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Balkan Nephropathy: Evolution of Our Knowledge

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Balkan endemic nephropathy (BEN), originally described in the late 1950s as a chronic tubulointerstitial kidney disease, is identified by its unique epidemiological features. The most remarkable characteristic of BEN is the focal topographical nature that characterizes its occurrence at the global, national, and even household level. BEN affects only certain endemic rural foci along tributaries of the Danube River in the Balkan countries of Bosnia, Bulgaria, Croatia, Romania, and Serbia. The spatial distribution has remained astonishingly unchanged with time because the disease affects the same endemic clusters as 50 years ago. The natural course of the disease is characterized by universal development of end-stage renal disease and the frequent development of upper urinary tract tumors, posing a substantial disease burden to the afflicted areas. The greatest challenge in the study of BEN has been the elucidation of its cause. The unique features of the disease, in particular its endemic nature and the long incubation period required for the disease to develop, have led to the proposal that BEN represents a unique environmental disease. The quest for the responsible environmental factor has been long and diverse, and although no definitive answer has been provided to date, converging lines of evidence support the theory that long-term consumption of food contaminated with aristolochic acid underlies the pathogenesis of BEN. The present review describes the evolution of our knowledge of BEN in relation to the development of the main theories for its pathogenesis.
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In 1956, the first description of a novel form of interstitial kidney disease appeared in the non-English literature. The story had started a decade earlier, when local physicians noted a high prevalence of kidney disease in certain settlements in northwest Bulgaria in the district of Vratza. This prompted a thorough investigation by Tanchev, who studied 664 patients with renal disease at the regional hospital from 1950 to 1954. He was the first to observe the remarkable clustering of the patients in villages, families, and even households. After presenting the condition at local meetings in 1953 and proposing the term "endemic Vratza nephritis" in 1955, Tanchev et al[1] published the first detailed clinical description of the new entity in 1956. A year later, a "family outbreak" of renal disease was noticed in the neighboring country of (what was at the time) Yugoslavia; a mother and 2 daughters had died of renal failure, and the father and son also had severe renal damage. It was soon recognized that a disease with almost identical clinical and epidemiological phenotype to the Vratza nephritis existed in confined rural areas of Yugoslavia.[2] This series of original publications was completed in 1961, as it became evident that a similar nephropathy was also prevalent in discrete regions of Romania.[3]

As a consequence of these early reports, 2 scientific conferences were organized to address the facts for the new disease. The first was held by the World Health Organization in 1965, and the second, 2 years later, by the CIBA Foundation.[4,5] As a result of these meetings, a novel nosological entity was recognized that was designated Balkan endemic nephropathy (BEN). The term epitomized the 2 predominant features of the new disease; first, it existed in the Balkans exclusively, and, second, it occurred in endemic clusters.

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In the following years, the clinical and epidemiological characteristics of BEN were clarified.[6-8] It represents a discrete form of tubulointerstitial nephropathy with insidious presentation and slow progression. Nonspecific symptoms and anemia typically develop before significant renal dysfunction.[9,10] The latter ensues eventually and manifests as reduced tubular transport, low-molecular-weight proteinuria, and an increase in serum urea nitrogen level, but no high blood pressure or edema, findings usually encountered in every form of advanced nephropathy.[11,12] Histologically, interstitial fibrosis and tubular atrophy are prominent features, as opposed to the absence of significant inflammatory changes (Fig 1).[13-15] In the final stages, the kidneys are reduced in size, sometimes weighing as little as 50 g and measuring just 2 to 3 cm.[12,13] The outcome is universally fatal unless renal dialysis therapy is introduced. It therefore is evident that from the clinical stand point, the new entity only added one more subcategory to the long list of interstitial renal diseases. BEN would have never achieved such scientific attention and such nominated adjectives as "mysterious" and "enigmatic" if not for its unique epidemiological characteristics.

From the very beginning, it was recognized that the most remarkable feature of BEN is its focal nature.[8,16-20] At the global level, the disease has been described in Balkan countries only: the aforementioned Bulgaria and Romania, as well as Bosnia, Serbia, and Croatia, countries formed after the division of the former Yugoslavia. On a national scale, BEN cases are not distributed evenly throughout each affected country. They are strictly confined in 142 settlements in the former Yugoslavia, 40 in Bulgaria, and 40 in southwest Romania, the total area not exceeding 500 miles in length or 20,000 km^2 in surface (Fig 2). This geographic distribution is so stable that 50 years after the original description, no new endemic areas have been reported and no endemic areas became free from BEN. All affected regions consist of villages or small towns built on the alluvial planes of tributaries of the Danube River. In accord with that observation, the disease affects only rural farming populations, but never inhabitants of big cities. The focal nature of BEN is so remarkably firm that afflicted villages are in close proximity to unaffected ones, only 2 to 3 km apart. Finally, the focal nature is even preserved at the local level. That means that within an affected village, one can find diseased households that exist next to disease-free ones. In a single household, only individuals "living under the same roof and eating the same food" may be affected. However, BEN does not show a preference for specific ethnic or religious groups. This was shown when Croatian aboriginals were compared with Ukrainian immigrants in the area of Slavonski Brod in regard to several parameters of renal function. It was clearly shown that the sole factor determining the presence or absence of pathological values was residency in an endemic versus a nonendemic area.[21]

Apart from its focal nature, BEN is also characterized by a long incubation period. For an

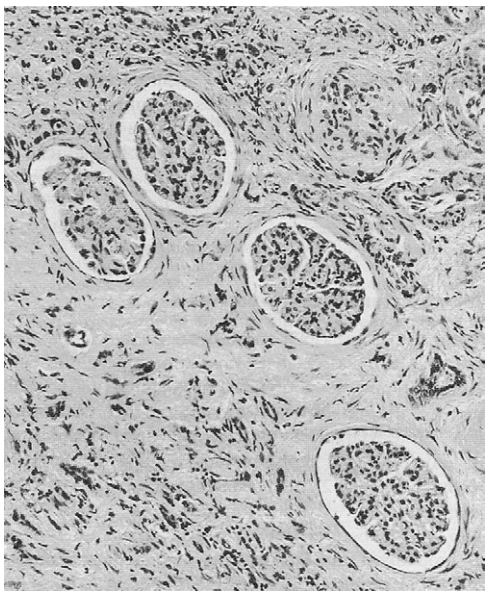

Figure 1. Histological features of Balkan endemic nephropathy. The dense interstitial fibrosis is in contrast to the preservation of glomeruli. (Adapted from Nadasdy and Sedmak,[15] with permission.)

Figure 2. Map shows the distribution of endemic foci of Balkan nephropathy. (Adapted with permission from Stefanovic and Cosyns.[8])

individual to have the disease, he or she must live in the endemic area for at least 15 to 20 years. This probably explains why the disease has never been diagnosed in children. Accordingly, a native who leaves the area before reaching the age of 20 years is spared from developing BEN. Conversely, immigrants into an afflicted focus become susceptible after living there for 15 to 20 years. After being exposed to the risk of developing BEN, it takes an equally long time before clinical manifestation occurs. Therefore, the typical peak of the disease takes place between the third and fifth decades. This may explain why BEN was not recognized before World War II; the average life expectancy at the time (40 to 50 years) may simply have been too short to allow the disease to reach the clinical stage.[22]

Finally, even in the first reports of BEN, it was recognized that such patients were at increased risk of developing upper urinary tract tumors.[23-26] This association was already known in the 1960s, but became more pronounced in later years when the life expectancy of patients with BEN increased significantly as a result of the broad use of dialysis therapy. The estimated incidence of tumors of urothelial origin has been 57 times greater in BEN endemic regions between 1969 and 1977 compared with nonendemic areas; similarly, bladder cancer was 12 times more frequent.[27] These epidemiological studies provided a strong link between BEN and upper urinary tract tumors.

THE SEARCH FOR A CAUSE

It was not long after the original description of BEN that the search for its cause started. For a disease with such an intriguing epidemiological profile, it was inevitable that there was room for wide speculation. As far as the inhabitants of the endemic villages were concerned, the reason was simple. The sudden misfortune that was laid upon their lives after the end of World War II could not have any other explanation but the interference of supernatural forces, divine or otherwise. It therefore was not unexpected that they tried to calm these forces with rituals and ceremonies and protect themselves by wearing amulets.

However, for the health scientists, the unique characteristics of BEN offered different opportunities. This was a disease with a distinct, homogeneous phenotype, affecting a small part of the world, confined to certain recognized areas, and with all the afflicted people easily identifiable and therefore amenable to thorough investigation and research. It therefore is not unexpected that one of the first reports of BEN also announced a definitive answer to its pathogenesis, because Serbian investigators considered it to be the result of lead poisoning of the flour in endemic regions.[28] Ironically, 50 years later, the precise cause of BEN has not been definitely established.

Meanwhile, perhaps no other human disease has produced so many hypotheses in an effort to elucidate its causal factors. The quest for the cause of BEN proved not to be an easy task because of many factors. First, the epidemiological data from the countries where the disease was prevalent were not of perfect quality. Second, collaboration between investigators from these different countries did not take place. As a

consequence, results from studies in one country were never confirmed in the others. Third, no matter how fascinating its nature may be, BEN is a rare disorder affecting only some 10,000s of people in confined Balkan villages, with a practical consequence being a lack of funding resources for its study. Finally, political and social events that took place in the troubled region of the Balkan Peninsula had their own negative effect on the efforts to find a solution to the mystery of BEN. One has to keep in mind that the political tensions that followed the end of World War II created a situation in the Balkan area that made a rare nephropathy much more a local disturbance than a national priority.

No matter what the obstacles were, research about the causative factor(s) of BEN started almost immediately after its recognition as a discrete nosological entity. For a disease with such a narrow, defined, and well-preserved geographic distribution, it was a logical assumption that the causative factor should be an environmental one endemic to the afflicted regions. Nonetheless, along with this ecological influence, other factors had to be taken into consideration. For example, an obvious question was how could such an environmental trigger selectively affect certain villages and households and leave the ones just next to them undisturbed? Could these differences be explained by the existence of genetic variability between diseased and healthy families? Or could it be an infectious agent prevalent to the inflicted foci? Another pivotal question related to the long incubation period of the disease, which pointed to a long exposure to a low-dose harming agent that existed in the environment for many years. If that is the case, why then did BEN appear only during the second half of the century in the endemic areas and not before? Such a temporal association requires a major local environmental change before the first appearance of the disease, and such an alteration is not easy to identify in the history of the region. In addition, any etiologic hypothesis should take into consideration not only the nephropathy itself, but also the increased incidence of upper urothelial neoplasms in the patients. Finally, if somebody were to solve the mystery of BEN, he or she should use information from both animal models of interstitial nephropathy and the recognized effects of the candidate environmental factors in human health and disease. Given all these varied questions, it is not surprising that throughout the years, the effort to solve the riddle of BEN involved not only nephrologists, but also scientists from such diverse fields as occupational medicine, animal models of human disease, environmental sciences and epidemiology, oncology, genetics, and geology.

One of the first comprehensive hypotheses regarding the pathogenesis of BEN appeared in the literature in the early 1970s. Akhmeteli and then Krogh proposed that BEN was the result of contamination of the food chain in endemic areas by ochratoxin A (OTA),[29] a toxic product of molds that belong to the *Aspergillus* or *Penicillium* fungal genera. This "mycotoxin" theory was built around the remarkable similarity between BEN and porcine nephropathy.[30] The latter has a similar confined geographic distribution because it mainly occurs in Northern Europe and shares many pathological characteristics with BEN. Porcine nephropathy is caused by OTA, raising the possibility for a similar connection between BEN and mycotoxins. Therefore, an effort to provide evidence for such an association was started.

From a general point of view, the establishment of a cause-and-effect link between an environmental factor and a disease, the so-called exposure analysis, consists of a stepwise approach.[31,32] Briefly, the agent has to be present in an endemic area and in quantities large enough to induce health damage. In addition, there should be evidence regarding how the offending agent moves from the environmental source to the human organism. Moreover, topographical specificity should exist, meaning that the agent must be more prevalent in endemic compared with nonendemic regions. Finally, if the same agent occurs in other areas of the world, a similar disease would be expected to be found there as well. If not, an explanation should be offered.

The application of these principles in the case of the ochratoxin/BEN association has produced ambivalent results. Contamination of food with OTA is very frequent, and

widespread exposure is common in several places in the world, including Western nations.[33,34] Some, but not all, studies have detected greater levels of OTA in the foodstuff consumed in endemic versus nonendemic areas, as well as in affected versus nonaffected households.[30,35-37] Other studies reported that patients with BEN have increased serum and urine concentrations of OTA, indicating greater consumption of the mycotoxin.[38] It therefore could be the case that (although the contamination of the food chain is ubiquitous) patients with BEN are exposed to greater quantities of OTA as a result of some still unidentified practice in food processing and consumption. Nonetheless, it was shown in the same studies that the variation in OTA values within the affected population was very high and often overlapped greatly with values from nonendemic areas of the world. Moreover, there was no constant association between consumption of OTA and its levels in serum or urine.

A strong argument against the mycotoxin theory is that OTA had never been linked to any type of nephropathy in humans. However, this argument may be subject to challenge after the proposal in the 1990s that 2 other forms of interstitial nephropathy may be associated with OTA: the first is endemic in Tunisia,[39,40] and the second is the karyomegalic interstitial nephropathy.[41] Both types of renal disease share clinicopathologic similarities with BEN and support the hypothesis that OTA toxicity may underlie the pathogenesis of the latter, as well. The evidence for the role of OTA as the causative factor for BEN was critically reviewed in a recent international symposium held in Zagreb.[42] A series of arguments against such a role were presented; the strongest were the uneven geographic distribution of food contamination with OTA and BEN and the lack of definitive proof for OTA-DNA adduct formation.

A different pathogenetic hypothesis was proposed by Kazantzis[43] in 1967 during the conference on BEN organized by the CIBA Foundation. He claimed that contamination of the baking flour in endemic areas by seeds of the birthwort *Aristolochia clematitis* was causing the disease (Fig 3). Apparently, a toxic constituent was contained in the seeds of the plant and induced the renal damage. The initial exploration of this theory is attributed to Ivić,[44] who analyzed available data and performed field and laboratory studies to provide a well-documented hypothesis on the pathogenesis of BEN. Again, animal studies set the background. Ivić was aware of publications of such Croatian scientists as Dumic[45] and Martincic[46] reporting that horses consuming hay that contained seeds of *A clematitis* experienced renal disease with proteinuria. More strikingly, when kidneys from these horses were examined histologically, they showed tubulointerstitial damage with minimal inflammation, a picture that was analogous to BEN.[45,46] To prove his hypothesis, Ivić performed field studies and observed that seeds from *A clematitis* were interspersed among wheat grains during the harvest. The local villagers did not make an effort to remove the contaminants during flour preparation. Because bread is the major constituent of the local diet, contamination of the wheat with even a few seeds of *A clematitis* could result in low-dose long-term intoxication with the offending agent. Continuing his sound scientific approach, Ivić went on and fed rabbits with flour prepared from *A clematitis* seeds. The rabbits developed nephropathy, which, at the histological level, resembled the findings of BEN. Ivić even proved the carcinogenetic potential of the plant because rats developed sarcomas at the site of injection of aqueous extracts of *A clematitis*.[47] It is surprising that Ivić's thorough approach and well-documented results failed to attract more interest from the scientific community until many years later.

However, this was to change in 1993, when many cases of Chinese herb nephropathy erupted.[48] Several hundred young Belgian women developed end-stage renal disease after receiving slimming pills at a single medical clinic in Brussels. The regimen contained 2 Chinese herbs, and it eventually was proved that it was contaminated with aristolochic acid, a main toxic product of *Aristolochia* species. Its presence in the slimming regimen was the result of accidental substitution of the prescribed herb *Stephania tetrandra* ('Han Fang-ji') by *Aristolochia fangchi* ('Guang Fang-ji'). Therefore, the name of the Belgian disorder changed

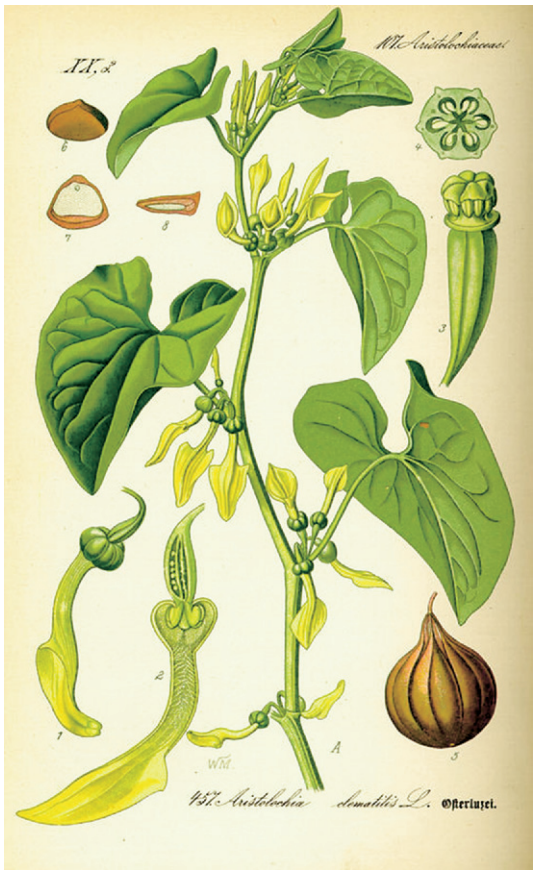

Figure 3. *Aristolochia clematitis* (birthwort) as shown in an illustration from Otto Wilhelm Thomé's *Flora von Deutschland, Österreich und der Schweiz*, reproduced from www.biolib.de with permission from Kurt Stueber.

to Chinese herb nephropathy, or aristolochic acid nephropathy. It was soon realized that this disease had many similarities to BEN, especially in the morphological characteristics, which were almost identical in the 2 conditions. This similarity suggested that the same factor, ie, aristolochic acid, could be responsible for both conditions.[49] This association was supported further by the development of renal failure in Japanese patients receiving an aristolochic acid–containing remedy for atopic dermatitis.[50] Interestingly, upper urothelial neoplasms also developed in some patients with Chinese herb nephropathy.[51,52]

These intriguing reports renewed scientific interest and stimulated new research that resulted in strong evidence supporting a dominant role for aristolochic acid in the pathogenesis of BEN. After its metabolic activation, aristolochic acid reacts with DNA to generate covalent aristolactam-DNA adducts. Some forms of these adducts are stable and can be detected in the affected tissues. Therefore, if aristolochic acid were to blame for BEN, renal tissue from these patients should contain such adducts. Arlt et al[53] were the first to prove that this hypothesis is correct by detecting aristolochic acid–specific adducts in all urinary tract tissues from patients with BEN that they examined. Conversely, OTA-specific adducts were detected with much lower density and in only some of the examined tissues. Recently, work by Grollman et al[42,54] expanded these results by showing that aristolochic acid–specific adducts were present in the renal cortex of 5 patients with BEN from an endemic region in Croatia, but not in 5 patients with other forms of chronic renal disease or 5 patients with upper urinary tract transitional cell cancer living in a nonendemic area of Croatia. In addition, when a p53 mutational spectra analysis was performed in urothelial cancer specimens from patients living in endemic foci, a "signature" mutation (A:T→T:A) was detected. Interestingly, this molecular mark is also induced by aristolochic acid in animal models of carcinogenesis and was observed in one patient with aristolochic acid nephropathy and ureteral tumor.[42] Therefore, aristolochic acid appears to be responsible not only for the development of renal injury, but also for the high incidence of urothelial cancer in patients with BEN. A recent publication by Lemy et al[55] provides convincing evidence for the carcinogenic potential of aristolochic acid. This was a case series in Belgian patients undergoing renal transplantation for end-stage renal failure caused by slimming pill–related aristolochic acid nephropathy. In the

pathological examination of the resected specimens, upper-tract urothelial carcinoma was diagnosed in 45% of patients. More interestingly, on long-term follow-up, there was a 40% incidence of bladder carcinoma. It was concluded from this study that the pathogenic properties of aristolochic acid exist for a long time after cessation of exposure.

To date, the aristolochic acid hypothesis perhaps offers the best-characterized model for the pathogenesis of BEN.[42,56] The pivotal work of Ivić combined with modern state-of-the-art biochemical and molecular techniques have promulgated a pathogenetic concept that explains most of the epidemiological and clinical parameters of BEN. However, a critical question still is unanswered; why do only 2% to 5% of the residents in an endemic area develop the disease?[22] This cannot be attributed easily to preferential exposure of such a small minority of the population to aristolochic acid, but it could result from genetic polymorphisms. Nevertheless, if the aristolochic acid hypothesis is true, the most important clinical conclusion is that BEN is preventable with simple measures. One can safely predict that contamination of the flour with *A clematitis* seeds has already been eliminated as a result of the changing ways of life and work in the endemic areas.[56] The widespread use of herbicides and use of new harvesting techniques has led to decreased growth of *A clematitis* in the harvesting fields. Nowadays, fewer and fewer families bake their own bread, rendering the making of contaminated flour highly improbable. If this hypothesis proves to be true, we can be optimistic that a decrease in the incidence of BEN should be expected in the years ahead.

No review of the evolution of our knowledge of BEN is complete if it does not address the so-called "lignite hypothesis." This theory was developed in the 1990s by scientists of the US Geological Survey.[57,58] It originated from the primary observation that there was a spatial relationship between the endemic villages with BEN and the locations of Pliocene lignite deposits in the Balkans. All endemic areas are in close vicinity to low rank coals. The lignite theory claims that toxic organic substances from these coals leak into groundwater and are transported to the wells that exist in the alluvial valleys, below the lignite deposits.[59] This was shown to be true because studies reported greater concentrations of these organic compounds in the water from wells in the endemic areas compared with those in nonendemic ones.[60] The final link in the exposure chain is that people in the villages use water from their wells for drinking and cooking purposes.[22,61,62] In line with this, settlements where most inhabitants use the same limited number of wells show hyperendemicity for BEN. The presence of small concentrations of organic toxic products in the water of the wells is compatible with a slow low-level, but relentless, poisoning of the villagers, a concept that fits very well with the natural history of BEN. The increased incidence of urothelial cancers also can be explained by this theory because these toxic organics are well-known carcinogenic factors. Maybe the most impressive application of the lignite hypothesis is that it may be used as a predictive model.[22] An area in Serbia that was not known to be endemic was proved to be such after it was recognized to lay close to a low rank coal field. Research on the role of the Pliocene lignites in the pathogenesis of BEN required a lot of field studies and thus it was abruptly interrupted when the war broke out in Yugoslavia in 1993.[63]

Although the environmental factor(s)–centered theories dominated the efforts to explain the etiopathogenesis of BEN, alternative explanations were sought, as well. The infection theory claims that BEN is caused by a coronavirus. Uzelac-Keserović et al[64] developed epithelial cell monolayer cultures from kidney biopsy specimens obtained from patients with BEN and control patients. They were able to detect a novel virus, named the EBN virus, that was present in BEN-derived epithelial cell cultures, but from none of the control cultures. It was proposed that the microorganism was a novel coronavirus based on its cross-reactivity with human coronaviruses OC43 and 229E, as well as a pig coronavirus known as transmissible gastroenteritis virus (TGEV).[64] When seroreactivity to antigens from the new coronavirus was tested, it was shown to be very high in patients with BEN on dialysis therapy (87% by

neutralization activity and 95% by immunofluorescent assay, respectively) and controls from endemic areas (74% and 80%), but not in controls from nonendemic regions (13.5% and 60.5%). Nonetheless, the coronavirus theory was put in dispute when Vero cells infected with EBN virus were tested further with various methods, including electron microscopy studies.[65] No similarities between EBN virus and coronaviruses were observed in that study.

The strict clustering of BEN cases in selected families has raised the possibility that genetic factors may determine its clinical and epidemiological profile. This theory was established by detailed family investigations in Bulgarian patients with BEN. After studying 4,077 patients from 417 BEN-affected families, Toncheva et al[66] were led to the conclusion that all patients with BEN belong to certain families. Even residents of nonendemic foci who were found to have BEN were identified to be members of BEN families and to have moved from their birthplaces. In addition, BEN shows some epidemiological characteristics typical of genetic disorders. For example, the proportion of sick offspring increases according to the number of parents affected. Accordingly, the risk of developing the disease is much greater in first-degree than second-degree relatives and decreases substantially in remote relatives.[66] To further elaborate the genetic component of BEN, cytogenetic studies were performed by the same group of scientists. They proposed that a specific BEN-associated locus exists in 3q25 combined with instability of the long arm of chromosome 3.[67,68] Interestingly, this alteration in 3q25 may also determine the genetic susceptibility for the development of the disease in relatives of patients with BEN.[69] Additional data generated from the same group link the increased prevalence of urothelial neoplasms that occurs in patients with BEN to genetically determined aberrations in oncogenesis. Patients with BEN were tested for chromosomal aberrations induced by X-rays or folic acid deprivation.[70] There was increased frequency of the aforementioned abnormality in 3q25, but also in 3 other areas that all contain oncogenes, namely c-src (*CSK*,1q36), raf-1 (*RAF1*, 3p25), and myb (*MYB*, 6q23).

It has been estimated that 100,000 people are at risk of BEN, whereas 25,000 have the disease. It is not clear what the current trend for BEN incidence is because results from studies performed in different endemic areas produced conflicting information. Some epidemiological reports reported an increase between 1967 and 1970, a plateau between 1970 and 1984, and a final decrease in disease prevalence in some endemic areas.[71] Similarly, a decreasing incidence with time was found in another endemic area during a surveillance period between 1978 and 1997.[72] Nevertheless, for certain endemic regions, BEN continues to pose a major health problem, and it seems that the incidence of new cases remains stable over time.[73] Probably, these differences are related to differences in the study design or true epidemiological differentiation between endemic areas.

Fifty years after its original description, BEN has shown impressive stability in regard to its epidemiological profile and clinical phenotype. Although this stability initially generated optimism for the identification of the causative agent(s), this proved to be a much more difficult task.[74] The most widely accepted theories have failed to date to answer the main critical question: what is the basis for the focal-topographical nature of BEN? BEN is definitely not a genetic disorder in that it does not follow a pattern of Mendelian inheritance. Conversely, BEN clusters in familial foci, indicating exposure to a common environmental offense. Nevertheless, it is difficult to appreciate which environmental agent could be so selective as to affect only a small minority of closely residing individuals. In regard to that question, one needs to consider the possibility of BEN-like diseases existing in other parts of the world. Supporters of the mycotoxins or the lignite hypothesis have already proposed that BEN is part of and may be the most obvious example of a panendemic nephropathy that takes place in locations characterized by the contamination of the food or water supplies by OTA or coal-derived organic toxic substances, respectively.[75,76]

As the case is for most human diseases, it is likely that BEN is a multifactorial disorder. An environmental factor probably is superimposed on a

certain genetic background to create the phenotype of the disease. A triggering agent must exist in the environment in sufficient quantity and with an available exposure route that allows it to be introduced to the human host. There should be a defined population focus isolated enough to render exposure to the agent to be universal in that population and constant. There should be stability of the population at risk, so that individuals are exposed for a long period and an average lifespan that should far exceed 50 years of age, allowing for the long incubation time of the disease. Finally, there should be a health care system with the ability for proper establishment of the diagnosis. It therefore is possible that the combination of all these prerequisites in the post–World War II Balkans resulted in the identification of BEN. From this perspective, it can be said that BEN represents a unique geophysical experiment in which all the components must meld for the disease to develop. The optimistic view from that perspective is that only one of these factors needs to be eliminated for the disease to disappear. We speculate that certain changes in the lifestyle of the people, agricultural practices, or dietary habits may remove one or more cofactor(s) for the development of BEN. Such a scenario eventually would eliminate the burden of this unfortunate condition from the Balkans.

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REFERENCES

1. Tanchev Y, Evstatiev Z, Dorossiev D, Pencheva J, Zvetkov G: Studies on the nephritides in the District of Vratza. Savremenna Medicina 7:14-29, 1956

2. Danilovic V, Djurisic M, Mokranjac M, Stojimirovic B, Zivojinovic J, Stojakovic P: Porodicna oboljenia bubrega u selu Sopic izvazvana hronicnom intoksikacijom olovom. Srpski Arh Tcelok Lek 85:1115-1125, 1957

3. Fortza N, Negoescu M: Nefrita cronica azotemia endo-epidemica. Stud Cercet Med 1:217-221, 1961

4. World Health Organization: Memorandum: The endemic nephropathy of South-Eastern Europe. Bull World Health Org 32:441-448, 1965

5. Wolstenholme GE, Knight J (eds): CIBA Foundation Study Group No. 30: The Balkan Nephropathy. Boston, MA, CIBA Foundation, 1967

6. Georgiev G: Clinical and genealogical investigation of Balkan endemic nephropathy [in Bulgarian]. Sophia, Bulgaria, Bulgarian Academy of Science, 1979, pp 1-55

7. Radonic M, Radosevic Z: Clinical features of Balkan endemic nephropathy. Food Chem Toxicol 30: 189-192, 1992

8. Stefanovic V, Cosyns JP: Balkan nephropathy, in Davison AM, Cameron JS, Grunfeld JP, Ponticelli C (eds): Oxford Textbook of Clinical Nephrology. New York, NY, Oxford University, 2005, pp 1095-2101

9. Pavlović-Kentera V, Clemons GK, Trbojević S, Dimković N, Djukanović L: Erythropoietin and anemia in the progression of Balkan endemic nephropathy and other renal diseases. Nephron 54:139-143, 1990

10. Plestina R: Some features of Balkan endemic nephropathy. Food Chem Toxicol 30:177-181, 1992

11. Hall PW III, Piscator M, Vasiljevic M, Popović N: Renal function studies in individuals with the tubular proteinuria of endemic Balkan nephropathy. Q J Med 41:385-393, 1972

12. Djukanovic L, Bukvic D, Maric I: Creatinine clearance and kidney size in Balkan endemic nephropathy patients. Clin Nephrol 61:384-386, 2004

13. Vukelic M, Sostaric B, Belicza M: Pathomorphology of Balkan endemic nephropathy. Food Chem Toxicol 30:193-200, 1992

14. Vukelic M, Sostaric B, Fuchs R: Some pathomorphological features of Balkan endemic nephropathy in Croatia, in Castegnaro M, Plestina R, Dirheimer G, Chernozemsky IN, Bartsch H (eds): Mycotoxins, Endemic Nephropathy and Urinary Tract Tumors. IARC Scientific Publications No 115. Lyon, France, International Agency for Research on Cancer, 1991, pp 37-42

15. Nadasdy T, Sedmak D: Acute and chronic tubulointerstitial nephritis, in Jennette JC, Olson JL, Schwartz MM, Silva FG (eds): Heptinstall's Pathology of the Kidney (ed 6). Philadelphia, PA, Lippincott Williams & Wilkins, 2006, pp 1124-1126

16. Hall PW, Batuman V: Balkan endemic nephropathy—Introduction. Kidney Int Suppl 34:S1-S3, 1991

17. Hall PW III, Dammin GJ: Balkan nephropathy. Nephron 22:281-300, 1978

18. Ceovic S, Hrabar A, Saric M: Epidemiology of Balkan endemic nephropathy. Food Chem Toxicol 30: 183-188, 1992

19. Cracium E, Rosculescu I: On Danubian endemic familial nephropathy (Balkan nephropathy). Am J Med 49:774-779, 1970

20. Austwick PKC: Balkan nephropathy. Practitioner 225:1031-1038, 1981

21. Ceović S, Hrabar A, Radonić M: An etiological approach to Balkan endemic nephropathy based on the investigation of two genetically different populations. Nephron 40:175-179, 1985

22. Tatu CA, Orem WH, Finkelman RB, Feder GL: The etiology of Balkan endemic nephropathy: Still more questions than answers. Environ Health Perspect 106:689-700, 1998

23. Sattler TA, Dimitrov T, Hall PW: Relation between endemic (Balkan) nephropathy and urinary tract tumours. Lancet 1:278-280, 1977

24. Petkovic S, Mutavdzic M, Petronic V, Markovic V: Les tumeurs du bassinet et de l'uretère. Recherches cliniques et étiologiques. J Urol Néphrol 77:429-439, 1971

25. Chernozemsky IN, Stoyanov IS, Petkova-Bocharova TK, et al: Geographic correlation between the occurrence of endemic nephropathy

and urinary tract tumours in Vratza district, Bulgaria. Int J Cancer 19:1-11, 1977

26. Stoyanov IS, Chernozemsky IN, Nicolov IG, Stoichev II, Petkova-Bocharova TK: Epidemiologic association between endemic nephropathy and urinary system tumors in an endemic region. J Chronic Dis 31:721-724, 1978

27. Markovic N, Ignjatovic I, Cukuranovic R, Petrovic B, Kocic B, Stefanovic V: Decreasing incidence of urothelial cancer in a Balkan endemic nephropathy region in Serbia. A surgery-based study from 1969 to 1998. Pathol Biol 53:26-29, 2005

28. Danilovic V, Djurisic M, Mokranjac M, Stojimirovic B, Zivojinovic J, Stojakovic P: Chronic nephritis caused by poisoning with lead via the digestive tract (flour). Presse Med 65:2039-2040, 1957

29. Krogh P, Hald B, Plestina R, Ceovic S: Balkan (endemic) nephropathy and foodborn ochratoxin A: Preliminary results of a survey of foodstuffs. Acta Pathol Microbiol Scand B 85:238-240, 1977

30. Krogh P: Mycotoxic porcine nephropathy: A possible model for Balkan endemic nephropathy, in Puchlev A (ed): Proceedings of the Second International Symposium on Endemic Nephropathy. Sofia, Bulgaria, Bulgarian Academy of Sciences, 1974, pp 26-270

31. Lioy PJ: Measurement methods for human exposure analysis. Environ Health Perspect 103:S35-S43, 1995 (suppl 3)

32. Long DT, Voice TC: Role of exposure analysis in solving the mystery of Balkan endemic nephropathy. Croat Med J 48:300-311, 2007

33. Kuiper-Goodman T, Scott PM: Risk assessment of the mycotoxin ochratoxin A. Biomed Environ Sci 2:179-248, 1989

34. Stefanaki I, Foufa E, Tsatsou-Dritsa A, Dais P: Ochratoxin A concentrations in Greek domestic wines and dried vine fruits. Food Addit Contam 20:74-83, 2003

35. Petkova-Bocharova T, Castegnaro M: Ochratoxin A contamination of cereals in an area of high incidence of Balkan endemic nephropathy in Bulgaria. Food Addit Contam 2:267-270, 1985

36. Abouzied MM, Horvath AD, Podlesny PM, et al: Ochratoxin A concentrations in food and feed from a region with Balkan endemic nephropathy. Food Addit Contam 19:755-764, 2002

37. Vrabcheva T, Petkova-Bocharova T, Grosso F, et al: Analysis of ochratoxin A in foods consumed by inhabitants from an area with Balkan endemic nephropathy: A 1 month follow-up study. J Agric Food Chem 52: 2404-2410, 2004

38. Radic B, Fuchs R, Peraica M, Lucic A: Ochratoxin A in human sera in the area with endemic nephropathy in Croatia. Toxicol Lett 91:105-109, 1997

39. Maaroufi K, Achour A, Betbeder AM, et al: Foodstuffs and human blood contamination by the mycotoxin ochratoxin A: Correlation with chronic interstitial nephropathy in Tunisia. Arch Toxicol 69:552-558, 1995

40. Maaroufi K, Achour A, Hammami M, et al: Ochratoxin A in human blood in relation to nephropathy in Tunisia. Hum Exp Toxicol 14:609-614, 1995

41. Godin M, Fillastre J-P, Simon P, Francois A, Le Roy F, Morin J-P: Is ochratoxin A a nephrotoxic in human beings? Adv Nephrol Necker Hosp 26:181-206, 1997

42. Grollman AP, Jelakovic B: Role of environmental toxins in endemic (Balkan) nephropathy. October 2006, Zagreb, Croatia. J Am Soc Nephrol 18:2817-2823, 2007

43. Kazantzis G: Comment in the general discussion: Possible nephrotoxic agents, in Wolstenholme GE, Knight J (eds): The Balkan Nephropathy. Boston, MA, CIBA Foundation, 1967, p 114

44. Ivić M: Etiology of endemic nephropathy. Lijec Vjesn 91:1273-1281, 1969

45. Dumic A: Horse poisoning with *Aristolochia clematitis* [in Croatian], in Zagreb: Izdanje Vojno tehnickog glasnika. 1954, pp 3-45

46. Martincic M: Toxic effects of *Aristolochia clematitis* on horses' kidneys [in German]. Vetererinarski Arhiv 27:51-59, 1957

47. Ivić M, Lovriæ B: Carcinogenic action of *Aristolochia* [in Serbian]. Acta Medica Med 5:1-3, 1967

48. Vanherweghem JL, Depierreux M, Tielmans C, et al: Rapidly progressive interstitial renal fibrosis in young women: Association with slimming regimen including Chinese herbs. Lancet 341:387-391, 1993

49. Cosyns JP, Jadoul M, Squifflet JP, van Cangh PJ, van Ypersele de Strihou C: Chinese herbs nephropathy: A clue to Balkan endemic nephropathy? Kidney Int 45:1680-1688, 1994

50. Tanaka A, Nishida R, Sawai K: Traditional remedy-induced Chinese herbs nephropathy showing rapid deterioration of renal function [in Japanese]. Nippon Jinzo Gakkai Shi 39: 794-797, 1997

51. Cosyns JP, Jadoul M, Squifflet JP, Wese FX, van Ypersele de Strihou C: Urothelial lesions in Chinese herb nephropathy. Am J Kidney Dis 33: 1011-1017, 1999

52. Nortier JL, Muniz MC, Schmeiser HH, et al: Urothelial carcinoma associated with the use of a Chinese herbs (*Aristolochia* species). N Engl J Med 342:1686-1692, 2000

53. Arlt VM, Pfohl-Leszkowicz A, Cosyns J, Schmeiser HH: Analyses of DNA adducts formed by ochratoxin A and aristolochic acid in patients with Chinese herbs nephropathy. Mutat Res 494:143-150, 2001

54. Grollman AP, Shibutani S, Moriya M, et al: Aristolochic acid and the etiology of endemic (Balkan) nephropathy. Proc Natl Acad Sci U S A 104:12129-12134, 2007

55. Lemy A, Wissing KM, Rovire S, et al: Late onset of bladder urothelial carcinoma after kidney transplantation for end-stage aristolochic acid nephropathy: A case series with 15-year follow-up. Am J Kidney Dis 51: 471-477, 2008

56. Hranjec T, Kovač A, Kos J, et al: Endemic nephropathy: The case for chronic poisoning by aristolochia. Croat Med J 46:116-125, 2005

57. Feder GL, Radovanovi Z, Finkelman RB: Relationship between weathered coal deposits and the aetiology of Balkan endemic nephropathy. Kidney Int Suppl 34:S9-S11, 1991

58. Orem WH, Feder GL, Finkelman RB: A possible link between Balkan endemic nephropathy and the leaching of toxic organic compounds from Pliocene lignites by groundwater: Int J Coal Geol 40:237-252, 1999

59. Orem WH, Tatu CA: Health Effects of Toxic Organic Compounds from Coal—The Case of Balkan Endemic Nephropathy (BEN), in USGS: USGS Fact Sheet FS–004–01. Reston, VA, April 2001

60. Goldberg MC, Feder GL, Radovanovic Z: Correlation of Balkan endemic nephropathy with fluorescent organic compounds in shallow ground water. Appl Hydrogeol 2:15-23, 1994

61. Niagolova N, McEllmurry SP, Voice TC, et al: Nitrogen species in drinking water indicate potential exposure pathway for Balkan endemic nephropathy. Envir Pollut 134:229-237, 2005

62. Voice TC, McElmurry SP, Long DT, Dimitrov P, Ganev VS, Petropoulos EA: Evaluation of the hypothesis that Balkan endemic nephropathy is caused by drinking water exposure to contaminants leaching from Pliocene coal deposits. J Expo Sci Environ Epidemiol 16:515-524, 2002

63. Palca J: Eastern Europe: Missing an opportunity. Science 248:20-22, 1991

64. Uzelac-Keserović B, Spasić P, Bojanić N, et al: Isolation of a coronavirus from kidney biopsies of endemic Balkan nephropathy patients. Nephron 81:141-145, 1999

65. Riquelme C, Escors D, Ortego J, et al: Nature of the virus associated with endemic Balkan nephropathy. Emerg Infect Dis 8:869-870, 2002

66. Toncheva D, Dimitrov T, Stojanova S: Etiology of Balkan endemic nephropathy: A multifactorial disease? Eur J Epidemiol 14:389-394, 1998

67. Toncheva D, Dimitrov T, Tzoneva M: Cytogenetic studies in Balkan endemic nephropathy. Nephron 48:18-21, 1988

68. Stefanović V: Balkan endemic nephropathy: A need for novel aetiological approaches. Q J Med 91:457-463, 1998

69. Toncheva D, Dimitrov T: Genetic predisposition to Balkan endemic nephropathy. Nephron 72:564-569, 1996

70. Toncheva DI, Gergov TD, Tzoneva MT, Bouchakliev ZP: Spontaneous and induced chromosome aberrations in Balkan endemic nephropathy. Kidney Int Suppl 34:S97-S101, 1991

71. Dimitrov PS, Simeonov VA, Ganev VS, Karmaus WJ: Is the incidence of Balkan endemic nephropathy decreasing? Pathol Biol (Paris) 50:38-41, 2002

72. Cukuranovic R, Petrovic B, Cukuranovic Z, Stefanovic V: Balkan endemic nephropathy: A decreasing incidence of the disease. Pathol Biol (Paris) 48:558-561, 2000

73. Djukanovic L, Radovic M, Bakovic J, et al: Epidemiology of end-stage renal disease and current status of hemodialysis in Yugoslavia. Int J Artif Organs 25:852-859, 2002

74. Stefanovic V, Toncheva D, Atanasova S, Polenakovic M: Etiology of Balkan endemic nephropathy and associated urothelial cancer. Am J Nephrol 26:1-11, 2006

75. Abid S, Hassen W, Achour A, et al: Ochratoxin A and human chronic nephropathy in Tunisia: Is the situation endemic? Hum Exp Toxicol 22: 77-84, 2003

76. Orem W, Tatu C, Pavlovic N, et al: Health effects of toxic organic substances from coal: Toward "panendemic" nephropathy. Ambio 36:98-102, 2007