ORIGINAL ARTICLE

UDC: 575:[616.379-008.64-06:616.61
DOI:10.2298/VSP1407627I

Association of renin-angiotensin system genes polymorphism with progression of diabetic nephropathy in patients with type 1 diabetes mellitus

Udruženost polimorfizma gena renin-angiotenzin sistema u razvoju dijabetesne nefropatije kod bolesnika sa dijabetesom tipa 1

Vesna Ilić*, Miroljub Ilić†, Ivan Soldatović‡, Srdjan Popović†, Zvonko Magić*§

***Institute for Medical Research, Military Medical Academy, Belgrade, Serbia; †Institute for Endocrinology, Diabetes and Medical Research, Clinical Center of Serbia, Belgrade, Serbia; ‡Institute for Medical Statistics, Faculty of Medicine, University of Belgrade, Belgrade, Serbia; §Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia**

Abstract

Background/Aim. Diabetic nephropathy (DN) as a major microvascular complication of diabetes mellitus (DM) include a progressive increase in urinary albumin excretion in association with an increase in blood pressure and to end stage renal failure. Hypertension connected with renin-angiotensin system (RAS) hyperactivity and corresponding genotypes, angiotensinogen (AGT), angiotensine-converting enzyme (ACE) and angiotensin II type 1 receptor (AT1R), predispose the increasing risk of DN. The aim of this study was to assess the distribution of AGT, ACE and AT1R gene polymorphisms in patients with type 1 DM according to the level of DN and patients clinical characteristics. **Methods.** The study included 79 type 1 diabetic patients. Inclusion criteria were: age between 20–40, duration of diabetes > 5 years, and no other severe diseases. Clinical characteristics were gained from interviewing the patients. Polymorphism was detected by polymerase chain reaction (PCR) and restriction fragment length polymorphism using restriction enzymes Psy I (Tth 111 I) and Hae III. **Results.** The patients with proteinuria compared with normo- and microalbuminuric patients, highly differed in age, diabetes duration, blood pressure level, hypertension, rethynopathy and urinary albumin excretion values ($p < 0.001$). No statistically significant difference between the groups was found for the ACE and AT1R gene polymorphisms distribution. The presence of TT genotype of the M235T polymorphism was significantly higher in the group with proteinuria ($p < 0.05$). The patients with hypertension raised nephropathy 5.2 times higher (OR = 5.20, $p < 0.05$) while carriers of TT allel developed nephropathy 28.38 times higher (OR = 28.389, $p < 0.01$) than those with MM genotype. **Conclusion.** Increased association of hypertension and TT angiotensinogen gene polymorphism in patients with diabetes mellitus with proteinuria could be a significant marker of diabetic nephropathy.

Key words:
diabetic nephropathies; renin-angiotensin system; diabetes mellitus, type 1; polymorphism, genetic.

Apstrakt

Uvod/Cilj. Dijabetesna nefropatija (DN) kao jedna od najznačajnijih komplikacija dijabetesa melitusa (DM) uključuje progresivno povećanje urinarne ekskrecije albumina koja udružena sa hipertenzijom, dovodi do terminalne bubrežne insuficijencije. Hipertenzija i povećana aktivnost renin-angiotenzin sistema (RAS) uz prisustvo određenih polimorfizama gena za RAS, angiotenzinogen (AGT), angiotenzin-konvertujući enzim (ACE) i angiotenzinski receptor tipa 1 (AT1R) mogu da ukažu na povećanu sklonost ka razvoju DN. Cilj rada bio je ispitivanje distribucije polimorfizma gena AGT, ACE i AT1R u grupi bolesnika sa DM tipa 1 u odnosu na stepen razvoja nefropatije, definisane kliničke parametre i faktore rizika. **Metode.** Ispitivanjem je bilo obuhvaćeno 79 bolesnika sa DM tipa 1, starosti 20–40 godina, trajanjem dijabetesa duže od 5 godina i bez drugih hroničnih bolesti. Polimorfizam gena RAS ustanovljen je metodom PCR-RFLP korišćenjem restrikcione endonukleaze Psy I (Tth 111I) i Hae III. **Rezultati.** Bolesnici sa proteinurijom u odnosu na normo- i mikroalbuminurične ispitanike značajno su se razlikovali po godinama starosti, trajanju dijabetesa, vrednosti krvnog pritiska, hipertenzije, retinopatije i urinarne ekskrecije albumina ($p < 0,001$). U ispitivanim grupama, razlike u učestalosti genskih polimorfizama za ACE i AT1R nisu bile statistički

Correspondence to: Vesna Ilić, Institute for Medical Research, Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Phone: +381 11 3608 857. E-mail: vesnailic@hotmail.com

značajne. Prisustvo TT genotipa u M235T polimorfizmu gena za AGT bio je značajno viši u grupi bolesnika sa proteinurijom ($p < 0,05$). Učestalost nefropatije bila je 5,2 puta veća (OR = 5,20, $p < 0,05$) kod dijabetičara sa hipertenzijom, dok je TT genotip ukazivao na 28.38 puta veću sklonost (OR = 28,389, $p < 0,01$) ka razvoju nefropatije u odnosu na MM genotip. **Zaključak.** Povećanje učestalosti hipertenzije i TT alelske forme u angiotenzinogen genskom polimorfizmu kod bolesnika u terminalnom stadijumu nefropatije mogu imati značaj za određivanje markera procene rizika od nastanka komplikacija u dijabetesu melitusu tipa 1.

Ključne reči:
dijabetičke nefropatije; renin-angiotenzin sistem; dijabetes melitus, tip 1; polimorfizam, genetički.

Introduction

Diabetic nephropathy (DN) is an important microvascular complication of a long-standing type 1 and type 2 diabetes mellitus (DM) associated with considerable morbidity and mortality. In spite of the main risk factors for the development of DN as hypertension and poor glycemic control, genetic susceptibility in both type 1 and type 2 DM is also of the great importance. Other risk factors that relate to the development of nephropathy are smoking, obesity and dyslipidemia [1]. Once DN is present, risk factors favorise evolution to more advanced stage. Recent studies suggest that antihypertensive drugs (angiotensine converting enzymes – ACE-inhibitors) should be used in prevention of DN even in normotensive patients.

DN is defined as increased protein excretion in urine. Early stage is characterized by a small increase in urinary albumin excretion (UAE), also called microalbuminuria or incipient DN. More advanced disease is defined by the presence of macroalbuminuria or proteinuria. The latter is named overt or "manifested" DN.

Patients with elevated level of urinary albumin excretion are at high risk of cardiovascular complications and need frequent and carefull examination for early detection of nephropathy and cardiovascular and lipid abnormalities [2].

Hemodynamic changes in DN, as systemic and glomerular hypertension, lead to the initiation and progression of nephropathy and may be explained by alteration in the renin-angiotensine system (RAS).

The prominent role of the component genes of the RAS in cardiovascular regulation sugest the possibility that polymorphism of the genes of the RAS might be involved in the genetic predisposition to develop DN. Among the candidate genes of the RAS, the angiotensinogen (AGT), ACE and angiotensin II type 1 receptor (AT1R) genes seem to be particulary relevant to renal disease and related with predisposition for renal complications [3, 4]. The genetic polymorphisms of these key components of RAS provide a basis for studying the relationship between genetic variants and the development of vascular and renal damage in patients with DM.

Several polymorphisms were identified in the AGT gene wich are linked to essential hypertension. Of those, M235T polymorphism (methionine substituted by threonine) resulting in M and T alel forms, was extensively studied in cardiovascular and renal diseases [4, 5]. A TT genotype is associated with the progression of limited hypertension into manifested hypertension as well as with increasing risk for the diabetic nephropathy level [6].

Polymorphism of the ACE gene has a frequent insertion-deletion (I/D) polymorphism characterized by the 278-bp insertion (allele I) or deletion (allele D) variant in intron 16 associated with serum and tissue ACE levels. The I/D polymorphism, wich determine most of ACE individual variance was proposed as a genetic marker for DN. The level of ACE in plasma is in direct correlation with the genotype, and plays a critical role in determining intrarenal angiotensin and kinin concentrations [7].

The AT1R polymorphism located at the position 1166 (A/C) in 3´ untranslated region has been considered as a risk factor for hypertension and cardiovascular disease [5].

In the present study we examined the distribution of the AGT, ACE and AT1R gene polymorphisms in patients with type I DM devided into three groups according to the level of DN – normoalbuminuric, microalbuminuric or incipient nephropathy and proteinuric or macroangiopathy level. Also, polymorphisms of three studied genes were correlated with clinical characteristics of patients with DN.

This study was approved by the Ethic Committee of the Institute of Diabetes and Metabolic Diseases, Clinical Center of Serbia, Belgrade.

Methods

All the patients with type 1 DM were presented with certain criteria and hospitalised in the Institute of Diabetes and Metabolic Diseases from 2008 to 2010.

The prospective observational study included 79 type 1 diabetic patients, among them 33 had normoalbuminuria, 21 were with microalbuminuria, while 25 patients had proteinuria. The patients were classified according to the amount of albuminuria using the median result of the three 24 h urine collections. Urinary albumin excretion (UAE) was determined with the nephelometric technique. Normoalbuminuric level was defined as UAE < 30 mg/24 h, microalbuminuria as UAE 30–300 mg/24 h and macroalbuminuria or manifested proteinuria as UAE over 300 mg/24 h. Inclusion criteria were age (20–40 years), duration of diabetes (> 5 years) and the absence of other severe diseases.

Clinical characteristics were gathered from interviewing the patients (duration of diabetes, family history for hypertension, cardiovascular diseases, diabetes, smoking duration). The defined risk factors (arterial blood pressure and complications) were measured on the first day of visit. Blood samples were drawn for measurement of serum cholesterol, HbA1c and for the determination of genotypes.

Determination of genotypes

Individual genomic DNA samples were extracted from periferal blood with Applied Biosystems 6100 Nucleic Acid prep Station instrument.

The AGT (M235T) gene polymorphism and AT1R (A1166C) gene polymorphisms were analysed by polymerase chain reaction (PCR) and subsequent restriction-endonuclease digestion (Tth 111I and Hae III) [8]. To determine the ACE I/D genotype, DNA (100 ng) was amplified using two insertion-specific primers described by Bonnardeaux et al. [9]. The reaction products were visuelised on 2% agarose gel stained with ethidium bromide. Final analysis of the genotype was performed after 10% PAGE stained with silver nitrate.

Statistical analysis

All statistical analyses were performed in SPSS 12.0 (SPSS Inc, Chicago, Illinois) statistical package). The results are presented as frequency, percent and mean ± SD. The χ^2, Kruskal-Wallis and ANOVA test were used to compare the 3 groups, while Mann-Whitney *U*-test was used to compare the 2 groups. A logistic regression model was performed to asses associations of nephropathy and other variables. All *p*-values that were less than 0.05 were considered significant.

Results

Patient characteristics (total number 79) are given in Table 1.

crovascular complication showed high statistically significant difference in the group of patients with proteinuria (Table 1).

We genotyped all the patients for the AGT, ACE and AT1R gene polymorphisms. The distribution of genotype and allele frequencies in the examined groups are presented in Table 2.

No statistically significant difference among the examined groups was found in the genotype and allele distribution for the ACE and AT1R gene polymorphisms.

The carriers of the MT and TT genotype of the AGT gene polymorphism were more frequent in the group of patients with elevated UAE. The distribution of homo- and heterozygous genotypes for the T allele (MT and TT) was significantly higher in the group of patients with proteinuria when compared to normoalbuminuric patients.

The presence of homozygous TT genotype of the AGT M235T polymorphism, compared among the 3 devided groups according to UAE level, was significantly higher in the group of patients with high albumin excretion.

The genotype and allele distribution for the ACE and AT1R gene polymorphisms, among the examined groups according to elevated albumin excretion, showed no statistically significant difference.

We found the tendency for statistical significance between the pressence of TT and I/D gene polymorphisms and increased blood pressure in patients with proteinuria. The pressence of MT/TT genotype of AGT M235T gene and AC/CC genotype of AT1R gene did not reveal a significant association with retinopathy in the group of patients with

Table 1

Characteristics of diabetes mellitus (DM) patients

Patients characteristics	Normoalbuminuria (n = 33)	Microalbuminuria (n = 21)	Proteinuria (n = 25)	*p*-value
Age (years), $\bar{x}$ ± SD	28.1 ± 5.8	25.8 ± 6.8	34.2 ± 6.8	< 0.001[a]
Duration of DM (years), $\bar{x}$ ± SD	9.6 ± 4.6	11.3 ± 4.4	21.9 ± 5.8	< 0.001[a]
Family history of HTA, n (%)	22 (42.3)	14 (26.9)	16 (30.8)	ns[c]
Family history of DM, n (%)	11 (52.4)	14 (19)	6 (28.6)	ns[c]
Family history of CVD, n (%)	16 (43.2)	13 (35.1)	8 (21.6)	ns[c]
Blood pressure (mmHg), $\bar{x}$ ± SD				
systolic	121 ± 12	126 ± 14	148 ± 26	< 0.001[a]
diastolic	79 ± 7	85 ± 9	92 ± 15	< 0.001[a]
Hypertension, n (%)	9 (24.3)	9 (24.3)	19 (51.4)	< 0.001[c]
Polineuropathy, n (%)	13 (30.2)	14 (32.6)	16 (37.2)	ns[c]
Retinopathy, n (%)	7 (18.9)	11 (29.7)	19 (51.4)	< 0.001[c]
HbA1c (%)	9.3	9.7	10.4	ns[c]
Cholesterol, n (%)	11 (39.3)	7 (25.0)	10 (35.7)	ns[c]
HDL cholesterol (mmol/L), $\bar{x}$ ± SD	1.25 ± 0.39	1.18 ± 0.44	1.28 ± 0.38	ns[a]
LDL cholesterol (mmol/L), $\bar{x}$ ± SD	3.14 ± 1.06	3.05 ± 1.07	4.00 ± 1.58	ns[b]
UAE (mg/24 h)	13.1 ± 5.2	128.1 ± 87.3	368.3 ± 223.8	< 0.001[b]
Smokers, n (%)	14 (34.1)	12 (29.3)	15 (36.6)	ns[c]

HTA – hypertension; CVD – cardiovascular disease; BP – blood pressure; HbA1c – glycohemoglobin A1c; HDL – high density lipoprotein; LDL – low density lipoprotein; UAE – urinary albumin excretion; n – number of patients in examined groups; ns – not significant; values are calculated by [a]ANOVA, [b]Kruskal Wallis; [c]χ^2 test.

The patients with proteinuria, compared with normo- and microalbuminuric patients, highly differed in age and diabetes duration, blood pressure level and hypertension.

There was no statistically significant diference in the occurence of polyneuropathy among the examined groups of patients, while the presence of rethinopathy as a frequent miproteinuria, but the homozygous DD genotype of the ACE gene polymorphism showed an increasing frequency in the proteinuric group compared with normoalbuminuric patients (Table 3).

Using the Backward logistic regression model, after obtaining all predictors associated with renal failure, we

Table 2

Distribution of genotypes and allele frequencies of AGT, ACE nad AT1R genes in the examined patients

Genotype	Normoalbuminuria (n = 33)	Microalbuminuria (n = 21)	Proteinuria (n = 25)	*p*-value
AGT (M235T), n (%)				
M allele	37 (56)	20 (47.6)	19 (38)	ns[a]
T allele	29 (44)	22 (52.4)	31 (62)	
MM	7 (21.2)	5 (23.8)	3 (12.0)	
MT	23 (69.7)	10 (47.6)	13 (52.0)	< 0.05[b]
TT	3 (9.1)	6 (28.6)	9 (36.0)	
MM+MT	30 (90.1)	15 (71.4)	16 (64)	< 0.05[c]
TT	3 (9.9)	6 (28.6)	9 (36)	
ACE (I/D), n (%)				
I allele	34 (51.5)	25 (59.5)	24 (48)	ns[a]
D allele	32 (48.5)	17 (40.5)	26 (52)	
II	11 (33.3)	7 (33.3)	6 (24.0)	
ID	12 (36.4)	11 (52.4)	12 (48.0)	ns[ab]
DD	10 (30.3)	3 (14.3)	7 (28.0)	
II+ID	23 (69.7)	18 (85.7)	18 (72)	ns[c]
DD	10 (30.3)	3 (14.3)	7 (28)	
AT1R (A1166C), n (%)				
A allele	47 (71)	28 (67)	33 (66)	ns[a]
C allele	19 (29)	14 (33)	17 (34)	
AA	16 (48.5)	12 (57.1)	10 (40.0)	ns[b]
AC	15 (45.5)	4 (19)	13 (52)	
CC	2 (6.1)	5 (22.8)	2 (8.0)	
AA+AC	31 (93.9)	16 (76.2)	23 (92)	ns[c]
CC	2 (6.1)	5 (23.8)	2 (8)	

[a]Mann-Whitney U test; [b]Kruskal-Wallis test; [c]χ^2 test;
AGT – angiotensinogen; ACE – angiotensin-converting enzyme; AT1R – angiotensin II type I receptor; n – number of patients in examined groups according to albuminuria (percentages are shown in parentheses); ns – not significant.

Table 3

Genotype distribution according to hypertension, polyneuropathy and rethinopathy in the examined groups of patients with diabetes mellitus

Genotype distribution	Yes, n (%)	No, n (%)	*p*-value
Hypertension			
AGT			
MM	8 (53.3)	7 (46.7)	ns
MT/TT	34 (53.1)	30 (46.9)	ns
ACE			
II	13 (54.2)	11 (45.8)	ns
ID/DD	29 (52.7)	26 (47.3)	ns
AT1R			
AA	20 (52.6)	18 (47.4)	ns
AC/CC	22 (53.7)	19 (46.3)	ns
Polyneuropathy			
AGT			
MM	8 (53.3)	7 (46.7)	ns
MT/TT	28 (43.8)	36 (56.3)	ns
ACE			
II	10 (41.7)	14 (58.3)	ns
ID/DD	26 (47.3)	29 (52.7)	ns
AT1R			
AA	16 (42.1)	22 (57.9)	ns
AC/CC	20 (48.8)	21 (51.2)	ns
Rethynopathy			
AGT			
MM	5 (33.3)	10 (66.7)	ns
MT/TT	37 (57.8)	27 (42.2)	ns
ACE			
II	14 (58.3)	10 (41.7)	ns
ID/DD	28 (50.9)	27 (49.1)	ns
AT1R			
AA	19 (50.0)	19 (50.0)	ns

χ^2 test; AGT – angiotensinogen; ACE – angiotensin-converting enzyme; AT1R – angiotensin II type I receptor; n – number of patients in examined groups according to albuminuria (percentages are shown in parentheses); ns – not significant.

found that duration of diabetes, high blood pressure, rethinopathy and AGT polymorphism were highly associated with nephropathy in patients with DM (Table 4).

Table 4

Correlation of diabetes mellitus (DM) duration, hypertension (HT), rethinopathy, AGT and ACE gene polymorphism with nephropathy in the examined group of patients

Predictor	*p*-value	OR (95% CI)
Duration of:		
DM	< 0.01	1.192 (1.057–1.345)
HT	< 0.05	5.200 (1.301–20.790)
rethinopathy	< 0.05	2.840 (1.288–6.261)
AGT polymorphism		
MM	< 0.05	-
MT	ns	1.358 (0.234–7.885)
TT	< 0.01	28.389 (2.422–332.724)
ACE polymorphism		
II	ns	-
ID	ns	2.113 (0.435–10.258)
DD	ns	0.307 (0.053–1.780)

AGT – angiotensinogen; ACE – angiotensin-converting enzyme; ns – not significant; OR – odds ratio; CI – confidence interval.

It was shown that as the duration of diabetes increases by one unit, the chances of developing nephropathy were 1.192 times higher being statistically significant. If a patient had hypertension, the likelihood for nephropathy was 5.2 times higher. A patient with TT allele proned to develop nephropathy 28.38 times higher than the carrier of MM genotype. If a patient had the MT allele, the chance to develop nephropathy was 1.358 times greater, but this was not statistically significant. In fact, only the TT genotype showed notable importance.

The univariate analyses did not show a statistically significant association for ACE/II genotype according to increasing renal complications. The DD genotype in our study is associated with 0.307 lower risk for nephropathy.

Discussion

We examined the association between the DN level and genotype distribution of the RAS genes in normoalbuminuric, micro and proteinuric groups of patients with DM type 1. Also, we analysed main clinical complications in patients with DM type 1 and correlated them with the studied gene polymorphisms.

Patients with microalbuminuria are at high risk of cardiovascular complications and need frequent and careful examination for early detection of nephropathy, retinopathy, cardiovascular and lipid abnormalities. Identification of factors related to the development of microalbuminuria leads to the development of strategies to prevent nephropathy and reduce the occurence of new cases [8].

Our data suggest a statisticaly significant correlation between blood pressure level (systolic and diastolic) and rethinopathy as more prominent microvascular complication in patients with terminal renal insuficiency. Both systolic and diastolic hypertension accelerate the progression of diabetic nephropathy, and agressive antihypertensive management successed to decrease the rate of fall of glomerular filtration rate (GFR) [10].

In patients with type 1 diabetes, the median life expectancy can significantly increase using appropriate antihypertensive intervention, with the reduction of mortality from 94% to 45%, and the reduction in the need for dialysis and transplantation from 73 to 31% 16 years after the development of overt nephropathy [11]. For patients with nephropathy, treatment with ACE inhibitors is indicated as a part of initial therapy concerning previous genotyping analysis of the ACE gene polymorphism.

In the present study, due to a rather small number of patients, T allele of the M235T polymorphism and D allel of the ACE I/D polymorphism tend to be increased in patients with elevated blood pressure in the proteinuric type 1 diabetes group. Furthermore, current literature suggests that the ACE D/D genotype predicts poor renal response to ACE inhibitors and to agents that do not block the RAS in subjects with DN. Two longitudinal observational studies pointed out that GFR decreased rapidly in proteinuric type 1 DM patients with the DD genotype [12, 13]. In the other study, the albumin excretion rate of type 1 DM patients with the II genotype increased more rapidly than in those with the DD genotype, while the response to ACE inhibitor was better in patients with II than in those with the DD genotype [14].

In some follow-up studies, implementation of RAS blockade therapy in type 1 diabetic patients reduced the progression to overt DN to 3.4% *per* year. It should be notified that 45% of patients in progression of DN decrease microalbuminuria by intensified antihypertensive treatment [15].

Several data suggest that hyperglicaemia may be very important factor for the increasing incidence of cardiovascular complications and progression from microalbuminuric to DN. Despite previous observational studies findings about association of poor glycaemic control and progression in albuminuria and the rate of progression to DN, we found no statistically significant correlations in HbA1c values and quality of glycoregulation between the normoalbuminuric and microalbuminuric patients. There was no significant increasing serum levels of total cholesterol, low density hipoprotein (LDL), high density lipoprotein (HDL) cholesterol in our examined group although it has been reported that dia-

betic patients could manifest alterations in serum lipid level ranging from 30 to 90% [16].

Polymorphisms for different components of RAS have been described with controversal results according to the different ethnic backgrounds of the study populations, for example Asian carriers of 235T allel appear to have a higher risk of hypertension than white carriers [17].

On the basis of the previous studies, we cannot conclude that there is a link between the ACE I/D polymorphism and DN. The results differ in a number of studies and data do not exclude a pathogenic role of mutations somewhere else in the ACE gene, but prevaling conclusion that the ACE I/D polymorphism is not a reliable marker for the prediction of DN in patients with type 1 diabetes [18]. Our data do not indicate an independent effect of ACE and AT1R polymorphism on the risk for DN, so we are unable to confer the interaction between the ACE I/D and M235T polymorphism, suggesting that geneticaly determined AGT levels can cause risk for DN through Ang I generation.

Previous results in type 1 diabetic patients showed an association between DN and the T allele of AGT M235T polymorphism. In the article of Walder et al. [18], the autors therefore found that the TT genotype was 3 times more common in the group of 98 nephropathic than in 98 normoalbuminuric type 1 DM patients, indicating a link to this genotype and elevated serum angiotensinogen with the possible risk for DN conferred by this genotype. In the another study group of hypertensive patients at a start of dialysis, the carriers of the T allele of the AGT gene were more frequent in the group of patients compared to controls [6, 19]. The results of our study showed that in patients with hypertension, a likelihood for nephropathy is 5.2 times higher, while in the group of patients with TT allele risk to develop nephropathy is 23.38 times higher than in the carriers of MM genotype.

Our data designate that the T allele occurs more frequently among patients with proteinuria compared with those with normoalbuminuria, and the level of evidence shows statistically highly significant difference.

In our study, the T allele of the AGT-M235T polymorphism and CC genotype of the AT1-A1166C polymorphism was not associated with retinopathy.

A recent meta-analysis, including two studies with type 1 diabetic patients, did not find corellation between the AGT M235T gene polymorphism and diabetic retinopathy. Nevertheless, the associations between RAS activity and retinopathy have been previously reported [20–23].

Local changes as a result of the presence of different polimorphisms of RAS are manifested as changes in renal hemodynamics, increased intraglomerular pressure and glomerular filtration rate, as the major determinants of renal function. The interactions between the alleles of the RAS probably play a major role in determining the development of DN. Experimental data might allow identification of groups with high risk of developing diabetic nephropathy and, therefore, the analysis of RAS system genes plays an important role in providing novel therapeutic targets or individualized treatment strategies for both the prevention and treatment of these complications.

Conclusion

The increased association of hypertension and the TT AGT gene polymorphism in patients with diabetes mellitus with proteinuria could be a significant marker of diabetic nephropathy.

REFERENCES

1. *Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T.* Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes Care 2005; 28(1): 164−76.
2. *Parving HH, Chaturvedi N, Viberti G, Mogensen CE.* Does Microalbuminuria Predict Diabetic Nephropathy Diab Care 2002; 25(2): 406−7.
3. *Chawla T, Sharma D, Singh A.* Role of the renin angiotensin system in diabetic nephropathy. World J Diabetes 2010; 1(5): 141−5.
4. *Cherney DZ, Lai V, Scholey JW, Miller JA, Zinman B, Reich HN.* Effect of Direct Renin Inhibition on Renal Hemodynamic Function, Arterial Stiffness, and Endothelial Function in Humans With Uncomplicated Type 1 Diabetes: A pilot study. Diab Care 2010; 33(2): 361−5.
5. *Kim S, Iwao H.* Molecular and cellular mechanisms of angiotensin II-mediated cardiovascular and renal diseases. Pharmacol Rev 2000; 52(1): 11−34.
6. *Buraczynska M, Ksiazek P, Drop A, Zaluska W, Spasiewicz D, Ksiazek A.* Genetic polymorphisms of the renin-angiotensin system in end-stage renal disease. Nephrol Dial Transplant 2006; 21(4): 9791−83.
7. *Murphey LJ, Gainer JV, Vaughan DE, Brown NJ.* Angiotensin-converting enzyme insertion/deletion polymorphism modulates the human in vivo metabolism of bradykinin. Circulation 2000; 102(8): 829−32.
8. *Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F.* An insertion/deletion polymorphism in the angiotensin 1-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 1990; 86(1): 1343−6.
9. *Bonnardeaux A, Davies E, Jeunemaitre X, Féry I, Charru A, Clauser E*, et al. Angiotensin II type 1 receptor gene polymorphisms in human essential hypertension. Hypertension 1994; 24(1): 63−9.
10. *Jacobsen P, Tarnow L, Carstensen B, Hovind P, Poirier O, Parving H.* Genetic variation in the Renin-Angiotensin system and progression of diabetic nephropathy. J Am Soc Nephrol 2003; 14(11): 2843−50.
11. *Molitch ME, DeFronzo RA, Franz MJ, Keane WF, Mogensen CE, Parving HH,* et al. Nephropathy in diabetes. Diabetes Care 2004; 27: 79–83.
12. *Jacobsen PK, Tarnow L, Parving HH.* Time to consider ACE insertion/deletion genotypes and individual renoprotective treatment in diabetic nephropathy. Kidney Int 2006; 69(8): 1293−5.
13. *Andersen S, Tarnow L, Cambien F, Rossing P, Juhl TR, Deinum J,* et al. Long-Term Renoprotective Effects of Losartan in Diabetic Nephropathy: Interaction with ACE insertion/deletion genotype. Diab Care 2003; 26(5): 1501−6.
14. *Penno G, Chaturvedi N, Talmud PJ, Cotroneo P, Manto A, Nannipieri M*, et al. Effect of angiotensin-converting enzyme (ACE)

gene polymorphism on progression of renal disease and the influence of ACE inhibition in IDDM patients: findings from the EUCLID Randomized Controlled Trial. EURODIA Controlled Trial of Lisinopril in IDDM. Diabetes 1998; 47(9): 1507−11.

15. *Ritz E, Dikow R.* Hypertension and antihypertensive treatment of diabetic nephropathy. Nat Clin Pract Nephrol 2006; 2(10): 562−7.
16. *American Diabetes Association.* Standards of medical care in diabetes-2010. Diabetes Care 2010; 33(Suppl 1): S11−61.
17. *Fang YJ, Deng HB, Thomas GN, Tzang CH, Li CX, Xu ZL,* et al. Linkage of angiotensinogen gene polymorphisms with hypertension in a sibling study of Hong Kong Chinese. J Hypertens 2010; 28(6): 1203−9.
18. *Walder B, Spanaus KS, Weinreich T, Widmer U.* Genetic heterogeneity in the renin-angiotensin system and the risk of diabetic nephropathy: association with the angiotensinogen gene but not with the ACE gene. J Clin Cardiol Basic Cardiol 1998; 1(1): 55−8.
19. *Wu S, Chiang F, Chen WJ, Liu P, Hsu K, Hwang J,* et al. Three single-nucleotide polymorphisms of the angiotensinogen gene and susceptibility to hypertension: single locus genotype vs. haplotype analysis. Physiol Genomics 2004; 17(2): 79−86.
20. *Franken AA, Derkx FH, Man VA, Hop WC, Rens GH, Peperkamp E*, et al. High plasma prorenin in diabetes mellitus and its correlation with some complications. J Clin Endocrinol Metab 1990; 71(4): 1008−15.
21. *Danser AH, Dorpel MA, Deinum J, Derkx FH, Franken AA, Peperkamp E*, et al. Renin, prorenin, and immunoreactive renin in vitreous fluid from eyes with and without diabetic retinopathy. J Clin Endocrinol Metab 1989; 68(1): 160−7.
22. *Ittersum FJ, de Man AM, Thijssen S, Knijff P, Slagboom E, Smulders Y,* et al. Genetic polymorphisms of the renin-angiotensin system and complications of insulin-dependent diabetes mellitus. Nephrol Dial Transplant 2000; 15(7): 1000−7.
23. *Schjoedt KJ, Hansen HP, Tarnow L, Rossing P, Parving HH.* Long-term prevention of diabetic nephropathy: an audit. Diabetologia 2008; 51(6): 956−61.

Received on October 19, 2012.
Revised on January 4, 2013.
Accepted on February 18, 2013.