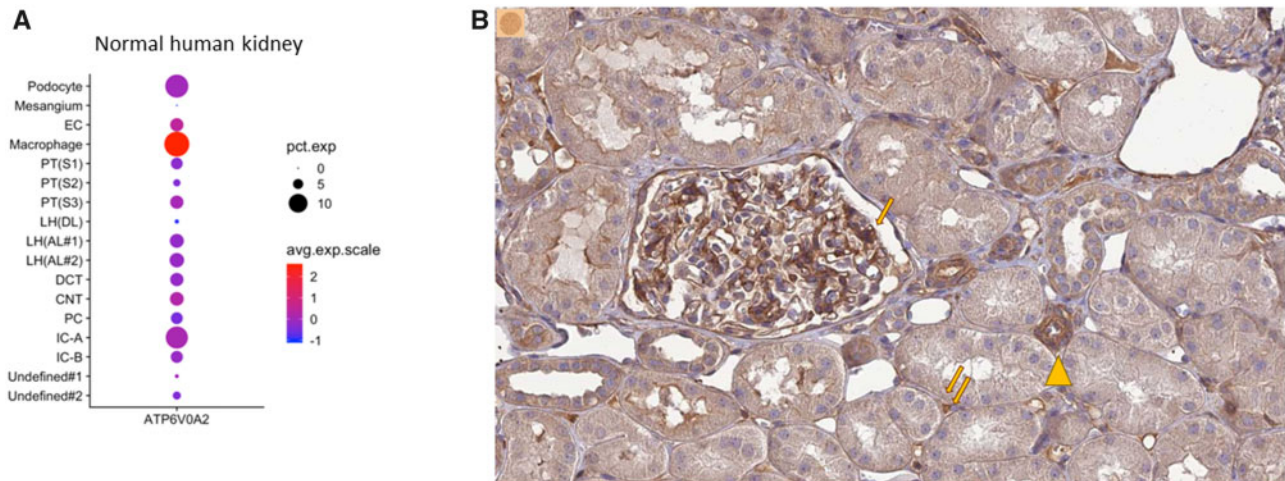


FIGURE 4: Kidney expression of ATP6V0A2. (A) Single-cell transcriptomics of human kidney tissue identified kidney ATP6V0A2 mRNA expression mainly in podocytes and distal tubules. Sources: [39] and [41]. (B) ATP6V0A2 immunostaining in the kidney. Note the high expression in the glomerular capillary walls, potentially corresponding to podocytes (arrows) and distal tubules (arrowheads) as well as interstitium (double arrow). Image credit: Human Protein Atlas. Image available from version 20.1 at <https://www.proteinatlas.org/ENSG00000185344-ATP6V0A2/tissue/kidney#img> [42] (accessed 20 June 2021).

Table 3. Analytical correlates of kidney ATP6V0A2 mRNA expression in human kidney disease according to the Nephroseq database of kidney transcriptomics studies [43]

Sample	Kidney disease or condition	R-value	P-value	References
eGFR				
Tubulointerstitium	IgA nephropathy	-0.540	0.007	[44]
Tubulointerstitium	Lupus nephritis	-0.793	0.033	[45]
Glomeruli	Lupus nephritis	-0.706	0.034	[45]
Kidney	Transplant kidney	-0.521	0.038	[46]
Proteinuria				
Tubulointerstitium	FSGS	0.672	0.012	[45]
Glomeruli	Collapsing FSGS	0.963	0.037	[47]

The municipality with the highest prevalence of KRT is Calvillo, which could be a good starting point. Calvillo is one of the richest counties in Aguascalientes and the largest guava producer in Mexico. *Psidium guajava*, the guava tree, is considered a medicinal plant and contains several compounds with described medicinal properties, i.e. it contains potentially bioactive molecules [48]. Guava is a widely consumed fruit and its leaves are used to make tea and traditional remedies such as antidiarrhoeals. However, some of the isolated bioactive compounds are cytotoxic [49]. Increased serum urea in male rats and nephrocalcinosis and chronic pyelonephritis in female rats were described in low-quality nephrotoxicity studies [50]. Increased creatinine was also described in mice and rats ingesting guava leaves [51, 52]. Furthermore, guava could be a potential source of heavy metals if the soil and irrigation water are contaminated, as recently reported in South Africa, where the total target hazard quotient values for heavy metals in guava were within unsafe limits for children [53]. Moreover, the issue of overuse or misuse of pesticides for guava farming (Fulidol, Slaughter, Furadan, malathion and parathion among others) leading to ground and water contamination or unsafe direct exposure has been raised as potentially contributing to CKDu in Calvillo [54]. Overexposure to malathion [55, 56] and cypermethrin [57, 58], used as pesticides on guava farms in Calvillo, has also been reported as potentially associated with impaired kidney function and nephrotic syndrome [56]. The guava

hypothesis, while far-fetched, cannot be discarded outright. There is precedent for unexpected toxicities of edible fruits. Litchi (*Litchi chinensis* Sonn.) is a tropical and subtropical fruit belonging to the genus *Litchi* in the soapberry family, Sapindaceae. Several episodes of litchi toxicity leading to the deaths of children have been reported and the toxicity was variously attributed to pesticides or to a toxin, methylene cyclopropyl-glycine, found in unripe litchi fruit, that depletes glucose reserves in the body, making it more toxic for undernourished children, in whom it may trigger encephalitis-related deaths [59–61]. If such an acute severe event was not identified until the end of 20th century, more insidious kidney toxicity may be even more difficult to detect.

Contamination of water used for drinking or crop irrigation is another potential concern. Aguascalientes sits just north of Jalisco and both the Calvillo and San Pedro rivers flow into Jalisco. In Calvillo, Gutierrez-Peña et al. [18] report a high proportion (44%) of wells with fluoride levels above the Mexican standard of <1.5 mg/L; although lead, arsenic, aluminium, barium, cadmium, copper, total chromium, iron, manganese, mercury and zinc were within safe limits. Specifically, according to the Department of Sanitary Regulation of the State of Aguascalientes, levels of fluoride were 1.86 ± 1.8 mg/L in Calvillo wells. This raises the issue of potential fluoride toxicity to the kidneys. In mice, intake of water with fluoride levels of 1.5 mg/L synergized with water containing the World Health

Organization (WHO) maximum recommended levels for cadmium and hardness to cause kidney injury characterized by interstitial fibrosis, mononuclear cell interstitial infiltration, tubular atrophy and, interestingly, FSGS [62]. Thus the fluoride content in drinking water in Calvillo may theoretically synergize with other components of water or with a genetic predisposition to cause kidney injury. In India, high serum and/or urine fluoride levels were found in 20% of children with nephrotic syndrome [63]. Indeed, foot process effacement, indicative of podocyte injury, was observed in mice exposed to fluoride [64]. Fluoride is the nephrotoxic component in methoxyflurane [65] and high bone fluoride (and lead) were found in Sri Lanka CKDu, although it was unclear to what extent they were caused or a consequence of CKD [66]. However, urinary and serum fluoride were also increased, suggesting increased exposure and potential contribution to nephrotoxicity [67]. Thus fluoride is an obvious candidate nephrotoxic agent to be studied in Aguascalientes CKDu.

Despite current evidence of a safe water supply for other heavy metals in Calvillo, past exposure cannot be discarded as contributing to CKDu. Several occurrences of contaminated water have been described in Aguascalientes. Calvillo sits on the Calvillo riverbank, which runs parallel to the San Pedro River, the main body of water in the state. Both rivers draw water from the same mountain range. Marked deterioration in water quality was found in the San Pedro River as it passed by Aguascalientes in 2011 [68]. In northern Aguascalientes, 42 communities were exposed to high concentrations of arsenic in drinking water. In Tepezala and Asientos, high lead concentrations in blood were observed ($>10 \mu\text{g}/\text{dL}$). The infant populations exposed to metals in drinking water showed a statistically significant correlation between exposure to cadmium or lead and urinary protein; however, the cadmium and lead concentrations in the water supplies were only slightly higher than the levels recommended by the WHO. Heavy contamination of the San Pedro River was linked to the discharge of domestic and industrial effluents with high concentrations of organic matter, nutrients (total phosphorus and total nitrogen), organic

xenobiotics and faecal matter. Sediments were high in organic xenobiotics (anilines and detergents), copper and zinc of anthropogenic origin, as well as contamination of natural origin by arsenic. Natural arsenic contamination is likely linked to the volcanic activity and thermal waters that originated the name of Aguascalientes [69]. Groundwater contained high concentrations of fluorides, arsenic, mercury, chromium, iron, manganese and lead. According to the US Environmental Protection Agency criteria, all the sediment samples in one study were polluted by arsenic, 50% by lead and zinc, 25% by copper and ~13% by manganese and chromium. Three sediment samples presented moderate pollution by iron and another three by mercury. However, wells did not show conclusive evidence of pollution of the aquifer and individual wells duplicated the safe limit for arsenic or mercury in drinking water [70].

Lastly, air pollutants such as 2.5- μm particulate matter are high in Aguascalientes, and these pollutants have been related to low birthweight, a potential cause of CKD [71].

In summary, there is evidence for potential exposure to heavy metals and/or pesticides. Potential interactions and synergy between them in causing nephrotoxicity should be explored. In this regard, exposure may have occurred years before and sources of exposure may already have been corrected. In pre-clinical studies, maternal exposure to excess fluoride may cause nephrotoxicity in offspring that becomes evident during puberty [64] and bone accumulation of lead and fluoride in persons may cause a slow release from endogenous sources and nephrotoxicity [66].

WHAT ARE THE NEXT STEPS?

According to the GBD 2017 Study, the age-standardized CKD mortality in Aguascalientes is below the national average [8]. This should be corrected in light of the evidence presented by Gutierrez-Peña *et al.* [18]. Furthermore, given the extremely high incidence of KRT in young people, understanding the cause of Aguascalientes CKDu should be viewed as a research priority to which international funders of health research should

Table 4. Potential research agenda to unravel the drivers of CKDu in Aguascalientes

Stage 1. Basic epidemiology

1. What has been the timeline of CKDu incidence in Aguascalientes?
 - a. Was it always there, albeit undiagnosed?
 - b. Can its appearance as a frequent condition be traced to any point of time in recent decades that can in turn be associated with any environmental change?
2. Is there a familial clustering of CKDu that may point to genetic or shared environmental factors?
3. Is there a geographical clustering that may point to genetic or shared environmental factors?
4. Is there early evidence of kidney disease in children that would argue against occupational exposure?
5. Is there evidence of occupational exposure as a risk factor?
6. What is the birth weight and gestational age of persons with CKDu?

Stage 2. Search for genetic factors, depending in part on results from Stage 1

1. GWAS and/or exome sequencing of bona fide CKDu and unaffected family members

Stage 3. Search for environmental factors, depending in part on results from Stage 1

1. Careful analysis of shared environmental exposures (air, food, water and occupational) for chemicals or heavy metals
 - a. Is there evidence of fluoride-induced nephropathy?
 - b. Is there any evidence of arsenic toxicity?
 - c. Is there evidence for toxicity related to guava farming or consumption?
2. Systems biology approaches in kidney and urine samples for clues on cause: kidney transcriptomics and/or single-cell transcriptomics, urine peptidomics, metabolomics and heavy metal screens

contribute. Several potentially interacting genetic and environmental factors have been identified. Unravelling their contributions to CKD in Aguascalientes may benefit CKD patients throughout the world. The need for urgent action to identify and stem the cause of the high incidence of CKD extends to other CKD hotspots in Mexico, including Tierra Blanca in Veracruz and Poncitlan in Jalisco. [Table 4](#) presents a draft research agenda.

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CONFLICT OF INTEREST STATEMENT

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REFERENCES

- Ferguson R, Leatherman S, Fiore M et al. Prevalence and risk factors for CKD in the general population of southwestern Nicaragua. *J Am Soc Nephrol* 2020; 31: 1585–1593
- Chandrajith R, Nanayakkara S, Itai K et al. Chronic kidney diseases of uncertain etiology (CKDu) in Sri Lanka: geographic distribution and environmental implications. *Environ Geochem Health* 2011; 33: 267–278
- Wijkström J, Leiva R, Elinder CG et al. Clinical and pathological characterization of mesoamerican nephropathy: a new kidney disease in Central America. *Am J Kidney Dis* 2013; 62: 908–918
- Leibler JH, Ramirez-Rubio O, Velázquez JJA et al. Biomarkers of kidney injury among children in a high-risk region for chronic kidney disease of uncertain etiology. *Pediatr Nephrol* 2021; 36: 387–396
- González-Quiroz M, Pearce N, Caplin B et al. What do epidemiological studies tell us about chronic kidney disease of undetermined cause in Meso-America? A systematic review and meta-analysis. *Clin Kidney J* 2018; 11: 496–506
- Perez-Gomez MV, Martin-Cleary C, Fernandez-Fernandez B et al. Meso-American nephropathy: what we have learned about the potential genetic influence on chronic kidney disease development. *Clin Kidney J* 2018; 11: 491–495
- GBD Compare | IHME Viz Hub. <https://vizhub.healthdata.org/gbd-compare/> (16 April 2021, date last accessed)
- Agudelo-Botero M, Valdez-Ortiz R, Giraldo-Rodríguez L et al. Overview of the burden of chronic kidney disease in Mexico: secondary data analysis based on the Global Burden of Disease Study 2017. *BMJ Open* 2020; 10: e035285
- Boletín Estadístico Informativo del Centro Nacional de Trasplantes (BEI-CENATRA) Volumen V, No. 1, Periodo: Enero-junio, 2020. https://www.gob.mx/cms/uploads/attachment/file/590075/BEI-CENATRA_03-11-2020.pdf (14 April 2021, date last accessed)
- INEGI. Instituto Nacional de Estadística y Geografía. Censo Población y Vivienda 2020. <https://www.inegi.org.mx/programas/ccpv/2020/default.html#Documentacion> (20 April 2021, date last accessed)
- Méndez-Durán A, Francisco Méndez-Bueno J, Tapia-Yáñez T et al. Epidemiología de la insuficiencia renal crónica en México. *Dial Trasplante* 2010; 31: 7–11
- United States Renal Data System (USRDS). *USRDS Home Page*. <https://www.usrds.org/> (16 April 2021, date last accessed)
- Obrador GT, García-García G, Villa AR et al. Prevalence of chronic kidney disease in the Kidney Early Evaluation Program (KEEP) México and comparison with KEEP US. *Kidney Int* 2010; 77: S2–S8
- Centro Nacional de Trasplantes. *Estado Actual de Donación y Trasplantes en México Anual 2013*. <http://www.gob.mx/cenatra/documentos/estadisticas-50060> (28 January 2017, date last accessed)
- De Arrigunaga SA, Martínez, MAC, Ortega, C et al. Clinical and pathological characterization of patients with ESKD from a potential Mexican CKD of undetermined etiology (CKDu) hotspot. *J Am Soc Nephrol* 2019; 30: 220
- Correa-Rotter R. Report From the Third International Workshop on Chronic Kidney Diseases of Uncertain/Non-Traditional Etiology in Mesoamerica and Other Regions Workshop Organizing Committee: Technical Series SALTRA. NIEHS. www.saltra.una.ac.cr (16 April 2021, date last accessed)
- File:Mexico States blank map.svg - Wikipedia. https://en.m.wikipedia.org/wiki/File:Mexico_States_blank_map.svg (22 June 2021, date last accessed)
- Gutiérrez-Peña M, Zuñiga-Macias L, Marin-García R et al. High prevalence of end-stage renal disease of unknown origin in Aguascalientes Mexico: role of the registry of chronic kidney disease and renal biopsy in its approach and future directions. *Clin Kidney J* 2021; 14: 1197–1206
- United States Renal Data System. End-stage Renal Disease, International Comparisons. <https://adr.usrds.org/2020/end-stage-renal-disease/11-international-comparisons> (16 April 2021, date last accessed)
- Weaver VM, Fadrowski JJ, Jaar BG. Global dimensions of chronic kidney disease of unknown etiology (CKDu): a modern era environmental and/or occupational nephropathy? *BMC Nephrol* 2015; 16: 145
- González-Quiroz M, Camacho A, Faber D et al. Rationale, description and baseline findings of a community-based prospective cohort study of kidney function amongst the young rural population of northwest Nicaragua. *BMC Nephrol* 2017; 18: 16
- Gonzalez-Quiroz M, Smpokou ET, Silverwood RJ et al. Decline in kidney function among apparently healthy young adults at risk of Mesoamerican nephropathy. *J Am Soc Nephrol* 2018; 29: 2200–2212
- Laws RL, Brooks DR, Amador JJ et al. Biomarkers of kidney injury among Nicaraguan sugarcane workers. *Am J Kidney Dis* 2016; 67: 209–217
- Gunasekara T, De Silva PMCS, Herath C et al. The utility of novel renal biomarkers in assessment of chronic kidney disease of unknown etiology (CKDu): a review. *Int J Environ Res Public Health* 2020; 17: 9522
- López-Marín L, Chávez Y, García XA et al. Histopathology of chronic kidney disease of unknown etiology in Salvadoran agricultural communities. *MEDICC Rev* 2014; 16: 49–45

26. Nanayakkara S, Komiya T, Ratnatunga N et al. Tubulointerstitial damage as the major pathological lesion in endemic chronic kidney disease among farmers in North Central Province of Sri Lanka. *Environ Health Prev Med* 2012; 17: 213–221
27. Carriazo S, Perez-Gomez MV, Ortiz A. Hypertensive nephropathy: a major roadblock hindering the advance of precision nephrology. *Clin Kidney J* 2020; 13: 504–509
28. Couturier A, Ferlicot S, Chevalier K et al. Indirect effects of severe acute respiratory syndrome coronavirus 2 on the kidney in coronavirus disease patients. *Clin Kidney J* 2020; 13: 347–353
29. Sethi S, Zand L, Nasr SH et al. Focal and segmental glomerulosclerosis: clinical and kidney biopsy correlations. *Clin Kidney J* 2014; 7: 531–537
30. Lepori N, Zand L, Sethi S et al. Clinical and pathological phenotype of genetic causes of focal segmental glomerulosclerosis in adults. *Clin Kidney J* 2018; 11: 179–190
31. Conti G, De Vivo D, Fede C et al. Low birth weight is a conditioning factor for podocyte alteration and steroid dependence in children with nephrotic syndrome. *J Nephrol* 2018; 31: 411–415
32. Riedhammer KM, Braunisch MC, Günthner R et al. Exome sequencing and identification of phenocopies in patients with clinically presumed hereditary nephropathies. *Am J Kidney Dis* 2020; 76: 460–470
33. Kopp JB, Anders HJ, Susztak K et al. Podocytopathies. *Nat Rev Dis Prim* 2020; 6: 1–24
34. Ahn W, Bombback AS. Approach to diagnosis and management of primary glomerular diseases due to podocytopathies in adults: core curriculum 2020. *Am J Kidney Dis* 2020; 75: 955–964
35. Franco CPT. Matrimonio entre parientes. Causas y causas de dispensa en la parroquia de La Encarnación, 1778–1822. *Let Hist* 2015; 13: 59–85
36. Esparza, VMG. La erosión de la endogamia o de la dinámica del mestizaje. Aguascalientes, Nueva Galicia, siglos XVII y XVIII. *Relac Estud Hist Soc* 2019; 40: 148–177
37. Bahena-Bahena D, López-Valdez J, Raymond K et al. ATP6V0A2 mutations present in two Mexican Mestizo children with an autosomal recessive cutis laxa syndrome type IIA. *Mol Genet Metab Rep* 2014; 1: 203–212
38. 12-124203239-C-T | gnomAD v2.1.1 | gnomAD. https://gnomad.broadinstitute.org/variant/12-124203239-C-T?dataset=gnomad_r2_1 (21 June 2021, date last accessed)
39. Wu H, Uchimura K, Donnelly EL et al. Comparative analysis and refinement of human PSC-derived kidney organoid differentiation with single-cell transcriptomics. *Cell Stem Cell* 2018; 23: 869–881e8
40. Wilson PC, Wu H, Kirita Y et al. The single-cell transcriptomic landscape of early human diabetic nephropathy. *Proc Natl Acad Sci USA* 2019; 116: 19619–19625
41. Kidney Interactive Transcriptomics. <http://humphreyslab.com/SingleCell/displaycharts.php> (21 June 2021, date last accessed)
42. Uhlén M, Fagerberg L, Hallström BM et al. Proteomics. Tissue-based map of the human proteome. *Science* 2015; 347: 1260419
43. Nephroseq. <https://www.nephroseq.org/resource/login.html> (21 June 2021, date last accessed)
44. Reich HN, Tritchler D, Cattran DC et al. A molecular signature of proteinuria in glomerulonephritis. *PLoS One* 2010; 5: e13451
45. Cohen CD, Frach K, Schlöndorff D, Kretzler M. Quantitative gene expression analysis in renal biopsies: a novel protocol for a high-throughput multicenter application. *Kidney Int* 2002; 61: 133–140
46. Flechner SM, Kurian SM, Solez K et al. De novo kidney transplantation without use of calcineurin inhibitors preserves renal structure and function at two years. *Am J Transplant* 2004; 4: 1776–1785
47. Hodgkin JB, Borczuk AC, Nasr SH et al. A molecular profile of focal segmental glomerulosclerosis from formalin-fixed, paraffin-embedded tissue. *Am J Pathol* 2010; 177: 1674–1686
48. Gutiérrez RMP, Mitchell S, Solis RV. Psidium guajava: a review of its traditional uses, phytochemistry and pharmacology. *J Ethnopharmacol* 2008; 117: 1–27
49. Feng XH, Wang ZH, Meng DL et al. Cytotoxic and antioxidant constituents from the leaves of *Psidium guajava*. *Bioorganic Med Chem Lett* 2015; 25: 2193–2198
50. Attawish A, Chavalittumrong P, Rugsamon P et al. Toxicity study of *Psidium guajava* Linn. leaves 1995. *Warasan Krom Withhayasat Ka Phaet* 2005; 37: 289–305
51. Adeyemi OS, Akanji MA. Biochemical changes in the kidney and liver of rats following administration of ethanolic extract of *Psidium guajava* leaves. *Hum Exp Toxicol* 2011; 30: 1266–1274
52. Díaz-de-Cerio E, Verardo V, Gómez-Caravaca AM et al. Health effects of *Psidium guajava* L. leaves: an overview of the last decade. *Int J Mol Sci* 2017; 18: 897
53. Gupta SK, Ansari FA, Nasr M et al. Multivariate analysis and health risk assessment of heavy metal contents in food-stuffs of Durban, South Africa. *Environ Monit Assess* 2018; 190: 151–151
54. Cardona JHA, Violante RE, Vazquez JR et al. The ingestion of contaminated water with pesticides and heavy metals as probable cause to chronic renal failure. *Int J Pure App Biosci* 2015; 3: 423–426
55. Wan E, Darssan D, Karatela S et al. Association of pesticides and kidney function among adults in the US population. <https://www.researchsquare.com/article/rs-452983/v1> (4 July 2021, date last accessed)
56. Yokota K, Fukuda M, Katafuchi R, Okamoto T. Nephrotic syndrome and acute kidney injury induced by malathion toxicity. *BMJ Case Rep* 2017; 2017: bcr201722073
57. Inayat Q, Ilahi M, Khan J. A morphometric and histological study of the kidney of mice after dermal application of cypermethrin. *J Pak Med Assoc* 2007; 57: 587–591
58. Haque SM, Sarkar CC, Khatun S et al. Toxic effects of agro-pesticide cypermethrin on histological changes of kidney in Tengra, Mystus tengara. *Asian J Med Biol Res* 2018; 3: 494–498
59. Sinha SN, Ramakrishna UV, Sinha PK et al. A recurring disease outbreak following litchi fruit consumption among children in Muzaffarpur, Bihar—a comprehensive investigation on factors of toxicity. *PLoS One* 2020; 15: e0244798
60. Shrivastava A, Kumar A, Thomas JD et al. Association of acute toxic encephalopathy with litchi consumption in an outbreak in Muzaffarpur, India, 2014: a case-control study. *Lancet Glob Heal* 2017; 5: e458–66
61. Vaux DL, Silke J. IAPs, RINGs and ubiquitylation. *Nat Rev Mol Cell Biol* 2005; 6: 287–297
62. Wasana HMS, Perera GDRK, Gunawardena PDS et al. WHO water quality standards vs synergic effect(s) of fluoride, heavy metals and hardness in drinking water on kidney tissues. *Sci Rep* 2017; 7: 42516

63. Curnutte JT, Babior BM, Karnovsky ML. Fluoride-mediated activation of the respiratory burst in human neutrophils. A reversible process. *J Clin Invest* 1979; 63: 637–647
64. Tian X, Xie J, Chen X et al. Deregulation of autophagy is involved in nephrotoxicity of arsenite and fluoride exposure during gestation to puberty in rat offspring. *Arch Toxicol* 2020; 94: 749–760
65. Allison SJ, Docherty PD, Pons D et al. Methoxyflurane toxicity: historical determination and lessons for modern patient and occupational exposure. *N Z Med J* 2021; 134: 76–79
66. Ananda Jayalal T, Mahawithanage S, Senanayaka S et al. Evidence of selected nephrotoxic elements in Sri Lankan human autopsy bone samples of patients with CKDu and controls. *BMC Nephrol* 2020; 21: 384
67. Fernando WBNT, Nanayakkara N, Gunarathne L et al. Serum and urine fluoride levels in populations of high environmental fluoride exposure with endemic CKDu: a case-control study from Sri Lanka. *Environ Geochem Health* 2020; 42: 1497–1504
68. Avelar-González FJ, Ramírez LE, Martínez SM et al. Water quality in the State of Aguascalientes and its effects on the population's health. In: Úrsula Oswald Spring, ed. *Water Resources in Mexico: Scarcity, Degradation, Stress, Conflicts, Management, and Policy*. Berlin: Springer, 2011, pp. 217–229
69. University of Maine. Arsenic. Where Arsenic Comes From. <https://umaine.edu/arsenic/where-arsenic-comes-from/> (21 June 2021, date last accessed)
70. Guzmán-Colis G, Ramírez-López EM, Thalasso F et al. Evaluation of pollutants in water and sediments of the San Pedro river in the state of Aguascalientes. *Univ Cienc* 2011; 27: 17–32
71. Secretaría de Sustentabilidad, Medio Ambiente y Agua (SSMAA). *Programa Cielo Claro para la Mejora en la Calidad del Aire del Estado de Aguascalientes 2018–2028*. <https://www.aguascalientes.gob.mx/SSMAA/pdf/ProgramaCieloClaroProAireAguascalientes201820281eraEdicion29nov18.pdf> (4 July 2021, date last accessed)