



Angiotensin II type 1 receptor gene polymorphism could influence renoprotective response to losartan treatment in type 1 diabetic patients with high urinary albumin excretion rate

Uticaj polimorfizma gena za AT1 receptor na renoprotektivnu efikasnost losartana kod bolesnika sa dijabetesom tip I i povišenom urinarnom ekskrecijom albumina

Tamara Dragović*, Boris Ajdinović†, Rajko Hrvačević‡, Vesna Ilić§, Zvonko Magić§, Zoran Andelković*, †Nikola Kocev

Military Medical Academy, *Clinic of Endocrinology, †Institute of Nuclear Medicine, ‡Clinical of Nephrology, §Institute for Medical Research, Belgrade, Serbia; †School of Medicine, Belgrade University, Institute for Informatics and Statistics, Belgrade, Serbia

Abstract

Background/Aim. Diabetic nephropathy (DN) is a clinical syndrome characterized by persistent albuminuria, increasing arterial blood pressure and progressive decline in glomerular filtration rate (GFR). When persistent albuminuria is established, antihypertensive treatment becomes most important factor in slowing the progression of diabetic glomerulopathy. The aim of this study was to examine if renoprotective response to a short-term losartan therapy depends on 1166 A/C gene polymorphism for its target receptor. **Method.** The study included 35 patients with diabetes mellitus type 1 and persistently high urinary albumin excretion rate (UAE: > 30 mg/24 h), genotyped for the 1166 A/C gene polymorphism for the angiotensin II type 1 receptor (AT1R). The participants were segregated into 3 genotype groups according to combinations of A or C allele: AA(16%), AC(15%) and CC(11%). The patients received losartan 50 mg daily for 4 weeks, following 100 mg daily for another 8 weeks. At baseline and after 12 weeks of the treatment period UAE, blood pressure, GFR and filtration fraction (FF) were determined. **Results.** After 12 weeks of the treatment with losartan, albuminuria was reduced from baseline by 9% [95% confidence interval (CI): 1–17, $p = 0.039$] in the AA genotype, and by 11% (95% CI: 6–17, $p = 0.0001$) in the AC genotype. Losartan

treatment reduced albuminuria in the CC group by 5% (95%CI: -13–22, $p = 0.47$). Glomerular filtration rate remained unchanged in all genotype groups. Filtration fraction was significantly reduced from baseline by 0.018 ± 0.024 ($p = 0.012$) only in the AC genotype. In the AA genotype, FF was reduced from baseline by 0.017 ± 0.03 ($p = 0.052$), and in the CC genotype by 0.01 ± 0.008 ($p = 0.092$). In the AA group, systolic blood pressure declined from 136 ± 24 mmHg at baseline, to an average of 121 ± 18 mmHg at the end of the study ($p = 0.001$). The AC group achieved reduction from 131 ± 10 mmHg at baseline to 115 ± 7 mmHg ($p = 0.001$) during the investigation period. In the AA genotype group losartan reduced diastolic blood pressure from 86 ± 13 mmHg at baseline to 78 ± 8 mmHg ($p = 0.004$), and in the AC genotype from 88 ± 5 mmHg at baseline to 11.7 ± 5.6 mmHg during the investigation period ($p = 0.001$). In the CC genotype diastolic blood pressure reduction remained nonsignificant ($p = 0.066$). **Conclusion.** The results of our small sample size study provide the evidence that 1166 A/C AT1R polymorphism could be associated with the renoprotective response to losartan therapy.

Key words: diabetes mellitus, type 1; diabetic nephropathies; polymorphism, genetic; angiotensin II; losartan.

Apstrakt

Uvod/Cilj. Dijabetesna nefropatija (DN) je klinički sindrom koji karakterišu trajna albuminurija, povišeni krvni pritisak i progresivno sniženje jačine glomerulske filtracije. Kada se pojavi trajna albuminurija, uvođenje antihipertenzivne terapije predstavlja najvažniji faktor za usporavanje

progresije dijabetesne glomerulopatije. Naša studija imala je za cilj da ispita u kolikoj meri je renoprotektivni odgovor na lečenje losartanom, blokatorom receptora za angiotenzin II, uslovljen polimorfizmom gena za njegov ciljni receptor. **Metode.** Ispitivanjem je bilo obuhvaćeno 35 bolesnika sa dijabetesom tip 1 i trajno povišenom urinarnom ekskrecijom albumina od preko 30 mg/24 h. Ispitanici su lečeni lo-

sartanom u dozi od 50 mg dnevno tokom četiri sedmice, nakon čega je doza povećana na 100 mg dnevno, narednih osam sedmica. Na početku ispitivanja i nakon 12 sedmica lečenja izmerene su vrednosti dnevne urinarne ekskrecije albumina (UEA), jačine glomerulske filtracije (JGF), glomerulske filtracione frakcije (FF) i sistemskog krvnog pritiska. U zavisnosti od postojanja A i C alela u okviru 1166A/C polimorfizma gena za receptor tip 1 angiotenzina II (AT1R), ispitanici su bili podeljeni u tri grupe (AA, AC i CC) radi utvrđivanja razlike ispitivanih parametara na početku i na kraju lečenja. **Rezultati.** Posle 12 sedmica lečenja, losartan je snizio albuminuriju za 9% [interval poverenja (IP) 95%: 1–17, $p = 0,039$] u AA homozigotnoj grupi i za 11% (IP 95%: 6–17, $p = 0,0001$) u AC heterozigotnoj grupi. Redukcija albuminurije kod CC homozigota nije bila značajna. Jačina glomerulske filtracije ostala je nepromenjena u sve tri genotip-

ske grupe. Terapija losartanom dovela je do značajnog smanjenja FF za $0,018 \pm 0,024$ ($p = 0,012$) jedino u AC grupi, dok se redukcija FF u AA grupi za $0,017 \pm 0,03$ ($p = 0,052$) približila statističkoj značajnosti. Kod CC homozigota smanjenje FF nije bilo značajno. U grupama AA i AC, postignuto je visoko statistički značajno sniženje i sistolnog i dijastolnog krvnog pritiska ($p < 0,01$), dok sniženje arterijskog pritiska u CC grupi nije bilo značajno ($p > 0,05$). **Zaključak.** Individualni renoprotektivni odgovor na terapiju losartanom, bar jednim delom mogao bi biti uslovljen 1166 A/C polimorfizmom gena za AT1R.

Ključne reči:
dijabetes melitus, insulin-zavisni; dijabetesne nefropatije; polimorfizam, genetički; angiotenzin II; losartan.

Introduction

Diabetic nephropathy (DN) is a clinical syndrome characterized by persistent albuminuria, increasing arterial blood pressure and progressive decline in glomerular filtration rate (GFR)¹. When persistent albuminuria is established (over 30 mg/24 h), antihypertensive treatment becomes most important factor in slowing the progression of diabetic glomerulopathy. It is recommended that this therapy should be started as early as possible, at the microalbuminuric stage, even if the hypertension is absent².

Extensive investigations have documented the key role of the renin-angiotensin-aldosterone system (RAAS) in the pathogenesis and pathophysiology of diabetic renal disease. Angiotensin II (Ang II), the major effector of this system, acts as the circulating vasoconstrictory hormone, as well as paracrine peptide that modulates renal function. Most of its effects are mediated via Ang II type 1 receptors, localised in glomerular vascular smooth muscle cells, mesangial cells and on the luminal surface of tubular cells. This hormone also has the fundamental role in starting and progression of DN by its hemodynamic and non-hemodynamic effects³.

Angiotensin II type receptor 1 (AT1R) antagonists are a group of antihypertensive drugs that act on the terminal cascade of RAAS by selective binding to AT1R. This provides more complete blockade of Ang II actions in systemic and local manner, which is especially important in diabetic state where the intrarenal RAAS activity is enhanced⁴.

Despite good glycemic control, lipid lowering agents and antihypertensive treatment, these patients have 3-6 times faster decline in renal function comparing to non-diabetic patients⁵. One of the possible reasons for currently nonadequate renoprotective treatment and bad prognostic values, are individual variations in response to the first-line therapy of these patients. This variations could be genetically influenced, due to the existence of polymorphisms within the genes that encode for enzymes related with drug metabolism, or genes for proteins of the physiological pathway on which the drug acts^{6,7}. Because of the central role of RAAS in pathogenesis of DN, it would be important to evaluate the

type of genetic polymorphisms binding to this system, that could influence individual renoprotective efficacy of drug treatment.

As we mentioned before, most of the actions of Ang II are mediated by stimulation of AT1R, including vasoconstriction, regulation of the vascular tone and proliferative processes in glomerule. Moreover, genetic variations of this specific receptor can alter AT1R-mediated reactions by altering its expression or structure^{7,8}.

This human receptor gene is composed of 5 exons and 4 introns with a length of more than 50 kb. So far, various genetic polymorphisms of this receptor gene have been reported, but the best evaluated of all is the 1166 A/C single nucleotide polymorphism (SNP). This SNP is an A/C transversion in the 3' untranslated region of the gene⁹. There is a growing evidence to suggest that the 1166 A/C AT1R polymorphism is implicated in higher risk for cardiovascular events, and the C allele became serious candidate for genetic variations that lead to enhanced activity of systemic and/or paracrine RAAS¹⁰⁻¹³. Considering previous studies, treatment with AT1R antagonists could overcome some other forms of polymorphisms^{14,15}. We, therefore, wanted to evaluate if the renoprotective effect of losartan could be influenced by polymorphism of its target receptor gene.

Methods

Thirty-five patients, men ($n = 20$) and women ($n = 15$), with diabetes mellitus type 1 and persistently high urinary albumin excretion rate (UAE > 30 mg/24h)¹⁶ were included in this study. Each patient underwent a detailed history, physical and laboratory examination, in order to evaluate inclusion and exclusion criteria. Before enrollment, each patient was examined for urinary albumin excretion rate. All patients fulfilled the following inclusion criteria: diabetes mellitus type 1 more than 5 years; of over 18 years age and persistently high albuminuria. The patients were excluded if they had a history of congestive heart failure, malignant hypertension, valvular heart and aortic disease, renal artery stenosis, creatinin clearance less than 60 mL/min and earlier

established persistent erythrocyturia and/or urinary infection. Dietary intake of protein or salt was not restricted.

After evaluation for the inclusion criteria, the patients underwent the measurement of renal hemodynamic parameters and blood pressure. Then, the participants received two daily oral doses of losartan: 50 mg daily in the first 4 weeks, followed by 100 mg for another 8 weeks. After 12 weeks of treatment, renal hemodynamics and measurement of blood pressure and albuminuria were repeated. The subjects were genotyped for 1166 A/C AT1R polymorphism, and subdivided into three groups (AA, AC and CC) in order to establish statistically significant differences in the examined parameters, before and after the treatment period.

Urinary albumin excretion was determined as the mean values obtained in the two separate 24 h urine collections using an immunonephelometric assay (BN 100 Dade Behring analyzer)¹⁶. It was measured at baseline and again after 12 weeks of the treatment.

Blood pressure was measured using mercury sphygmomanometer in the seated position after resting for at least 10 minutes and was determined as the average of three measurements taken 5 minutes apart¹⁷. During the treatment, blood pressure was measured 24 h after the last dose.

Genomic DNAs were extracted with Applied Biosystems 6100 Nucleic Acid prep Station instrument. Concentration of DNA was determined by measuring the optical density at 260 nm. The A1166C gene polymorphism was analyzed by polymerase chain reaction (PCR) and subsequent restriction-endonuclease digestion according to description of Dzida et al¹⁸. Polymerase chain reaction product

intercept. The results were standardized for 1.73 m² body surface area, using the patients surface area at the start of the study.

Effective renal plasma flow (ERPF) was measured as the uptake of 131-iodine-labeled hippuran in the kidney, 1 to 2 minutes after the *i.v.* injection, with a correction for renal depth and with background subtraction²⁰. Filtration fraction (FF) was determined by dividing GFR by ERPF¹².

All data were presented as mean \pm SD except for albuminuria, which was given as median (interquartile range). The one-way ANOVA test was used to analyse between-group and within-group differences. The paired Student's *t*-test was used to test the differences between the baseline values and those after the treatment with losartan. The values of albuminuria were logarithmically transformed, owing to its skewed distribution, and then tested by the Student's *t*-test. A *p*-value less than 0.05 was considered statistically significant.

Results

The average age of the 35 patients with diabetes mellitus type 1 and high values of albumin excretion rate was 33 \pm 9 years, and the average diabetes duration was 16 \pm 7 years. All patients were genotyped for 1166 A/C AT1R polymorphism. Sixteen subjects had the AA genotype, 15 subjects had the AC genotype, while the rest four subjects had the CC genotype in 1166 A/C AT1R polymorphism.

There were no significant differences in the clinical parameters before the treatment with losartan among three genotype groups (*p* > 0.05) (Table 1).

Table 1
Baseline clinical characteristics of 35 type 1 diabetic patients with high values of daily urinary albumin excretion segregated by genotype

Patients' characteristics	AA (n = 15)	AC (n = 16)	CC (n = 4)
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
Sex (men/women)	12/3	12/4	1/3
Age (years)	32 \pm 11	34 \pm 8	23 \pm 3
DM duration (years)	18 \pm 8	14 \pm 7	13 \pm 2
BMI (kg/m ²)	22.8 \pm 11.28	22.8 \pm 2.33	22.4 \pm 1.95
HbA1c (%)	9.34 \pm 1.82	8.83 \pm 1.22	9.88 \pm 1.62
Albuminuria (mg/24h) [§]	99 (43-5838)	87 (45-830)	190 (45-854)
GFR (mL/min/1.73m ²)	97 \pm 19	96 \pm 20	104 \pm 20
FF	0.18 \pm 0.04	0.17 \pm 0.03	0.19 \pm 0.05
Systolic BP (mmHg)	136 \pm 24	131 \pm 10	133 \pm 19
Diastolic BP (mmHg)	86 \pm 13	88 \pm 5	95 \pm 6

All data are expressed as means \pm SD except albuminuria. DM – diabetes mellitus; BMI – body mass index; HbA1c – hemoglobin A1c; GFR – glomerular filtration rate; FF – filtration fraction; BP – blood pressure; [§] median (minimum – maximum)

was visualized by electrophoresis on 2% agarose gel stained with 2 μ L of 10 mg/mL ethidium bromide solution.

Glomerular filtration rate was measured as the renal uptake of 99mTc-diethylenetriaminepentaacetic acid (DTPA), 2 to 3 minutes after traces arrival in the kidney by the method of Goates et al¹⁹. A total GFR was calculated using a formula derived from regression analysis comparing 24 h creatinine clearance to percent renal uptake:

GFR (mL/min) = % renal uptake \times 9.8127 – 6.82519; where 9.8127 is the regression coefficient and 6.8219 the *y*-

intercept. After 12 weeks of the treatment with losartan, the mean values of daily urinary albumin excretion was significantly reduced in the AA genotype and in the AC genotype group, while the changes of albuminuria in the CC genotype group remained nonsignificant (Table 2). Albuminuria was reduced from the baseline by 9% [95% confidence interval (CI): 1–17, *p* = 0.039] in the AA group, and by 11% (95%CI: 6–17, *p* = 0.0001) in the AC group. The losartan treatment reduced albuminuria in the CC group by 5% (95%CI: -13–22, *p* = 0.47). There were no significant differences between the

Table 2
Comparison of clinical findings among the three different genotypes after 12 weeks treatment with losartan

Clinical findings	AA	AC	CC
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
Albuminuria (mg/24h) §	96 (18 – 7335) †	55 (16 – 783) ‡	154 (17 – 1130)
GFR(mL/min/1.73m ²)	93 ± 18	92 ± 23	99 ± 20
FF	0.16 ± 0.02	0.15 ± 0.03 †	0.18 ± 0.04
Systolic BP (mmHg)	121 ± 18 ‡	115 ± 7 ‡	120 ± 12
Diastolic BP (mmHg)	78 ± 8 ‡	75 ± 8 ‡	85 ± 11
Reduction rate			
albuminuria (%) (95% CI)	9 (1–17) †	11(6–17) ‡	4 (-13–22)
SGF (mL/min)	3.4 ± 10.8	4.0 ± 15.4	4.7 ± 5.7
FF	0.017 ± 0.03	0.018 ± 0.024 †	0.01 ± 0.008
systolic BP (mmHg)	15.5 ± 9.4 ‡	10.3 ± 21.5 ‡	12.5 ± 13.2
diastolic BP (mmHg)	8.2 ± 9.2 ‡	11.7 ± 5.6 ‡	10.3 ± 6.2

All data are expressed as means ± SD except albuminuria. BMI – body mass index; HbA1c – hemoglobin A1c; GFR – glomerular filtration rate; FF – filtration fraction; BP – blood pressure; CI – confidence interval; § median (minimum-maximum); † $p < 0.05$ vs baseline; ‡ $p < 0.01$ vs baseline

albuminuria reduction among three different genotype groups during the study.

Glomerular filtration rate remained unchanged in all examined groups ($p > 0.05$).

After 12 weeks of the losartan treatment, FF was significantly reduced from the baseline by 0.018 ± 0.024 ($p = 0.012$) only in AC genotype. In the AA genotype, FF was reduced from baseline by 0.017 ± 0.03 ($p = 0.052$), and in the CC genotype by 0.01 ± 0.008 ($p = 0.092$) (Table 2). There were no significant differences between GFR and FF reduction in the three examined groups during the study.

The mean values of systolic blood pressure were significantly lowered in the AA and in the AC genotype groups, while the CC genotype group achieved nonsignificant reduction. In the AA group, systolic blood pressure declined from 136 ± 24 mmHg (mean ± SD) at baseline to an average of 121 ± 18 mmHg at the end of the study ($p = 0.001$). The AC group achieved reduction from 131 ± 10 mmHg at baseline to 115 ± 7 mmHg ($p = 0.001$) during the investigation period. Similar results were obtained in the diastolic blood pressure reduction. In the AA genotype losartan reduced diastolic blood pressure from 86 ± 13 mmHg at baseline to 78 ± 8 mmHg ($p = 0.004$) during the investigation period, and in the AC genotype from 88 ± 5 mmHg at baseline to 11.7 ± 5.6 mmHg during the study ($p = 0.001$). In the CC genotype, diastolic blood pressure reduction remained nonsignificant ($p = 0.066$) (Table 2).

There were no significant differences between systolic or diastolic blood pressure reduction among three different genotype groups during the study.

Discussion

The intrarenal RAAS may be activated early in the course of diabetes mellitus, despite normal or suppressed levels in plasma²¹. Increased intraglomerular capillary hydraulic pressure, as a result of Ang II mediated efferent arteriolar vasoconstriction has been identified as a potential therapeutic target for the prevention of progressive diabetic renal damage. So, the beneficial effect of AT1R antagonism is a result of predominant efferent arteriolar vasodilatation,

tending to lower filtration fraction and intraglomerular hypertension. The result of this change is the reduction of albumin urinary loss²². The reduction in arterial blood pressure is also important, because the transmission of blood pressure on glomerules becomes smaller. Losartan is an antihypertensive drug which acts by directly blocking AT1 receptors²³.

Genetic variations of AT1R genes can alter AT1R-mediated reactions by altering its expression or structure. To date, 1166 A/C SNP has been the best evaluated of all AT1R polymorphisms with an A to C transversion in the 3' untranslated region of the gene. Within this SNP, the C allele remains a candidate for genetic variations that leads to an enhanced activity of systemic and/or paracrine RAAS⁷⁻⁹.

In patients with diabetes type 1, during hyperglycemic clamp conditions, Miller et al¹², demonstrated that only the C allele carriers exhibited a significantly augmented systemic pressor response to high glucose. This observation may indicate that the C allele predicts enhanced Ang II responsiveness. In another study with healthy subjects, the same author demonstrated that the C allele is associated with enhanced intrarenal and peripheral Ang II activity, resulting in an augmented efferent arteriolar resistance¹³. The baseline values of vascular tone in AC/CC subjects were augmented with the associated higher values of FF and intrarenal vascular resistance compared to AA carriers.

Measurement of FF provides a good estimate of intraglomerular pressure; higher values of this parameter are associated with higher values of urinary albumin loss. Twelve weeks of the losartan therapy in our study group decreased FF in all participants, but the most pronounced reduction was observed in AC carriers. In AA homozygous subjects, a change of FF reached bordered values of statistical significance, while CC homozygous subjects responded by modest dilatation of efferent arteriolas. Nevertheless, GFR remained unchanged in all participants. Stabilization of GFR despite the reduction of intraglomerular and systemic arterial pressure is another approval of beneficial renoprotective effect of chronic AT1R blockade.

Similar results have been demonstrated in the study with hypertensive patients, measuring the acute renovascular

response to an active metabolite of losartan - EXP3174²⁴. This study confirmed the association of the C allele with higher sensitivity to Ang II. When EXP3174 was infused, CC homozygous subjects revealed enhanced rigidity of efferent arteriolas, compared to AA carriers, expressed by smaller reduction in FF with unchanged SGF. It is therefore somewhat unexpected to find the strongest hemodynamic response in the AC group in our study population. Nevertheless, Miller et al.¹² observed the AC group associated with CC carriers (as AC/CC group), while Spiering et al.²⁴ pointed their examination only on homozygous subjects (AA or CC). So, the AC genotype results in these studies remained unavailable.

In our study, losartan therapy significantly reduced daily albuminuria in the AA genotype and AC genotype groups. Reduction of albuminuria after a short-term treatment is a hemodynamic phenomenon reflecting the highest reduction in FF and intraglomerular pressure at the same genotype²⁵. Redon et al.²⁶ did not reveal any association of 1166 A/C AT1R polymorphism with reduction of albuminuria in telmisartan treated hypertensive patients. Among patients with non-diabetic renal disease Coto et al.⁶ also did not confirm any influence of 1166 A/C polymorphism on losartan-induced reduction of proteinuria. Considering previous results, a more pronounced difference in antiproteinuric response could be expected in AA and CC genotype. However, we found the best losartan-induced antiproteinuric response in AC group, by the mechanisms that still could not be easily understood.

In our study, AA and AC carriers experienced the greater values of systolic and diastolic blood pressure reduction, while homozygous for the C allele, expressed weaker antihypertensive response during the study.

Good antihypertensive response in the AA carriers group is consistent with observations of Spiering et al.²⁴. In that study of hypertensive patients it was demonstrated that homozygous for the C allele experienced modest decrease in blood pressure during intravenous administration of the active metabolite of losartan. In another study, de Nus et al.²⁷ also observed more pronounced mean arterial blood pressure reduction in AA carriers comparing to the AC group after a short-term treatment with telmisartan. In hypertensive patients with non-diabetic renal disease, treated with losartan, significantly decreased diastolic blood pressure in AA carriers comparing to the AC group was observed⁶.

Some other studies did not confirm any association of this gene polymorphism with antihypertensive effect of

AT1R antagonists. Kurland et al.¹⁷ and Redon et al.²⁶ did not find any connection between 1166 A/C AT1R polymorphism and individual response to irbesartan and telmisartan therapy. Unlike the previous studies, in Russian population of hypertensive patients, C allele carriers were more sensitive to candesartan antihypertensive effect²⁸. Miller et al.¹³ also found that healthy normotensive C allele carriers expressed a more pronounced reduction in blood pressure following the administration of single oral dose of losartan.

The reasons for these discrepancies are still not well understood, as well as the clinical meaning of A/C AT1R polymorphism. The 1166 AT1R allele is located in the 3' uncoding region, so the polymorphism of this gene does not affect the binding of Ang II to AT1R or signal transduction directly. So, the A/C1166 AT1R polymorphism may be linked to another coding region of AT1R by linkage disequilibrium, or the stability of DNA transcript of AT1R may be altered by this polymorphism²⁹. Furthermore, diabetes mellitus is associated with local increase in the intrarenal Ang II formation, which appears to be more dependent on paracrine factors rather than on circulating concentrations of other RAAS components. Because of the compensatory suppression of systemic RAAS in diabetics, this could have completely different effects on blood pressure regulation, comparing to healthy or hypertensive individuals. On the other hand, it is not well defined if the increased sensitivity of vascular structures to Ang II stimulation in C allele carriers arises from differences in the number of AT1R binding sites, or from the functional activity of this receptor^{30,31}.

Conclusion

We can conclude that examination of 1166 A/C polymorphism-dependent individual, systemic or paracrine responsiveness to losartan therapy must be considered as an interreaction between systemic and intrarenal RAAS activity. In addition, our small sample size of homozygous C allele carriers, limits the conclusions that could be drawn from the obtained issues of CC genotype. Nevertheless, the results from CC genotype group are almost equalized in suggestion that these patients may be less sensitive to the AT1R antagonistic effects of losartan. Finally, our study provides the evidence that 1166A/C AT1R polymorphism could influence the renoprotective response to treatment with losartan, making the rational basis for future longitudinal examination of individualized therapy with AT1R blockers.

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