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In-depth Review

Balkan endemic nephropathy—current status and future perspectives

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Abstract

Balkan endemic nephropathy (BEN), originally described in 1956, is a unique familial, chronic renal disease encountered with a high-prevalence rate in Serbia, Bulgaria, Romania, Croatia and Bosnia and Herzegovina. The most prominent features of the disease are its endemic nature, long-incubation period, familial clustering of the disease and an unusually high incidence of associated upper urothelial cancer (UUC). There are no clear-cut data on BEN incidence and prevalence, since the studies carried out in different endemic areas yielded contradictory information. In spite of intermittent variations, the incidence of new cases has remained stable over time. It has been estimated that almost 100 000 people are at risk of BEN, whereas 25 000 have the disease. The clinical signs and symptoms of BEN are non-specific and often remain unrecognized for years. There are no pathognomonic diagnostic features of BEN, but the set of epidemiological, clinical and biochemical data along with the pattern of pathologic injury in the absence of any other renal diseases are highly suggestive of this entity. 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 poisoning with aristolochic acids (AAs). In light of these later studies, one can raise the question whether AAs could be responsible for previously and currently widespread unrecognized global renal disease and UUC.

Keywords: aristolochic acid; Balkan endemic nephropathy; aetiology; hypothesis; urothelial cancer

Introduction

Balkan endemic nephropathy (BEN) is a familial, slowly progressive, chronic renal disease with insidious onset in the fifth decade of life and terminal renal failure in the sixth or seventh decade. The occurrence of BEN has been recorded with a high prevalence rate in Serbia, Bulgaria, Romania, Bosnia and Herzegovina and Croatia (Figure 1). The first cases were described in 1956 in Bulgaria, where Tanchev *et al*. [1] published the first detailed clinical description of the new entity. A year later, a renal disease with almost identical clinical and epidemiological characteristics was reported in Yugoslavia (Serbia) [2]. In 1961, it was found that a similar nephropathy was also prevalent in Romania [3]. The most prominent features of the disease are its endemic nature, the long latent period before development of the disease, familial clustering of the disease and the remarkably high incidence of upper urothelial cancer (UUC) associated with BEN or occurring in the population at risk [4–6]. No cases have been documented in children or adolescents. The aetiology of BEN has been the subject of many published studies resulting in the generation of several hypotheses. Although data published on these various hypotheses have presented some indication of their relevance to the aetiology of BEN, only one of them, dealing with chronic aristolochic acid (AA) poisoning, has provided conclusive evidence related to the aetiology of BEN and its clinical characteristics.

Epidemiology

There are multiple challenges complicating the study of the epidemiology of BEN. Data on the incidence of BEN have been controversial. There are no clear-cut data on the current trend for the incidence and prevalence of BEN. The studies carried out in different endemic areas have produced conflicting information. 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 [7, 8]. Similarly, in another endemic area, a decreasing incidence over time was found during a follow-up period spanning the time period 1978 to 1997 [9].

Assessments are frequently based on the number of BEN patients undergoing haemodialysis treatment. In Serbia, BEN patients represent an average of 6.5% (5–46%) of the haemodialysis population. The high

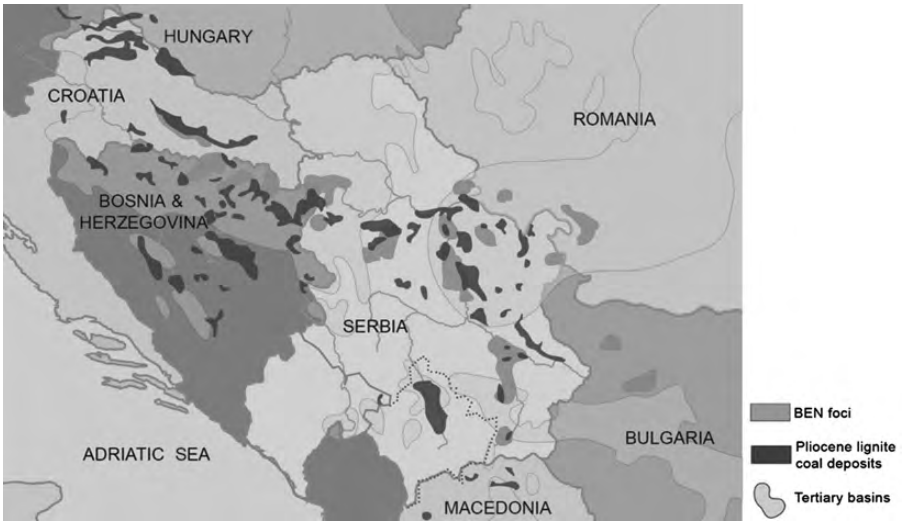

Fig. 1. Geographical distribution of endemic foci and pliocene lignite coal deposits.

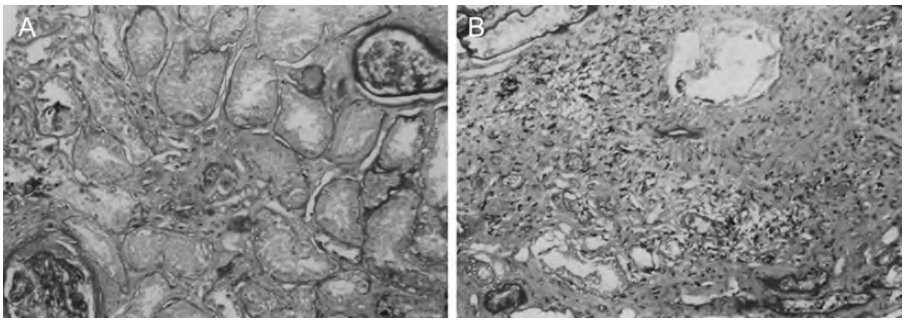

Fig. 2. (**A**) Advanced glomerular sclerosis (initial obsolescence), with interstitial sclerosis and tubular atrophy (PAS, ×250); (**B**) Focal tubular atrophy with prominent interstitial sclerosis and clearly delineated mononuclear infiltrate (PAS, ×250) [R. Čukuranović. Genetic and morphophysiologic study of BEN. Doctoral Thesis. Medical Faculty, University of Niš, 1–169, 1992 (in Serbian)].

prevalence rate of haemodialysed patients with BEN indicates that BEN is not disappearing [10].

It has been estimated that almost 100 000 people are at risk of BEN, whereas 25 000 have the disease. Despite intermittent variations, the incidence of new cases has remained stable over time [11]. These differences may also be related to changes in the study design or true epidemiological differences between sequential time frames and endemic areas and can also be attributed to the natural course of the disease [7].

Clinical features

The clinical signs and symptoms of BEN are non-specific and often remain unrecognized for years [12]. The initial asymptomatic period is followed by weakness and lassitude, mild lumbar pain, pallor of the skin and a copper-brownish discoloration of the palms and soles. At this stage, which occurs at an older age [13, 14], anaemia is associated with a significant loss of renal function and indicates the presence of chronic kidney disease (CKD). Blood pressure is usually normal, although in the advanced phase it may be elevated but at a lower incidence than in other forms of CKD. Intermittent proteinuria of the tubular type may be found early while in the uraemic phase, it becomes permanent. The urinary sediment shows sparse white and red blood cells. In the early stages of BEN, the loss of urine concentration capacity precedes the decline in the glomerular filtration rate [15–17]. Serum proteins and immunoglobulins are unchanged.

Kidney imaging reveals a variable decrease in kidney size with very small contracted kidneys in the end stage [18]. Extreme kidney atrophy has been proposed as a criterion for the clinical diagnosis of BEN. Some studies suggest that the smallest kidneys are found in advanced stages of BEN, whereas others have reported that this occurs in the earlier stages. In BEN patients, it has been found that the dimensions of the kidneys depend on the parental status and the age of offspring. In the offspring of a mother with BEN, ultrasound measurements of the kidney cortex thickness seem to portend a prognostic value [19]. In conclusion, adult offspring in BEN families can be characterized by shorter kidney length and an increased excretion of albumin, total protein and β2-microglobulin, in particular, when the mother had BEN [19, 20].

Pathology

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 (Figure 2).

The consistent renal histological findings characterizing BEN are extensive hypocellular interstitial fibrosis associated with tubular atrophy involving medullary rays, the attending outer medulla and the cortical labyrinth, where it decreases typically from the outer to the inner cortex.

Glomerular and vascular lesions are associated with periglomerular fibrosis, glomerular lesions, including ischaemic, microcystic, obsolescent glomeruli, occasional thrombotic microangiopathy-like lesions and focal segmental sclerosis-like lesions. Vascular lesions include arteriolar hyalinosis, intimal fibrous hyperplasia, occasional mucoid arterial intimal fibrosis and multifocal thickening and splitting of peritubular capillary basement membranes detected by electron microscopy [21, 22].

Diagnostic criteria

There are no diagnostic features which could be marked as pathognomonic of BEN but the set of epidemiological, clinical and biochemical data along with the pattern of pathologic injury, in the absence of any other renal disease, is highly suggestive of this entity. Initially, the criteria proposed by Danilovic *et al*. were widely used [23]. Over time, these criteria evolved and contributed to a better diagnosis of BEN [24–26].

Updated recommendations [27] developed during the 'International Workshop on the Diagnostic Criteria in BEN', held in Brač, Croatia, in 2008, and at a meeting organized in 2012 (Skopje, FYR of Macedonia) aimed at providing recommendations for the screening, diagnosis and therapy of patients with BEN [21]. The consensus statement is intended to assist scientists in speaking the same 'diagnostic language', thus enabling the comparison of results obtained in different countries.

Review of various aetiological hypotheses

All hypotheses related to BEN can be classified into three main groups (Table 1).

Exogenous factors

Lead intoxication. In one of the first original papers on BEN published by Danilovic *et al*., lead was incriminated as the causative agent of BEN, having been found in the flour used for baking bread in the affected villages [2]. However, this hypothesis was not substantiated by later studies.

Table 1. Various aetiological hypotheses related to BEN classified into three main groups

Exogenous factors	Endogenous factors
(i) Lead intoxication	(i) Genetic predisposition
(ii) Metals and metalloids	(ii) Changes in enzyme activity—LCAT deficiency
(iii) Intoxication with *A. clematitis*	(iii) Genetic polymorphism
(iv) Ochratoxin A	(iv) Chromosomal aberrations
(v) Pliocene lignite	(v) Viral disease
	(vi) Immunological factors
Miscellaneous—multifactors	
(i) Lecithin cholesterol acyl transferase and organic substances from coal	

Metals and metalloids. Several publications have suggested the possibility that a deficiency of some essential trace elements, e.g. selenium, might be involved in the aetiology of BEN [28]. The concentrations and the extent of selenium deficiency are well documented in rocks, soil, water, food stuffs and in serum samples collected from endemic and non-endemic regions of Serbia [29]. However, no association between selenium deficiency and a high incidence of BEN and UUC in endemic areas was confirmed. The findings of a systematic 2-year follow-up study indicated that metals (cadmium and lead) and metalloids (arsenic and selenium) do not play a role in the aetiology of BEN [30].

Chronic intoxication with Aristolochia clematitis. *Aristolochia clematitis* is one of the old healing plants already in use by the ancient Egyptians and Greeks (Figure 3). The Greek name 'Aristolochia' is a compound word made up of áristos—fair, beautiful, and lochia—menstrual periods. It refers to the use of the plant in aiding childbirth and was prescribed by the Greeks to women following delivery to facilitate a resuming of their periods. The species name, '*Clematitis*' derives from the Greek 'klema' for tendril, the growth form of this species of *Aristolochia*. The English name 'birthwort' likewise refers to the plant's use as an aid to birth [31]. Although herbal drugs derived from *Aristolochia* spp. have been known since antiquity and were used in obstetrics and in the treatment of snake bites (Rosenmund and Reichstein, 1943) [32] in 1981, the use of *Aristolochia* spp. was forbidden in many countries due to possible carcinogenic effects. Peters and Hedwall in 1962 indicated that AA was known to be nephrotoxic in the rabbit as early as in 1892, in the horse in 1893 and in the rabbit and the mouse in 1958 [33].

In 1969, Ivić proposed that the aetiology of BEN could be related to chronic *A. clematitis* poisoning in which seeds from these plants, which are encountered abundantly in local wheat fields, intermingle with wheat grain during the harvesting process [34]. He speculated that human exposure to a toxic component of *Aristolochia* might occur through ingestion of bread prepared from flour derived from contaminated grain. He demonstrated in rabbit models that flour prepared from *A. clematitis* seeds induced a nephropathy, which resembled the findings in BEN. Ivić even proved the carcinogenetic potential of the plant as rats developed sarcomas of the skin at the site of injection of aqueous extracts of *A. clematitis* [35]. Ivić concluded that on the basis of geographical, epidemiological and laboratory investigations, there are strong arguments suggesting that endemic nephropathy is essentially caused by chronic poisoning with the seeds of *A. clematitis* [34]. Although his classic paper presented the brilliant examples of drawing correct conclusions regarding the aetiology of a complex human disease from simple but well thought out experimental observations, this clever hypothesis has never been adequately pursued. His well-documented results attracted more interest from the scientific community many years later.

The ever-growing interest in unravelling the mystery of BEN and the role of AA was prompted by reports of a high incidence of CKD that developed in a group of otherwise healthy Belgian women [36]. In 1990, a clinic in Brussels began prescribing capsules as part of a slimming regimen

Fig. 3. Post harvests second generation *Aristolochia Clematitis* growing in the wheat field (**A**), with ripe seeds in the soil (**B**) and wheat grain from that field (**C**) (hyperendemic village Petka, Serbia, 2011).

consisting of Chinese herbal remedies believed to contain, in part, *Stephania tetrandra*. Unintentionally, *S. tetrandra* (in Mandarin Han Fang-Ji) was replaced by *Aristolochia fangchi* (Guang Fang-Ji in Mandarin) since both of the plants are used in Chinese traditional medicine carrying similar names (*Fang-Ji)* [37, 38]. The Oriental Materia Medica [39] specifies that the Chinese prescribe *A. fangchi*, *Aristolochia heterophylla, Cocculus trilobus* or *S. tetrandra* indiscriminately, obviously not taking into account the possible presence of AA.

The observed nephropathy has been ascribed to the ingestion of Chinese herbal remedies that have included species of the genus *Aristolochia* which were positively classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC) [40]. The outbreak of the so-called Chinese herb nephropathy (CHN) in Belgium in 1993 affected more than 100 patients, mostly women, half of them requiring renal replacement therapy [41, 42]. It was found that, in the majority of cases, progression to end-stage renal disease occurred despite discontinuation of Chinese herbal remedies [38], and invasive urothelial carcinoma after exposure to Chinese herbal remedies containing AAs may occur even without severe renal failure [43].

The CHN reported in Belgium in 1993 presented as a rapidly progressive renal interstitial fibrosis leading to end-stage renal disease [44]. Within a few years, it emerged that CHN patients developed a high risk of UUC. Urothelial malignancy of the upper urinary tract developed in almost half of the patients [42, 45]. Later, it was found that even patients who did not display the characteristic histological features of CHN were also exposed to a high risk of UUC [46]. 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) [47, 48].

Following these publications, some research groups conducted pioneering studies on the molecular mechanism of AA-induced carcinogenesis [49, 50]. Later, methods developed in the course of this research were applied to the identification of AA–DNA adducts in tissues of Belgian women with CHN [51].

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 by the method of ^{32}P-post-labelling in urothelial tissue of these patients [42, 52–54]. AAs I and II are the most abundant of the AAs and are found in almost all *Aristolochia* species [43]. Once established, AA–DNA adducts persist for years in the renal cortex, serving as reliable biomarkers of exposure to AA [42]. Arlt *et al*. were the first to prove that this hypothesis is correct by detecting specific AA–DNA adducts in all urinary tract tissues from patients with CHN that they examined [55], as well as in invasive liver metastases and various human tissues outside the urinary tract [47].

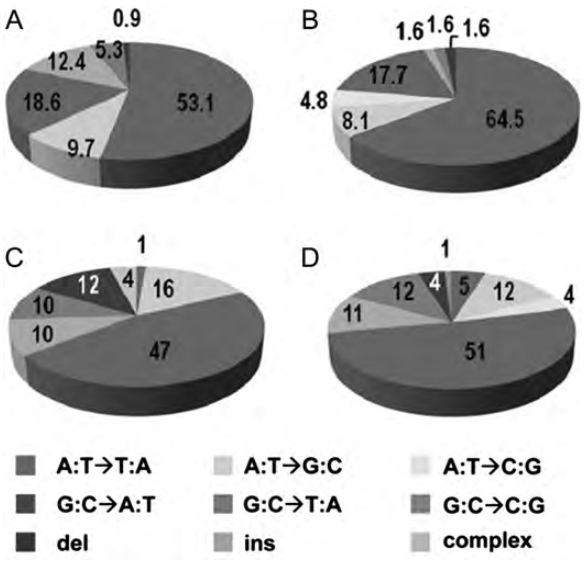

Fig. 4. TP53 mutational spectra in urothelial carcinomas. (**A**) TP53 mutations in DNA obtained from UUC in endemic regions of Bosnia, Croatia and Serbia (62 mutations); (**B**) TP53 mutations in DNA obtained from UUC in Taiwan (113 mutations); (**C**) TP53 mutations in urothelial carcinomas of the 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)[61].

Cosyns first raised awareness of the unique renal histopathology of CHN with its striking similarity to BEN [56].

These fascinating reports renewed scientific interest and inspired new research that produced unequivocal evidence supporting the role of AA in the pathogenesis of BEN and UUC.

The similarities between CHN and BEN have led to the hypothesis of a common aetiological agent for both diseases. This hypothesis implies that BEN, CHN and AAN are the same disease [57] and that dietary ingestion of AA, in conjunction with individual genetic susceptibility, accounts for all epidemiological, clinical and pathophysiologic features of BEN and associated UUC [58].

A recent publication by Grollman *et al*. [59, 60] presented results showing that the accumulation of AA–DNA adducts was present in the renal cortex and upper urinary tract of five patients with BEN from an endemic region in Croatia, but not in five patients with other forms of chronic renal disease or five patients with upper urinary tract transitional cell cancer living in a non-endemic area of Croatia. A study of the incidence of UUC and urinary bladder tumours (UBTs) associated with BEN in the 30-year follow-up revealed a total of 575 urothelial neoplasms during the

10-year period, 1989–1998, compared with 659 in the period 1969–1988. Remarkably, UUC had an 11.2-fold higher incidence in endemic than in non-endemic areas. However, this was far less than in the period 1969–1988, when UUC was 57.1 times more frequent. UBT were 2.3 times more frequent in endemic than in non-endemic areas, but 11.9 times more frequent in the previous period 1969–1988 [61]. It seems that the frequency of neoplasms is decreasing from the upper towards the lower urothelium.

Immunosuppression in BEN transplant patients comprises an even greater increased risk of developing UUC requiring nephroureterectomy. Thus, 33.3% BEN of patients develop UUC, compared with 0.67% affected transplanted patients with other causes of CKD [62].

Cytological screening procedures of UUC in AAN using fresh voided urine or cystoscopy with bilateral ureteral washing and brushing characterize the finding of giant multinucleated cells and/or nucleolar atypias [63]. According to these results, the specificity of cytological analysis on voided urine and brushing samples is 100 and 96%, respectively, and the sensitivity 17 and 54%, respectively.

The finding by Grollman *et al.* of AA-derived DNA adducts in renal cortical and urothelial tumour tissue of patients with documented BEN, associated with the dominance of the A:T → T:A transversions in the p53 tumour suppressor gene mutational spectrum, was a breakthrough in the identification of AA as an aetiological agent of the upper tract malignancy observed in BEN [64]. The TP53 mutation spectrum in AA-induced UUC displays an unusual pattern that is readily distinguished from all other spectra that have emerged to date from among the 27 000 tumour mutations in the IARC TP53 database. 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 [49], are similarly found in Taiwanese patients with UUC (Figure 4).

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 raise the question whether AA could be responsible for a previously and currently widespread unrecognized global renal disease and UUC [65].

Ochratoxin A. The finding that porcine nephropathy has many characteristics in common with BEN led some researchers to propose that ochratoxin A (OTA)-induced renal disease and urothelial tumours in humans are highly similar. It was one of the first well-elaborated hypotheses regarding the aetiology and pathogenesis of BEN that appeared in the literature in the early 1970s. Akhmeteli and later Krogh proposed that BEN was the result of contamination of the food chain in endemic areas by OTA [66].

Numerous surveys conducted in North America, Asia and Europe have revealed that OTA, a toxic product of molds that belong to the *Aspergillus* or *Penicillium* fungal genera, is a natural contaminant of plant products. Contamination frequencies of up to 40% have been encountered, at levels in the range of 5–500 μg/kg. OTA is a significant causal determinant of porcine nephropathy. Renal lesions in porcine nephropathy include degeneration of the proximal tubules, interstitial fibrosis and hyalinization of the glomeruli. The disease is endemic, and outbreaks have been associated with weather conditions [67].

OTA is considered to be a human carcinogen, an opinion based on sufficient evidence of carcinogenicity in experimental animals. Detection and quantification of DNA adducts related to OTA in human kidney tissues and urothelial tumours of BEN patients from Bulgaria, Croatia and Serbia were the subject of many studies, aimed at finding the molecular evidence for OTA role in the aetiology of BEN. Extensive field research analysing the OTA in foods consumed by inhabitants from the area with BEN aimed at providing results confirming the high exposure of this population to OTA, and thus strengthening the hypothesis of the involvement of this mycotoxin in BEN aetiology, has been performed. Although several authors published data on new molecular and field [68] evidence for the implication of mycotoxin OTA in the aetiology of human nephropathy and urinary tract tumours [55, 68], no adequate human studies of the relationship between exposure to OTA, the human renal disease and cancer have been reported.

Analysis of tissue specimens from CHN for the presence of DNA adducts related to both OTA and AA exposure revealed that AA-specific DNA adducts were detectable in all five urinary tract tissues from five patients, whereas OTA-related DNA adducts were detectable in two kidneys and only one ureter. Furthermore, OTA-related DNA adduct levels were ∼50 times lower than AA–DNA adduct levels. In female and male rats treated with the same slimming regimen as the CHN patients, but with a 10-times higher level of Chinese herbs, AA–DNA adducts were found in kidney tissues but adducts derived from OTA were not observed. These results demonstrate that presumably OTA-related DNA adducts do not play a key role in CHN or CHN-associated UUC [52, 55] and consequently in BEN and associated UUC.

Pliocene lignite. This hypothesis was proposed in 1991 by scientists from the US Geological Survey [69, 70] based on the geographical matching between the location of Pliocene lignite deposits in the Balkans and the location of endemic areas (Figure 1), as well as preliminary geochemical analyses of well water from villages located in endemic areas of former Yugoslavia, which showed the presence of toxic organic compounds not observed in well water from non-endemic villages [71, 72].

The lignite hypothesis is based on the assumption that toxic organic compounds in lignite, or in weathered lignite, may be released by groundwater and thus contaminates drinking water wells. Although the concentrations of these organic molecules in well water may be low, long exposure and/or accumulation in body tissues over time may lead to kidney lesions. The development of UUC in some individuals can also be explained by this hypothesis because most of these toxic organics are well-known carcinogenic factors [73, 74].

This hypothesis emphasizes the role of environmental factors involved in the aetiology of BEN, accounts for the peculiar geographical restriction of BEN and provides a basis for investigating the possible wider impact of coal-derived toxic organic compounds in groundwater on human health [75].

Endogenous factors

Genetic predisposition. The familial clustering of the disease was indicative of the role of a hereditary

predisposition in the aetiology of BEN and prompted many genetic investigations. The hypothesis implicating the multifactorial nature of the BEN aetiology assumes that genetic factors create a predisposition to BEN [76]. The combined action of genetic and environmental factors may result in the development and determination of clinical and epidemiological characteristics and the progression of the disease.

This hypothesis was substantiated by family investigations in Bulgarian patients with BEN. A study by Toncheva *et al*. in 4077 subjects from 417 families affected with BEN led to the conclusion that all patients with BEN belong to certain families [77]. Even residents from non-endemic villages diagnosed to have BEN were found to be members of BEN families that had migrated from their birthplaces. Furthermore, some epidemiological characteristics of BEN are indicative of the involvement of genetic disorders, i.e. the proportion of the affected offspring increases in accordance with the number of parents affected. Consequently, the risk of developing BEN is much higher in first-degree than second-degree relatives and decreases substantially in remote relatives [77].

Changes in enzyme activity. Norum and Gjone proposed in 1967 a familial deficiency of lecithin-cholesterol acyltransferase (LCAT) as a primary disorder. They showed that familial renal disease can develop secondarily to LCAT deficiency and lipid abnormalities [78]. Familial LCAT deficiency is an autosomal recessive disorder.

LCAT deficiency is associated with the percentage increase of free cholesterol followed by the proportional decrease of esterified cholesterol and abnormalities in the structure of lipoprotein particles. As a consequence of lipid disorders, other organs can also be involved, such as the kidneys, cornea and erythrocytes, with the clinical manifestations of proteinuria, usually associated with renal insufficiency, corneal opacities and haemolytic anaemia. The gene encoding LCAT is localized in region q 21–22 of chromosome 16 which consists of six exons. In LCAT-deficient patients, several mutations in all six exons have been described. Biochemical and clinical manifestations of familial LCAT deficiency are highly variable. The finding of no or extremely low LCAT activity in affected patients suggests that expression of the disease is modulated by additional environmental factors and genes of minor importance [79, 80].

A study by Pavlovic in 1991 showed that a certain proportion of healthy subjects from BEN families had a peculiar form of lipid abnormalities associated with an abnormal LCAT activity, and a possible association between these abnormalities and the aetiology of BEN was raised for the first time. The contribution of genetic and/or environmental factors to these abnormalities in BEN has not yet been elucidated [81].

Genetic polymorphism. The hypothesis of a multifactorial aetiology of BEN anticipates that an interaction of polymorphic gene variants and various environmental factors causes an increased risk of renal disease and cancer [77]. Xenobiotic metabolizing enzymes are known to play a role in the metabolic activation of environmental mutagens and carcinogens to exert their carcinogenic effects as well as detoxification by increasing their hydrophilia [82]. It has been shown that genetic variants of these enzymes involved in the uptake, conversion and excretion of xenobiotics determine individual levels of detoxification and are modifiers of an increased/decreased risk of chronic diseases and/or cancer [83, 84].

Extensive studies conducted in experimental settings and in BEN patients have investigated the role of several genetic polymorphisms in a number of enzymes (CYP2D6, CYP3A4, CYP3A5, NQO1, GSTT1, GSTM1, GSTP1, NAT1 and NAT2) from a detoxification system [85–87].

Some studies presented results demonstrating that the CYP3A5*1 allele, previously reported as a marker for CYP3A5 expression in the human kidney, is associated with increased risk of BEN, while CYP3A4*1B and CYP2D6 genotypes do not significantly modify the risk for the disease [85]. It has also been found that the GSTM1 wt allele associates with BEN. The significantly lower prevalence of the GSTM1 deletion homozygotes among BEN patients suggests that individuals bearing the GSTM1-null genotype could be better protected [86]. Additionally, some results established that alleles NQO1*2 and NQO1*3, as well as lack of GSTT1 and GSTM1, did not influence the BEN risk [87].

Chromosomal aberrations. It was hypothesized that the occurrence and frequent association of BEN and cancer can be explained by the chromosomal hypothesis of oncogenesis [88]. The first cytogenetic investigation in healthy relatives of patients with BEN born in non-endemic areas was done in 1996. Characteristics of BEN No. 3 chromosomal anomalies in terms of extremely high frequencies of 3q25 homologue discordance, chromosome breaks at the 3q25 band, structural aberrations affecting the 3q25 band, unusually high frequency of acquired chromosomal aberrations and a family history with 1 or 2 BEN parents were identified in five relatives. It is proposed that they are at a high risk for developing the disease and that a genetic mechanism might be involved in the aetiology of BEN [76]. The additional finding of a specific chromosome marker 3q in BEN was reported, characterized by a discordance in the banding patterns of the long arms, shortening band 3q25, faster fusion of sub-bands q26.1 and q26.3 and lack of differentiation of q24 [89].

Viral disease. In 1975, some evidence of a viral involvement in the aetiology of BEN was published, suggesting that a slow coronavirus infection causes BEN in humans [90]. Data substantiating this hypothesis remained unconvincing. It was more recently documented when some authors were able to detect a novel corona virus, using primary kidney tissue cultures established as explants from tissue obtained at operations from five BEN patients with urinary tract tumours. Four out of the five biopsy specimens on extended culture yielded a coronavirus virus which was cytopathogenic for human fibroblast and Vero cells, but none from the control cultures. It was proposed that the microorganism was a novel coronavirus based on its cross-reactivity with the human coronaviruses OC43 and 229E, as well as a porcine-transmissible gastroenteritis virus [91].

Nonetheless, the coronavirus hypothesis was challenged when Vero cells infected with the BEN virus were additionally tested with various methods, including electron microscopy studies. It was found that the only virus

detected in the cell cultures infected with the BEN-associated virus was unrelated to coronaviruses, since coronaviruses caused a cytopathic effect on infected cells differed from those observed after infection by the BEN-associated virus. Importantly, they highlighted that the involvement of a coronavirus should no longer be considered in BEN induction. Further studies are needed to clarify the nature of the 28.4 nm, non-enveloped virus particles found in the kidney cells of patients with BEN and to determine whether this virus is the causal agent of the disease [92].

Immunological changes. This hypothesis supports the presence of an inflammatory pathway in BEN through the involvement of polymorphic enhancer hs1.2 influencing different binding complexes and consequently the 3D structure of the 3′ regulatory region of IgH. This study is the first that clearly links BEN to a gene involved in the regulation of immune response [93].

Miscellaneous multifactorial aetiologies

Pavlovic *et al*. [94] tested for the first time the possible simultaneous role of coal-derived toxic organic compounds and decreased enzyme LCAT activity in the aetiology of BEN, using water concentrates from both endemic and non-endemic areas. It has been shown that well water from BEN villages contains higher numbers and concentrations of both extractable and high-molecular weight organic compounds compared with controls. The authors presumed that in this study, organic compounds contributed to higher LCAT-inhibiting activity of Serbian and Romanian drinking water samples from both non-BEN and BEN villages. On the other hand, the higher abundance of organic compounds in drinking water samples from BEN villages caused much higher LCAT-inhibiting activity compared with non-BEN ones. This finding indicates that there is presumably a clear-cut distinction between drinking water qualities coming from those two localities.

On the basis of these results showing higher organic compound LCAT-inhibiting activity, the authors proposed the concept of cause and effect events in the pathogenesis of BEN. Thus, the organic compounds in drinking water from BEN villages inhibit LCAT activity, which may cause plasma lipid abnormalities and consequent changes of intracellular and cellular membrane lipid composition [95] that can trigger the pathogenic mechanisms responsible for the development of BEN.

Conclusions

Based on all of these previously published hypotheses, we think that a multifactorial aetiology is the best match with regard to BEN pathophysiology, with a genetic predisposition being the ‘condicio sine qua non’ for the development of BEN. Accepting the concept of a multifactorial set of causes seems to be a valid hypothesis, as this would help explain why the BEN aetiology has been so difficult to decipher for >50 years. It seems that this multifactorial character of BEN aetiology is associated with the multilevel distribution of causative agents within a pathogenetic chain of events. A significant proportion of the pathogenetic chain of events does not only take place in the renal and upper urothelial tissue as target organs. It is likely that the intrinsic environmental aetiological factor never reaches kidney structures in its original form but only after being metabolized in the liver.

The genetic predisposition is presumably responsible for providing the key circumstances for imposing the action of other environmental and endogenous aetiological factors. Further work aimed at exploring the contribution of factors mentioned in some hypotheses to a multifactorial aetiology and occurrence of BEN is necessary to determine the role of the environment in the aetiology of BEN and UUC.

In light of the worldwide distribution of *Aristolochia* spp. and the widespread use of herbal remedies in traditional Chinese medicine, as well as recently published data that some crops can take up AA from the soil, there is a possibility that diseases similar to BEN and UUC exist elsewhere as unrecognized disease entities.

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References

1. Tanchev Y, Evstatiev Z, Dorossiev D *et al*. Studies on the nephritides in the District of Vratza. *Savremena Medicina* 1956; 7: 14–29
2. Danilovic V, Djurisic M, Mokranjac M *et al*. Chronic nephritis due to lead poisoning by digestive route (flour). *Presse Med* 1957; 65: 2039–2040 (in French)
3. Fortza N, Negoescu M. Nefrita cronica azotemia endo-epidemica. *Stud Cercet Med* 1961; 1: 217–221
4. Stefanovic V, Polenakovic M, Toncheva D. Urothelial carcinoma associated with Balkan endemic nephropathy. A worldwide disease. *Pathol Biol (Paris)* 2011; 59: 286–291
5. Dinev I, Dimitrov TS, Doytchinov D. Clinical study of tumors in Balkan endemic nephropathy (BEN). Current research in endemic (Balkan) nephropathy, Proceedings of the 5th symposium on endemic (Balkan) nephropathy. *Nis* 1983; 5: 253–256
6. Radovanović Z, Janković S, Jevremović I. Incidence of tumors of urinary organs in a focus of Balkan endemic nephropathy. *Kidney Int* 1991; 34: S75–S76
7. Dimitrov PS, Simeonov VA, Ganev VS *et al*. Is the incidence of Balkan endemic nephropathy decreasing? *Pathol Biol (Paris)* 2002; 50: 38–41
8. Dimitrov PS, Simeonov VA, Stein AD. Balkan endemic nephropathy in Vratza, Bulgaria, 1964–1987: an epidemiologic analysis of population-based disease registers. *Eur J Epidemiol* 2001; 17: 847–853
9. Cukuranovic R, Petrovic B, Cukuranovic Z *et al*. Balkan endemic nephropathy: A decreasing incidence of the disease. *Pathol Biol (Paris)* 2000; 48: 558–561
10. Janković S, Bukvić D, Marinković J *et al*. Time trends in Balkan endemic nephropathy incidence in the most affected region in Serbia, 1977–2009: the disease has not yet disappeared. *Nephrol Dial Transplant* 2011; 26: 3171–3176
11. Djukanović L, Radović M, Baković J *et al*. Epidemiology of end-stage renal disease and current status of hemodialysis in Yugoslavia. *Int J Artif Organs* 2002; 25: 852–859

12. Stefanovic V, Cukuranovic R, Miljkovic S *et al*. Fifty years of Balkan endemic nephropathy: challenges of study using epidemiological method. *Ren Fail* 2009; 31: 409–418
13. Djukanović L, Marić I, Marinković J *et al*. Evaluation of criteria for the diagnosis of Balkan endemic nephropathy. *Ren Fail* 2007; 29: 607–614
14. Cukuranovic R, Jovanovic I, Miljkovic S *et al*. Hemodialysis treatment in patients with Balkan endemic nephropathy: an epidemiological study. *Ren Fail* 2007; 29: 805–810
15. Dimitrov P, Tsolova S, Georgieva R *et al*. Clinical markers in adult offspring of families with and without Balkan endemic nephropathy. *Kidney Int* 2006; 69: 723–729
16. Arsenović A, Bukvić D, Trbojević S *et al*. Detection of renal dysfunctions in family members of patients with Balkan endemic nephropathy. *Am J Nephrol* 2005; 25: 50–54
17. Alecković M, Mesić E, Trnacević S *et al*. Glomerular filtration rate in examined population of Bosnian Posavina—region of Balkan Endemic Nephropathy. *Bosn J Basic Med Sci* 2010; 10: S68–S72
18. Radonić M, Radosević Z. Clinical features of Balkan endemic nephropathy. *Food Chem Toxicol* 1992; 30: 189–192
19. Hanjangsit K, Dimitrov P, Karmaus W *et al*. Reduced kidney size in adult offspring of Balkan endemic nephropathy patients and controls: a prospective study. *Am J Med Sci* 2010; 340: 94–102
20. Hanjangsit K, Karmaus W, Dimitrov P *et al*. The role of a parental history of Balkan endemic nephropathy in the occurrence of BEN: a prospective study. *Int J Nephrol Renovasc Dis* 2012; 5: 61–68
21. Jelaković B, Radovanović Z, Cosyns JP *et al*.. Consensus statement on screening, diagnosis classification and treatment of endemic (Balkan) nephropathy. *Nephrol Dial Transplant* (in press)
22. Čukuranović R. Genetic and morphophysiologic study of Balkan endemic nephropathy. Doctoral Thesis. Medical Faculty, University of Niš, 1–169, 1992 (in Serbian)
23. Danilović V. Diagnosis of endemic nephropathy. *Papers in XLVII AN BiH* 1973; 17: 53–64
24. Stefanović V. Diagnostic criteria for endemic (Balkan) nephropathy. In: Strahinjić S, Stefanović V, eds. *Current Research in Endemic (Balkan) Nephropathy*. Niš University Press, Niš: 1983; 351–363
25. Djukanović L, Marinković J, Marić I *et al*. Contribution to the definition of diagnostic criteria for Balkan endemic nephropathy. *Nephrol Dial Transplant* 2008; 23: 3932–3938
26. Stefanović V, Jelaković B, Čukuranović R *et al*. Diagnostic criteria for Balkan endemic nephropathy: proposal by an international panel. *Ren Fail* 2007; 29: 867–880
27. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Chapter 1: definition and classification of CKD. *Kidney Int Suppl* 2013; 3: 19–62
28. Maksimovic ZJ. Selenium deficiency and Balkan endemic nephropathy. *Kidney Int Suppl* 1991; 34: S12–S14
29. Maksimović ZJ, Djujić I. Selenium deficiency in Serbia and possible effects on health. *Biomed Environ Sci* 1997; 10: 300–306
30. Karmaus W, Dimitrov P, Simeonov V *et al*. Metals and kidney markers in adult offspring of endemic nephropathy patients and controls: a two-year follow-up study. *Environ Health* 2008; 7: 11
31. Vogel A. Plant Encyclopaedia. '*Aristolochia clematitis* L. (Common Birthwort)'. Available at http://www.avogel.com/plant-encyclopaedia/aristolochia_clemastitis.php (8 May 2013, date last accessed)
32. Rosenmund H, Reichstein T. Zur Kenntnis der Aristolochiasäure. *Pharm Acta Helv* 1943; 18: 243–261
33. Peters G, Hedwall PR. Aristolochic acid intoxication: a new type of impairment of urinary concentrating ability. *Arch Int Pharmacodyn* 1963; 145: 334–355
34. Ivić M. Etiology of endemic nephropathy. *Lijec Vjesn* 1969; 91: 1273–1281 (in Serbian)
35. Ivić M, Lovrić B. Carcinogenic action of Aristolochia. *Acta Medica Med* 1967; 5: 1–3 (in Serbian)
36. Vanherweghem JL, Depierreux M, Tielemans C *et al*. Rapidly progressive interstitial renal fibrosis in young women: Association with slimming regimen including Chinese herbs. *Lancet* 1993; 341: 387–391
37. Vanherweghem JL. A new form of nephropathy secondary to the absorption of Chinese herbs. *Bull Mem Acad R Med Belg* 1994; 149: 128–135; discussion 135–140 (in French)
38. Vanherweghem JL. Misuse of herbal remedies: the case of an outbreak of terminal renal failure in Belgium (Chinese herb nephropathy). *J Altern Complement Med* 1998; 4: 9–13
39. Hsu HY. *Oriental Materia Medica, A Concise Guide*. Oriental Healing Arts Institute, Long Beach, CA: 1986
40. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. *IARC Monogr Eval Carcinog Risks Hum* 2002; 82: 1–556
41. Martinez MC, Nortier J, Vereerstraeten P *et al*. Progression rate of Chinese herb nephropathy: impact of Aristolochia fangchi ingested dose. *Nephrol Dial Transplant* 2002; 17: 408–412
42. Nortier JL, Martinez MC, Schmeiser HH *et al*. Urothelial carcinoma associated with the use of a Chinese herb (Aristolochia fangchi). *N Engl J Med* 2000; 342: 1686–1692
43. Nortier JL, Schmeiser HH, Muniz Martinez MC *et al*. Invasive urothelial carcinoma after exposure to Chinese herbal medicine containing aristolochic acid may occur without severe renal failure. *Nephrol Dial Transplant* 2003; 18: 426–428
44. Cosyns JP. Aristolochic acid and 'Chinese herbs nephropathy': a review of the evidence to date. *Drug Saf* 2003; 26: 33–48
45. Cosyns JP, Jadoul M, Squifflet JP *et al*. Urothelial lesions in Chinese-herb nephropathy. *Am J Kidney Dis* 1999; 33: 1011–1017
46. Nortier JL, Vanherweghem JL. Renal interstitial fibrosis and urothelial carcinoma associated with the use of a Chinese herb (Aristolochia fangchi). *Toxicology* 2002; 181–182: 577–580
47. Arlt VM, Alunni-Perret V, Quatrehomme G *et al*. Aristolochic acid (AA)–DNA adduct as marker of AA exposure and risk factor for AA nephropathy-associated cancer. *Int J Cancer* 2004; 111: 977–980
48. Debelle FD, Vanherweghem JL, Nortier JL. Aristolochic acid nephropathy: a worldwide problem. *Kidney Int* 2008; 74: 158–169
49. Moriya M, Slade N, Brdar B *et al*. TP53 Mutational signature for aristolochic acid: an environmental carcinogen. *Int J Cancer* 2011; 129: 1532–1536
50. Arlt VM, Stiborová M, vom Brocke J *et al*. Aristolochic acid mutagenesis: molecular clues to the aetiology of Balkan endemic nephropathy-associated urothelial cancer. *Carcinogenesis* 2007; 28: 2253–2261
51. Arlt VM, Schmeiser HH, Pfeifer GP. Sequence-specific detection of aristolochic acid–DNA adducts in the human p53 gene by terminal transferase-dependent PCR. *Carcinogenesis* 2001; 22: 133–140
52. Schmeiser HH, Bieler CA, Wiessler M *et al*. Detection of DNA adducts formed by aristolochic acid in renal tissue from patients with Chinese herbs nephropathy. *Cancer Res* 1996; 56: 2025–2028
53. Bieler CA, Stiborova M, Wiessler M ^{32}P-post-labelling analysis of DNA adducts formed by aristolochic acid in tissues from patients with Chinese herbs nephropathy. *Carcinogenesis* 1997; 18: 1063–1067
54. Pfau W, Schmeiser HH, Wiessler M. ^{32}P-postlabelling analysis of the DNA adducts formed by aristolochic acid I and II. *Carcinogenesis* 1990; 11: 1627–1633

55. Arlt VM, Pfohl-Leszkowicz A, Cosyns J *et al*. Analyses of DNA adducts formed by ochratoxin A and aristolochic acid in patients with Chinese herbs nephropathy. *Mutat Res* 2001; 494: 143–150
56. Cosyns JP, Jadoul M, Squifflet JP *et al*. Chinese herbs nephropathy: a clue to Balkan endemic nephropathy? *Kidney Int* 1994; 45: 1680–1688
57. Grollman AP, Scarborough J, Jelaković B. Aristolochic acid nephropathy: an environmental and iatrogenic disease. In: Fishbein J, ed. *Advances in Molecular Toxicology 3*. Elsevier, Amsterdam: 2009; 211–222
58. Hranjec T, Kovac A, Kos J *et al*. Endemic nephropathy: the case for chronic poisoning by aristolochia. *Croat Med J* 2005; 46: 116–125
59. Grollman AP, Jelaković B. Role of environmental toxins in endemic (Balkan) nephropathy. October 2006, Zagreb, Croatia. *J Am Soc Nephrol* 2007; 18: 2817–2823
60. Grollman AP, Shibutani S, Moriya M *et al*. Aristolochic acid and the etiology of endemic (Balkan) nephropathy. *Proc Natl Acad Sci USA* 2007; 104: 12129–12134
61. Chen CH, Dickman KG, Moriya M *et al*. Aristolochic acid-associated urothelial cancer in Taiwan. *Proc Natl Acad Sci USA* 2012; 109: 8241–8246
62. Markovic N, Ignjatovic I, Cukuranovic R *et al*. Decreasing incidence of urothelial cancer in a Balkan endemic nephropathy region in Serbia. A surgery based study from 1969 to 1998. *Pathol Biol (Paris)* 2005; 53: 26–29
63. Basic-Jukic N, Hrsak-Puljic I, Kes P *et al*. Renal transplantation in patients with Balkan endemic nephropathy. *Transplant Proc* 2007; 39: 1432–1435
64. Nortier JL, Zlotta A, Petein M *et al*. Upper urinary tract carcinoma after intake of Aristolochia Fangchi: value of urinary cytology in end-stage 'Chinese-herb nephropathy'. In: Nunez KR, ed. *Trends in Kidney Cancer Research*. Nova Science Publishers, Inc, New York, NY: 2006; 77–88
65. Pavlović NM, Maksimović V, Maksimović JD *et al*. Possible health impacts of naturally occurring uptake of aristolochic acids by maize and cucumber roots: links to the etiology of endemic (Balkan) nephropathy. *Environ Geochem Health* 2013; 35: 215–226
66. Krogh P, Hald B, Plestina R *et al*. Balkan (endemic) nephropathy and foodborn ochratoxin A: preliminary results of a survey of foodstuffs. *Acta Pathol Microbiol Scand B* 1977; 85: 238–240
67. Hald B. Porcine nephropathy in Europe. *IARC Sci Publ* 1991; 115: 49–56
68. Pfohl-Leszkowicz A, Tozlovanu M, Manderville R *et al*. New molecular and field evidences for the implication of mycotoxins but not aristolochic acid in human nephropathy and urinary tract tumor. *Mol Nutr Food Res* 2007; 51: 1131–1146
69. Feder GL, Radovanović Z, Finkelman RB. Relationship between weathered coal deposits and the etiology of Balkan endemic nephropathy. *Kidney Int Suppl* 1991; 34: S9–S11
70. Orem WH, Feder GL, Finkelman RB. A possible link between Balkan endemic nephropathy and the leaching of toxic organic compounds from Pliocene lignite by groundwater: preliminary investigation. *Int J Coal Geol* 1999; 40: 237–252
71. Feder GL, Tatu CA, Orem WH *et al*. Weathered coal deposits and balkan endemic nephropathy. *Facta Universitatis Ser Med Biol* 2001; 9: 34–38
72. Orem WH, Tatu CA, Lerch III HE *et al*. Identification and environmental significance of the organic compounds in water supplies associated with a Balkan endemic nephropathy region in Romania. *J Environ Health Res* 2004; 3: 53–61
73. Orem W, Tatu C, Pavlovic N *et al*. Health effects of toxic organic substances from coal: toward 'panendemic' nephropathy. *Ambio* 2007; 36: 98–102
74. Bunnell JE, Tatu CA, Lerch HE *et al*. Evaluating nephrotoxicity of high-molecular-weight organic compounds in drinking water from lignite aquifers. *J Toxicol Environ Health A* 2007; 70: 2089–2091
75. Tatu CA, Orem WH, Finkelman RB *et al*. The etiology of Balkan endemic nephropathy: still more questions than answers. *Environ Health Perspect* 1998; 106: 698–700
76. Toncheva D, Dimitrov T. Genetic predisposition to Balkan endemic nephropathy. *Nephron* 1996; 72: 564–569
77. Toncheva D, Dimitrov T, Stojanova S. Etiology of Balkan endemic nephropathy: a multifactorial disease? *Eur J Epidemiol* 1998; 14: 389–394
78. Norum KR, Gjone E. Familial serum-cholesterol esterification failure. A new inborn error of metabolism. *Biochim Biophys Acta* 1967; 144: 698–700
79. Idzior-Waluś B. Familial LCAT deficiency. *Przegl Lek* 2001; 58: 919–923 (in Polish)
80. Calabresi L, Pisciotta L, Costantin A *et al*. The molecular basis of lecithin: cholesterol acyltransferase deficiency syndromes: a comprehensive study of molecular and biochemical findings in 13 unrelated Italian families. *Arterioscler Thromb Vasc Biol* 2005; 25: 1972–1978
81. Pavlović NM, Varghese Z, Persaud JW *et al*. Partial lecithin: cholesterol acyltransferase (LCAT) deficiency in Balkan endemic nephropathy. *Kidney Int Suppl* 1991; 34: S102–S104
82. Ozawa S. Genetic polymorphisms in xenobiotic metabolizing enzymes as a determinant of susceptibility to environmental mutagens and carcinogens in humans. *Yakugaku Zasshi* 1997; 117: 895–909 (in Japanese)
83. Wolf CR. Individuality in cytochrome P450 expression and its association with the nephrotoxic and carcinogenic effects of chemicals. *IARC Sci Publ* 1991; 115: 281–287
84. Ginsberg G, Guyton K, Johns D *et al*. Genetic polymorphism in metabolism and host defense enzymes: implications for human health risk assessment. *Crit Rev Toxicol* 2010; 40: 575–619
85. Atanasova SY, von Ahsen N, Toncheva DI *et al*. Genetic polymorphisms of cytochrome P450 among patients with Balkan endemic nephropathy (BEN). *Clin Biochem* 2005; 38: 223–228
86. Andonova IE, Sarueva RB, Horvath AD *et al*. Balkan endemic nephropathy and genetic variants of glutathione S-transferases. *J Nephrol* 2004; 17: 390–398
87. Toncheva DI, Von Ahsen N, Atanasova SY *et al*. Identification of NQO1 and GSTs genotype frequencies in Bulgarian patients with Balkan endemic nephropathy. *J Nephrol* 2004; 17: 384–389
88. Tsoneva M, Dimitrov Ts, Toncheva D. Familial cytogenetic studies in Balkan endemic nephropathy. *II. Vutr Boles* 1985; 24: 61–66 (in Bulgarian)
89. Toncheva D, Dimitrov T, Tzoneva M. Cytogenetic studies in Balkan endemic nephropathy. *Nephron* 1988; 48: 18–21
90. Apostolov K, Spasic P. Evidence of a viral aetiology in endemic (Balkan) nephropathy. *Lancet* 1975; 2: 1271–1273
91. Uzelac-Keserović B, Spasić P, Bojanić N *et al*. Isolation of a coronavirus from kidney biopsies of endemic Balkan nephropathy patients. *Nephron* 1999; 81: 141–145
92. Riquelme C, Escors D, Ortego J *et al*. Nature of the virus associated with endemic Balkan nephropathy. *Emerg Infect Dis* 2002; 8: 869–870
93. Frezza D, Serone E, Lolli S *et al*. Balkan endemic nephropathy risk associates to the hs1,2 Ig enhancer polymorphism. *Eur J Inflamm* 2012; 10: 393–403
94. Pavlovic NM, Orem WH, Tatu CA *et al*. The role of lecithin cholesterol acyltransferase and organic substances from coal in the etiology of Balkan endemic nephropathy: a new hypothesis. *Food Chem Toxicol* 2008; 46: 949–954
95. Spector AA, Yorek MA. Membrane lipid composition and cellular function. *J Lipid Res* 1985; 26: 1015–1035

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