ETIOLOGY AND EPIDEMIOLOGY OF BALKAN ENDEMIC NEPHROPATHY (BEN)

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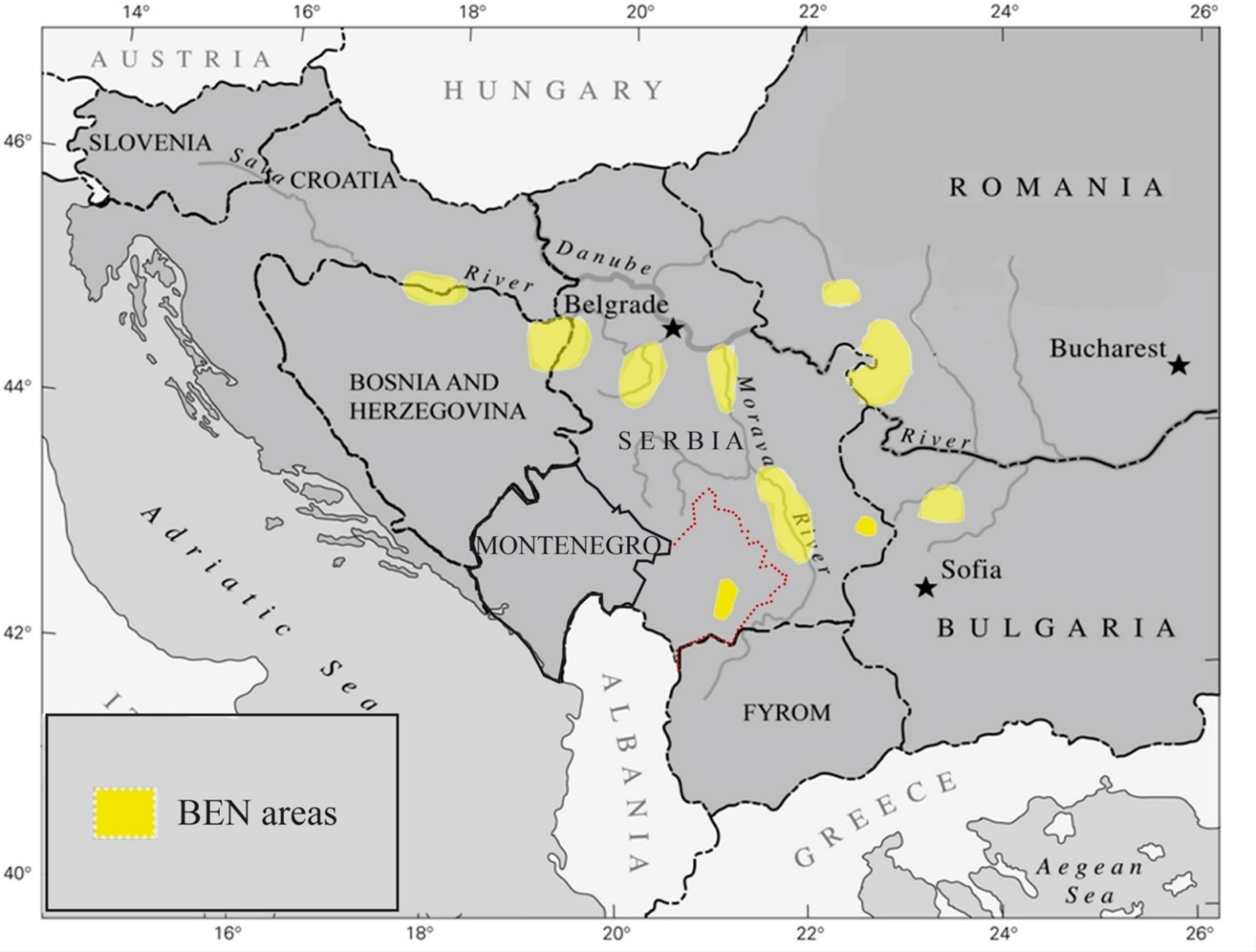
BEN, originally described in 1956, is a unique familial, chronic renal disease encountered with a high prevalence rate in Bosnia and Herzegovina, Bulgaria, Croatia, Romania, and Serbia.

The first cases were described in:

1. Bulgaria:	Tanchev Y, et al. Studies on the nephritides in the District of Vratza. Savremena Medicina 1956; 7: 14-29
2. Serbia:	Danilovic V, et al. Chronic nephritis due to lead poisoning by digestive route (flour). Presse Med. 1957; 65: 2039-2040 (in French)
3. Romania:	Fortza N, et al. Nefrita cronica azotemia endo-epidemica. Stud Cercet Med 1961; 1: 217-221

Geographical distribution of BEN



Epidemiology

1. There are no clear-cut data on the current trend for the incidence and prevalence of BEN
2. The studies carried out in different endemic areas have produced conflicting information
3. Some epidemiological studies reported
   - An increase in the prevalence of BEN between 1967 and 1970
   - A steady state between 1970 and 1984 and
   - Ultimately a decrease in some endemic areas
   - Similarly, in another endemic area, a decreasing incidence over time was found during a follow-up period from 1978 to 1997

Epidemiology

1. Assessments are frequently based on the number of BEN patients on dialysis
2. In Serbia, BEN patients represent an average of 6.5% (5–46%) of the dialysis population
3. Estimations: almost 100 000 people are at risk, 25 000 have BEN
4. Despite intermittent variations, the incidence remained stable over time.
5. Differences may be related to

   - Changes in the study design
   - True epidemiological differences between sequential time frames and BEN areas
   - Consequence of the natural course of the disease

BALKAN ENDEMIC NEPHROPATHY (BEN)

The main features of the disease are:

- Endemic nature
- Long incubation period
- Familial clustering of the disease, and
- Unusually high incidence of associated upper urothelial cancers (UUC)

Clinical signs and symptoms

Non-specific and often remain unrecognized for years

The initial asymptomatic period is followed by:

- Weakness and lassitude,
- Mild lumbar pain,
- Pallor of the skin and
- Copper brownish discoloration of the palms and soles

Blood pressure is usually normal

Anaemia

Intermittent proteinuria

Sparse urinary sediment

Loss of urine concentration capacity

Very small contracted kidneys

Diagnostic criteria

There are no pathognomonic diagnostic features of BEN

The set of:

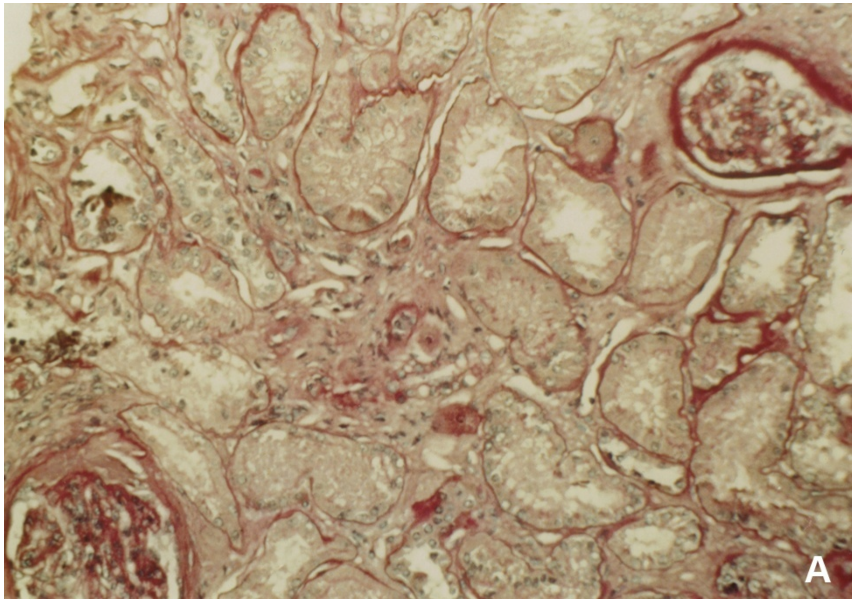
- Epidemiologic
- Clinical and biochemical data
- Pattern of pathologic injury
- Absence of any other renal diseases
- Familial clustering of the disease

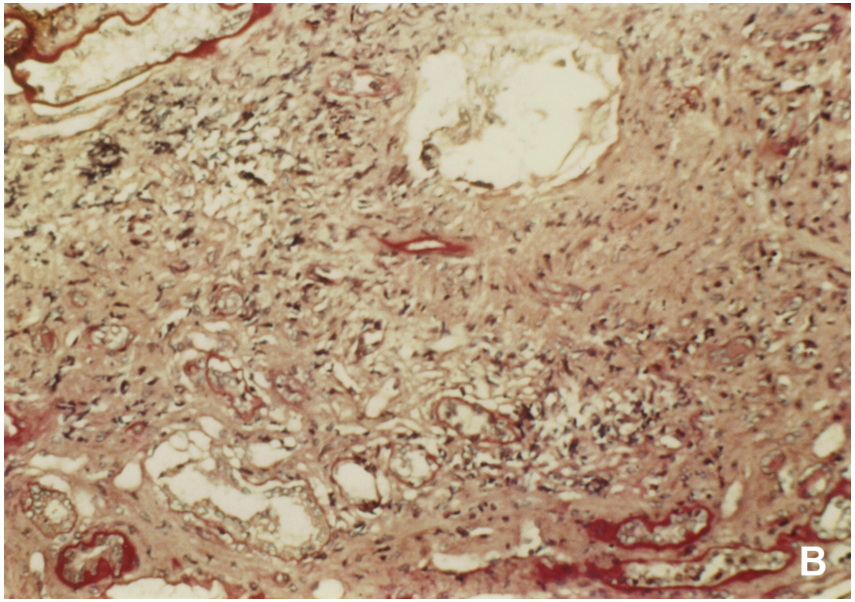
Are highly suggestive of this entity

Pathology of BEN

The pathology of BEN is characterized by a progressive atrophy and sclerosis of all structures of the kidney, and it shares similarities with tubulointerstitial kidney diseases

Pathology of BEN





A - Advanced glomerular sclerosis (initial obsolescence), with interstitial sclerosis and tubular atrophy (PAS, x250).

B - Focal tubular atrophy with prominent interstitial sclerosis and clearly delineated mononuclear infiltrate (PAS, x250)

Čukuranović R. Genetic and morphophysiologic study of Balkan endemic nephropathy. Doctoral Thesis. Medical Faculty, University of Niš, 1-169, 1992 (in Serbian)

All hypotheses related to BEN can be classified into three main groups

A. Exogenous factors

(a) Lead intoxication

(b) Selenium deficiency

(c) Intoxication with Aristolochia Clematitis

(d) Ochratoxin A

(e) Pliocene lignite

B. Endogenous factors

(a) Genetic predisposition

(b) Changes in enzyme activity;
- LCAT deficiency
- Decreased erythrocyte ALA-D activity
- Changes of CYP2D6 activity

(c) Genetic polymorphism

(d) Chromosomal aberrations

(e) Viral disease

C. Miscellaneous factors - Multifactor

Lecithin cholesterol acyltransferase (LCAT) - Organic substances from coal

CHN = AAN = BEN

Although the aetiology has been extensively studied, fostering the publication of various hypotheses, only one of them has provided conclusive evidence related to the aetiology of BEN.

Studies conducted over the past decade have provided particularly strong arguments that BEN and UUC are caused by chronic poisining with Aristolochic acids (AAs)

CHN = AAN = BEN

1. In 1969, Ivić proposed that the aetiology of BEN could be related to chronic A. Clematitis poisoning in which seeds from these plants intermingle with wheat grain during the harvesting process

2. He speculated that human exposure to a toxic component of Aristolochia might occur through ingestion of bread prepared from flour derived from contaminated grain.

3. These well-documented results attracted more interest from the scientific community many years later

Aristolochia Clematitis

(A) A. Clematitis growing in the wheat field

(B) Post harvests second generation A. Clematitis

(C) Ripe seeds in the soil

(D) Wheat grain from that field

Hyper endemic village Petka, Serbia, 2011

CHN = AAN = BEN

1. In 1990, a clinic in Brussels began prescribing capsules as part of a slimming regimen consisting of Chinese herbal remedies believed to contain in part, Stephania tetrandra (in Mandarin Han Fang-Ji)

2. Unintentionally, it was replaced by Aristolochia fangchi (Guang Fang-Ji in Mandarin) since both plants are used in Chinese traditional medicine carrying similar names (Fang-Ji)

3. CHN reported in Belgium in 1993 presented as a rapidly progressive renal interstitial fibrosis leading to end-stage renal disease

4. It emerged that almost half of the CHN patients developed UUC.

CHN = AAN = BEN

1. Exposure of CHN patients to AA that belongs to the family of carcinogenic, mutagenic and nephrotoxic compounds was substantiated by the identification of AA-DNA adducts

2. Once established, AA–DNA adducts persist for years in the renal cortex, serving as reliable biomarkers of exposure to AA

3. The outbreaks of AA-associated renal failure have been subsequently reported in several other countries, and the name was replaced by Aristolochic acid nephropathy (AAN)

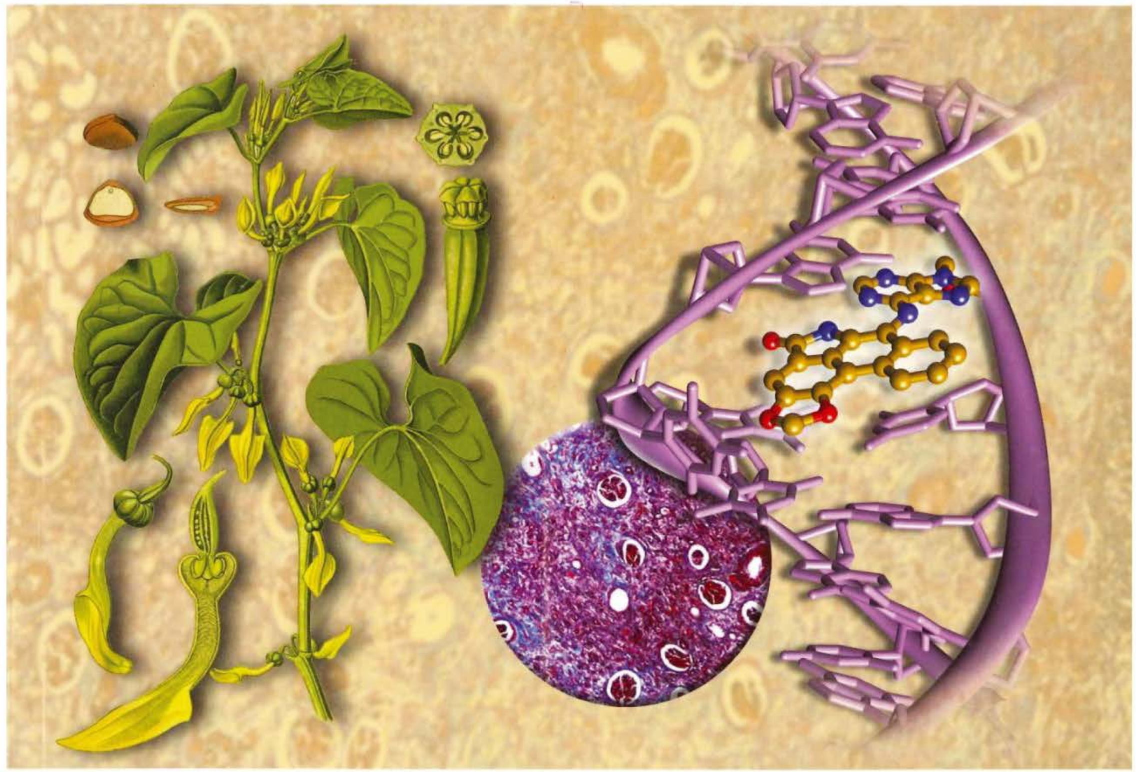
CHN = AAN = BEN

1. Cosyns first raised awareness to the unique renal histopathology of CHN with its striking similarity to BEN

2. The similarities between CHN and BEN have led to the hypothesis of a common etiological agent for both diseases. This hypothesis implies that:

a. BEN, CHN and AAN are the same disease
b. Dietary ingestion of AA, in conjunction with individual genetic susceptibility, accounts for all-epidemiological, clinical, and pathophysiologic features of BEN and associated UUC

kidney INTERNATIONAL



Volume 81 Issue 6 MARCH (2) 2012

AP Grollman et al

M.E. DeBroe (commentary)
Chinese herbs nephropathy and Balkan endemic nephropathy: toward a single entity, aristolochic acid nephropathy
Kid Int (2012) 81, 513-515

CHN = AAN = BEN

A recent publication presented results showing:
*That the accumulation of AA–DNA adducts was present in the renal cortex and UUT of five patients with BEN from an endemic region in Croatia

* But not in five patients with other forms of CKD or five patients with UUC living in a non-endemic area of Croatia

*The finding by Grollman et al. of AA-derived DNA adducts in renal cortical and urothelial tumor tissue of patients with documented BEN,
*Association with the dominance of the A:T → T:A transversions in the TP53 tumor suppressor gene mutational spectrum

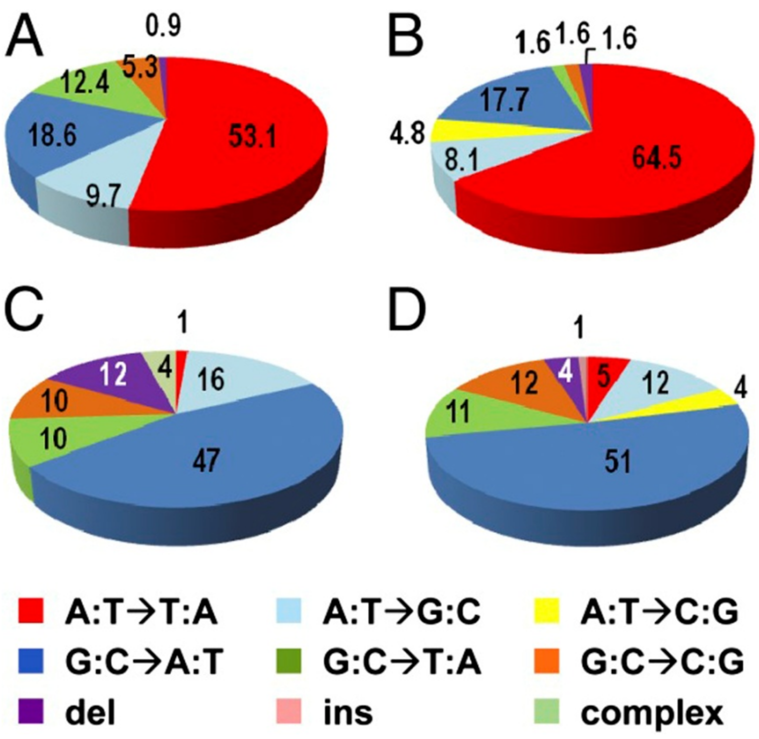
was a breakthrough in the identification of AA as an aetiological agent of the UUC observed in BEN

CHN = AAN = BEN

Unique features of this spectrum, including the predominance of A:T→ T:A transversions found also in Taiwanese patients with UUC confirmed the hypothesis that:

*All components of the AA signature TP53 mutational spectrum, established in the context of UUC associated with BEN, are similarly found in Taiwanese patients with UUC

CHN = AAN = BEN



TP53 mutational spectra in urothelial carcinomas:

A - TP53 mutations in DNA obtained from UUC in endemic regions of Bosnia, Croatia and Serbia (62 mutations);

B - TP53 mutations in DNA obtained from UUC in Taiwan (113 mutations).

C - TP53 mutations in urothelial carcinomas of the renal pelvis and ureter, worldwide (73 mutations).

D - TP53 mutations in urothelial carcinomas of the renal pelvis, ureter, bladder, and nonspecified urinary organs, worldwide (696 mutations).

Chen CH, Dickman KG, Moriya M, et al. Aristolochic acid-associated urothelial cancer in Taiwan. Proc Natl Acad Sci USA 2012; 109: 8241-8246)

Possible health impacts of naturally occurring uptake of aristolochic acids by maize and cucumber roots: links to the etiology of endemic (Balkan) nephropathy

Nikola M. Pavlović, Vuk Maksimović, Jelena Dragišić Maksimović, William H. Orem, Calin A. Tatu, Harry E. Lerch, Joseph E. Bunnell, et al.

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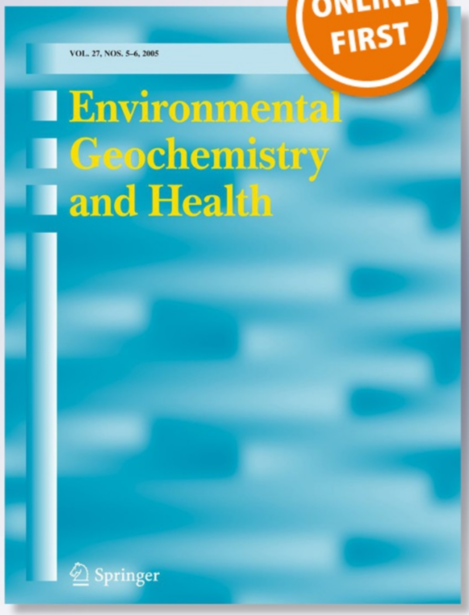

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ORIGINAL PAPER

Possible health impacts of naturally occurring uptake of aristolochic acids by maize and cucumber roots: links to the etiology of endemic (Balkan) nephropathy

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Received: 21 March 2012 / Accepted: 20 July 2012

Abstract Aristolochic acids (AAs) are nephrotoxic and carcinogenic derivatives found in several *Aristolochia* species. To date, the toxicity of AAs has been inferred only from the effects observed in patients suffering from a kidney disease called "aristolochic acid nephropathy" (AAN, formerly known as "Chinese herbs nephropathy"). More recently, the chronic poisoning with *Aristolochia* seeds has been considered to be the main cause of Balkan endemic nephropathy, another form of chronic renal failure resembling AAN. So far, it was assumed that AAs can enter the human food chain only through ethnobotanical use (intentional or accidental) of herbs containing self-produced AAs. We hypothesized that the roots of some crops growing in fields where *Aristolochia* species grew over several seasons may take up certain amounts of AAs from the soil, and thus become a secondary source of food poisoning. To verify this possibility, maize plant (*Zea mays*) and cucumber (*Cucumis sativus*) were used as a model to substantiate the possible significance of naturally occurring AAs' root uptake in food chain contamination. This study

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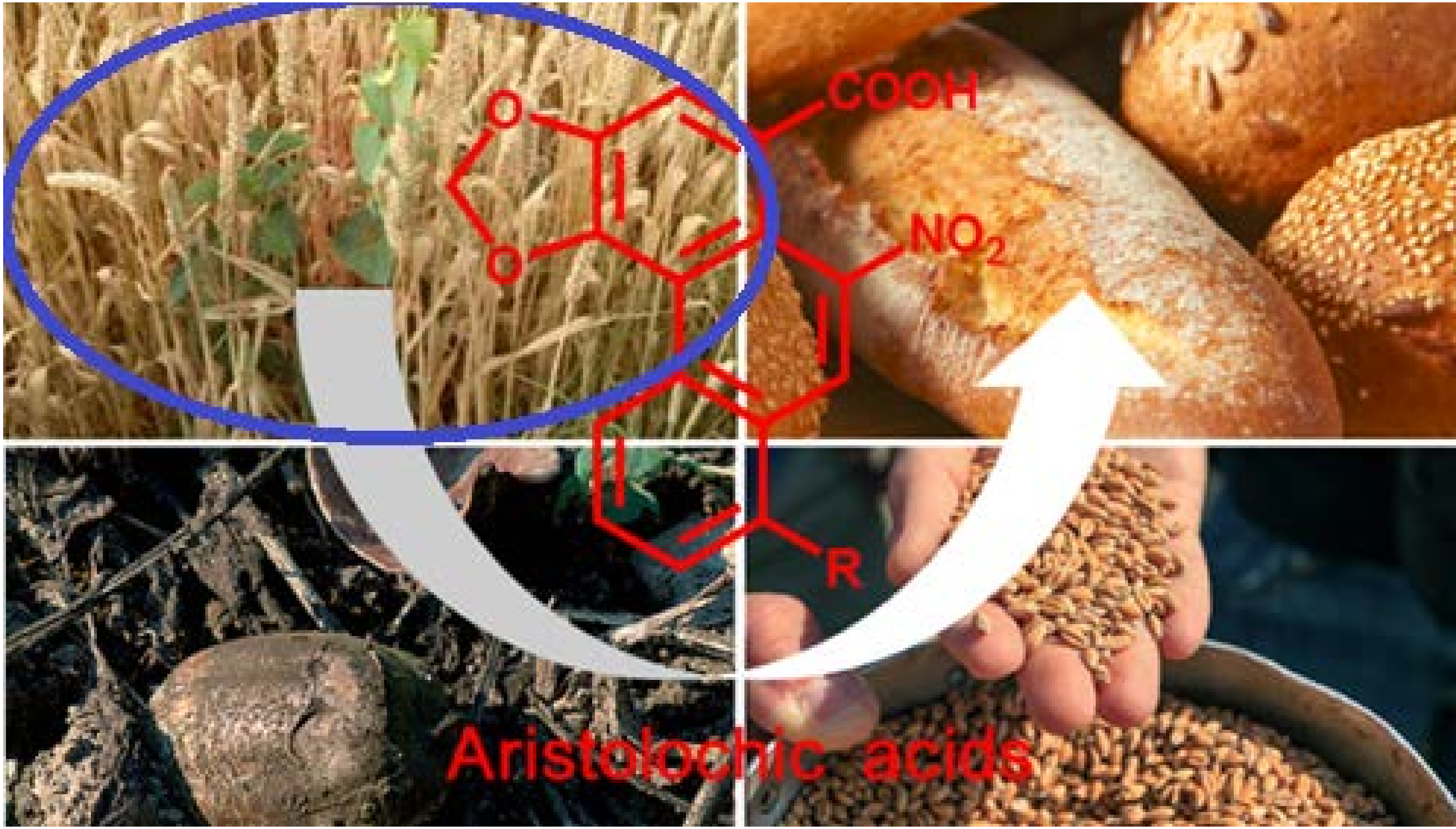

Fig. 1 The pathway from the wheat filed to the bread leading to covert chronic intoxication with AAs.

Research team of the China-Hong Kong-Serbia project: "The etiology and exposure pathways in the etiology of Aristolochic acid nephropathy (AAN) and/or Balkan endemic nephropathy (BEN)"

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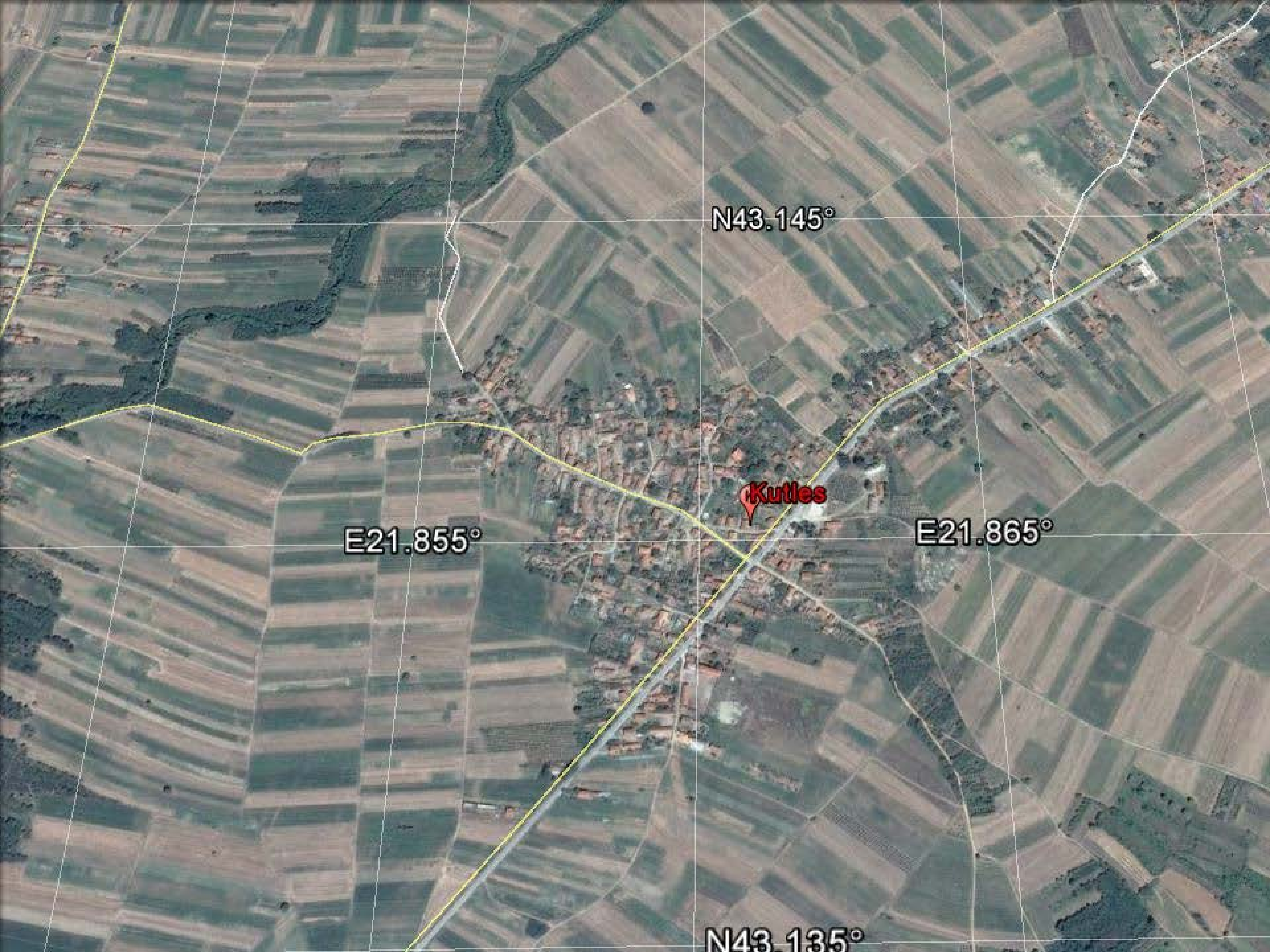
The goal of this study is to test the hypothesis that environmental pollution by AAs and root uptake of AAs in the polluted soil may be one of the major pathways by which AAs enter the human food chain.

The hypothesis driving this study was that the decay of Aristolochia Clematitis, an AA-containing herbaceous plant that is found growing widespread in the endemic regions, could release free AAs to the soil, which could be taken up by food crops growing nearby, thereby transferring this potent human nephrotoxin and carcinogen into their edible parts.

Using the highly sensitive and selective high-performance liquid chromatography coupled with fluorescence detection method, **we identified and quantitated in this study for the first time AAs in corn, wheat grain, and soil samples collected from the endemic village Kutles in Serbia.**

Our results provide **the first direct evidence that food crops and soil in the Balkans are contaminated with AAs**. It is possible that the presence of **AAs in edible parts of crops originating from the AA-contaminated soil could be one of the major pathways by which humans become exposed to AAs worldwide.**


N43.145°
Kutles
E21.855°
E21.865°
N43.135°

Concentrations of AA1 and AA2 in wheat and maize grain samples from endemic and non-endemic areas

	Food crop from endemic area				Food crop from non-endemic area			
	Wheat grain		Maize grain		Wheat grain		Maize grain	
	AA-I	AA-II	AA-I	AA-II	AA-I	AA-II	AA-I	AA-II
# of samples analyzed	80	80	80	80	/	/	55	55
# of positive identifications	58	34	35	19	/	/	5	0
concentration range, ng/g	11.2-642.3	12.7-102.4	15.8-336.6	16.3-141.7	/	/	10.9-17.0	/
Mean concentration, ng/g[c]	113.6	33.9	53.6	22.0	/	/	13.5	/
Average daily exposure, µg/day	56.0	16.7	4.4	1.8	/	/	1.1	/

Concentrations of AA1 and AA2 in soil samples from endemic and non-endemic areas.

	Soil samples from endemic area				**Soil samples from non-endemic area**			
	from wheat field		from maize field		from wheat field		from maize field	
	AA-I	AA-II	AA-I	AA-II	AA-I	AA-II	AA-I	AA-II
# of samples analyzed	80	80	80	80	55	55	55	55
# of positive identifications	49	45	26	19	4	2	2	0
concentration range, ng/g	12.7-775.5	12.5-218.1	11.8-510.4	19.7-250.1	11.1-36.4	115.0-17.2	12.4-17.3	/
Mean concentration, ng/g[c]	135.5	68.5	105.3	67.3	21.5	16.1	14.8	/

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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Quantitation of Aristolochic Acids in Corn, Wheat Grain, and Soil Samples Collected in Serbia: Identifying a Novel Exposure Pathway in the Etiology of Balkan Endemic Nephropathy

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Supporting Information

ABSTRACT: While to date investigations provided convincing evidence on the role of aristolochic acids (AAs) in the etiology of Balkan endemic nephropathy (BEN) and upper urothelial cancer (UUC), the exposure pathways by which AAs enter human bodies to cause BEN and UUC remain obscure. The goal of this study is to test the hypothesis that environmental pollution by AAs and root uptake of AAs in the polluted soil may be one of the pathways by which AAs enter the human food chain. The hypothesis driving this study was that the decay of *Aristolochia clematitis* L., a AA-containing herbaceous plant that is found growing widespread in the endemic regions, could release free AAs to the soil, which could be taken up by food crops growing nearby, thereby transferring this potent human nephrotoxin and carcinogen into their edible parts. Using the highly sensitive and selective high-performance liquid chromatography coupled with fluorescence detection method, we identified and quantitated in this study for the first time AAs in corn, wheat grain, and soil samples collected from the endemic village Kutles in Serbia. Our results provide the first direct evidence that food crops and soil in the Balkans are contaminated with AAs. It is possible that the presence of AAs in edible parts of crops originating from the AA-contaminated soil could be one of the major pathways by which humans become exposed to AAs.

KEYWORDS: *Balkan endemic nephropathy, upper urothelial cancer, aristolochic acid nephropathy, aristolochic acids, exposure pathways, root uptake*

INTRODUCTION

Aristolochic acids (AAs; Figure 1) are a group of nitrophenanthrene carboxylic acids found in the herbal genus *Aristolochia* and are classified by the International Agency for Research on Cancer (IARC) as a Group 1 carcinogen.[1−5] Although AAs are banned from use in many countries, misuse of AA-containing herbs occurs.[5−10] For example, the misuse of AA-containing herbs in the preparation of slimming drugs has caused numerous end-stage kidney diseases in Belgian women that participated in a slimming regime in the 1990s.[5,8−10] Accumulating data also indicate AAs as the causative agent of Balkan endemic nephropathy (BEN),[10−15] a chronic kidney disease affecting numerous farmers living in the rural area of the Balkan Peninsula.[16] It has been argued that the problem is caused by the commingling of the fruits of *Aristolochia clematitis* L. with commercial grains.[17] However, the pathways by which AAs enter the human food chain to cause BEN has remained obscure.[3,16,18]

Recently, it was disclosed that *A. clematitis* L., an AA-containing herbaceous plant, is widespread in some areas of the endemic region.[13,19,20] Despite the identification of AAs in the soil samples that has yet to be reported, it is postulated that AAs may have entered the environment from the decay of *A. clematitis* L. Furthermore, we discovered that food crops grown in AA-contaminated environments would uptake and accumulate AAs in the edible parts of the plant.[13,21] We thus proposed root uptake of AAs from the environment may be one of the exposure pathways by which the nephrotoxic AAs enter our food chain.

In this study, we try to confirm root uptake as one of the pathways by which AAs enter human bodies to cause BEN by analyzing soil, corn, and wheat grain samples collected from a well-known BEN-endemic village (Kutles; latitude, 43.140; longitude, 21.862) in Serbia. Using our recently developed high-performance liquid chromatography coupled with fluorescence detection (HPLC−FLD) method, we could detect aristolactams, the fluorescent nitroreduction products of AAs (Figure 1),[22] which allowed us to test for the presence of AAs in wheat grain and soil samples from where the wheat was grown. Further, we have conducted a surveillance analysis of AAs in wheat grain and corn seed samples collected by farmers

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JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Thomas Hofmann
Editor-in-Chief
The Journal of Agricultural and Food Chemistry

March 9, 2017

Dear Drs. Chan and Pavlović,

CONGRATULATIONS! Your published article entitled "Quantitation of Aristolochic Acids in Corn, Wheat Grain, and Soil Samples Collected in Serbia: Identifying a Novel Exposure Pathway in the Etiology of Balkan Endemic Nephropathy" was selected for the **2017 *Journal of Agricultural and Food Chemistry* Research Article of the Year Award** (AGRO division).

This award comes with the following honors:

1) An invitation by the *Journal of Agricultural and Food Chemistry* and the ACS Division of Agrochemistry (AGRO) for one person to give a lecture on this topic at the 254th ACS National Meeting, which takes place August 20-24, 2017 in Washington, DC.
2) A plaque and a cash award of $1,000.
3) Travel and lodging cost reimbursement of up to $1,250 by ACS Publications for one person to attend the meeting, and meeting registration coverage for one person by AGRO division.
4) The opportunity to have your article featured on the cover of the *Journal of Agricultural and Food Chemistry* in recognition of the Research Article of the Year Award.
5) An invitation to prepare a Perspective article for publication in the *Journal of Agricultural and Food Chemistry* that places the context of your research in the field.

A prerequisite of receiving this award is attending the 254th ACS National Meeting and giving a lecture on your research. If you or a co-author is unable to attend this meeting, unfortunately we will have to re-assign the award.

I hope that you will be able to accept this award. **Please let Dr. Ariel Grostern** (Managing Editor; a_grostern@acs.org) **know by March 13, 2017 if you will be able to attend the 254th ACS National Meeting and be presented with this award.**

Attached, please find the same e-mail in letter form for your records.

We look forward to hearing from you.

Sincerely,

Thomas Hofmann

Editor-in-Chief
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In light of the persistent widespread use of Aristolochia herbal remedies in traditional Chinese medicine and recently published data that some crops can take up AA from the soil, one can accept that AA could be responsible for a previously and currently widespread unrecognized global renal disease and UUC

It is now clear that BEN represents a form of AAN, from which it is pathologically indistinguishable and we can with highest confidence apply the equation: CHN=AAN=BEN

Prevention of exposure to AA is a key public health priority

Prevention of exposure to AA in parts of the Balkans where BEN is prevalent, and elsewhere has not received sufficient attention since establishment of the etiologic relationship between AA and the disease

There is an urgent need for research addressing many key areas:

a. Determining the true worldwide extent of exposure

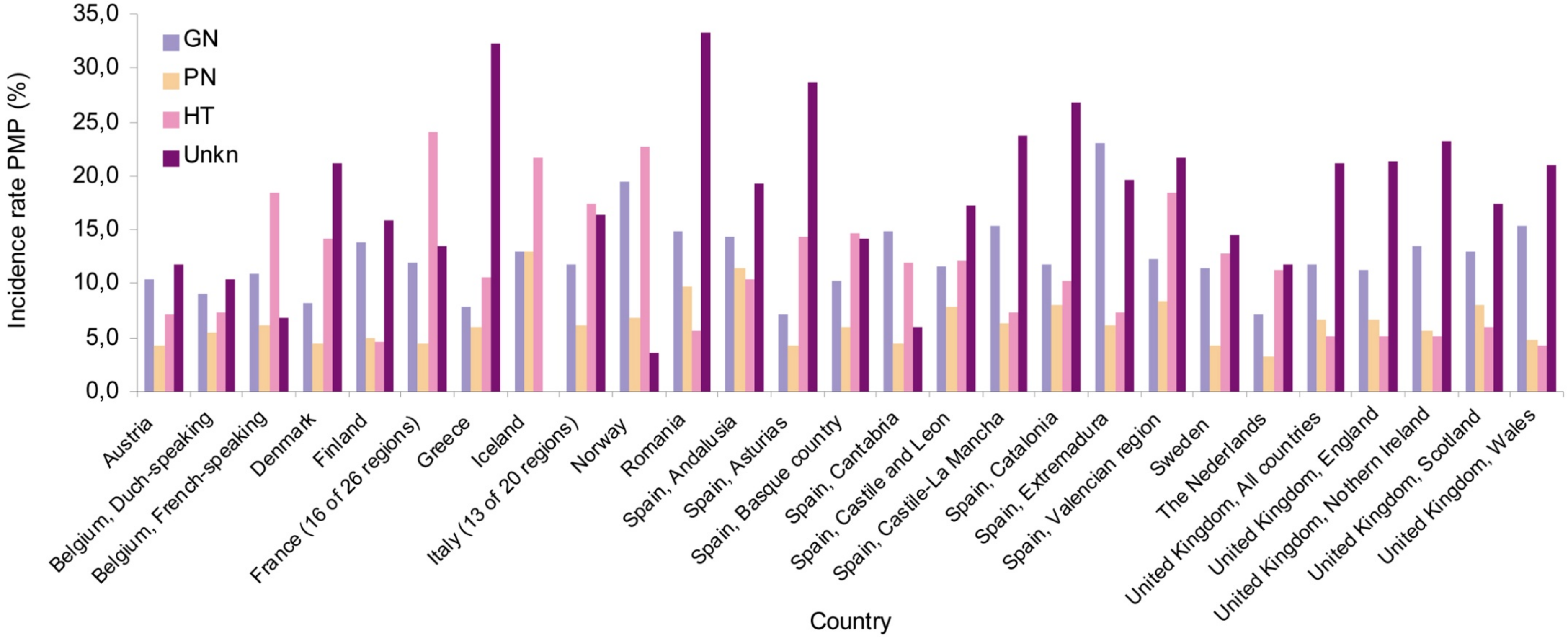
b. Defining of genetic variants that might be responsible for increased sensitivity or resistance to the nephrotoxic effects of AA

c. Testing the accuracy and usefulness of diagnostic criteria and finding the optimal screening protocols

d. Developing therapeutic agents that can reverse or delay progression of the disease.

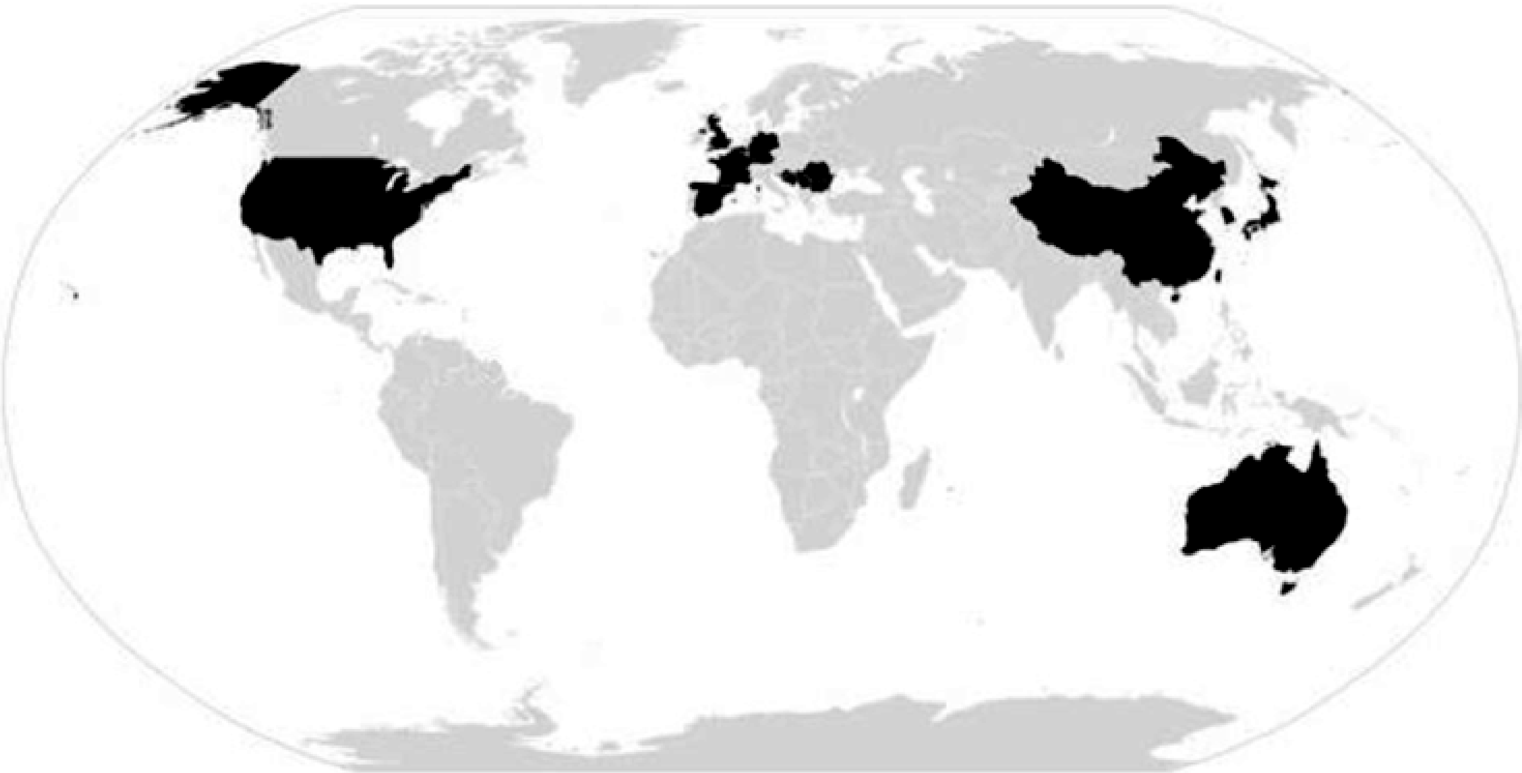
Incident rates per million population, unadjusted

at day 1, by cause of renal failure



EUROPEAN RENAL ASSOCIATION ERA-EDTA Registry. Annual Report 2008.

World map showing the epidemiology of AAN or BEN



Countries in which cases of AAN or BEN have been reported in the literature are highlighted. It is likely that the true worldwide distribution of the diseases extends beyond the countries highlighted, especially in the Far East and South Asia.

Gökmen MR, Cosyns JP, Arlt VM, et al. The epidemiology, diagnosis, and management of aristolochic Acid nephropathy: a narrative review. Ann Intern Med. 2013; 158(6): 469–477.