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Genetic variants of GRK4 influence circadian rhythm of blood pressure and response to candesartan in hypertensive patients

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Abstract

Background: Genetic variants of coding genes related to blood pressure regulation participate in the pathogenesis of hypertension and determines the response to specific antihypertensive drugs. G protein-coupled receptor kinase 4 (GRK4) and its variants are of great importance in pathogenesis of hypertension. However, little is known about role of GRK4 variants in determine circadian rhythm of blood pressure and response to candesartan in hypertension. The aim of this study was to analyze the correlation of GRK4 variants and circadian rhythm of blood pressure, and to explore their effect on antihypertensive efficiency of candestartan.

Methods: In this study, a total of 1239 cases were eligible, completed ambulatory blood pressure monitoring (ABPm) observation and exon sequencing of G protein-coupled receptor kinase 4 (GRK4). ABPm was obtained before and after 4-week treatment of candesartan. Diurnal variation of systolic blood pressure and antihypertensive effect of candesartan were then assessed.

Results: Compared to GRK4 wild type (GRK4-WT), patients with GRK4 variants were more likely to be non-dippers (*odds ratio (OR) 6.672, 95% confidence interval (CI) 5.124–8.688, P <*

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Ethics statement

This study was approved by the Ethics Committee of Daping Hospital (Ethics Committee of Daping Hospital 2015 No. 93). Informed written consents were obtained from each subject.

Disclosure statement

The author(s) have no conflicts of interest to disclose.

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.001), with GRK4 A142V (OR 5.888, 95% CI 4.332–8.003, $P < .001$), A486V (OR 7.102, 95% CI 5.334–9.455, $P < .001$) and GRK4 R65L (OR 3.273, 95% CI 2.271–4.718, $P < .001$), respectively. Correlation analysis revealed that non-dippers rhythm of blood pressure were associated with GRK4 variants (r = .420, $P < .001$), with GRK4 A142V (r = .416, $P < .001$), A486V (r = .465, $P < .001$) and GRK4 R65L (r = .266, $P < .001$), respectively. When given 4-week candesartan, patients with GRK4 variants showed better antihypertensive effect as to drop in blood pressure (24 h mSBP, *21.21 ± 4.99 vs 12.34 ± 4.78 mmHg, P < .001*) and morning peak (MP-SBP, *16.54 ± 4.37 vs 11.52 ± 4.14 mmHg, P < .001*), as well as greater increase in trough to peak ratio (SBP-T/P, *.71 ± .07 vs .58 ± .07, P < .001*) and smoothness index (SBP-SI, *1.44 ± .16 vs 1.17 ± .11, P < .001*) than those with GRK4 WT.

Conclusion: This study indicates that hypertensive patients with GRK4 variants are more likely to be non-dippers. What's more, patients with GRK4 variants possess a significantly better antihypertensive response to candesartan than those with GRK4 WT.

Keywords

Essential hypertension; GRK4; circadian rhythm; candesartan

Introduction

Hypertension is a major risk factor for cardiovascular disease. By 2025, the number of hypertensive patients is expected to exceed 1.5 billion worldwide, with the number of hypertensives in China reaching 300 million (1–3). Interaction of gene and environment contribute to the development of essential hypertension. The pathogenesis of hypertension mainly includes abnormal renal water and sodium excretion, arterial dysfunction, excessive sympathetic activation, endocrine and immune disorders (4–6). Generally, it is hard to ascertain the influence of one single gene on hypertension, unless this gene interacts with others that are germane to blood pressure regulation. The G protein-coupled receptor kinase 4 (GRK4) is such a gene. Expression of GRK4 is limited to only a few organs, such as the brain, kidney and vascular system, implying its crucial role in long term control of BP and natriuresis. Actually, GRK4 plays critical roles in the activity of the peripheral dopaminergic and angiotensin system. Previous studies have shown that genetic mutants of GRK4 can lead to an increase of its activity, and further increase the function of angiotensin receptor type 1 (AT_1R) in kidney, and participate further in the pathogenesis of hypertension (7–10). Antihypertensive effects of drugs, including AT_1R blockers, may vary significantly among hypertensive patients with different genetic variations (11–13). Considering, that the AT_1R is the target of candesartan, we speculate that GRK4 variants may play a crucial role in the different responses to candesartan in hypertensive patients. Therefore, we identified genotype of GRK4 in hypertensive patients through exon sequencing, and then analyzed the influence of GRK4 variants on the blood pressure Circadian Rhythm and antihypertensive efficiency of candesartan.

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Methods

Study population

From September 2015 to December 2019, 3651 outpatients or inpatients with untreated hypertension in the Department of Cardiology, Third Military Medical University, were invited and 2303 cases agreed to participate in this project. Among this cohort, 1239 cases were eligible and completed the whole study. Inclusion criteria were confined to untreated hypertensive patients, Han Chinese, 40–70-years-old with blood pressure ≥140/90nmmHg. Exclusion criteria were secondary hypertension, combined with liver and kidney dysfunction, severe diabetes mellitus, multiple infections, chronic wasting diseases, malignant tumors, or intolerance to testing. All candidates were voluntary participants, and a signed unified consent form was obtained. In addition, they were given a standard set of questionnaire and follow-up survey which included demographic data, family history, profession, history of drinking and smoking.

This study was approved by the Ethics Committee of Daping Hospital (Ethics Committee of Daping Hospital 2015 No. 93). Informed written consents were obtained from each subject. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

General parameters for assessment

Height and weight were measured for body mass index (BMI) assessment. Blood were obtained between 8:00 and 10:00 in the morning with fasting overnight (at least 8 h), for blood sodium, chloride, potassium, fasting plasma glucose (FPG), triglyceride (TG), total cholesterol (TC), creatinine and genotype analysis. General information was collected, including sex, age, and history of hypertension. All clinical biochemical items, including FPG, TG, TC, and creatinine were tested in clinical laboratory of DaPing Hospital, The Third Military Medical University.

Arterial blood pressure monitoring

Baseline arterial blood pressure was measured before-treatment by a noninvasive portable ambulatory blood pressure monitoring (ABPm) (SunTech Medical Inc, Morrisville, US). Measurement was performed once every 20 min automatic in daytime (06:00 am to 22:00 pm) and once every 30 min at night (22:00 pm to 06:00 am). Data with efficiency and accuracy of >90% was collected for further analysis. Parameters for analysis include mean systolic blood pressure for 24 hours (24 h mSBP) and mean diastolic blood pressure for 24 hours (24 h mDBP), mean daytime systolic blood pressure (mdSBP) and mean daytime diastolic blood pressure (mdDBP), mean night systolic blood pressure (mnSBP) and mean night diastolic blood pressure (mnDBP), the diurnal variation rate of systolic blood pressure (BPvR) namely [(mdSBP-mnSBP)/mdSBP] ×100%. According to BPvR, circadian rhythm of blood pressure was classified into dippers and non-dippers category. BPvR of dippers is defined as 10–20% drop from the determined average. Otherwise they were classified as non-dippers (14,15).

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Genotyping

Genomic DNA was isolated using a commercial kit (QIAamp DNA Blood Mini Kit; Qiagen, Tokyo, Japan) from the peripheral blood of participants. A thermal cycler (PC-700; Astec, Fukuoka, Japan) PCR was used to amplifiy DNA fragments according to the recommended protocol (rTAQ, Toyobo, Osaka, Japan).

Primers used in this project are listed as follow: GRK4 R65L, rs2960306-F 5′ATGTGGTGGACTGTAATGATTCT3′, rs2960306-R 5′AGCATAAGATTGGGTGGTTG3′. GRK4 A142V, rs1024323-F 5′AAGGGTCAACTACACTCTAGGAAC3′, rs1024323- R 5′TGGTTTGTACTGTATTAAGTTGGC3′. GRK4 A486V, rs1801058-F 5′TCTTGGGAACTGAGGGAGC3′, rs1801058-R 5′GTGCTGGAAGGGCAGTACC3′. The program consisted of 39 cycles serialy of denaturation at 94°C for 10s, annealing at 57°C for 35s, and extension at 72°C for 20s followed by a final extension at 72°C for 5 min. The PCR products were ran in 1.0% agarose gels under 150 V 100 mA for 20 min, and were further analyzed according to the DNA marker after electrophoretic imaging. PCR products were then purified and collected for further sequencing (Sangon Biotech Co. Ltd, Shanghai, PRC). Exon sequencing of GRK4 was performed on the Illumina HiSeq 2000 platform (Illumina Inc. San Diego, CA, USA) and analyzed by SeqMAN software.

Assessment of antihypertensive effect of candesartan

Participants were given candesartan at a dose of 4 mg/day. ABPm of each patient was obtained after 4 weeks of treatment with candesartan. Calculate the drops in BP and morning peak (MP), and the value of trough-to-peak (T/P) ratio and smoothness index (SI) of blood pressure. MP was calculated as awakening-value (mean blood pressure within 2 hours after waking up) minus trough (mean of the values of the lowest BP during nighttime sleep and two values immediately before and after). The MP value consisted of the MP of the systolic blood pressure (SBP-MP) and MP of diastolic blood pressure (DBP-MP). T/P ratios were calculated by peak (mean of max-two values of BP drop in 2–6 hours after medication) to trough (mean of BP drop during 2 hours before the next medication). SI of blood pressure was defined as mean value of BP change every hour for 24 hours divided by their standard deviation.

Statistical analysis

SPSS 17.0 (SPSS, Inc., Chicago, IL) was used for statistical analysis. Categorical variables were examined using the χ^2 test. Continuous variables were expressed in the form of mean ± standard deviation. The χ^2 test and correlation analyze were used to evaluate the relation between circadian rhythm of blood pressure and GRK4 variants. T-test was used to compare the difference in the antihypertensive response between the different genotypes. Statistical difference was defined by a P value of ≤.05.

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Results

Basic clinical characteristics and blood pressure of patients

A total of 1239 subjects were grouped as dippers or non-dippers according to BPvR. No significant differences were found with regards to age, gender, history of hypertension, FPG, BMI, and Creatinine, between groups ($P > .05$) (Table 1).

24-hour ABPm showed that mnSBP and 24 h mSBP of non-dippers group were significantly higher than those of dippers group, while dSBP and dDBP showed no difference ($P > .05$) (Table 1). Compared with dippers, smaller reduction of blood pressure at night resulted in significantly higher in mnSBP and 24 h mSBP in non-dippers.

Distribution of GRK4-WT and GRK4 variants in dippers and non-dippers hypertensive patients

To determine variation of GRK4 in enrolled patients, we executed genotyping and exon sequencing using peripheral blood of participants and identified 3 GRK4 variants, including A142V, A486V and R65L. GRK4 A142V mutated at rs1024323, whose genotype was A/G or A/A (Figure 1A and B). GRK4 A486V mutated at rs1801058, whose genotype was C/T or T/T (Figure 1C and D). GRK4 R65L mutated at rs2960306, whose genotype was G/T and T/T (Figure 1E and F). Specifically, there were 409 patients with GRK4 wild-type (GRK4-WT), 209 patients with splice A142V mutation, 381 and 71 patients with splice A486V and R65L mutation separately (Table 2). Furthermore, 60 patients were double-site mutation with A142V+A486V, 109 patients were triple-site mutation with A142V+A486V+R65L.

Among the 1239 subjects, 698 patients were non-dippers, of which 84% were GRK4-variants (Figure 2A). However, GRK4-WT accounted for 56% in dippers hypertensive patients. Data analysis exhibited that hypertensive patients with GRK4 variants were more likely to be non-dippers than those with GRK4-WT (*odds ratio (OR) 6.672, 95% confidence interval (CI) 5.124–8.688, $P < .001$*). Correlation analysis further exhibited that non-dippers was positively correlated with GRK4 variants (r = .420, *$P < .001$*).

We then investigated the role of different genotypes and alleles of GRK4 variants in determine the circadian rhythm of blood pressure. Compared with that of GRK4-WT, circadian rhythm of blood pressure in GRK4 A142V was more likely to be non-dippers in both genotype and allele (*all $P < .001$*). Correlation analysis identified that non-dippers was positively correlated with A142V genotype (r = .416, *$P < .001$*) and allele (r = .288, *$P < .001$*) (Table 2A). However, circadian rhythm distribution between A142V homozygous and heterozygous mutations showed no difference ($P > .05$). Similar with the GRK4 A142V, circadian rhythm of blood pressure in GRK4 A486V and R65L subgroup were both more likely to be non-dippers in both genotype and allele (*all $P < .001$*). Further analysis exhibited that non-dippers was positively correlated with A486V genotype (r = .465, *$P < .001$*), allele (r = .293, *$P < .001$*) and with R65L genotype (r = .266, *$P < .001$*), allele (r = .163, *$P < .001$*), respectively (Table 2B,C). No difference between A486V homozygous and heterozygous mutations was found in circadian rhythm ($P > .05$).

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Effect of GRK4 variants on variable response to candesartan in hypertensive patients

Participants were then grouped according to the GRK4 genotype, including GRK4-WT and GRK4 variants. There was no difference between groups as to BMI, FPG, cardiac-renal function, et al ($P > .05$). 24 h mSBP in patients with GRK4 variants was significantly higher than that of GRK4-WT (Table 3). To investigate the effect of GRK4 variants on their response to candesartan in hypertensive patients, 24 h ABPm of each patient was detected again after 4-week treatment of candesartan. We assessed the response to candesartan through parameters, including drop in BP and MP, the increase in values of T/P ratio and SI.

Compared with blood pressure in pre-treatment state, blood pressure in both GRK4-WT and GRK4 variants were significantly lower. Moreover, drop in 24 h mSBP (*21.21 ± 4.99 vs 12.34 ± 4.78 mmHg, P < .001*) and 24 h mDBP (*17.56 ± 4.65 vs 9.28 ± 2.56 mmHg, P < .001*) in GRK4 variants showed significantly greater than those in GRK4-WT. Consistent with 24 h blood pressure, decrease in mdSBP, mdDBP, mnSBP and mnDBP in GRK4 variants showed significantly greater than those in GRK4-WT (all $P < .001$) (Figure 2B). However, there was no significant statistic differences among A142V, A486V and R65L patients as to the above parameters.

In addition, degrees of MP decline in GRK4-WT and GRK4 variants exhibited significant differences. Data revealed that the decrease in both SBP-MP (*16.54 ± 4.37 vs 11.52 ± 4.14 mmHg, P < .001*) and DBP-MP (*13.17 ± 3.05 vs 9.41 ± 3.05 mmHg, P < .001*) in GRK4 variants were greater than those found in GRK4-WT group (Figure 2C). As to the T/P ratio, patients with GRK4 variants revealed greater increase in T/P ratio in both SBP (SBP-T/P, *.71 ± .07 vs .58 ± .07, P < .001*) and DBP (*.60 ± .06 vs .51 ± .05, P < .001*) after 4-week treatment of candesartan than those found in GRK4-WT (Figure 2D). Beside, increase invalues of SBP-SI(*1.44 ± .16 vs 1.17 ± .11, P < .001*) and DBP-SI (*1.38 ± .11 vs 1.13 ± .10, P < .001*) in patients with GRK4 variants were also found greater than those with GRK4-WT (Figure 2E). Nevertheless, no statistical differences were found among A142V, A486V and R65L variants, concerning changes in MP, T/P ratio and SI of blood pressure ($P > .05$).

Discussion

There are several variants of GRK4, among which A142V, A486V and R65L are most closely related to the occurrence and development of hypertension. In the present study, GRK4 A142V, A486V and R65L are identified, and account for 67% of all the participants. Correlation analysis identifies that non-dippers circadian rhythm are positively associated with GRK4 variants, including GRK4 A142V, A486V and R65L, which implies GRK4 variants can significantly influence the fluctuation of blood pressure in hypertensive patients. Thus, our present study reveals, for the first time, that GRK4 variants affect the circadian rhythm fluctuation of blood pressure. Hypertensive patients with these 3 variants are more likely to be non-dippers. Physiologic fluctuation of blood pressure shows a phenomenon of circadian changes. Patients with dipper hypertension exhibit lower blood pressure at night than that of daytime, while non-dipper hypertension maintains the blood pressure at a high-level during the night (16,17). It will not only cause corresponding symptoms, but also leads to structural and functional remodeling of the cardiovascular system and damages of

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target organs (18–20). Although the mechanism on how blood pressure circadian rhythm is regulated is not clear, the renin-angiotensin system has been found to play a crucial role (21–24). It has been identified that the level of angiotensin II at night in non-dipper hypertensive patients is significantly higher than that of the dippers. Circadian rhythm of blood pressure in non-dipper hypertensive patients can be improved by lowering the level of angiotensin II, implying the crucial role of angiotensin system in determine of circadian rhythm. It has been identified that genetic variants of GRK4 can enhance the function of AT_1 R. Thus, GRK4 variants may influence circadian rhythm of blood pressure through abnormal regulation of angiotensin II-AT_1R signaling.

A series of clinical studies show that effect of the same antihypertensive drug at the same dose may vary greatly among population (25–27). However, previous investigations on pharmacogenetics imply that only a small fraction of genes probably contribute to the antihypertensive responses. It is found that therapeutic effect of candesartan varies among the population (12,28). Considering the regulation of GRK4 on AT_1R expression and function, we wonder whether the patients with genetic variants of GRK4 possess distinguished antihypertensive effect of candesartan. As expected, Candesartan has a stronger anti-hypertensive effect in patients with GRK4 variants compared GRK4-WT patients, with regards absolute value of 24 h mSBP. To better assess and explore the different antihypertensive effect of candesartan in GRK4-WT and GRK4 variants, we introduce MP, T/P ratio and SI. More than 60% of sudden deaths, heart attacks and strokes happen in the morning (29,30). Excessive elevation in MP has also been identified to increase the incidence of atherosclerosis and left ventricular abnormalities, implying the great importance of controlling MP of blood pressure (31,32). T/P ratio and SI, as independent indicators of effects of antihypertensive treatment, was used to evaluate the sustainability of BP reduction. In this investigation, patients with GRK4 variants possess a greater decline in MP and increase in values of T/P ratio and BP-SI. This indicates that when given candesartan, patients with GRK4 variants possess a better antihypertensive effect and may gain greater protection against hypertension and even better prognosis.

Conclusions

This study provides evidence that genetic variants of GRK4 can influence circadian rhythm fluctuation of blood pressure and possess a better antihypertensive effect of candesartan. Further studies are still needed, including investigations on exactly how GRK4 variants influence the fluctuation of blood pressure, as well as a thorough assessment of the response of GRK4 variants to candesartan in a larger population.

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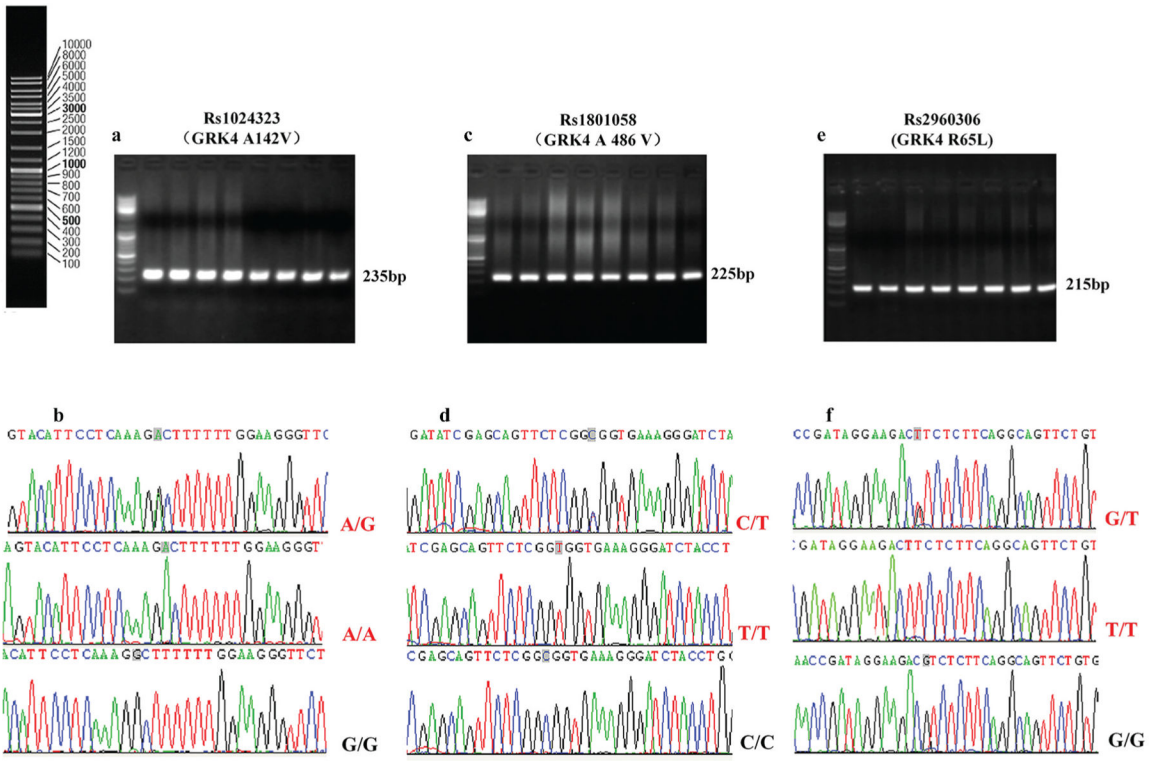

Figure 1.

Genotype identification of GRK4. A) Identification of GRK4 A142V mutant at rs1024323, detected at 235 bp. B) Genotype of GRK4 A142V variants, A/G and A/A in red mean variant genotype and G/G in black stands for wild type. C) Identification of GRK4 A486V mutant at rs1801058, detected at 225 bp. D) Genotype of GRK4 A486V variants, C/T and T/T in red mean variant genotype and C/C in black stands for wild type. E) Identification of GRK4 R65L mutant at rs2960306, detected at 215 bp. F) Genotype of GRK4 R65L variants, T/T and G/T in red mean variant genotype and G/G in black stands for wild type.

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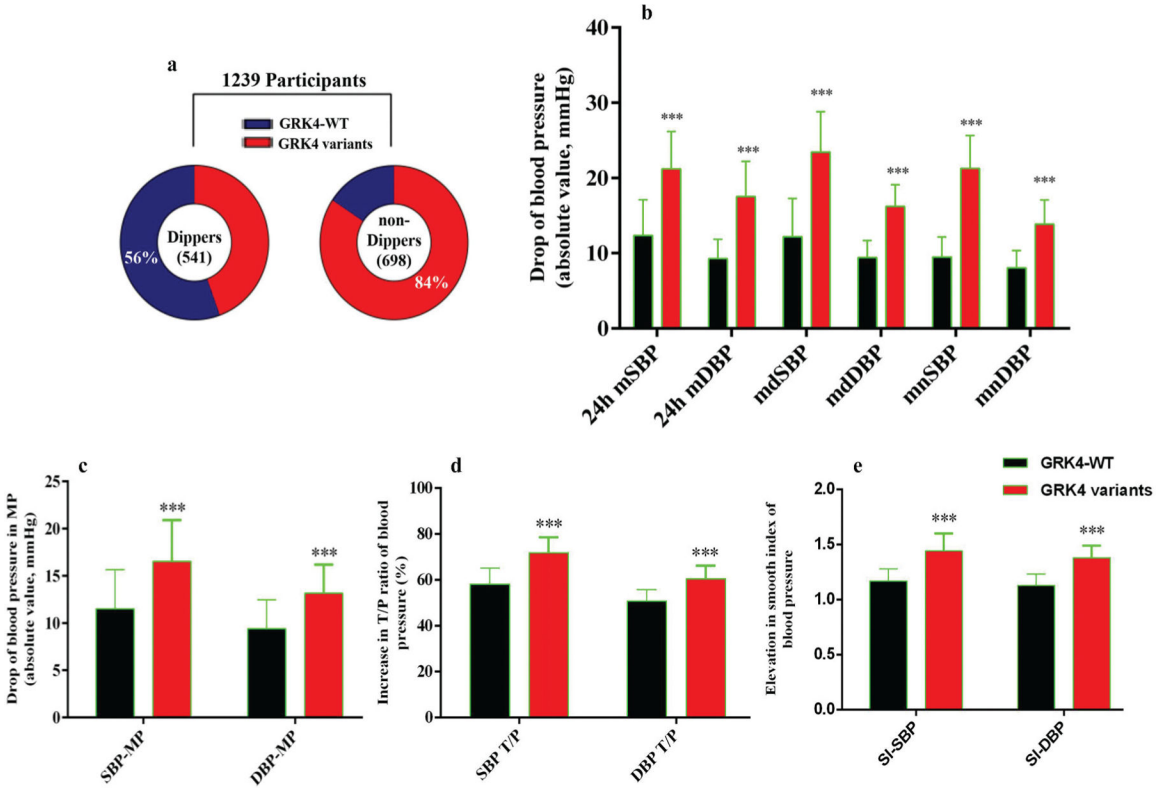

Figure 2.

Distribution of GRK4 variants and effect of GRK4 variants in response to candesartan in hypertensive patients. A) propotion of GRK4-WT and GRK4 variants in dippers and non-dippers. B) difference in drop of blood pressure. C) difference in drop of MP. D) difference in elevation of T/P ratio. E) difference in elevation of SI. 24 h mSBP, mean systolic blood pressure of 24 hours. 24 h mDBP, mean diastolic blood pressure of 24 hours. mdSBP, mean daytime systolic blood pressure. mdDBP, mean daytime diastolic blood pressure. nSBP, night systolic blood pressure. mnDBP, night diastolic blood pressure. MP, morning peak. T/P, trough to peak ratio. SI, smooth index. All data were presented as means±SD. ***, $P < .001$, versus GRK4-WT group.

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Table 1.

General parameters of participants.

	Dippers (n = 541)	Non-Dippers (n = 698)	*P*-value
Age (year)	54.2 ± 14.5	55.7 ± 14.1	.067
Sex (M/F)	292/249	355/343	.701
BMI (kg/m^2)	22.51 ± 4.22	23.01 ± 4.72	.053
FBG (mmol/L)	5.31 ± .82	5.32 ± .74	.822
TG (mmol/L)	1.18 ± .71	1.22 ± .82	.367
TC (mmol/L)	4.94 ± 2.21	5.03 ± 2.16	.471
Creatinine (mg/dL)	74.21 ± 13.37	75.35 ± 16.24	.186
History of HP (year)	2.42 ± 1.51	2.23 ± 1.85	.053
HR (beat/min)	74.63 ± 9.21	75.21 ±10.15	.299
24 h mSBP	148.13 ±7.44	157.24 ±7.22	<.001
24 h mDBP	87.22 ± 6.41	87.56 ± 8.55	.440
mdSBP	157.32 ± 10.96	156.84 ± 7.44	.360
mdDBP	89.51 ± 8.43	89.21 ± 6.48	.479
mnSBP	136.51 ± 8.23	147.07 ± 6.71	<.001
mnDBP	81.93 ± 5.61	82.31 ± 6.51	.280

All data were presented as means±SD. BMI, body mass index. FBG, fasting blood glucose. TG, Triglyceride. TC, Total cholesterol. HR, heart rate. 24 h mSBP, mean systolic blood pressure for 24 hour. 24 h mDBP, mean diastolic blood pressure for 24 hour. mdSBP, mean daytime systolic blood pressure. mdDBP, mean daytime diastolic blood pressure. mnSBP, mean night systolic blood pressure. mnDBP, mean night diastolic blood pressure.

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Table 2.

Correlation analysis of non-dippers hyperension and GRK4 variants.

					χ^2 test	Correlation analysis		
			Dippers	Non-dippers	OR (95%CI)	P-value	r	P-value
A	Genotype	GG	303(56%)	112(16%)	5.888 (4.332–8.003)	<.001	.416	<.001
vs. A142V		AG/AA	81(15%)/38(7%)	182(26%)/77(11%)				
	Aelle	G	687(63.5%)	405(29%)	3.630 (2.896–4.550)	<.001	.288	<.001
		A	157(14.5%)	336 (24%)				
B	Genotype	CC	303(56%)	112(16%)	7.102 (5.334–9.455)	<.001	.465	<.001
vs. A486V		CT/TT	103(19%)/49(9%)	287(41%)/112(16%)				
	Aelle	C	720(65.5%)	510(36.5%)	3.600 (2.950–4.393)	<.001	.293	<.001
		T	200(18.5%)	510(36.5%)				
C	Genotype	GG	303(56%)	112(16%)	3.273 (2.271–4.718)	<.001	.266	<.001
vs. R65L		GT/TT	49(9%)/32(6%)	77(11%)/21(3%)				
	Aelle	G	655(60.5%)	301(21.5%)	2.272 (1.698–3.039)	<.001	.163	<.001
		T	114(10.5%)	119(8.5%)				

Genotypes or alleles in red represent GRK4 variants. OR, odds ratio.CI, confidence interval.

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Table 3.

General parameters of participant with GRK4 WT or GRK4 variants.

Group	GRK4-WT	GRK4-V	*P-value*
Case (M/F)	409 (215/194)	830 (432/398)	.120
Age (year)	54.31 ± 12.22	55.03 ± 14.18	.380
BMI (kg/m^2)	23.84 ± 4.12	24.05 ± 4.77	.447
24 h mSBP (mm Hg)	151.38 ± 6.46	157.12 ± 5.74	<.001
24 h mDBP (mm Hg)	89.91 ± 7.24	91.23 ± 8.39	.510
FBG (mmol/L)	5.65 ± .77	5.48 ± .58	.074
TC (mmol/L)	4.86 ± 1.33	5.02 ± 1.87	.122
TG (mmol/L)	1.03 ± .81	1.01 ± .78	.675
HR (beat/min)	74.41 ± 10.72	75.04 ± 9.37	.289

All data were presented as means±SD. BMI, body mass index. 24 h mSBP, mean systolic blood pressure of 24 h. 24 h mDBP, mean diastolic blood pressure of 24 h. FBG, fasting blood glucose. TG, Triglyceride. TC, Total cholesterol. HR, heart rate.