

## Original Article

# GRK4 variant influences the antihypertensive effect and target organ protection of losartan

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**Abstract:** Background: The control of essential hypertension (EP) is not ideal. G protein-coupled receptor kinase 4 (GRK4) is found to play an important role in the development of hypertension. The role of genetic variants in the efficacy of antihypertensive drugs has attracted more attention. The goal of this study was to analyze the effects of GRK4 variants on the antihypertensive efficacy of losartan and the precise treatment of target organ protection. Methods: The untreated and diagnosed hypertensive patients in our hospital were selected. The distribution of GRK4 variants was detected by polymerase chain reaction (PCR) amplification method. Losartan was administered to patients for 4 weeks. The patients were divided into wild type and mutant type groups according to the GRK4 mutation. The blood pressure drop amplitude, blood pressure peak-to-peak ratio (T/P), blood pressure morning peak, and blood pressure smoothing index (SI) were recorded. Echocardiographic ejection fraction (EF), left ventricular end-systolic diameter (LVESD), left ventricular thickness, left ventricular end-diastolic diameter (LVEDD), and alanine aminotransferase (ALT) were measured. Aspartate aminotransferase (AST), total cholesterol (TG), triglyceride (TC), blood urea nitrogen (BUN), serum creatinine (Scr), blood  $\beta$ 2-microglobulin ( $\beta$ 2-MG), and uric acid were also tested. Results: A total of 312 patients were enrolled in the study, including 72 (72/312, 23.1%) of GRK4 wild-type and 240 (240/312, 76.9%) of GRK4 variants. After treatment with losartan, the blood pressure drop and blood pressure T/P, blood pressure morning peak, and blood pressure SI in GRK4 variant patients were significantly changed compared with GRK4 wild-type group ( $P < 0.05$ ). Among them, the change of GRK4 variant A142V was more significant. Conclusion: Losartan showed more significant antihypertensive effect on patients with GRK4 variants. The most significant improvement was observed in patients with GRK4 variant A142V, indicating that the target organs in patients with GRK4 variants might be protected after administration.

**Keywords:** GRK4, variant, essential hypertension, losartan, target organ

## Introduction

Essential hypertension (EP) refers to the increase in blood pressure that cannot determine the exact causes. It is one of the most common diseases in the cardiovascular system. The prevalence of EP is obviously increased around the world, and it is as high as 20% in China [1, 2]. EP can cause complications in heart, liver, and kidney, which can lead to left ventricular hypertrophy, congestive heart failure, renal arteriosclerosis, renal failure, and liver damage [3]. Hypertension and its complications have become the leading disease affecting human health. Prevention and treatment of hypertension can significantly reduce the incidence of cardiovascular and cerebrovascular diseases [4, 5]. Although there are many methods such

as step-by-step treatment with drugs and combination therapy, the current control of hypertension is not ideal. The control rate of hypertension in China is only 6.1%. Even in the developed countries such as the United States, the control rate of hypertension is only 34% [6, 7].

The pathogenesis of EP is related to several factors, including neurohumoral factors, physical and chemical factors, genetic factors, and environmental factors [8]. Humoral factors, such as angiotensin, dopamine, natriuretic peptides, and vasopressin are regulated by G-protein coupled receptors, while G-protein coupled receptor kinase (GRK) is a key kinase that regulates G-protein coupled receptor [9]. GRK is a serine/threonine protein kinase family with seven GRK subtypes. It is found to be widely

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expressed in tissues and organs except GRK4 [10, 11]. Although GRK4 is expressed only in a few tissues, such as the brain, kidney, myometrium, and testis, it is found to play a crucial role in the development of EP [12]. It was confirmed that the role of genetic variants in influencing the efficacy of antihypertensive drugs has attracted attention [13]. At least six variants of GRK4 have been found (A142V, A486V, R65L, V247I, A253T, and G562D). It was confirmed that GRK4 variants A142V, A486V, and R65L can enhance GRK4 activity [14-16]. The goal of this study was to analyze the effects of GRK4 variants on the antihypertensive efficacy of losartan in the treatment of EP.

### Materials and methods

#### General information

312 cases of untreated hypertension patients in Bishan District People's Hospital from January 2017 to December 2017 were enrolled, including 186 males and 126 females with mean age of  $35.2 \pm 5.6$  (18-55) years old. Inclusion criteria [4]: systolic blood pressure > 140 mmHg and/or diastolic blood pressure < 90 mmHg, mild to moderate hypertension. Exclusion criteria [4]: history of secondary hypertension; malignant hypertension, stroke, peripheral vascular disease, old myocardial infarction; previous treatment of percutaneous coronary intervention (PCI); combined with cardiomyopathy, pericardium disease, or infective endocarditis; acute heart failure; presence of infectious diseases, malignant tumors, severe diabetes, severe liver and kidney disease, systemic immune diseases, and malignant tumor complications. This study was approved by the Medical Ethics Committee of Bishan District People's Hospital (Chongqing, China). All legal guardians who were selected for the study had signed the informed consent form.

#### Main reagents and instruments

Trizol reagent was purchased from Invitrogen. The genomic DNA extraction kit, the PCR product purification and recover kit, primers, and other commonly used reagents were purchased from Sangon. ABI7900 PCR was purchased from ABI. The Labsystem Version 1.3.1 microplate reader was purchased from Bio-rad Corporation. ABPM6100 non-invasive portable dynamic blood pressure monitor was purchased from China Meigao Company. The Kurt

AU860 automatic biochemical analyzer was purchased from Beckman.

#### PCR detection of GRK4 variant

In this experiment, genomic DNA was extracted from peripheral blood using a genomic DNA extraction kit. The sample was added with cell lysate and centrifuged at 3600 rpm for 5 min. DNA was extracted according to the kit instructions. Primer sequence was designed as follows: GRK4 R65L forward, 5'ATGTGGTGTGGACAATGATTCT3'; reverse, 5'AGCATAAGATTGGGTGGTTG3'; GRK4 A142V forward, 5'AAGGGTAACACTCTAGGAAC3'; reverse, 5'TGGGTACTGTTTTATTAAGTTGGC3'; GRK4A486V forward, 5'TCTTGTGGGAACAGGGAGC3'; reverse, 5'GTGCGGGCTGGAAAGTACC3'.

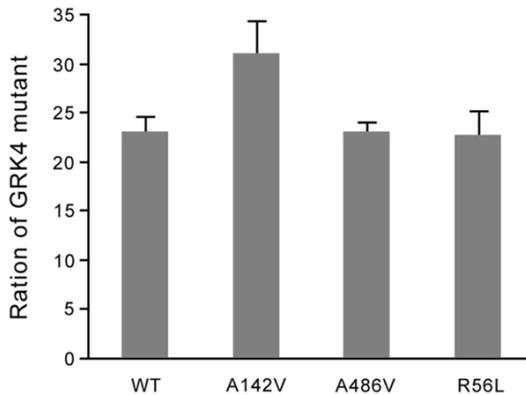
#### Administration

The dosage of losartan was 50 mg/time/day. The blood pressure was measured before and 4 weeks after taking medicine. The 24-h ambulatory blood pressure was monitored before and after the enrollment. According to the requirements of the Chinese Hypertension Guidelines, this study controlled the blood pressure reduction range below 140/90 mmHg. If the antihypertensive effect of losartan was not obvious after 1 week of treatment, the drug dose can be gradually increased to 100 mg. If the blood pressure was still poorly controlled, diuretics can be added.

#### Blood pressure monitor

After 4 weeks of treatment, blood pressure T/P, blood pressure morning peak, and blood pressure SI were recorded. For blood pressure T/P, during the 24 h ambulatory blood pressure test, the average value of the 1 h and adjacent of maximum blood pressure drop within 2-6 h after taking the drug was collected as the peak (P), and taking the blood pressure drop value 2 h before the next dose as the trough (T) to calculate blood pressure T/P. For blood pressure morning peak, taking difference between the highest value of blood pressure in the morning (6:00~10:00) and mean blood pressure at the nighttime (22:00~6:00), which is divided into the morning peak of systolic blood pressure and the morning peak of diastolic blood pressure. For the influence of blood pressure SI, it is calculated as the ratio of the mean and stan-

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**Figure 1.** GRK4 variant distribution.

standard deviation of blood pressure changes per hour within 24 h.

### *Analysis of heart, liver and kidney function*

EF, LVESD, left ventricular thickness, LVEDD, ALT, AST, TG, TC, BUN, Scr, blood  $\beta$ 2-MG, and uric acid changes were detected.

### *Statistical analysis*

All data analyses were performed using SPSS 19.0 software. The measurement data were presented as mean  $\pm$  standard deviation (SD) and compared by ANOVA.  $P < 0.05$  was considered as statistically significant difference.

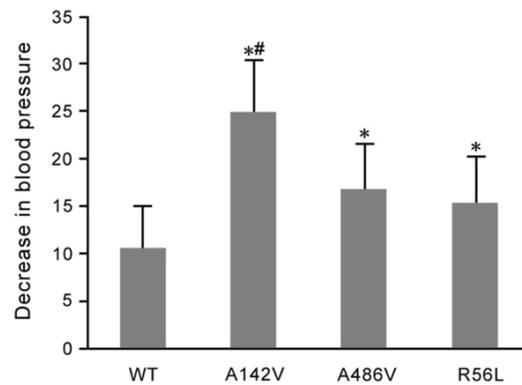
## Results

### *GRK4 variant distribution*

The distribution of GRK4 variants was detected by PCR amplification. A total of 312 patients were enrolled in the study, including 72 (72/312, 23.1%) of GRK4 wild type and 240 (240/312, 76.9%) of GRK4 variants. Among them, 97 cases (97/312, 31.1%) exhibited A142V point mutation, 72 cases (72/312, 23.1%) exhibited A486V mutation, and 71 cases (71/312, 22.8%) exhibited R65L mutation (**Figure 1**). It was suggested that the incidence of GRK4 variants in EP patients was high. However, there is no significant difference in the distribution of major mutation sites.

### *Losartan decreases blood pressure in patients with GRK4 variant*

Four weeks after losartan administration of losartan, the blood pressure decrease amplitude



**Figure 2.** The impact of losartan on the decrease of blood pressure in patients with GRK4 variant. \* $P < 0.05$ , compared with WT. # $P < 0.05$ , compared with A486V or R65L.

was measured. The amplitude of blood pressure in GRK4 variants was significantly decreased compared with that in the GRK4 wild type group ( $P < 0.05$ ), especially in the A142V group (**Figure 2**).

### *Losartan affects blood pressure T/P, morning peak, and SI in patients with GRK4 variant*

After treatment with losartan, the blood pressure T/P, blood pressure morning peak, and blood pressure SI changes in GRK4 variant patients were more significant compared with GRK4 wild-type group ( $P < 0.05$ ), especially in A142V group (**Figure 3**).

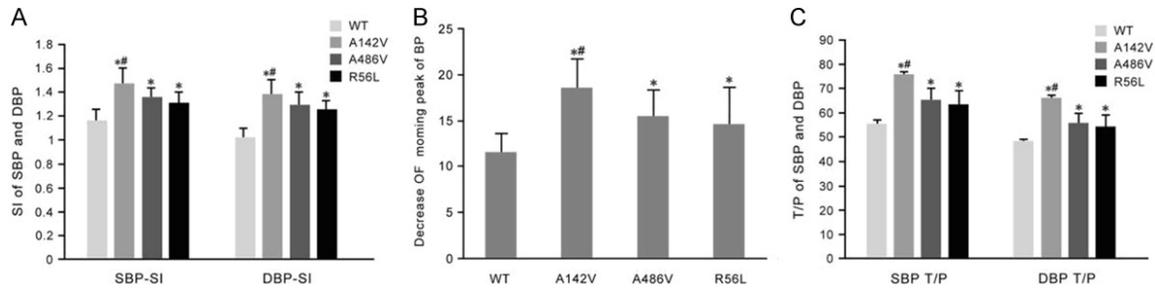
### *The cardiac protective effect of losartan on GRK4 variant patients*

The patients treated with losartan received echocardiography, including EF, LVESD, left ventricular thickness, and LVEDD. It was shown that the cardiac function indicators were significantly improved in patients with GRK4 variants after treated with losartan compared with the GRK4 wild-type group ( $P < 0.05$ ). There was no statistical difference among the three GRK4 variant subtypes (**Table 1**).

### *The liver protective effect of losartan on GRK4 variant patients*

The ALT, AST, TG, and TC of each group were detected by automatic biochemical analyzer. It was demonstrated that compared with the GRK4 wild type group, the ALT, AST, TG, and TC in the GRK4 variant patients were significantly

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**Figure 3.** The effect of losartan on blood pressure T/P, morning peak, and SI in patients with GRK4 variant. \* $P < 0.05$ , compared with WT. # $P < 0.05$ , compared with A486V or R65L.

**Table 1.** The cardiac protective effect of losartan on GRK4 variant

	WT	A142V	A486V	R65L
LVESD (mm)	31.37±3.26	38.72±5.81*	36.27±7.85*	36.11±4.67*
LVEDD (mm)	40.21±6.47	47.36±8.55*	45.31±7.51*	48.16±8.06*
EF (%)	50.72±7.31	43.12±6.68*	43.12±6.68*	43.12±6.68*
LVAWT (mm)	9.13±0.89	8.61±0.81*	8.59±0.79*	8.63±0.63*
LVPWT (mm)	9.08±0.62	8.14±0.43*	8.11±0.33*	8.09±0.55*

\* $P < 0.05$ , compared with WT.

**Table 2.** The liver protective effect of losartan on GRK4 variant

	WT	A142V	A486V	R65L
ALT (U/L)	75.52±5.26	38.15±4.43*	37.25±5.12*	41.31±2.57*
AST (U/L)	65.65±6.21	41.31±3.52*	40.35±5.35*	39.55±4.34*
TG (mmol/L)	1.97±0.31	1.22±0.68*	1.35±0.41*	1.41±0.51*
TC (mmol/L)	4.97±1.22	3.14±1.41*	3.11±1.35*	3.09±1.52*

\* $P < 0.05$ , compared with WT.

improved after treated with losartan ( $P < 0.05$ ). However, there was no statistical difference among the three GRK4 variant subtypes (Table 2).

### The renal protective effect of losartan on GRK4 variant patients

The BUN, Scr, blood  $\beta$ 2-MG, and uric acid of each group were detected by automatic biochemical analyzer. It was revealed that compared with the GRK4 wild type group, the renal function in the GRK4 variant patients were significantly improved after treated with losartan ( $P < 0.05$ ). However, there was no statistical difference among the three GRK4 variant subtypes (Table 3).

### Discussion

The rapid development of cloud data technology, biotechnology, imaging technology, and dete-

ction technology has promoted the modern medicine into the era of precision medicine. The search for genetic differences is the main means to achieve precision medicine. Whether different genetic types of hypertensive patients exhibited different treatment response attracted much more attention [17]. Previous studies found that calcium channel gene variants enhanced the antihypertensive effect of calcium channel blockers,  $\beta$ -receptor variants elevated the antihypertensive effect of  $\beta$ -blocker metoprolol, AT1 receptor variant A1166C improved the blood pressure lowering

effect of angiotensin-converting enzyme inhibitor (ACEI), whereas its ability to improve arteriosclerosis is reduced [18, 19]. The AT1 receptor plays an important role in the control of blood pressure. The kidney and blood vessels are key tissues that regulate blood pressure in the human body. They affect the blood pressure level by regulating the retention of water and sodium and the relaxation and contraction of blood vessels. Stimulating AT1 receptor can induce vasoconstriction and sodium retention to elevate blood pressure. AT1 receptor function is also increased under hypertensive states [20, 21]. Among the five major classes of antihypertensive drugs recommended by the World Health Organization (WHO), ACEI and AT1 receptor blockers (ARB) are targeting the angiotensin system (RAS) [22]. As an ARB drug, losartan presents different effect on different patients. The target of losartan is AT1R, while GRK4 mutation can affect the function of AT1R.

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**Table 3.** The renal protective effect of losartan on GRK4 variant

	WT	A142V	A486V	R65L
BUN ( $\mu\text{mmol/L}$ )	7.52 $\pm$ 0.76	3.22 $\pm$ 0.73*	3.29 $\pm$ 0.88*	3.95 $\pm$ 0.72*
Scr ( $\mu\text{mmol/L}$ )	65.65 $\pm$ 6.21	41.31 $\pm$ 3.52*	40.35 $\pm$ 5.35*	39.55 $\pm$ 4.34*
Blood $\beta$ 2-MG (mg/L)	3.31 $\pm$ 0.39	2.01 $\pm$ 0.61*	2.12 $\pm$ 0.47*	2.25 $\pm$ 0.59*
Uric acid (mmol/L)	131.22 $\pm$ 11.31	67.14 $\pm$ 15.48*	72.11 $\pm$ 12.35*	73.92 $\pm$ 9.58*

\* $P < 0.05$ , compared with WT.

Whether the GRK4 mutation is related to the antihypertensive effects of losartan has become a widespread concern [23, 24].

Previous studies have demonstrated that increased activity of GRK4 increased AT1 receptor function in hypertensive states. GRK4 variant is responsible for the increase in GRK4 activity. We speculated that GRK4 variant may affect the blood pressure. It was confirmed that GRK4 variants A142V, A486V, and R65L can increase GRK4 activity [8]. A large number of experimental studies proved that GRK4 is an important substance affecting the expression and function of AT1 receptor. In the animal model of GRK4 variant A142V transgenic mice, the blood pressure of A142V transgenic mice was significantly higher than that of wild type, accompanied with impaired urinary sodium excretion. Moreover, the plasma renin level was elevated, while vascular and renal AT1 receptor protein expressions were significantly increased in A142V transgenic mice. AT1 receptor blocker losartan effectively reduced the blood pressure of A142V transgenic mice. AT1 receptor knockout mice and A142V transgenic mice were crossed to obtain "A142V transgenic mice without AT1 receptor expression", and it was found that A142V transgenic mice exhibited normal blood pressure without AT1 receptor expression [25, 26]. Meta-analysis of multiple studies also revealed that GRK4 variants are closely related to hypertension [27]. In this study, 312 EP patients with essential hypertension were enrolled. PCR confirmed that there were 72 (23.1%) patients with wild-type GRK4 and 240 patients (76.9%) with GRK4 variants, suggesting that GRK4 variants account for the majority of EP patients. After treatment with losartan, the blood pressure dropped and blood pressure T/P, blood pressure morning peak, and blood pressure SI changes in GRK4 variant patients were significantly changed compared with GRK4 wild-type group. Among them, the change of GRK4 variant A142V was

more significant. The target organs in patients with GRK4 variants were obviously protected after administration, suggesting that GRK4 variants have significant antihypertensive influence on losartan and provide accurate medical care for target organ protection. In the further study, it is proposed to deeply analyze the related mechanism of losartan on the antihypertensive effect of patients with GRK4 variant, and provide relevant reference for clinical treatment.

### Conclusion

Losartan showed more significant antihypertensive effect on patients with GRK4 variants. The most significant improvement was observed in patients with GRK4 variant A142V, indicating that the target organs in patients with GRK4 variants might be protected after administration.

### Disclosure of conflict of interest

None.

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### References

- [1] Hong SH. Potential for physician communication to build favorable medication beliefs among older adults with hypertension: a cross-sectional survey. *PLoS One* 2019; 14: e0210169.
- [2] Thomopoulos C, Parati G and Zanchetti A. Effects of blood pressure lowering on outcome incidence in hypertension: 4. Effects of various classes of antihypertensive drugs—overview and meta-analyses. *J Hypertens* 2015; 33: 195-211.

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- [3] Angell SY, De Cock KM and Frieden TR. A public health approach to global management of hypertension. *Lancet* 2015; 385: 825-827.
- [4] Eadon MT and Chapman AB. A physiologic approach to the pharmacogenomics of hypertension. *Adv Chronic Kidney Dis* 2016; 23: 91-105.
- [5] Szczykowska J, Hryszko T and Naumnik B. Cardiac troponins in chronic kidney disease patients with special emphasis on their importance in acute coronary syndrome. *Adv Med Sci* 2019; 64: 131-136.
- [6] Kim H, Park J, Nam KH, Jhee JH, Yun HR, Park JT, Han SH, Chung W, Oh KH, Park SK, Kim SW, Kang SW, Choi KH, Ahn C and Yoo TH. The effect of interactions between proteinuria, activity of fibroblast growth factor 23 and serum phosphate on renal progression in patients with chronic kidney disease: a result from the KoreaN cohort study for Outcome in patients with chronic kidney disease study. *Nephrol Dial Transplant* 2019; [Epub ahead of print].
- [7] Dillman JR, Serai SD, Trout AT, Singh R, Tkach JA, Taylor AE, Blaxall BC, Fei L and Miethke AG. Diagnostic performance of quantitative magnetic resonance imaging biomarkers for predicting portal hypertension in children and young adults with autoimmune liver disease. *Pediatr Radiol* 2019; 49: 332-341.
- [8] Wang Z, Zeng C, Villar VA, Chen SY, Konkalmatt P, Wang X, Asico LD, Jones JE, Yang Y, Sanada H, Felder RA, Eisner GM, Weir MR, Armando I and Jose PA. Human GRK4 $\gamma$ 142V variant promotes angiotensin II type I receptor-mediated hypertension via renal histone deacetylase type 1 inhibition. *Hypertension* 2016; 67: 325-334.
- [9] Huang H, Li X, Zheng S, Chen Y, Chen C, Wang J, Tong H, Zhou L, Yang J and Zeng C. Down-regulation of renal G protein-coupled receptor kinase type 4 expression via ultrasound-targeted microbubble destruction lowers blood pressure in spontaneously hypertensive rats. *J Am Heart Assoc* 2016; 5.
- [10] Sanada H, Yatabe J, Midorikawa S, Katoh T, Hashimoto S, Watanabe T, Xu J, Luo Y, Wang X, Zeng C, Armando I, Felder RA and Jose PA. Amelioration of genetic hypertension by suppression of renal G protein-coupled receptor kinase type 4 expression. *Hypertension* 2006; 47: 1131-1139.
- [11] Yatabe J, Sanada H, Midorikawa S, Hashimoto S, Watanabe T, Andrews PM, Armando I, Wang X, Felder RA and Jose PA. Effects of decreased renal cortical expression of G protein-coupled receptor kinase 4 and angiotensin type 1 receptors in rats. *Hypertens Res* 2008; 31: 1455-1464.
- [12] Yang J, Villar VA, Jones JE, Jose PA and Zeng C. G protein-coupled receptor kinase 4: role in hypertension. *Hypertension* 2015; 65: 1148-1155.
- [13] Chen K, Fu C, Chen C, Liu L, Ren H, Han Y, Yang J, He D, Zhou L, Yang Z, Zhang L, Jose PA and Zeng C. Role of GRK4 in the regulation of arterial AT1 receptor in hypertension. *Hypertension* 2014; 63: 289-296.
- [14] Sanada H, Yoneda M, Yatabe J, Williams SM, Bartlett J, White MJ, Gordon LN, Felder RA, Eisner GM, Armando I and Jose PA. Common variants of the G protein-coupled receptor type 4 are associated with human essential hypertension and predict the blood pressure response to angiotensin receptor blockade. *Pharmacogenomics J* 2016; 16: 3-9.
- [15] Bhatnagar V, O'Connor DT, Brophy VH, Schork NJ, Richard E, Salem RM, Nievergelt CM, Bakris GL, Middleton JP, Norris KC, Wright J, Hiremath L, Contreras G, Appel LJ and Lipkowitz MS; AASK Study Investigators. G-protein-coupled receptor kinase 4 polymorphisms and blood pressure response to metoprolol among African Americans: sex-specificity and interactions. *Am J Hypertens* 2009; 22: 332-338.
- [16] Allen SJ, Parthasarathy G, Darke PL, Diehl RE, Ford RE, Hall DL, Johnson SA, Reid JC, Rickert KW, Shipman JM, Soisson SM, Zuck P, Munshi SK and Lumb KJ. Structure and function of the hypertension variant A486V of G protein-coupled receptor kinase 4. *J Biol Chem* 2015; 290: 20360-20373.
- [17] Ihm SH, Bakris G, Sakuma I, Sohn IS and Koh KK. Controversies in the 2017 ACC/AHA hypertension guidelines: who can be eligible for treatments under the new guidelines? - An Asian perspective. *Circ J* 2019; 83: 504-510.
- [18] Tan ST, Quek RYC, Haldane V, Koh JJK, Han EKL, Ong SE, Chuah FLH and Legido-Quigley H. The social determinants of chronic disease management: perspectives of elderly patients with hypertension from low socio-economic background in Singapore. *Int J Equity Health* 2019; 18: 1.
- [19] He DH, Lin JX, Zhang LM, Xu CS and Xie Q. Early treatment with losartan effectively ameliorates hypertension and improves vascular remodeling and function in a prehypertensive rat model. *Life Sci* 2017; 173: 20-70.
- [20] Li L, Guan Y, Kobori H, Morishita A, Kobara H, Masaki T, Nakano D and Nishiyama A. Effects of the novel nonsteroidal mineralocorticoid receptor blocker, esaxerenone (CS-3150), on blood pressure and urinary angiotensinogen in low-renin Dahl salt-sensitive hypertensive rats. *Hypertens Res* 2019; 42: 769-778.
- [21] Gatineau E, Cohn DM, Poglitsch M, Loria AS, Gong M and Yiannikouris F. Losartan prevents

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- the elevation of blood pressure in adipose-PRR deficient female mice while elevated circulating sPRR activates the renin-angiotensin system. *Am J Physiol Heart Circ Physiol* 2019; 316: H506-H515.
- [22] Akbari H, Asadikaram G, Vakili S and Masoumi M. Atorvastatin and losartan may upregulate renalase activity in hypertension but not coronary artery diseases: the role of gene polymorphism. *J Cell Biochem* 2019; 120: 9159-9171.
- [23] de Araujo LJ, Nagaoka MR, Borges DR and Kouyoumdjian M. Participation of hepatic alpha/beta-adrenoceptors and AT1 receptors in glucose release and portal hypertensive response induced by adrenaline or angiotensin II. *Braz J Med Biol Res* 2018; 51: e7526.
- [24] Zhao J, Cheng Q, Liu Y, Yang G and Wang X. Atorvastatin alleviates early hypertensive renal damage in spontaneously hypertensive rats. *Biomed Pharmacother* 2019; 109: 602-609.
- [25] Rayner B and Ramesar R. The importance of G protein-coupled receptor kinase 4 (GRK4) in pathogenesis of salt sensitivity, salt sensitive hypertension and response to antihypertensive treatment. *Int J Mol Sci* 2015; 16: 5741-5749.
- [26] Muskalla AM, Suter PM, Saur M, Nowak A, Hersberger M and Krayenbuehl PA. G-protein receptor kinase 4 polymorphism and response to antihypertensive therapy. *Clin Chem* 2014; 60: 1543-1548.
- [27] Nordestgaard BG, Kontula K, Benn M, Dahllof B, de Faire U, Edelman JM, Eliasson E, Fyhrquist F, Hille DA, Ibsen H, Lyle PA, Berg K, Sandberg M, Sethi AA, Wong PH; Os I. Effect of ACE insertion/deletion and 12 other polymorphisms on clinical outcomes and response to treatment in the LIFE study. *Pharmacogenet Genomics* 2010; 20: 77-85.