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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

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Abstract

Non-synonymous *GRK4* variants, *R65L*, *A142V*, and *A486V*, are associated with essential hypertension in diverse populations. This study replicated the association of GRK4 variants, including $GRK4^{142V}$, with human essential hypertension in a Japanese population (n=588; hypertensive, n=486 normotensive controls) and determined whether the presence of *GRK4* variants predicted the blood pressure response to angiotensin receptor blockers (ARBs) in patients with essential hypertension. We analyzed 829 patients and compared the response to ARBs between individuals with no *GRK4* variants (n=136) and those with variants at one or any of the

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Competing interests

Drs. Jose and Felder own Hypogen, Inc., which owns the US Patent (6,660,474B1) for GRK4.
Drs. Eisner and Williams are members of the Board of Hypogen, Inc.

Ethics Statement

The Institutional Review Board at the Fukushima Medical University approved all protocols.

Financial Disclosure

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three loci (n=693). Carriers of *hGRK4*^{142V} had a greater decrease in systolic BP in response to ARBs than non-carrier hypertensive patients. By contrast those with variants only at *GRK4*^{486V} were less likely to achieve the BP goal in response to an ARB than those with no variants. These studies showed for the first time the association between *GRK4*^{142V} and a larger decrease in BP with ARBs in hypertensive patients.

Introduction

Hypertension affects over a quarter of the people worldwide and about a third of the population in the USA, costing the USA about $94 billion dollars annually (1, 2). The treatment of hypertension has improved but the pharmacological reduction of blood pressure (BP) to systolic BP <140 mm Hg and diastolic BP <90 mm Hg is successful in only about 50% of patients, and more than one drug is usually required (3). The suboptimal control of essential hypertension may be related to the fact that the current treatment is empirical and not based on *a priori* knowledge of genetic and molecular mechanisms. A pharmacogenetic approach to hypertension treatment may provide more cost-effective therapy and allow patients to achieve BP control more promptly with reduction in side effects, morbidity, and mortality (4).

Essential hypertension results from the interplay of genetic and environmental influences. One such environmental influence is mineral intake, especially sodium chloride (5–7). The renal tubular dopaminergic system, which is critical in facilitating sodium chloride excretion, is impaired in essential hypertension due to an over activity of G protein-coupled receptor kinase type 4 (GRK4), an enzyme that regulates the dopamine D_1 receptor (8–11). Over-activity of GRK4 is caused by three activating non-synonymous variants of the gene, namely, *R65L*, *A142V*, and *A486V* (8, 9, 12), and by themselves or by interacting with other genes have been reported to be associated with essential hypertension in diverse populations (13–17).

The causal relationship between *GRK4*^{142V} or *GRK4*^{486V} and human essential hypertension is supported by studies in transgenic mice; human (*h) GRK4*γ^{142V}, *hGRK4*γ^{486V} transgenic mice are hypertensive (8, 18, 19). Only a few other gene variants associated with essential hypertension in humans (20, 21) have been shown to produce hypertension in transgenic animals, an essential test for the demonstration that genetic variants are causal of a complex trait, such as hypertension (22).

GRK4 and the angiotensin type 1 receptor (AT_1R) interact in the regulation of BP in rodents (23). Increased AT_1R function could be caused by *GRK4*γ^{142V} because the hypertension in *hGRK4*γ^{142V} transgenic mice, which responds to angiotensin receptor blockers (ARBs), is associated with increased AT_1R expression (24). Whether or not these non-human studies can be translated to humans has not been determined.

The present studies were performed to validate the association of *GRK4* variants, including *GRK4*^{142V} with human essential hypertension and to test the hypothesis that the presence of *GRK4*^{142V} predicts the BP response to ARBs in humans with essential hypertension.

METHODS

Association studies

Study design and participants. A case-control study was carried out in Fukushima Prefecture, Japan, from April 2006 to March 2009. The Institutional Review Board at the Fukushima Medical University approved all protocols. Newly diagnosed, randomly-selected, untreated essential hypertensive Japanese had their BPs verified by a mercury sphygmomanometer, at least twice before enrollment (Table 1A).

Variables—For the baseline study, participants' clinical data were obtained from medical files that included age, sex, height, weight, family history of hypertension, daily alcohol consumption, current smoking habits, diabetes mellitus status, systolic and diastolic BPs. Exclusion criteria included diabetes, renal dysfunction or abnormal urinalysis (25), or secondary hypertension (26, 27). Blood samples for genotyping were drawn after obtaining informed consent from the participant. All patients were genotyped for 11 variants in 8 genes (Supplementary Table 1). Variants of the genes studied were detected by fluorescent probe melting, as described previously (28).

Demographic and basic clinical data—Differences between cases and controls in the association study were tested for demographic and clinical variables. Tests performed were Chi-square, t-test, or Wilcoxon Rank Sum test, where appropriate. All continuous variables were tested for normality with Shapiro Wilks test prior to comparisons of groups. STATA 10 was used for all tests.

Single-locus case-control association analyses—Hardy-Weinberg Equilibrium (HWE) was tested to detect evidence for genotyping error (29). If both cases and controls deviated from HWE, the inbreeding coefficients, f, were estimated to assess if the direction of deviation was different in cases and controls because such a result may be a sign of single-locus association (30). PLINK software was used for all of the single locus association tests (31). All variants had an HWE *P*- value ≥ 0.001.

Single-locus case-control association analyses correction for multiple testing bias—Chi-square tests were performed at the genotypic and allelic level, using eleven selected variants to determine association with human essential hypertension for 22 total tests. The Bonferroni correction significance level with α=0.05 and 22 tests is 0.002; *P*-values below this level were considered significant after multiple testing correction. The Bonferroni correction assumes that all tests performed are completely independent. In our data, we ran two tests per marker (allelic and genotypic) that were correlated and there was linkage disequilibrium (LD) among the GRK4 markers, thus our tests were not independent. Therefore, this correction and the P-value threshold above are very conservative, but still serve as a guide to significance and P-values below this threshold, and are clearly indicative of significant findings.

Multilocus analyses—Haplotype analyses were performed using UNPHASED for variants in *GRK4* (32). Multilocus analyses were performed using MDR, a nonparametric

method that can identify gene-gene interactions (33). *P*-values presented for these analyses were permutation-based and therefore, were already adjusted for multiple testing.

Pharmacogenetic study

Study participants were randomly selected Japanese patients with newly diagnosed, untreated essential hypertension who were referred to the hospital by office or outpatient-clinic physicians. Blood pressure (16, 26) was verified at least twice before enrollment using Omron HEM-905, Omron HEM-907, or Nippon Colin BP203i and subjects with systolic ≥140 and/or diastolic BPs ≥90 mm Hg (n=883) were included in this retrospective study (Table 1B). The Institutional Review Board at the Fukushima Medical University approved all protocols. Data on physical examination, routine urine and plasma laboratory tests, electrocardiogram, and chest x-ray were collected from medical records. Blood sample for genotyping was drawn after obtaining informed consent from the participant. Variants of *GRK4 (R65L, A142V* and *A486V*) were detected by fluorescent probe melting as described previously (28). Patients were initially treated with candesartan (8 mg/day), losartan (50 mg/day), telmisartan (40 mg/day), or valsartan (80 mg/day) taken orally. Successful BP response was defined as clinic BP of both systolic BP <140 and diastolic BP <90 mm Hg. If the target BP was not reached with the ARB alone, a calcium channel blocker or diuretic was added and patients were classified as non-responders. Patients who did not have a consistent response to two different ARBs were excluded. Medical consultations were scheduled every month, according to the patients' own schedules and requests.

Determination of target BP levels and primary outcome—Success rates of achieving treatment goals were calculated according to JSH2009 (27). Single-locus response to ARB assessed genotypic association with an additive genetic model, using Whole-genome Association Study Pipeline software (34). Logistic regression, adjusted for salt intake, was performed to assess the odds ratio (**OR**) of response for subjects with *GRK4* variants compared to *GRK4* "wild-type". Exclusion criteria for logistic regression analyses included the following: 1) missing genotypes at any GRK4 site, 2) possession of GRK4 genotype category with too few individuals (n < 5), and 3) individuals who belonged to genotyped groups that displayed no variance in responder status. In cases where the GRK4 variant category was uncommon, GRK4 genotypes were pooled, such that individuals possessing one or two copies of a variant allele at the site were analyzed as a single group in the regression. Following the implementation of the aforementioned exclusion criteria, 829 individuals remained for inclusion in logistic regression analyses. Thorough detailing of logistic regression inclusion/exclusion status for all participants is presented in Supplementary Table 2.

In addition, the measured decrease in BP by genotype was calculated and compared by t-tests to the referent genotype (*GRK4* "wild-type"). This latter set of tests enabled the assessment of whether the response to ARB was purely an artifact of the higher pre-treatment blood pressure or a better effect of the drug in individuals with specific genotypes.

Both analyses described above tested the effect of one or two variants at each *GRK4* site and/or at multiple sites simultaneously. All analyses were performed using STATA 11.

Pharmacogenetic studies correction for multiple testing bias—In order to determine the association between genotype at the three *GRK4* variants and response to ARB treatment in patients with newly diagnosed essential hypertension, we tested six genotype models against the referent model using three analytical methods (logistic regression, t-test/Wilcoxon Rank Sum for mean difference in systolic BP, t-test/Wilcoxon Rank Sum for mean difference in diastolic BP) for a total of 18 tests. The Bonferroni corrected significance level with α=0.05 and 18 tests is 0.003; *P*-values below this level were considered significant. However, as stated above this correction is very conservative.

Results

Genetic association studies

We performed a case-control study involving a Japanese population to re-evaluate, in a larger cohort (n=1074; hypertensive =588, normotensive controls=486), the association of 11 single nucleotide polymorphisms (SNPs) or other genetic markers in eight genes with hypertension (Supplementary Table 1) (15). This design was intended to validate the previous studies of *GRK4* and related genes (13–17) performed with a limited number of subjects in an independent, much larger Japanese cohort (16). Ages (range 45 to 83 years) were similar among the groups except for a slightly lower age in the female control group. A family history of hypertension was significantly more frequent in the cases than controls and BMI was significantly higher in cases than in controls (Table 1A). Genotypes were quality-controlled using both percent calls (genotyping efficiency) and tests for Hardy-Weinberg Equilibrium (HWE). Although some variants deviated from HWE in either cases or controls, none deviated in both; noted were below our criteria of P<0.001. Therefore, all variants were included in subsequent analyses (Supplementary Tables 3 and 4).

Variants in *ACE*, *AGT*, *AGTR*, *and CYP11B2* were not associated with hypertension (Supplementary Table 5). $GRK4^{65L}$, $GRK4^{142V}$, *or* $GRK4^{486V}$ was highly associated with hypertension in both allelic and genotypic tests ($P<10^{-7}$ to $<10^{-12}$) (Table 2). *DRD1* also had significant allelic association with hypertension (P=0.018). *PAI-1* and *GNB3*, which have been previously associated with hypertension in some (35), but not our smaller Japanese cohort comparing salt-sensitive and salt-resistant hypertensive subjects (16), were borderline for allelic association (Table 2). Logistic regression analyses for the *GRK4*, *DRD1*, *PAI-I*, and *GNB3* SNPs, adjusted for age, BMI and family history of hypertension, indicated significant independent effects for $GRK4^{65L}$, $GRK4^{142V}$, *and* $GRK4^{486V}$ (10^{-6} < P<0.0006), *PAI-1* and *DRD1* (Supplementary Table 6). The effect sizes of the *GRK4* SNPs were similar to each other; using an additive model with the wild-type homozygote as the referent, odds ratio (OR) ranged from 1.42–2.33 for hypertension.

Haplotype and multilocus analyses

Haplotype and multilocus analyses using multifactorial dimensionality reduction (MDR) also revealed highly significant *GRK4* associations (Table 3 and Supplementary Table 7). In the haplotype analysis with wild-type G-C-C as the referent, the variant T-T-T haplotype (*65L*, *142V* and *486V*) had an OR of 3.47 for hypertension (Supplementary Table 7). In the multilocus analyses, $GRK4^{142V}$, by itself, was predictive of hypertension status 60% of the

time, while the best multilocus model included *GRK4*^{142V} and *GRK4*^{486V} was predictive of the hypertensive phenotype 62% of the time (P<0.001 for these two models) (Table 3), due primarily to an additive effect of the two SNPs (Supplementary Figure 1). *GRK4*^{142V} and *GRK4*^{486V} interacted with *GNB3* and *PAI-1*, although models including these markers did not have as high testing accuracy as *GRK4*^{142V} and *GRK4*^{486V} alone. Prevalence-based association tests (PRAT), using three population prevalence estimates for hypertension (0.15, 0.20, and 0.25), showed significance for *GRK4*^{65L} and *GRK4*^{142V} in both cases and controls, whereas *GRK4*^{486V} was highly significant only in cases (Supplementary Table 8). Therefore, the PRAT results support all three GRK4 SNPs significantly associating with hypertension.

Pharmacogenetics

We compared the response to ARBs in individuals with no *GRK4* variants (n=136) to those with variants at all three SNPs (n=693). Individuals with variants only at *GRK4*^{486V}, one copy (n= 277) or two copies (n=125), were less likely to respond adequately to an ARB (<140/90 mm Hg) compared to those with no variants (ORs 0.32 [0.20, 0.50] 95% CI, $P=1.2\times10^{-06}$; 0.38 [0.22, 0.67] 95% CI, P= 0.001, respectively) (Table 4). Individuals with one or two copies of *GRK4*^{142V} did not differ significantly from non-variant carriers when assessed by achievement of the BP goal (<140/90 mm Hg; P=0.20; Table 4). However, *GRK4*^{142V} carriers had a greater decrease in systolic BP in response to ARBs than non-carriers (19.36 mm Hg vs. 14.53 mm Hg, $P=1.1\times10^{-03}$). This apparent inconsistency was likely due to the fact that *GRK4*^{142V} carriers had higher BPs (157.82± 8.49 standard deviation) before treatment than those without *GRK4* SNPs (154.47± 6.48 standard deviation, Wilcoxon rank-sum test, P=0.0078), thereby requiring a greater decrease in BP to be defined as responders (BP< 140/90). Nonetheless, even with a greater decrease in blood pressure, these individuals were less likely to normalize their systolic BPs. Moreover, individuals with at least one variant at each of the *GRK4* sites (n=191) were significantly less likely to respond to ARBs (OR 0.17 [0.09–0.30]95% CI, $P=2.5\times10^{-09}$) and had smaller decreases in both systolic and diastolic BPs (Table 4), as compared to those with no variant at any site. Although all patients in this study had low PRAs (<1.0 ng/ml/h), those who responded to ARBs had a higher PRA than the non-responders (0.55 vs. 0.27 ng/ml/h; P< 0.0001) (Table 5).

Discussion

The genetic basis of human essential hypertension remains to be determined (5). *GRK4* at 4p16.3 has been linked to hypertension and the *GRK4* SNPs, by themselves, or in combination with *ACE*, *CYP11B2*, or *GNB3* polymorphisms, are associated with essential hypertension in several populations (African, European-American, Australian and European-Caucasian, and Japanese) (13–17). Polymorphisms in *TH*, *ADRB2*, and *GRK4*^{486V} were also independently associated with essential hypertension in a Han Chinese population (17, 36). The discordance between these studies and those reported for another group of European-Americans (37) and European-Caucasians (38) may be related to ethnic background and failure to study all the variants in the *GRK4* coding region that may

associate with hypertension (8, 13–17). In the current study, *GRK4*^{142V}, by itself, or in the presence of *GRK4*^{486V}, *GNB3* and *PAI-1*, was associated with hypertension.

GWAS, which have identified genetic factors believed to influence 2% of the BP variation (5), did not report association between *GRK4* and hypertension. However, the failure to identify *GRK4* in GWAS does not, by itself, eliminate *GRK4* gene variants as causative of hypertension because the GWAS studies often fail to report all truly associating variants if they do not meet arbitrary *P*-value cutoffs (39). The failure of GWAS to identify the association of *GRK4* with hypertension may also be due to a failure to examine gene-gene interaction (epistasis) (40) and absence of *GRK4*^{142V} in almost all the Affymetrix and Illumina chips, except for Illumina Human 1 M beadchip. Moreover, in order to make the data statistically relevant, using pooled data, the authors would need to determine the "lowest common denominator" for coverage and may not have included any of the 3 GRK4 variants.

The importance of epistasis is supported by the current data and reports of the interactions of *GRK4* variants with variants of other genes and hypertension (9, 13–17, 40, 41). In the current study, *GRK4*^{142V}, by itself, or in the presence of *GRK4*^{65L}, *GRK4*^{486V}, *GNB3*, or *PAI-1*, was associated with hypertension. A failure to study gene-gene or gene-environment interaction may also explain the absence of association of *GRK4*^{486V} with hypertension in some European-Caucasians (37, 38), even though association has been found in other cohorts (13–17, 41), it may also explain the failure to find a single gene that is associated with hypertension in some GWAS (5, 35), including a Japanese GWAS (42). The discordance of findings among these populations may indicate the influence of genetic and environmental background (e.g., salt intake) in the phenotypic expression of a quantitative trait such as essential hypertension.

The current treatment of hypertension is empirical and not based on pathogenesis. Although the treatment of hypertension to systolic BP <140 mm Hg and diastolic BP <90 mm Hg is successful in ~50% of patients, two drugs are usually required (3, 43). A pharmacogenetics approach in hypertension may provide more efficient and cost-effective therapy with reduction in morbidity and mortality (4). Hypertensive subjects with apolipoprotein *16730*$^{C>T}$ and *CYP11B* $^{-344T>C}$, but not *AGTR* $^{1166A>C}$ and *ACE*$^{I/D}$ had a significant response to ARBs in some but not all studies (44–47). The activity of GRK4, which can decrease plasma membrane β-adrenergic receptor expression, can be blocked by β-adrenergic receptor antagonists (48); *GRK4*^{142V}, by itself, was associated with a more rapid response to β-adrenergic blockade, while the presence of both *GRK4*^{65L} and *GRK4*^{142} was associated with a decreased response to β-adrenergic blockade in African-American males with early hypertensive nephrosclerosis (49). In a study in two cohorts with essential hypertension without renal disease, as the number of individual *GRK4* SNPs (*65L* and *142V*) increased, BP response to β-adrenergic blockade in a mixed population of black and white individuals decreased; *GRK4*^{486V} was associated with increased adverse cardiovascular risk (50). *GRK4*^{R65} or *GRK4*^{A142} predicted a good BP response to a decrease in salt intake, while *GRK4*^{65L} or *GRK4*^{142V} predicted a limited response to reduced salt intake (51). By contrast, the presence of at least three *GRK4* allele variants (*65L*, *142V*, and *486V*), relative to those with fewer than three was associated with a better response to diuretic therapy (52). The

expression of *hGRK4*^{486V}, but not *hGRK4*^{142V}, in transgenic mice conferred salt sensitivity (53) and predicted a response to diuretics in humans with essential hypertension (54). Thus, the specific *GRK4* gene variant can predict the response to a particular antihypertensive drug. The current study of hypertensive Japanese showed an allelic and genotypic association of *GRK4*^{142V} with enhanced response of BP to ARBs. Although individuals with one or two copies of *GRK4*^{142V} did not show a difference in the achievement of BP goal compared to those with no *GRK4* variants, they had a significantly greater decrease in systolic BP in response to ARBs than non-carriers (19.36 vs. 14.58 mm Hg, $P=1.1\times10^{-03}$). Hypertensive Japanese with *GRK4*^{142V} had low PRA (< 1.0 ng/ml/h) (16). All patients in this study had low PRA but those at the higher end (0.55 vs. 0.27 ng/ml/h) of this low PRA range had a more robust response to ARBs. This may explain why the ratio of plasma aldosterone to plasma renin did not predict the response to diuretics (55).

We conclude that the presence of *GRK4*^{142V} was strongly predisposing to essential hypertension and also predictive of the magnitude of the decrease in BP with ARB monotherapy. No other gene coding region variant has been reported to have this degree of predictive power (44, 56). Moreover, the reports of the association of gene variants with a good response to antihypertensive therapy do not have direct proof; those gene variants have not been shown to cause hypertension in mice.

There are limitations to our study. The pharmacogenetic data need to be replicated in a larger population and in other ethnic groups, taking into account that the allele frequency of *GRK4* SNPs differ among ethnic groups (57). Nonetheless, the association and specificity of the response of *GRK4*^{142V} to ARBs, *GRK4*^{486V} to diuretics (54) and *GRK4*^{65L} and *GRK4*^{142V} poor response to β-adrenergic blockade or low salt diet (49–51) provide strong support for the role of this gene in hypertension treatment variability. The relationship between genotype and response to multiple drug combinations needs to be studied. In conclusion, the current data suggest that the presence of *GRK4*^{142V} could be used to justify the use of ARBs as first line of treatment, instead of a low salt diet (6, 55, 58–60) or diuretic (61).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1A

Demographic description of subjects in the association studies

Categorical Variable	Total	Case[2]	Control[2]	P-value
Sex				
Male	408	233	175	0.22
Age, yr[1]		57.5(9.1)	56.2(8.7)	0.14
SBP[1]		148.7 (7.1)	120.8(8.6)	**<0.001**
DBP[1]		92.5(6.6)	75.7(6.2)	**<0.001**
Female	666	355	311	
Age, yr[1]		57.4(9.3)	54.8(8.4)	**6×10^{-04}**
SBP[1]		148.6(6.9)	122.0(8.4)	**<0.001**
DBP[1]		94.1(3.9)	76.0(5.5)	**1×10^{-04}**
Family History				**<0.001**[3]
−	495	215	280	
+	577	372	205	
Smoking				0.07[3]
No	677	385	292	
Yes	396	203	193	
Body-mass index				**<0.001**[3]
<25	586	221	365	
>25	483	365	118	

[1]Continuous variable is not normally distributed (Shapiro Wilks test *P*-value < 0.05); *P*-values for variables that are not normally distributed were determined with the Mann-Whitney U test

[2]Mean (Standard Deviation) for continuous variables

[3]Chi-square *P*-value is reported

Table 1B

Demographic description of subjects in the angiotensin receptor blocker pharmacogenetic studies

Categorical Variable	Total	Non-responders	Responders	P-value
Male	383	298	85	
Age, yr[1] (Mean (SD)	63.0(10.9)	62.8(11.3)	63.6(9.4)	0.71
SBP before treatment				
Mean (SD)	153.3(6.5)	153.7(6.4)	151.7(6.5	**8.5×10^{-03}**
Median	152	152	152	
SBP after treatment				
Mean (SD)	141.9(9.8)	144.9(8.5)	131.2(5.8)	**<0.001**
Median	143	145	132	
Δ SBP Mean (SD)	11.3(9.7)	8.2(8.1)	21.0(7.6)	**<0.001**
DBP before treatment				
Mean (SD)	86.3(7.6)	88.0(6.6)	80.4(8.1)	**<0.001**
Median	86	90	80	
DBP after treatment				
Mean (SD)	81.2(10.1)	84.6(8.3)	69.5(6.7)	**<0.001**
Median	83	86	70	
Δ DBP Mean (SD)	4.8(8.3)	3.3(7.4)	10.9(8.5)	**<0.001**
Female	498	377	121	
Age, yr[1] (Mean (SD)	64.9(10.9)	64.8(11.15)	65.3(9.9)	0.78
SBP before treatment				
Mean (SD)	152.8(6.1)	153.5(6.1)	150.7(5.5)	**<0.001**
Median	152	152	151	
SBP after treatment				
Mean (SD)	141.5(10.3)	145.3(8.3)	129.6(5.7)	**<0.001**
Median	142	146	130	
Δ SBP Mean (SD)	11.3(9.7)	8.2(8.1)	21.0(7.6)	**<0.001**
DBP before treatment				

Categorical Variable	Total	Non-responders	Responders	P-value
Mean (SD)	86.7(7.5)	88.7(6.4)	80.5(7.4)	**<0.001**
Median	89	90	81	
DBP after treatment				
Mean (SD)	81.2(10.0)	84.7(8.4)	70.5(6.2)	**<0.001**
Median	83	86	70	
Δ DBP Mean (SD)	5.5(6.5)	4.0(5.5)	10.0(7.3)	**<0.001**

[1]Continuous variable is not normally distributed; *P*-values for variables that are not normal were determined with the Wilcoxon Rank Sum test, otherwise Student's t-test *P*-value is reported

Note: Sex information was missing for two study participants.

Table 2

Analyses of single nucleotide polymorphisms and/or markers in selected genes associated with human essential hypertension.

SNP	Genotype	HT	NT	Genotype Frequency Cases	Genotype Frequency Controls	Genotype P-value[1]	Allelic P-value[1]
GRK4 p.65R/L	*T/T*	10	5	0.02	0.01	**8.24×10^{-12}**	**1.34×10^{-11}**
	T/G	153	45	0.26	0.09		
	G/G	424	433	0.72	0.90		
GRK4 p.142A/V	*T/T*	23	12	0.04	0.02	**1.13×10^{-11}**	**4.19×10^{-11}**
	T/C	204	79	0.35	0.16		
	C/C	361	393	0.61	0.81		
GRK4 p.486A/V	*T/T*	157	77	0.27	0.16	**6.65×10^{-07}**	**5.54×10^{-08}**
	T/C	286	227	0.50	0.47		
	C/C	145	181	0.25	0.37		
DRD1 g.-48G>A	*A/A*	6	8	0.01	0.02	**6.0×10^{-02}**	**1.8×10^{-02}**
	A/G	100	107	0.17	0.22		
	G/G	482	368	0.82	0.76		
PA1-1 g.25952766_25952767insG	*4G/4G*	86	84	0.15	0.17	0.28	0.10
	4G/5G	260	223	0.44	0.46		
	5G/5G	241	179	0.41	0.37		
GNB3 p.S275S	*C/C*	140	130	0.24	0.27	0.22	0.07
	C/T	259	221	0.44	0.46		
	T/T	189	133	0.32	0.28		

c.= coding region, g. = gene, p.= protein

HT = hypertensive, NT=normotensive

[1]When cell sizes are less than 5, the Fisher's Exact test was used instead of a Chi-square test

Table 3

Significant association of hypertension with GRK4 with MDR analysis

Model	Training Balance Accuracy	Test Balance Accuracy	CV consistency	Permutation P-value
GRK4 p.142V	0.5994	0.5994	10/10	**<0.001**
GRK4 p.142V, *GRK4* p.486V	0.6197	0.6177	10/10	**<0.001**
GRK4 p.142V, *GRK4* p.486V, *GNB3* p.S275S	0.6302	0.5983	7/10	**<0.001**
GRK4 p.142V, *GRK4* p.486V, *PAI-I* g.25952766_25952767insG, *GNB3* p.S275S	0.6591	0.5942	10/10	**<0.001**

Table 4

Prediction of antihypertensive response to ARBs.

SNP	MODEL (n)[1]	OR	OR *P*-value	Mean Difference in SBP*	Mean Difference SBP *P*- value	Mean Difference in DBP*	Mean Difference DBP *P*- value
No GRK4 variants (n=136)	Referent			14.53		9.96	
R65L	1 or 2 copies of R65L only (n=8)	Not Determined[2]	Not Determined[2]	13.63	**0.81**	4.75	**0.06**
142V	1 or 2 copies of *142V* only (n=68)	0.64 (0.33–1.26)	0.20	19.36	**1.1×10^{-03}**	7.07	**4.7×10^{-03}**
486V	1 copy of *486V* only (n=277)	0.32 (0.20–0.50)	**1.2×10^{-06}**	10.37	**1.0×10^{-04}**	3.82	**<0.0001**
	2 copies of *486V* only (n=125)	0.38 (0.22–0.67)	**1.0×10^{-03}**	13.97	0.65	6.56	**4.2×10^{-03}**
R65L 142V	1 variant at *65L* and 1 variant at *142V* only (n=5)	Not Determined[2]	Not Determined[2]	7.20	**2.5×10^{-02}**	2.80	**0.03**
142V 486V	1 variant at *142V* and 1 variant at *486V* only (n=32)	0.42 (0.16–1.08)	0.07	11.93	0.19	3.66	**<0.0001**
R65L 142V 486V	At least 1 variant at the 3 *GRK4* sites (n=191)	0.17 (0.09–0.30)	**2.5×10^{-09}**	6.02	**<0.0001**	2.90	**<0.0001**

Note: 54 individuals were excluded from logistic regression analysis as described in Supplementary Table 2.

[1] Model used to classify exposure status in logistic regression model containing exposure and responder status.

[2] Logistic regression OR and corresponding *P*-values could not be determined in this category because all GRK4variant carriers were non-responders

* When normally distributed difference in mean SBP (systolic blood pressure) or DBP (diastolic blood pressure) was assessed using Student's t-test; when non-normal Wilcoxon Rank Sum test was used. Appropriate *P*-values are reported.

Table 5

Renin and aldosterone in relation to ARB response

Variable	N	Mean (SD)	Median (Min, Max)	Shapiro Wilks *P*-value	Wilcoxon Rank Sum
Plasma aldosterone concentration					
Responder	118	20.5 (14.3)	14.65(1,67)	**<0.0001**	0.2540
Non-responder	436	17.1 (9.70)	13.0 (2.6,66)	**<0.0001**	
Plasma renin activity					
Responder	118	0.55 (0.75)	0.1 (0.1,3.4)	**<0.0001**	**<0.0001**
Non-responder	436	0.27 (0.45)	0.1 (0.1,2.6)	**<0.0001**	