G PROTEIN-COUPLED RECEPTOR KINASE TYPE 4 (GRK4) GENE VARIANTS AND RESPONSE TO ANGIOTENSIN II TYPE 1 RECEPTOR BLOCKER THERAPY.

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Abstract: Dopamine produced by the kidney counteracts the prohypertensive and sodium retaining actions of the renin-angiotensin system (RAS). In hypertension, the ability of dopamine to counteract the actions of the RAS is impaired by an increased constitutive activity of genetic variants of G protein-coupled receptor kinase type 4 (GRK4) that act to desensitize dopamine receptors, especially D_1 and D_3 dopamine receptors. In spontaneously hypertensive rats silencing the GRK4 gene in the kidney improves D_1R function, ameliorating the hypertension. More recently, we have reported that GRK4 and the angiotensin II type 1 receptor (AT_1R) expressed in the kidney interact to regulate blood pressure in both the normotensive Wistar-Kyoto and spontaneously hypertensive rats. The current study was designed to test the association between GRK4 gene variants R65L, A142V, and A486V and angiotensin II type 1 receptor blocker (ARBs) response in 161 Japanese with essential hypertension. We found that the response (≤140/90 mm Hg) to monotherapy with ARBs (losartan, candesartan, valsartan, or telmisartan) was highly associated with the GRK4 gene variant A142V. The absolute decrease in blood pressure in response to the ARB was also associated with GRK4 A142V only. GRK4 genotype was not associated with plasma aldosterone concentration, plasma renin activity or their ratio. We conclude that the GRK4 gene variant 142V may be both strongly predisposing to essential hypertension and predictive of the initial response to ARB monotherapy.

Introduction

Dopamine produced by the kidney is important in increasing sodium chloride excretion during conditions of moderate sodium chloride intake[1,2]. Dopamine inhibits ion transport by acting on two families of dopamine receptors, D_1-like

receptor family (D_1R and D_5R) and D_2-like receptor family, (D_2R, D_3R, and D_4R). In the proximal tubule D_1-like receptors and the D_3R inhibit sodium/hydrogen exchanger type 3 (NHE3), sodium/phosphate cotransporter type 2 (NaPi2), sodium amino acid cotransporters, Cl/HCO3 exchanger at the luminal membrane, and NaHCO3 cotransporter and Na^+K^+ATPase at the basolateral membrane[1,2]. Dopamine, via D_2-like receptors, probably via D_3R and D_4R, also inhibit sodium transport at more distal nephron segments[3-5]. Dopamine receptors also promote natriuresis by interacting with other natriuretic receptors. For example, D_1R heterodimerizes with angiotensin II type 2 receptors (AT_2R)[6] and endothelin B receptors (ETBR)[7]. Dopamine receptors may also increase sodium excretion by counteracting the stimulatory effect of AT_1R on Na^+K^+ATPase and transporters and exchangers inhibited by dopamine[1]. The ability of D_1R to inhibit AT_1R is, in part, due to D_1R and AT_1R heterodimerization[1]. However, dopamine and AT_1R can also regulate each others' expression. D_1-like receptors (probably D_1R) inhibits the transcription of AT_1R[8] and D_5R increases the degradation of AT_1R[9].

The G protein-coupled receptor kinases (GRK) are a seven-member family of serine/threonine protein kinases that regulate the intracellular trafficking of G protein-coupled receptors, including, AT_1R, D_1R, and D_3R. GRK4 is needed for the normal functioning of D_1R and probably for D_3R[10]. However, increased activity of GRK2 and GRK4 impairs D_1R and D_3R by keeping them in a state of desensitization, in part by decreasing their plasma membrane expression[10-12]. In the spontaneously hypertensive rat (SHR), renal cortical activity of GRK4 is increased due to increased protein expression[13]. Selectively decreasing GRK4 gene expression, in the renal cortex, improves the function of D_1R by increasing sodium excretion and decreasing the blood pressure in SHRs. In humans, this is due to increased constitutive activity of genetic variants of GRK4[10,11]. Thus, GRK4 gene variants, R65L, A142V, and A486V, impair the ability of D_1R to stimulate adenylyl cyclase activity in Chinese hamster ovary cells with heterologous expression of the variants and human renal proximal tubule cells endogenously expressing these variants[14]. Overexpression of human GRK4 gene variant, GRK4 142V, in mice produces hypertension on a normal salt diet and the overexpression of human GRK4 gene variant, GRK4 486V in mice also produces hypertension, but only on a high salt diet[14,15].

We have reported that GRK4 and AT_1R in the renal cortex interact to regulate sodium excretion and blood pressure in the SHR[16]. Thus, decreasing the renal cortical expression of both GRK4 and AT_1R increases sodium excretion and lowers blood pressure to a greater extent than decreasing the expression of either GRK4 or AT_1R alone. Hypertension in transgenic mice with human GRK4 142V is also not exclusively caused by an impairment in D_1R function. Recently, we found that increased expression and activity of AT_1R is also involved in the hypertension of these transgenic mice[17]. Angiotensin II increases and AT_1R blockade decreases arterial blood pressure to a greater extent in human GRK4 142V transgenic mice than wild-type littermates or human GRK 4wild-type transgenic mice. Moreover, the absence of even just one $AT_{1A}R$ allele prevents the hypertension caused by human GRK4 142V transgene.
We have reported that GRK4 gene variants, R65L, A142V, and A486V, are associated with salt-sensitive hypertension in a Japanese population[18]. In that study, we found that the presence of at least three GRK4 gene variants even in normotensive Japanese impaired the natriuretic effect of docarpamine, an orally active dopaminergic drug. Because the pro-hypertensive effect of GRK4 gene variants may also be related to increased AT_1R expression and function, at least in transgenic mice, we tested the hypothesis that subjects with essential hypertension and GRK4 gene variants may respond better to AT_1R blockers (ARBs) than those without GRK4 gene variants.

Materials and Methods:

All protocols were carried out at the Fukushima Medical University under the approval of its Institutional Review Board (IRB). One hundred sixty one newly diagnosed (# males = 76, average age = 53.2 ± 9.2 years, # females = 85, average age = 56.5 ± 10.4 years, M ± SD) randomly-selected, untreated essential hypertensive Japanese (referred to the hospital from physicians' offices or physicians working in outpatient clinics) had

their blood pressures verified by a mercury sphygmomanometer, at least twice before enrollment, as reported[18]. After providing informed consent, the subjects with systolic blood pressures ≥140 mm Hg and/or diastolic blood pressures ≥90 mm Hg were admitted to the Study Unit for physical examination, routine urine and plasma laboratory tests, electrocardiogram, and chest x-ray. Patients with diabetes mellitus, renal dysfunction (estimated glomerular filtration rate < 60mL/min/1.73m^2, and/or abnormal urinalysis), or secondary hypertension were excluded from the study. Before the evaluation of the relationship between genotype and phenotype, each newly diagnosed hypertensive patient was immediately started on antihypertensive medication in accordance with the IRB.

Genotyping analysis: Variants of angiotensin converting enzyme (ACE) Insertion/Deletion, angiotensinogen (AGT) A-6G, M235T, AT_1R A1166C, aldosterone synthase (CYP11B2) C-344T, D_1 dopamine receptor (D_1R) A-48G, G-protein β3 subunit (GNB3) C825T, GRK4 R65L,A142V, A486V, plasminogen activator inhibitor-1 (PAI-1) 4G/5G, and 11βHSD2 G534A were detected as reported[18].

Hypertensive patients were tested for response to ARBs (candesartan, losartan, telmisartan, or valsartan). Those whose systolic blood pressure dropped below 140 mm Hg and diastolic blood pressure below 90 mm Hg were considered to be responders (n = 67), while others were considered non-responders (n = 94). Based on these criteria single-locus response/non-response association tests were performed at the allelic and genotypic level, using Whole-genome Association Study Pipeline (WASP) software developed at the Center for Human Genetics Research of Vanderbilt University (http://chgr.mc.vanderbilt.edu/wasp/) as described previously[18-20]. Eleven variants in eight genes were tested (Table 1). Associations were tested using ^{2}, Fisher's exact test and/or Armitage trend tests where appropriate. For those markers found to be significant odds ratios were calculated to assess the allelic effect size. Tests for Hardy-Weinberg equilibrium were also performed using WASP. In addition to response as a dichotomous variable, we analyzed the association of genetic variation with the change in blood pressure as a continuous variable. However, since changes in both systolic and diastolic blood pressure were non-normal (P= 0.0004 for systolic blood pressure and P= 0.05 for diastolic blood pressure, Shapiro Wilks tests), analyses were done using the non-parametric Kruskal-Wallis test.

In addition, plasma renin activity (PRA), plasma aldosterone concentration (PAC) and the PAC/PRA ratios were tested for genetic association on a normal salt diet. The three GRK4 coding single nucleotide polymorphisms (SNPs) were tested using ANOVA. Because there were very few minor allele homozygous individuals for R65L (one) and A42V (four) only heterozygotes were compared to wild type homozygotes for all three SNPs. All three outcome variables were tested for normality using Shapiro Wilks test and all deviated significantly ($P<10^{-4}$). Therefore, PRA, PAC and PAC/PRA were all BoxCox transformed, which successfully normalized them. ANOVA were performed using STATA software.

Results:

Genotypes were tested for Hardy Weinberg equilibrium (HWE) to detect any evidence for genotyping error. Of the 11 variants only A486V in GRK4 showed any evidence of Hardy- Weinberg disequilibrium (P= 0.041), but this variant was included in all subsequent analyses as the deviation was not strong. With the exception of GRK4 variants, none of the gene variants associated with response to ARBs. All three GRK4 SNPs showed allelic association with response or resistance to ARB treatment (P< 0.025 for all three, Table 2a), and A142V and A486V also showed evidence for genotype association (P= 0.003 and 0.039, respectively, Table 2a). R65L was borderline for the genotypic test (P= 0.064). However, GRK4 R65 and GRK4 A486 were associated with resistance to ARB treatment while GRK4 142V was associated with a response to ARB treatment, supporting a role GRK4 142V in the regulation of the AT_1R[13,17].

No variants except those in GRK4 showed evidence for a quantitative response to ARB treatment. Only GRK4 A142V was associated with a decrease in blood pressure (P=0.007 and P=0.011 for diastolic blood pressure and systolic blood pressure, respectively, Table 2b). Thus, our data strongly support the role of GRK4 142V in ARB response, and indicate that the other

genetic variants have no such effect.

PRA, PAC and PAC/PRA ratios were measured under normal salt diet and tested for association with GRK4 variants. Our data suggest that none of these variables associated with GRK4 genotypes (Table 3). However, A486V did show evidence of marginal association with PRA (P= 0.086).

Discussion:

The current treatment of hypertension is empirical and not based on pathogenesis. Although the control of blood pressure with treatment (systolic blood pressure <140 mm Hg and diastolic blood pressure <90 mm Hg) has increased to 48%, more than one drug is usually required[21]. It has been suggested that a pharmacogenetic approach may provide more cost-effective therapy with reduction in morbidity and mortality[22-24]. A review on the pharmacogenomics of the treatment of hypertension in 2006 indicated a total of 160 possible gene polymorphism and drug interactions, a quarter of which showed a significant pharmacogenetic effect[24].

The influence of gene variants of alpha adducin and ACE insertion/deletion has been the subject of several studies. The initial positive association between adducin gene variations and hypertension, salt-sensitive hypertension, in particular, and response to diuretics has not been consistently replicated[24-27]. In an editorial commentary, De Buyzere concluded that the evidence for adducin-Trp as a predictor of diuretic response cannot be accepted at this time[28]. Polymorphisms of the $_1$-adrenergic receptor have also not been consistently shown to be a marker of a response to adrenergic blockers[24,29]

Inhibitors of the renin-angiotensin system have also not been consistently shown to be related to gene variants of ACE or angiotensinogen[24]. We now report in a limited number of subjects (161) allelic and genotypic association of the GRK4 gene variant A142V and response to ARB treatment. The absolute decrease in blood pressure in response to ARB was also associated with GRK4 A142V. The response to ARB was not associated with plasma aldosterone concentration, plasma renin activity or their ratio. In contrast, GRK4 R65 and GRK4 A486 may predict a poor response to ARB treatment. Based on these data we conclude that GRK4 142V variant may be strongly predisposing to essential hypertension that also predicts the initial response to ARB monotherapy.

Acknowledgment:

This work was supported in part by grants from the Salt Science Research Foundation No 06C6 and 0835, the United States National Institutes of Health, R01HL092196 and P01HL074940.

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Table 1. List of genetic variants studied

Gene	Variant/Common Name	rs number
ACE	Intron 16 I/D	rs4646994
Angiotensinogen	A-6G	rs5051
	M235T(C4072T)	rs699
AT_1R	A1166C	rs5186
CYP11B2	C-344T	rs1799998
D_1R	A-48G	rs4532
GNB3	C825T	rs5443
GRK4	R65L (G448T)	rs1801058
	A142V (C679T)	rs1024323
	A486V (C1711T)	rs2960306
PAI-1	4G/5G	rs1799768

ACE: angiotensin converting enzyme, AT_1R: angiotensin II type 1 receptor,
CYP11B2: aldosterone synthase, D_1R: D_1 dopamine receptor,
GNB3: G protein 3 subunit, GRK4: G protein-coupled receptor kinase 4,
PAI-1: plasminogen activator inhibitor-1

Table 2. Genetic Association with response to angiotensin II type 1 receptor blockers a. Response vs. non response

Gene – Variant	Allelic Odds ratio[1]	95% CI	Allelic p value	Genotype p value
GRK4 – R65L	0.42	0.22, 0.83	0.011	0.064
GRK4 – A142V	1.86	1.08, 3.20	0.024	0.003
GRK4- A486V	0.58	0.36, 0.93	0.023	0.039

1- OR is measured relative to the common allele

b. Change in blood pressure

Gene – Variant	Diastolic Blood Pressure Kruskal Wallis P value	Systolic Blood Pressure Kruskal Wallis P value
GRK4 – R65L	0.546	0.627
GRK4 – A142V	0.007	0.011
GRK4- A486V	0.184	0.190

Table 3. Association of GRK4 polymorphisms and PAC, PRA, and PRC/PRA ratio on normal salt diet.

Variable/SNP	**ANOVA P-value**
PAC/PRA	
R65L	0.8483
A142V	0.8253
A486V	0.1441
PAC	
R65L	0.3782
A142V	0.8438
A486V	0.3924
PRA	
R65L	0.7811
A142V	0.8693
A486V	0.0859

PAC: plasma aldosterone concentration, PRA: plasma renin activity