Arch. Biol. Sci., Belgrade, 62 (4), 957-965, 2010 DOI:10.2298/ABS1004957K

GENETIC CLUES TO THE ETIOLOGY OF BALKAN ENDEMIC NEPHROPATHY: INVESTIGATING THE ROLE OF ACE AND AT1R POLYMORPHISMS

ZORICA KRCUNOVIĆ[1,5], IVANA NOVAKOVIĆ[1], NELA MAKSIMOVIĆ[1], DANICA BUKVIĆ[2], SANJA SIMIĆ-OGRIZOVIĆ[3], SLAVENKA JANKOVIĆ[4], LJUBICA ĐUKANOVIĆ[3] and DRAGANA CVETKOVIĆ[5]

[1] *Institute of Human Genetics, School of Medicine, University of Belgrade,* 11000 Belgrade, Serbia
[2] *Institute of Endemic Nephropathy,* 11000 Lazarevac, Serbia
[3] *Institute of Nephrology CCS,* 11000 Belgrade, Serbia
[4] *Institute of Epidemiology, School of Medicine, University of Belgrade,* 11000 Belgrade, Serbia
[5] *Faculty of Biology, University of Belgrade,* 11000 Belgrade, Serbia

Abstract - Balkan endemic nephropathy (BEN) was recognized as a distinct entity more than 50 years ago, but the exact environmental and genetic causes of the disease remain elusive. Considering the role of the renin-angiotensin system (RAS) in the emergence of various nephropathies, in the present study we evaluated the possible association with BEN of polymorphisms in two RAS genes: I/D ACE (an angiotensin-converting enzyme) and A1166C AT1R (an angiotensin type 1 receptor). The study groups consisted of 48 BEN patients from the endemic region in the district of Kolubara, Serbia, 33 patients with other nephropathies and 42 healthy individuals. The ACE DD genotype was significantly more represented in the NBEN group (OR=5.447; 95%CI=1.862-15.932, p<0.01). The frequency of the AT1R CC genotype was higher in BEN patients compared to controls (0.104 *vs.* 0.048), but the difference was not significant. Though the analyzed polymorphisms are associated with certain nephropathies, we found no support for their specific role in BEN susceptibility.

Key words: Balkan endemic nephropathy, candidate gene polymorphism, ACE I/D, AT1R A1166C, RAS system

UDC 616.61(497):597.21

INTRODUCTION

Although Balkan endemic nephropathy (BEN) was recognized as a distinct entity more than 50 years ago (Tanchev et al., 1956; Danilović et al., 1957), today it is in the focus of renewed research interest (e.g. Dimitrov et al., 2006; Grollman et al., 2007; Bamias and Boletis, 2008; Đukanović et al., 2009; Karmaus et al., 2009; Slade et al., 2009; Stefanović et al., 2009; Đukanović et al., 2010).

Balkan endemic nephropathy is a chronic non-inflammatory tubulointerstitial kidney disease characterized by slow progression to renal failure and shrinkage of the kidneys. It is frequently associated with urothelial carcinoma of the upper urinary tract. The characteristic feature of the disease is its spatial distribution – BEN occurs in certain rural areas of Serbia, Bulgaria, Romania, Croatia, and Bosnia and Herzegovina (e.g. Radovanović, 1991). The endemic villages are situated at low altitudes along the tributaries of the Danube; in Serbia, 73 endemic villages were found in the Kolubara valley, Podrinje, Mačva and Pomoravlje (Stefanović, 1998). The disease shows a mosaic pattern of distribution of endemic settlements within the area and familial clustering within the villages.

The etiology of BEN has been the subject of numerous studies, but despite decades of extensive research the exact causes remain elusive. Several lines of evidence confirm the concept of BEN as an environmentally induced disease, i.e. that at least

one environmental factor with known nephrotoxic (and cancerogenic) effects plays a key role in its development (Stefanović, 1998; Batuman, 2006; Long and Voice 2007). The main hypotheses include: chronic dietary exposure to aristolochic acid (AA) or mycotoxin ochratoxin A (OTA); exposure to metals and metalloids; the presence of soluble organic compounds in drinking water, such as polycyclic aromatic hydrocarbons (PAH) leached from Pliocene lignites.

The hypothesis that aristolochic acid (found in wheat contaminated with the seeds of *Aristolochia clematitis*) is responsible for BEN has gained new support recently. A number of studies (Arlt et al., 2007; Grollman et al., 2007; Slade et al., 2009) have provided molecular evidence that chronic exposure to AA is a significant risk factor for the development of nephropathy and the associated urothelial cancer. Another food contaminant, ochratoxin A, is also known for its nephrotoxic and carcinogenic effect (Petkova-Bocharova et al., 1991); however, it has not been confirmed as the primary cause of BEN (Long and Voice, 2007).

Exposure to metals and metalloids (cadmium, lead, arsenic, selenium) was also proposed as having a significant role in the etiology of BEN, but this hypothesis has subsequently been rejected (Karmaus et al., 2008). One of the most intriguing approaches is based on the geographic correlation of BEN-endemic areas with Pliocene lignite deposits. It is suggested that BEN is caused by long-term exposure to drinking water contaminated with toxic organic compounds leaching from lignite deposits; so far the evidence for this has been equivocal (Feder et al., 1991; Voice et al., 2006; Pavlović et al., 2008).

Along with environmental causes, genetic contributions to the etiology of BEN have been investigated as well. Since the first reports of familial clustering of the disease, the issue of a genetic predisposition to BEN has attracted much attention. Recently, various polymorphisms have been investigated in the context of interaction between an individual genetic variation in susceptibility and exposure to environmental toxic agents that can trigger the disease or influence its progression. So far, a small number of candidate genes have been found to be associated with an increased risk for BEN, mostly those involved in xenobiotic detoxification mechanisms that can result in nephrotoxic products. Andonova et al. (2004) analyzed glutathione S-transferase (GST) polymorphisms and found that the GSTM1 wt- allele was associated with an increased risk for BEN; the same was found for the CYP3A5*1 allele of cytochrome P450 (Atanasova et al., 2005). Toncheva et al. (2004) found no significant association of polymorphisms in NAD(P)H:quinone oxidoreductase (NQO1) with BEN, while MDR1 12 haplotype appeared to protect against BEN (Atanasova et al., 2004). Also, recent analysis of p53 single-base changes has suggested that these changes might be involved in genetic pathways leading to BEN (Krasteva and Georgieva, 2006).

Considering the role of the renin-angiotensin system (RAS) in the emergence of nephropathies, we have chosen candidate gene polymorphisms for our study: I/D ACE (angiotensin-converting enzyme) and A1166C AT1R (angiotensin type 1 receptor). These polymorphisms of two of the main RAS genes have been studied previously with respect to various diseases, but not to BEN.

Angiotensin-converting enzyme (ACE) acts on two important hormonal regulatory systems. It cleaves angiotensin I to generate the active angiotensin II, a potent vasoconstrictor that plays an important role in the homeostatic control of arterial pressure. ACE also metabolizes the vasodilators bradykinin and kallidin to inactive metabolites. Thus, functionally, the actions of ACE potentially result in increased vasoconstriction or decreased vasodilation (Atlas, 2007). The ACE insertion/deletion (I/D) polymorphism results from the presence/absence of a 287 bp DNA fragment, representing an Alu repetitive sequence, in intron 16. A strong association of the this polymorphism with the level of circulating enzyme was found: the mean plasma ACE level of DD subjects was about twice that of the II subjects, with heterozygotes having intermediate levels (Rigat et al., 1990; Tiret et al., 1992).

Type I receptor for angiotensin II (AT1R) mediates the effects of angiotensin II on a number of functions, including vasoconstriction and kidney functions, such as renal tubular sodium reabsorption and inhibition of renin release (Atlas, 2007). Several variants in the human AT1R gene may affect blood pressure; specifically, the AT1R A1166C variant that shows a significantly higher frequency in patients with essential hypertension (Bonnardeaux et al., 1994).

The aim of the present study was to examine the possible association between the candidate gene polymorphisms ACE I/D and AT1R A1166C and susceptibility to Balkan endemic nephropathy, as well as to other types of nephropathies.

MATERIALS AND METHODS

Subjects

The study was carried out on 123 unrelated individuals: 48 patients with BEN (BEN group) from the endemic region in Kolubara district**,** 33 patients with other types of nephropathies (NBEN group) and 42 healthy persons (control – CTR group). The patients were treated at the Institute of Endemic Nephropathy in Lazarevac and the Institute of Nephrology CCS in Belgrade. BEN was diagnosed according to the criteria of Danilović (1973): place of birth and residence in an endemic village, family history, anemia, specific color of face and palms, absence of arterial hypertension, mild proteinuria, low specific gravity of urine, retention of nitrous compounds in the blood and shrunken kidneys. The NBEN group consisted of patients with glomerulonephritis, polycystic kidney disease, rapidly progressive glomerulonephritis and system diseases. Written informed consent was obtained from all participants involved in this study. Genetic analyses were performed at the Institute of Human Genetics, School of Medicine, Belgrade.

Genetic analyses

DNA for gene analysis was extracted from peripheral blood leukocytes, from 10 mL whole blood, by a standard salting out procedure for DNA isolation.

ACE I/D genotyping

The insertion/deletion DNA polymorphism was detected using polymerase chain reaction (PCR) and polyacryilamide gel electrophoresis.

PCR conditions

Reactions were performed using 0.5 μl of each primer (Fw: 5'-CTGGAGACCACTCCCATCCTTTCT-3' and Rev:5'-GATGTGGCCATCACATTCGTCAGAT-3') in a final volume of 25 μl, containing 25 mM $MgCl_2$, 10mM of each dNTP, 10xB (8500 mM KCl, 100 mM TRIS HCl, pH 8.3, 15 mM $MgCl_2$, 0.01% gelatin), 1 unit of Taq polymerase. The DNA was amplified for 30 cycles with denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min using an Applied Biosystem Thermocycler. The PCR product is a 203 bp long amplificon in the absence of the insertion, or 490 bp long amplificon in the presence of the insertion.

AT1R genotyping

The single nucleotide polymorphism A1166C, an A-to-C variant in the 3' UTR at nucleotide 1166, was detected using PCR and restriction fragment length polymorphism (RFLP). *PCR conditions:* Reactions were performed using 0.5 μl of each primer (Fw: 5'-GCAGCACTTCACTACCAAATGGGC-3' and Rev: 5'-CAGGACAAAAGCAGGCTAGGGAGA-3') in a final volume of 25 μl, containing 25 mM $MgCl_2$, 10mM of each dNTP, 10xB (8500 mM KCl, 100 mM TRIS HCl, pH 8,3, 15 mM $MgCl_2$, 0,01% gelatin), 1 unit of Taq polymerase. The DNA was amplified for 30 cycles with denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 1 min using an Applied Biosystem Thermocycler. The PCR product is

Table 1. Demographic and clinical characteristics of patients with nephropathies (BEN – Balkan endemic nephropathy, NBEN – other types of nephropathies) and healthy control subjects (CTR).

	BEN			NBEN			CTR		
	n	mean ± SE	range	n	mean ± SE	range	n	mean ± SE	range
					Age (years)				
total	48	72.6 ± 1.2	51-88	33	52.4 ± 2.3	21-82	42	51.8 ± 2.0	22-82
males	30	71.9 ± 1.6	51-85	16	49.8 ± 3.8	21-82	22	58.3 ± 2.5	33-82
females	18	73.8 ± 1.9	57-88	17	56.9 ± 2.5	37-73	20	44.6 ± 2.4	22-61
					Age at first dialysis (years)				
	29	63.2 ± 1.97	42-80	33	45.0 ± 2.32	20-71			
					Months on dialysis				
	29	104.0 ± 10.9	6-264	33	101.8 ± 12.1	14-283			

a 255 bp long amplificon, which is futher digested using *HaeIII* restriction enzyme; the presence of C variant results in restriction fragments of 231 bp and 24 bp.

Statistical analyses

Statistical analyses were performed using the statistical package Statistica (v. 5.1, Statsoft Inc, Tulsa, OK, USA). To test for association with the disease, odds ratios with 95% confidence intervals were calculated.

RESULTS

The demographic and clinical characteristics of the study participants (patients with nephropathies and healthy control subjects) are given in Table 1. With respect to age distribution, the BEN group differed significantly from both the NBEN and the CTR (Kolmogorov Smirnov test, $p<0.01$); no significant differences were found between males and females in the patient groups (KS test, $p>0.2$).

Genotype and allele frequencies of ACE I/D and AT1R A1166C polymorphisms in the analyzed groups are given in Table 2; genotype distributions did not differ significantly between the sexes (results not shown; chi square, $p>0.05$). An electropherogram showing the AT1R A1166C genotypes in the group of BEN patients is given in Fig. 1.

To test for association with the disease, odds ratios were calculated; first, we tested a DD *vs.* (ID + II) model for ACE I/D polymorphism, and a CC *vs.* (AC + AA) model for AT1R A1166C polymorphism. A statistically significant association was found for ACE, but not for AT1R polymorphism. The frequency of the ACE DD genotype was significantly elevated only in the NBEN group (Table 2). Although D-allele carriers were more represented in the BEN patients than in the healthy subjects, this was not significant (D- *vs.* II, OR=1.23; 95%CI=0.67-2.27; $p>0.05$). The frequency of the AT1R C allele, as well as the CC genotype, was higher in the BEN patients compared to the controls, but not significantly; the difference between the NBEN and CTR was even smaller (Table 2). The CC genotype was not significantly associated with the disease (BEN *vs.* CTR: OR=2.326; 95% CI = 0.427-12.672; $p>> 0.05$).

When both loci were compared, in the DD CC *vs.* (ID + II) (AC + AA) model the frequency of DD CC was higher in patients with nephropathies than in healthy controls (0.129, 0.250 and 0.08 in BEN, NBEN and CTR group, respectively), but the small number of carriers precludes conclusions about the significance of this difference.

We also tested if the carriers of different ACE I/D genotypes differed in age at first dialysis, taken as the indicator of disease progression, i.e. timing of

Table 2. Genotype and allele frequencies of ACE I/D and AT1R A1166C polymorphisms in patients with nephropathies and healthy control subjects (BEN – Balkan endemic nephropathy; NBEN – other types of nephropathies; CTR – controls; OR – odds ratio, CI – confidence interval).

Genotype	allele	BEN	NBEN	CTR
ACE		*frequency (n)*	*frequency (n)*	*frequency (n)*
DD		0.417 (20)	0.818 (27)	0.452 (19)
ID		0.500 (24)	0.121 (4)	0.333 (14)
II		0.083 (4)	0.061 (2)	0.214 (9)
	D	0.667	0.879	0.619
	I	0.333	0.121	0.381
AT1R				
AA		0.479 (23)	0.606 (20)	0.642 (27)
AC		0.417 (20)	0.333 (11)	0.310 (13)
CC		0.104 (5)	0.061 (2)	0.048 (2)
	A	0.688	0.773	0.798
	C	0.312	0.227	0.202
ACE DD *vs.* ID + II		OR	95% CI	p
BEN *vs.* C		0.865	0.375-1.994	p>> 0.05
NBEN *vs.* C		**5.447**	**1.862-15.932**	**p < 0.01**

end-stage renal failure (Fig. 2). In both groups, the II carriers started with dialysis later. Though this difference was not significant, it is worth further testing on a larger sample of patients.

DISCUSSION

Many studies indicate that genetic susceptibility plays an important role in the rate of renal function decline. Considering the genetic component of BEN etiology, previous studies have focused mainly on the genes involved in xenobiotic detoxification mechanisms (e.g. Andonova et al., 2004). On the other hand, the RAS system is one of the key mediators in kidney fibrosis. The molecular mechanisms of this process are both the induction of matrix synthesis and the inhibition of matrix degradation in renal tubulointerstitium. Moreover, the RAS system has a crucial role in human kidney development. Homozygous or compound heterozygous mutations in the genes encoding renin, angiotensinogen, ACE, or ATR1 have been found in cases of renal tubular dysgenesis (Gribouval et al., 2005). As a result, polymorphisms in the genes that control the RAS system have been extensively studied in various renal disorders (e.g. Doria et al., 1994; Marre et al., 1997; Coll et al., 2003; Gribouval et al., 2005; Lee et al., 2008; Ahluwalia et al., 2009), but not in BEN so far.

In the present study, we analyzed polymorphisms in two of the main genes of the RAS system, in patients with BEN, as well as in patients with other types of nephropathies (NBEN). Contrary to expectations, our results did not show any statistically significant association between ACE genotypes and BEN; however, we found a significantly higher frequency of the ACE DD genotype in the NBEN group compared to the control. In addition, it should be noted that, although the D allele is considered a "risk allele" for a number of diseases, in our healthy control group both the D allele and DD genotype were represented at higher frequencies compared to the data reported for other populations (e.g. Schena et al., 2001; Cooper Worobey et al., 2009; Fatini et al., 2009). The obtained values are in accordance with the highest frequencies reported for this region of Europe in the meta-analysis of Lehmann et al. (2005) and with our previous findings (Novaković et al., 2010).

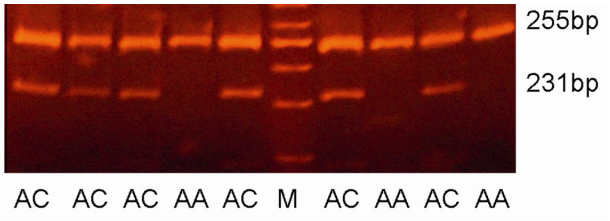

Fig. 1. Electropherogram of AT1R A1166C genotypes in BEN patients: 255bp and 231bp fragments represent A and C alleles, respectively (M – DNA ladder).

Our results contribute to a growing body of data, collected over 15 years, and to the debate about the association of ACE and AT1R gene polymorphisms with various renal disorders. The results are still controversial. Doria et al. (1994) reported that the I/D polymorphism of the ACE gene is associated with diabetic nephropathy (DN), but this was disputed by others (e.g. Tarnow et al., 1995). The large-scale, multicenter study on individuals with insulin-dependent diabetes mellitus at risk of kidney complications, called GENEDIAB (GEnetique de la NEphropathie DIABetique), concluded that the ACE gene is involved in both the susceptibility to DN and its progression toward renal failure (Marre et al., 1997). In addition, Jacobsen et al. (2003) observed that ACE polymorphism alone and in interaction with other polymorphisms of the RAS genes plays a role in the progression of DN. However, a recent study of 115 candidate genes did not support the significance of this association (Ewens et al., 2005).

The AT1R A1166C polymorphism has also been extensively studied with regard to its association with renal function decline, as well as with essential hypertension (e.g. Bonnardeaux et al., 1994). However, the issue of the "risk allele" is still controversial. Though some studies indicate that the A allele is associated with the risk of renal function decline (Jacobsen et al., 2003; Lee et al., 2009), most data point to the C allele. Thus, Coll et al. (2003) showed that the faster progression to end-stage renal disease is associated with the C allele, and this association remained significant after

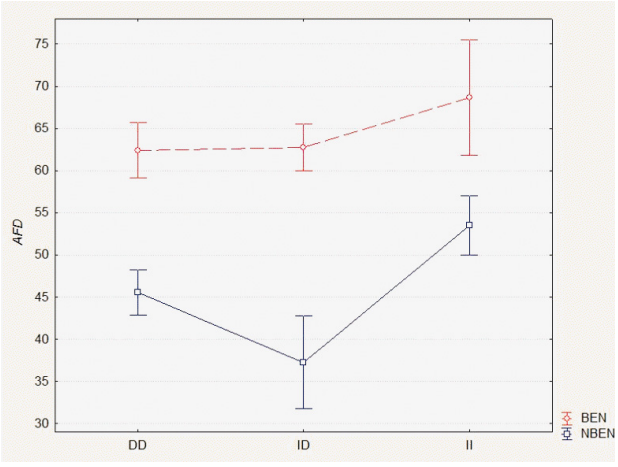

Fig. 2. Age at first dialysis in carriers of different ACE I/D genotypes (AFD – age at first dialysis in years, mean and standard error; dashed line – patients with Balkan endemic nephropathy, BEN group; solid line – patients with other types of nephropathies, NBEN group).

adjustment for relevant covariates. Lin et al. (2009) confirmed the association of the C allele with renal dysfunction in diabetics, while Cooper Worobey et al. (2009) found a marginally significant association of the CC genotype with a higher risk of renal function decline in female Caucasians. In our study, the frequencies of the C allele and CC genotype were higher in both the BEN and NBEN groups compared to the controls; though this difference was not significant, it should be further tested on a larger number of patients.

This study is the first that deals with RAS gene polymorphisms in renal disease patients in Serbia, and specifically in patients with BEN. As a conclusion, the genetic component of BEN remains unclear – though the analyzed polymorphisms were found to be associated with certain nephropathies, we found no support for their specific role in BEN susceptibility. More studies are needed on genetic variants in other members of the RAS system and on larger groups of patients in order to further contribute to what Batuman (2006) called the 'deciphering a complex mystery' – i.e. the solving the problem of the complex etiology of Balkan endemic nephropathy.

Acknowledgment - This study was supported by Ministry of Science and Technological Development of Republic of Serbia (Grant No. 145037).

REFERENCES

Ahluwalia, T.S., Ahuja, M., Rai, T.S., Kohli, H.S., Bhansali, A., Sud, K., and *M. Khullar* (2009). ACE variants interact with the RAS pathway to confer risk and protection against type 2 diabetic nephropathy. *DNA Cell Biol.* **28**, 141-150.

Andonova, I.E., Sarueva, R.B., Horvath, A.D., Simeonov, V.A., Dimitrov, P.S., Petropoulos, E.A., and *V.S. Ganev* (2004). Balkan endemic nephropathy and genetic variants of glutathione S-transferases. *J. Nephrol.* **17**, 390-398.

Arlt, V.M., Stiborová, M., Vom Brocke, J., Simões, M.L., Lord, G.M., Nortier, J.L., Hollstein, M., Phillips, D.H., and *H.H. Schmeiser* (2007). Aristolochic acid mutagenesis: molecular clues to the aetiology of Balkan endemic nephropathy-associated urothelial cancer. *Carcinogenesis,* **28**, 2253-2261.

Atanasova, S., Von Ahsen, N., Dimitrov, T., Armstrong, V., Oellerich, M., and *D. Toncheva* (2004). MDR1 haplotypes modify BEN disease risk: a study in Bulgarian patients with Balkan endemic nephropathy compared to healthy controls. *Nephron Exp. Nephrol.* **96**, e7-13.

Atanasova, S., Von Ahsen, N., Toncheva, D.I., Dimitrov, T.G., Oellerich, M., and *V.W. Armstrong* (2005). Genetic polymorphisms of cytochrome P450 among patients with Balkan endemic nephropathy (BEN). *Clinical Biochemistry,* **38**, 223–228.

Atlas, S.A. (2007). The Renin-Angiotensine Aldosterone System: Pathophysiological Role and Pharmacologic Inhibition. Supplement to Journal of Managed Care Pharmacy *JMCP,* **13**, No. 8, S-b.

Bamias, G.,and *Boletis, J.* (2008). Balkan nephropathy: evolution of our knowledge. *Am. J. Kidney Dis.* **52**, 606-616.

Batuman, V. (2006). Fifty years of Balkan endemic nephropathy: daunting questions, elusive answers. *Kidney International,* **69,** 644–646.

Bonnardeaux, A., Davies, E., Jeunemaitre, X., Fery, I., Charru, A., Clauser, E., Tiret, L., Cambien, F., Corvol, P., and *F. Soubrier* (1994). Angiotensin II type 1 receptor gene polymorphisms in human essential hypertension. *Hypertension,* **24**, 63-69.

Coll, E., Campos, B., González-Núñez, D., Botey, A., and *E. Poch* (2003). Association between the A1166C polymorphism of the angiotensin II receptor type 1 and progression of chronic renal insufficiency. *J. Nephrol.* **16**, 357-364.

Cooper Worobey, C., Fisher, N.D., Cox, D., Forman, J.P., and *G.C. Curhan* (2009). Genetic polymorphisms and the risk of accelerated renal function decline in women. *PLoS One,* **4**, e4787.

Danilović, V. (1973). Dijagnoza endemske nefropatije. *Radovi – XLVIII Od med nauka Akad nauka i umj BiH, Sarajevo,* **17**, 53-64.

Danilović, V., Đurišić, M., Mokranjac, M., Stojimirović, B., Živojinović, J., and *P. Stojaković* (1957). Chronic nephritis caused by poisoning with lead via the digestive tract (flour). *Presse Med.* **65**, 2039-2040.

Dimitrov, P., Tsolova, S., Georgieva, R., Bozhilova, D., Simeonov, V., Bonev, A., and *W. Karmaus* (2006). Clinical markers in adult offspring in families with and without Balkan Endemic Nephropathy. *Kidney International,* **69**, 723–729.

Doria, A., Warram, J.H., and *A.S. Krolewski* (1994). Genetic predisposition to diabetic nephropathy. Evidence for a role of the angiotensin I - converting enzyme gene. *Diabetes,* **43**, 690-695.

Đukanović, Lj., Ležaić, V., Miljković, Đ., Momčilović, M., Bukvić, D., Marić, I., Miljković, Ž., Marinković, J., and *M. Mostarica-Stojković* (2009). Transforming Growth Factor- 1 in Balkan Endemic Nephropathy. *Nephron Clin. Pract.* **111**, c127-c132 (DOI: 10.1159/000191206).

Đukanović, Lj., Stefanović, V., Basta-Jovanović, G., Bukvić, D., Glogovac, S., Dimitrijević, J., Đurić, S., Janković, S., Lukić, Lj., Marić, I., Nikolić, J., Novaković, I., Pejović, V., Radisavljević, S., Rakić, N., and *V. Savić* (2010). Investigation of Balkan endemic nephropathy in Serbia: How to proceed? *Srpski arhiv za celokupno lekarstvo,* **138**, 256-261.

Ewens, K.G., George, R.A., Sharma, K., Ziyadeh, F.N., and *R.S. Spielman* (2005). Assessment of 115 candidate genes for diabetic nephropathy by transmission/disequilibrium test. *Diabetes,* **54**, 3305-3318.

Fatini, C., Sticchi, E., Sofi, F., Said, A.A., Pratesi, G., Pulli, R., Pratesi, C., and *R. Abbate* (2009). Multilocus analysis in candidate genes ACE, AGT, and AGTR1 and predisposition to peripheral arterial disease: role of ACE D/-240T haplotype. *J. Vasc. Surg.* **50**, 1399-1404.

Feder, G. L., Radovanović, Z., and *R. B. Finkelman* (1991). Relationship between weathered coal deposits and the etiology of Balkan endemic nephropathy. *Kidney International,* **40,** (Suppl. 34), S3-S11.

Gribouval, O., Gonzales, M., Neuhaus, T., Aziza, J., Bieth, E., Laurent, N., Bouton, J.M., Feuillet, F., Makni, S., Ben Amar, H., Laube, G., Delezoide, A.L., Bouvier, R., Dijoud, F., Ollagnon-Roman, E., Roume, J., Joubert, M., Antignac, C., and *M.C. Gubler* (2005). Mutations in genes in the renin-angiotensin system are associated

with autosomal recessive renal tubular dysgenesis. *Nat. Genet*. **37**, 964-968.

Grollman, A. P., Shibutani, S., Moriya, M., Miller, F., Wu, L., Moll, U., Suzuki, N., Fernandes, A., Rosenquist, T., Medverec, Z., Jakovina, K., Brdar, B., Slade, N., Turesky, R.J., Goodenough, A.K., Rieger, R., Vukelić, M., and *B. Jelaković* (2007). Aristolochic acid and the etiology of endemic (Balkan) nephropathy. *Proc. Natl. Acad. Sci. U S A,* **104**, 12129-12134.

Jacobsen, P., Tarnow, L., Carstensen, B., Hovind, P., Poirier, O., and *H.H. Parving* (2003). Genetic variation in the renin-angiotensin system and progression of diabetic nephropathy. *J. Am. Soc. Nephrol*. **14**, 2843-2850.

Karmaus, W., Dimitrov, P., Simeonov, V., Tsolova, S., Bonev, A., and *R. Georgieva* (2008). Metals and kidney markers in adult offspring of endemic nephropathy patients and controls: a two-year follow-up study. *Environmental Health*, **7**, 11.

Karmaus, W., Dimitrov, P., Simeonov, V., Tsolova, S., and *V. Batuman* (2009). Offspring of parents with Balkan Endemic Nephropathy have higher C-reactive protein levels suggestive of inflammatory processes: a longitudinal study. *BMC Nephrol*. **28**, 10,10.

Krasteva, M.E., Georgieva, E.I. (2006). Germline p53 single-base changes associated with Balkan endemic nephropathy. *Biochem. Biophys. Res. Commun*. **342**, 562-567.

Lee, Y.T., Chiu, H.C., Su, H.M., Voon, W.C., Lin, T.H., Lai, W.T., and *S.H. Sheu* (2008). The D allele of angiotensin-converting enzyme polymorphism as a predictor of renal function decline over 4 years in an apparently healthy Chinese population aged 60 and older. *J. Am. Geriatr. Soc*. **56**, 718-723.

Lee, Y.T., Chiu, H.C., Huang, C.T., Su, H.M., Wang, C.L., Lin, T.H., Voon, W.C., Chen, H.C., Lai, W.T., and *S.H. Sheu* (2009). The A1166C polymorphism of angiotensin II Type 1 receptor as a predictor of renal function decline over 4 years follow-up in an apparently healthy Chinese population. *Clin. Nephrol.* **72**, 457-467.

Lehmann, D.J., Cortina-Borja, M., Warden, D.R, Smith, A.D., Sleegers, K., Prince J.A., van Duijn, C.M., and *P.G. Kehoe* (2005). Large meta-analysis establishes the ACE insertion-deletion polymorphism as a marker of Alzheimer's disease. *Am. J. Epidemiol*. 162, 305-317.

Lin, J., Hu, F.B., Qi, L., and *G.C. Curhan* (2009). Genetic polymorphisms of angiotensin-2 type 1 receptor and angiotensinogen and risk of renal dysfunction and coronary heart disease in type 2 diabetes mellitus. *BMC Nephrol*. **10**, 9.

Long, D.T., and *T.C. Voice* (2007). Role of exposure analysis in solving the mystery in Balkan endemic nephropathy. *Croat. Med. J*. **48**, 300-311.

Marre, M., Jeunemaitre, X., Gallois, Y., Rodier, M., Chatellier, G., Sert, C., Dusselier, L., Kahal, Z., Chaillous, L., Halimi, S., Muller, A., Sackmann, H., Bauduceau, B., Bled, F., Passa, P., and *F. Alhenc-Gelas* (1997). Genetique de la Nephropathie Diabetique (GENEDIAB) study group: Contribution of genetic polymorphism in the renin-angiotensin system to the development of renal complications in insulin-dependent diabetes. *J. Clin. Invest*. **99**, 1585-1595.

Novaković, I., Maksimović, N., Cvetković, S., and *D. Cvetković* (2010). Gene polymorphisms as markers of disease susceptibility. *JMB* **29,** (3) (DOI: 10.2478/v10011-010-0022-y).

Pavlović, N.M., Orem, W.H., Tatu, C.A. Lerch, H.E., Bunnell, J.E., Feder, G.L., Kostić, E.N., and *V.L. Ordodi* (2008). The role of lecithin cholesterol acyltransferase and organic substances from coal in the etiology of Balkan endemic nephropathy: a new hypothesis. *Food Chem. Toxicol.* **46**, 949-954.

Petkova-Bocharova, T., Castegnaro, M., Michelon, J., and *V. Maru* (1991). Ochratoxin A and other mycotoxins in cereals from an area of Balkan endemic nephropathy and urinary tract tumours in Bulgaria. In: Mycotoxins, Endemic Nephropathy and Urinary Tract Tumours. (Eds. Castegnaro, M., Plestina, R., Dirheimer, G., Chernozemsky, N. I., and H. Bartsch) *IARC Sci Publ*. **115**, 83–87.

Radovanović, Z. (1991). Epidemiological characteristics of Balkan Endemic Nephropathy in Eastern regions in Yugoslavia. In: Mycotoxins, Endemic Nephropathy and Urinary Tract Tumours. (Eds. Castegnaro, M., Plestina, R., Dirheimer, G., Chernozemsky, N. I., and H. Bartsch) *IARC Sci Publ*. **115**, 11-20.

Rigat, B., Hubert, C., Alhenc-Gelas, F., Cambien, F., Corvol, P., and *F. Soubrier* (1990). An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J. Clin. Invest*. **86**, 1343-1346.

Schena, F.P., D'Altri, C., Cerullo, G., Manno, C., and *L. Gesualdo* (2001). ACE gene polymorphism and IgA nephropathy: an ethnically homogeneous study and a meta-analysis. *Kidney Int*. **60**, 732-740.

Slade, N., Moll, U.M., Brdar, B., Zorić, A., and *B. Jelaković* (2009). p53 mutations as fingerprints for aristolochic acid: an environmental carcinogen in endemic (Balkan) nephropathy. *Mutat. Res*. **663**, 1-6.

Stefanović, V. (1998). Balkan endemic nephropathy: a need for novel aetiological approaches. *Q.J. Med*. **91**, 457-463.

Stefanović, V., Cukuranović, R., Miljković, S., Marinković, D., and *D. Toncheva* (2009). Fifty years of Balkan endemic nephropathy: challenges of study using epidemiological method. *Ren. Fail.* **31**, 409-418.

Tanchev, Y., Evstatiev, Z., Dorossiev, D., Pencheva, J., and *G. Tzvetkova* (1956). Studies on the nephritides in the District of Vratza. *Savremenna Med.* **7**, 14–29.

Tarnow, L., Cambien, F., Rossing, P., Nielsen, F.S., Hansen, B.V., Lecerf, L., Poirier, O., Danilov, S., and *H.H. Parving* (1995). Lack of relationship between an insertion/deletion polymorphism in the angiotensin I-converting enzyme gene and diabetic nephropathy and proliferative retinopathy in IDDM patients. *Diabetes,* **44**, 489-494.

Tiret, L., Rigat, B., Visvikis, S., Breda, C., Corvol, P., Cambien, F., and *F. Soubrier* (1992). Evidence, from combined segregation and linkage analysis, that a variant of the angiotensin I-converting enzyme (ACE) gene controls plasma ACE levels. *Am. J. Hum. Genet.* **51**, 197-205.

Toncheva, D.I., Von Ahsen, N., Atanasova, S.Y., Dimitrov, T.G., Armstrong, V.W., and *M. Oellerich* (2004). Identification of NQO1 and GSTs genotype frequencies in Bulgarian patients with Balkan endemic nephropathy. *J. Nephrol.* **17**, 384-389.

Voice, T.C., McElmurry, S.P., Long, D.T., Dimitrov, P., Ganev, V.S., and *E.A. Petropoulos* (2006). 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.

УДЕО ГЕНЕТИЧКЕ ОСНОВЕ У ЕТИОЛОГИЈИ БАЛКАНСКЕ ЕНДЕМСКЕ НЕФРОПАТИЈЕ: ИСПИТИВАЊЕ УЛОГЕ ПОЛИМОРФИЗАМА ГЕНА *ACE* И *AT1R*

ЗОРИЦА КРЦУНОВИЋ[1,5], ИВАНА НОВАКОВИЋ[1], НЕЛА МАКСИМОВИЋ[1], ДАНИЦА БУКВИЋ[2], САЊА СИМИЋ-ОГРИЗОВИЋ[3], СЛАВЕНКА ЈАНКОВИЋ[4], ЉУБИЦА ЂУКАНОВИЋ[3], и ДРАГАНА ЦВЕТКОВИЋ[5]

[1] *Институт за хуману генетику, Медицински факултет Универзитета у Београду,* 11000 Београд, Србија
[2] *Институт за ендемску нефропатију,* 11000 Лазаревац, Србија
[3] *Институт за нефрологију КЦС,* 11000 Београд, Србија
[4] *Институт за епидемиологију, Медицински факултет Универзитета у Београду,* 11000 Београд, Србија
[5] *Биолошки факултет Универзитета у Београду,* 11000 Београд, Србија

Балканска ендемска нефропатија (БЕН) позната је као засебан ентитет већ више од 50 година; ипак, средински и генетички фактори који доводе до болести нису довољно јасни. Имајући у виду улогу система ренин-ангиотензин (РАС) у етиопатогенези различитих нефропатија, у овој студији смо анализирали могуће асоцијације са БЕН полиморфизама главних РАС гена: I/D ACE (ангиотензин конвертазе) и A1166C AT1R (тип 1 рецептора за ангиотензин). Испитиване групе чинило је 48 болесника са БЕН из ендемске области у Колубарском региону, 33 болесника са другим нефропатијама (НБЕН) и 42 здраве особе. ACE DD генотип је био значајно чешћи само у НБЕН групи (OR=5.447; 95%CI=1.862-15.932, p<0.01). Учесталост AT1R CC генотипа је била већа у БЕН групи него код контроле (0.104 *vs.* 0.048), али разлика није статистички значајна. Иако се за анализиране полиморфизме већ показала асоцијација са одређеним нефропатијама, у нашем истраживању није доказана њихова специфична повезаност са БЕН.