The etiology of Balkan endemic nephropathy (BEN) is not yet fully understood but there appears to be a polygenic susceptibility to the disease involving interactions between multiple environmental factors and multiple genes (1). In blood samples of Bulgarian BEN patients, and in some of their healthy relatives, 3q24 - 3q26 abnormalities have been established (2). The frequency of acquired chromosomal anomalies is considerably higher than in healthy persons. Oncogenic bands are more frequently involved in structural aberrations and spontaneous chromosomal/chromatid breaks in BEN patients than in the healthy population (3).

Most bladder neoplasms are transitional cell carcinomas that are either papillary noninvasive (stage pTa) or invasive (stage pT1 or higher). Comparative genomic hybridization studies have shown that the most frequently deleted chromosomal regions in bladder cancer are 2q, 4q, 5q, 6q, 8p, 9p, 9q, 11p, 11q, and 13q, and the most frequently over-represented are 1q, 3p, 5p, 6p, 8q, 17q, and 20q (4). The deleted regions may carry tumor suppressor genes, and the regions with gains oncogenes. Recently, the loss of heterozygosity (LOH) in three BEN-associated tumors at seven microsatellite loci at 3q21.3 - 3q27.3 was studied (5). Comparative genomic hybridization (CGH) was also done and one of the tumors was investigated by 24-color FISH as well.

LOH in locus D3S1299 (3q24) was established in one case. CGH showed genetic gains at 1q, 3q, 7p, 7q, 15q, and 19q in at least two of the three tumors. Genetic loss was found in one case at 4q. Most frequent aberrations detected by 24-color FISH were der(X), der(X)t(X;18), der(16), der(3)t(3;15) and der(12). In conclusion, the LOH suggested the presence of a new, so far unidentified tumor-suppressor gene at 3q24. In pTa BEN tumor CGH showed that genome instability was extremely high. The 24-color FISH indicated highly complex chromosomal rearrangements.

Recently, the concept that the disease is caused by a virus, has been strongly supported by the isolation of a virus from kidneys, tumors and metastatic lymph glands obtained at operations for tumors of the urinary tract (6,7). BEN-associated virus was reported to share serologic homology with human corona viruses, as well as the porcine transmissible corona virus (TGEV). In another study, using the same material the size and the morphology of TGEV clearly differed from that of the BEN-associated virus that had no peplomers. The dominant and only virus detected in the cell cultures infected with the BEN-associated virus was unre-
of epidemiological criteria, proteinuria of the tubular type is acceptable. For early cases, diagnosis is made on the ground of unequivocal diagnosis of BEN and in control patients. Detection of these AA-specific DNA adducts in human tissue samples is achieved by the 32P-postlabeling method.

Two recent epidemiological studies carried out in Serbia and Croatia have suggested that the incidence of BEN may decrease in the near future (19,20). A study was performed to investigate the incidence of BEN patients on dialysis, and BEN-associated mortality in endemic areas around the South Morava River in Serbia from 1978 to 1997 (21). In the last ten years a marked decrease in the incidence of ESRD and BEN-induced mortality has been documented in the region. The marked decrease in the incidence of BEN, as observed in South Serbia, may give the impression that it no longer exists. However, the etiological factor(s) are still present, as noted in a recent study (22). In the South Morava River region urinary excretion of albumin was studied in 703 healthy children, age 9-13, from endemic and non-endemic settlements around the South Morava River. Since BEN is environmentally induced disease, with possible seasonal variation of toxicant(s), children were studied three times a year: spring, autumn and winter. Evidence is presented that in autumn children from families with BEN excreted significantly more albumin than those from non-endemic families but living in the same settlements, or from children living outside of the endemic region in the city of Nis.

A high incidence of BEN is still observed in the Kolubara River region in Serbia (23). This recent report favors the hypothesis that different endemic regions have certain local characteristics. The disease seems to have had an endemic-epidemic profile in the past, and the possibility of another epidemic occurring in the future cannot be excluded.

Further research
Etiology remains the main problem for research in BEN. Since research restricted to a single village frequently brought about inferences that could not hold true elsewhere, it is important to test etiological hypotheses in different endemic foci, i.e., to run studies at different sites, preferably as a multicentric research.

Frequent sources of errors in the past were poorly selected cases and controls. Individual diagnosis of BEN may well be wrong. BEN cases: inhabitants of endemic settlements and from families with documented BEN cases (ill or dead), with exclusion of known interstitial and glomerular kidney disease. Early stages of disease are prefered, however, for most studies patients in incipient renal failure will be acceptable. For early cases, diagnosis is made on the ground of epidemiological criteria, proteinuria of the tubular type (increased beta-2-microglobulin excretion) and scarce urinary deposit.

For any environmental (as well as most other) research, households should be considered as affected only if at least three members developed BEN and/or upper urothelial tumors. Two groups of control households should be selected – one in endemic and another in a neighboring non-endemic settlement. None of the control household members should have had a kidney disease or tumors of urinary organs.

The present state of knowledge on BEN etiology does not warrant any prophylactic trial. It applies to supplementing selenium, as well as any similar idea that may be attempted.

References
12. Petkova-Bocharova T, Castegnaro M. Ochratoxin A contamination of cereals in an area of high incidence.


