Single-Nucleotide Polymorphisms for Diagnosis of Salt-Sensitive Hypertension

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Background: Salt-sensitive (SS) hypertension affects >30 million Americans and is often associated with low plasma renin activity. We tested the diagnostic validity of several candidate genes for SS and low-renin hypertension.

Methods: In Japanese patients with newly diagnosed, untreated hypertension (n = 184), we studied polymorphisms in 10 genes, including G protein–coupled receptor kinase type 4 (GRK4), some variations of which are associated with hypertension and impair D1 receptor (D1R)-inhibited renal sodium transport. We used the multifactor dimensionality reduction method to determine the genotype associated with salt sensitivity (≥10% increase in blood pressure with high sodium intake) or low renin. To determine whether the GRK4 genotype is associated with impaired D1R function, we tested the natriuretic effect of docarpamine, a dopamine prodrug, in normotensive individuals with or without GRK4 polymorphisms (n = 18).

Results: A genetic model based on GRK4 R65L, GRK4 A142V, and GRK4 A486V was 94.4% predictive of SS hypertension, whereas the single-locus model with only GRK4 A142V was 78.4% predictive, and a 2-locus model of GRK4 A142V and CYP11B2 C-344T was 77.8% predictive of low-renin hypertension. Sodium excretion was inversely related to the number of GRK4 variants in hypertensive persons, and the natriuretic response to dopaminergic stimulation was impaired in normotensive persons having ≥3 GRK4 gene variants.

Conclusions: GRK4 gene variants are associated with SS and low-renin hypertension. However, the genetic model predicting SS hypertension is different from the model for low renin, suggesting genetic differences in these 2 phenotypes. Like low-renin testing, screening for GRK4 variants may be a useful diagnostic adjunct for detection of SS hypertension.

Hypertension (blood pressure ≥140/90 mmHg) affects more than 65 million adult Americans, and prehypertension (blood pressure between 120/80 mmHg and 139/89 mmHg) affects millions more (1). Salt sensitivity, defined as a 10% increase in mean arterial blood pressure after consumption of a diet high in salt (2–9), affects 58 million Americans without hypertension and can lead to morbidity and mortality rates similar to those of hypertension. New methods for patient identification and risk stratification should be developed for these chronic disease risk factors (10). Low plasma renin activity (PRA)* is used as a diagnostic adjunct for determining salt sensitivity despite the fact that salt sensitivity and low PRA do not correlate well (11, 12). The discovery of single-nucleotide

* Nonstandard abbreviations: PRA, plasma renin activity; SNP, single-nucleotide polymorphism; D1R, D1-dopamine receptor; GRK, G protein–coupled receptor kinase; ADD, α-adducin; GNβ3, G-protein β3 subunit; CYP11B2, aldosterone synthase; ACE, angiotensin-converting enzyme; AGT, angiotensigen; AT1R, angiotensin II type 1 receptor; 11βHSD2, 11β-hydroxysteroid dehydrogenase 2; SS, salt sensitive; PAI-1, plasminogen activator inhibitor-1; IRB, Institutional Review Board; BMI, body mass index; SR, salt resistant; BUN, blood urea nitrogen; and MDR, multifactor dimensionality reduction.
polymorphisms (SNPs) in genes that encode proteins involved in sodium regulatory pathways could lead to new diagnostic tools for salt sensitivity with or without hypertension.

Renal dopamine receptors are responsible for more than 50% of sodium excretion during moderate sodium surfeit (13). Renal paracrine inhibition of sodium transport by dopamine is impaired in genetically hypertensive rats and humans with essential hypertension (13–15) because of decreased dopamine D1 receptor (D1R) function not related to a primary defect of the D1R but rather to its uncoupling from second messengers. This uncoupling is caused by activating variants of the G protein–coupled receptor kinase type 4 (GRK4) (13,16), a gene involved in the desensitization of the D1R (16–19). Three variants of GRK4 (R65L, A142V, and A486V) impair D1R stimulation of renal cAMP production (16). Preventing renal GRK4 expression normalizes D1R function in renal proximal tubule cells from hypertensive persons (16) and ameliorates hypertension in spontaneously hypertensive rats (20). In mice, overexpression of human GRK4 142V but not wild-type GRK4 impairs the natriuretic response to D1R stimulation and produces hypertension (16).

To determine whether certain genotypes are predictive of salt sensitivity and/or low-renin hypertension, we studied the association between polymorphisms in the genes GRK4 (16, 21, 22), D1R (23), α-adducin (ADD) (24–26), G-protein β3 subunit (GNB3) (27), CYP11B2 (aldosterone synthase) (28), angiotensin-converting enzyme (ACE) (26, 29), angiotensinogen (AGT) (21, 25, 30), angiotensin II type 1 receptor (AT1R) (31), and 11β-hydroxysteroid dehydrogenase 2 (11βHSD2) (32), all of which have been reported to be associated with low-renin and/or salt-sensitive (SS) hypertension. Because plasma plasminogen activator inhibitor-1 (PAI-1) concentrations also correlate with low-renin hypertension (33), we also examined variants of this gene in newly diagnosed, untreated hypertensive Japanese patients.

Our analyses included investigation of multilocus genetic models for these 2 phenotypes. As a physiologic correlate to our genetic studies, we measured the renal responses to doxycarpine, a dopamine produg (34), in normotensive Japanese persons with or without GRK4 variants to test the hypothesis that GRK4 variants are related to the impairment of sodium excretion in response to D1R stimulation even in the absence of expression of the hypertensive phenotype.

The selection of cohorts for genetic studies can have a profound effect on study outcomes (35). We therefore selected a cohort of Japanese persons with a relatively homogeneous genetic background (30).

**Patients and Methods**

All protocols were carried out at the Fukushima Medical University with the approval of its Institutional Review Board (IRB).

**Hypertensive Patients**

Study participants were randomly selected Japanese patients with newly diagnosed, untreated essential hypertension who were referred to the hospital by office or outpatient-clinician physicians. We verified blood pressure with a mercury sphygmomanometer at least twice before enrollment. After providing informed consent, patients with systolic blood pressures ≥140 mmHg and/or diastolic blood pressures ≥90 mmHg were admitted to the study unit for physical examination, routine urine and plasma laboratory tests, electrocardiography, and a chest x-ray. Patients with diabetes mellitus, renal dysfunction (serum creatinine >11 mg/L; creatinine clearance <70 mL/min; microalbuminuria >30 mg/g of creatinine; and/or abnormal urinalysis), or secondary hypertension were excluded from the study. A total of 184 patients (104 females, 80 males; mean (SE) age, 54.8 (0.8) years; mean (SE) body mass index (BMI), 23.1 (0.2) kg/m²) were admitted into the study. After the diagnosis of essential hypertension was established, the study patients were admitted to the hospital for 5 weeks. After completion of the study of salt sensitivity and before evaluation of the relationship between genotype and phenotype, each patient was started on antihypertensive medication in accordance with the IRB.

**Salt sensitivity.** We assessed salt sensitivity of blood pressure by determining the blood pressure responses to changes in dietary sodium (36, 37) in 83 study patients who were willing to comply with the dietary protocol to assess salt sensitivity in the hospital. Patients received a normal-sodium diet (153 mmol/day) and 50 mmol potassium/day for 2 weeks, followed by a published dietary protocol (2, 9, 36, 37) consisting of 2 weeks of normal sodium intake followed by a low-sodium diet (51 mmol/day) for 5 days, a high-sodium diet (340 mmol/day) for 5 days, and a normal sodium diet (153 mmol/day) for another 5 days. Adherence to the diet was evident because sodium balance was achieved and sodium intake was matched by sodium excretion (Fig. 1). As expected, during the periods of high salt intake the sodium excretion in the SS patients did not match the sodium intake, but sodium balance was restored when normal sodium intake was re instituted. On the final day of each regimen, we monitored ambulatory blood pressure (Nippon Colin ABPM-630). Patients were considered to be SS if mean arterial pressure increased (≥10%) after the change in sodium intake from low to high; otherwise they were considered to be salt resistant (SR) (2, 9, 11, 37) (see Fig. 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/ vol52/issue3/). Mean arterial pressure was calculated as: diastolic blood pressure + 1/3 (systolic blood pressure – diastolic blood pressure).
Heart rates were not different between SS and SR individuals and were not affected by sodium intake. Therefore, heart rate was not taken into consideration in the calculation of mean arterial pressure.

We measured PRA in 2 groups of patients: 101 patients who remained on a normal sodium diet and 75 of the 83 patients studied for salt sensitivity (43 SR and 32 SS). In both groups, PRA was measured while patients were on a normal sodium diet and 75 of the 83 individuals and were not affected by sodium intake. Therefore, heart rate was not taken into consideration in the calculation of mean arterial pressure.

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NORMOTENSIVE PERSONS
Response to dopaminergic stimulation. We tested the effect of GRK4 variants on the natriuretic effect of docarpamine (34) in normotensive persons homozygous for the wild-type GRK4 polymorphisms [n = 8; mean (SE) age, 38.0 (2.7) years; BMI, 25.0 (1.1) kg/m²] and those heterozygous for each of the GRK4 polymorphisms [n = 10; age, 32 (1.8) years; BMI, 23.9 (0.8) kg/m²]. These persons had serum creatinine and blood urea nitrogen (BUN) concentrations within the reference intervals, as did the hypertensive patients, but had higher BMIs than the hypertensive patients. Each participant rested in the study unit for 30 min and then ingested 500 mL of distilled water, after which blood pressure and heart rate monitoring were begun. Urine was collected each hour. A total of 5 samples were obtained: 1 before and 4 after the oral ingestion of 750 mg of docarpamine.

Fig. 1. Effect of dietary sodium on sodium excretion in SR (○) and SS (■) hypertensive Japanese.

Sodium excretion during an intake of 340 mmol sodium chloride/day in SR patients (○) was significantly different from sodium excretion in SS patients (■) or with normal or low sodium intake in either group (factorial ANOVA, Newman–Keuls test). In SS patients, sodium excretion during the high sodium intake or during the second time on a normal sodium intake was significantly higher than sodium excretion during the initial period of normal sodium intake or during the low sodium intake (repeated measures ANOVA, Newman–Keuls test). Data are the mean (SE; error bars) for each of the

STATISTICAL ANALYSIS
Data are reported as the mean (SE). The effects of different sodium diets in SS and SR patients were evaluated by repeated-measures ANOVA. The linear contrast low/normal/high sodium was tested separately within each group and as a first-order interactive contrast in a two-way (factorial by repeated measures) ANOVA. No adjustments for multiple comparisons were applied to P values in Table 1 (also see Table 1 in the online Data Supplement). In survey tables such as Table 1 and Table 1 in the online Data Supplement, P values are descriptive rather than inferential. Thus, P values should be interpreted in light of the possibility of type 1 errors. Nevertheless, significance with Bonferroni correction for multiple comparisons was also noted (Table 1 and Table 1 in the online Data Supplement). We determined differences between groups by t-test or χ² test. Statistical analyses were performed with SPSS, Release 11.0.1 (SPSS Inc.), or SigmaPlot.

Allele frequency differences between SS and SR individuals and deviations from Hardy–Weinberg equilibrium were determined by TFPGA (http://bioweb.usu.edu/mpmbio/) (41). Associations of multilocus genotypes with salt sensitivity and PRA concentrations were assessed by multifactor dimensionality reduction (MDR). MDR assesses all possible genetic models and provides the best genetic model for differentiating salt sensitivity from salt resistance or low PRA from normal PRA based on cross-validation consistency and prediction error (42, 43). The best genetic model for salt sensitivity was determined by the highest classification success and the lowest prediction error (42, 43). To determine the single best genetic model, we repeated the MDR analysis with all sets of loci with significant findings in the original analyses. Significance of the models was determined by permutation testing. Classic diagnostic measures of sensitivity, specificity, positive predictive value, negative
predictive value, and diagnostic odds ratio were also calculated.

**Results**

Age [SS group, 59.3 (2.1) years; SR group, 57.1 (1.7) years], sex distribution (SS group, 26 females and 22 males; SR group, 21 females and 14 males), height [SS group, 158.4 (1.5) cm; SR group, 160.1 (1.5) cm], BMI [SS group, 22.7 (0.4) kg/m²; SR group, 22.6 (0.4) kg/m²], weight, and blood pressures (normal sodium diet, 153 mmol/day; see Table 1 in the online Data Supplement) did not differ between SS and SR hypertensive individuals. Increasing sodium intake from low to high increased blood pressures between SS and SR hypertensive individuals. Increasing Table 1 in the online Data Supplement) did not differ blood pressures (normal sodium diet, 153 mmol/day; see

Table 1. Genotype and allele frequencies of GRK4 gene variants in Japanese with SR, SS, low-renin, or normal-renin hypertension.

<table>
<thead>
<tr>
<th>GRK4 variant</th>
<th>SR (n = 48)</th>
<th>SS (n = 35)</th>
<th>Normal renin (n = 82)</th>
<th>Low renin (n = 94)</th>
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<tr>
<td>R65</td>
<td>48</td>
<td>7</td>
<td>77</td>
<td>37</td>
</tr>
<tr>
<td>R65L</td>
<td>0</td>
<td>23</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>65L</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>A142</td>
<td>43</td>
<td>7</td>
<td>70</td>
<td>26</td>
</tr>
<tr>
<td>A142V</td>
<td>5</td>
<td>20</td>
<td>10</td>
<td>11</td>
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<tr>
<td>142V</td>
<td>0</td>
<td>8</td>
<td>2</td>
<td>1</td>
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<tr>
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<td>30</td>
<td>0</td>
<td>39</td>
<td>12</td>
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<td>23</td>
<td>29</td>
<td>47</td>
</tr>
<tr>
<td>486V</td>
<td>5</td>
<td>12</td>
<td>14</td>
<td>35</td>
</tr>
</tbody>
</table>

* Allele frequency comparisons are significant for SS vs SR (and low- vs normal-renin) classes at all GRK4 sites in all cases (P < 0.001).

b Allele frequency comparisons are significant for SS vs SR (and low- vs normal-renin) classes at all GRK4 sites in all cases (P < 0.001).

changing sodium chloride intake from 340 to 153 mmol/day decreased mean blood pressure in SS but not in SR patients. Sodium excretion converged in the 2 groups 5 days after the decrease in sodium intake, from 340 to 153 mmol/day, but more sodium (81.9 mmol) was still retained in SS patients than in SR patients.

Urinary potassium excretion and the urinary sodium-to-potassium ratios were similar in both groups and positively correlated with sodium intake (data not shown). When evaluated with all the other variables listed in Table 1 in the online Data Supplement, urinary norepinephrine concentrations in both groups were found to be unaffected by sodium intake. However, when we examined the effect of dietary change on urinary norepinephrine separately from the other variables (Table 1 in the online Data Supplement), we found that urinary norepi-

[Image: Clinical Chemistry 52, No. 3, 2006 355]

**Fig. 2. Effect of dietary sodium on mean arterial blood pressure.**

Changing sodium chloride intake from 50 to 340 mmol/day increased mean blood pressure in SS (□) but not in SR (■) patients. Decreasing sodium chloride intake from 340 to 153 mmol/day decreased mean blood pressure in SS but not in SR patients. Error bars, SE. #, P < 0.05 for SR vs SS; *, P < 0.05 vs others in SS group (repeated-measures ANOVA, Newman–Keuls test).
nephrine decreased with high sodium intake in the SR group but not in the SS group, in agreement with other reports (44–46). In contrast, urinary dopamine increased with sodium intake in both the SR and SS groups (Table 1 in the online Data Supplement), suggesting that decreased renal production of dopamine is not involved in the hypertension of our study participants (13).

The allele and genotype frequencies of ACE, AGT, AT4R, PAI-1, CYP11B2, D1R, GNβ3, and ADD did not differ between SR and SS patients (Table 1 shows variants giving significant differences. The full data are shown in Table 2 in the online Data Supplement). In contrast, in SS patients, variant alleles at all 3 polymorphic GRK4 sites were more common and genotype frequencies differed between groups (P<10⁻⁴ for all comparisons; Table 1; also see Table 2 in the online Data Supplement). Most sites were in Hardy–Weinberg equilibrium except for AT4R genotypes, which had deficiencies of heterozygotes in both SS and SR patients (P<10⁻⁴ and P = 0.0003 for SR and SS patients, respectively), GRK4 A486V in the SS group (P = 0.006), and ACE I/D in the SR group (P = 0.04). The GRK4 A486V deviation is the result of a deficiency in the number of wild-type homozygotes and the ACE I/D deviation is attributable to a deficiency of heterozygotes.

The multisite genetic model that best predicted salt sensitivity included only the GRK4 variants R65L, A142V, and A486V and predicted SS status correctly 94.4% of the time (P < 0.001), based on the MDR analysis. The model using the 3 GRK4 variants for Japanese patients with SS hypertension had a sensitivity of 83% and a specificity of 100% with an infinite positive likelihood ratio, a 17% hypertension had a sensitivity of 83% and a specificity of 94.4% (P < 0.001), based on the MDR analysis. For SS status, the presence of 3 variants with a predictive success of 78.4% (P < 0.001). All variants that show single-site association, except GRK4 A142V, were not found in the best genetic model, indicating that the strongest effects are for this site. As opposed to the SS model, the GRK4 variants do not interact to increase disease predisposition. A 2-locus model that included both GRK4 A142V and CYP11B2 was also highly statistically significant (P < 0.001) with prediction success at 77.8%, indistinguishable from the GRK4 A142V model.

![Fig. 3. Best model for SS hypertension in Japanese patients using 13 polymorphisms from 10 genes.](image)

The best SS model incorporated only the GRK4 variants with a predictive accuracy of 94.4% (P < 0.001), based on the MDR analysis. High-risk genotypes are in darkly shaded cells and the low-risk genotypes in lightly shaded cells. The number of SS individuals is represented within each cell as the left-hand column of the histogram, and the number of SR patients is the right-hand column. The unshaded cells have no SS or SR individuals. Genotypes are most easily read by determining first GRK4 R65L, then GRK4 A142V, and finally GRK4 A486V. For example, the group in the top left-hand cell is low risk for salt sensitivity, with 25 SR patients and no SS patients. The genotype of this cell is RR (GRK4 65L, AA (GRK4 142V), and AA (GRK4 486V). For SS status, the presence of 3 variants is always associated with high risk. However, the accuracy of the risk assignment in cells with small numbers (n = 1) should be kept in mind.

![Fig. 4. Relationship between the increase in mean arterial blood pressure (ΔMAP) and fold increase in sodium excretion (UnNa fold Δ) with the change in salt intake from low to high in hypertensive patients classified according to the number of GRK4 variant alleles.](image)

The increase in mean arterial blood pressure is indicated by the columns, whereas the fold increase in sodium excretion is depicted by the circles and line. In each pair of numbers in parentheses inside the columns, the values given are for SR/SS. *, P < 0.05 vs ≥3 alleles, factorial ANOVA, Newman–Keuls test. Data are the mean (SE; error bars). There are no error bars in instances where the symbol is larger than the SE.
Because salt sensitivity was always found in hypertensive patients with 3 or more GRK4 gene variants (Fig. 4), we studied the effect of docarpamine on electrolyte excretion in normotensive patients without any or with all 3 GRK4 gene variants (Fig. 5; also see Table 3 in the online Data Supplement). One oral dose of docarpamine (750 mg), which minimally affected blood pressure, induced natriuresis, kaliuresis, and calciuresis in patients without GRK4 gene variants but not in those with all 3 GRK4 gene variants (Fig. 5; also see Table 3 in the online Data Supplement).

**Discussion**

We report several novel findings. First, 3 of the 6 GRK4 variants (R65L, A142V, and A486V, but not the variants V247I, A253T, and G562D) were more frequent in SS than in SR hypertensive Japanese patients and predicted salt sensitivity 94.4% of the time. Second, there were no differences in the allele frequencies of other genes previously thought to be important in the etiology of salt sensitivity. Third, hypertensive Japanese patients with 3 or more GRK4 gene variant alleles at any of the 3 sites (R65L, A142V, and A486V) were always sensitive to salt, although some patients with <3 GRK4 variants were also salt sensitive. Fourth, the ability to excrete a salt load was inversely related to the number of GRK4 variant alleles (R65L, A142V, and A486V), and the natriuretic effect of a dopaminergic drug was abrogated when 3 GRK4 gene variants were expressed, even in normotensive Japanese persons. Fifth, the same 3 GRK4 gene variants were individually associated with low-renin hypertension, but in addition low- and normal-renin participants showed differences in ADD allele and genotype frequencies. Sixth, the underlying genetic models for salt sensitivity and low-renin hypertension were different, indicating that they have different etiologies and are not clinically equivalent among Japanese. These findings support the notion that even in the event of a single gene being critical in the development of a phenotype, multiple polymorphic sites should be considered in detection of at-risk persons.

Patients with essential hypertension and low PRA are presumed to have SS hypertension. Our SS hypertensive patients had low PRA and aldosterone concentrations, similar to those described for SS hypertensive patients in other populations (25). This profile in SS patients is in keeping with decreased D1R-mediated inhibition of renal sodium transport and sodium and fluid retention in SS hypertension (13, 15). However, our findings and those of others indicate that low specificity precludes the use of PRA concentrations as an effective way to distinguish SS from SR individuals (2, 11, 12).

The usefulness of blood pressure sensitivity to salt intake as an intermediate phenotype has been reported to be reduced by the variability in blood pressure response to increased sodium intake (47), but GRK4 variants were not investigated in that study. In our study, GRK4 variants were not only more frequent in SS than in SR hypertensive Japanese patients, but homozygous gene variants for GRK4 R65L and A142V were seen only in SS hypertensive Japanese patients. Among our hypertensive patients, only SS hypertensive patients had at least 3 GRK4 variant alleles (65L, 142V, and 486V).

Three GRK4 gene variants (R65L, A142V, and A486V; allele and genotype frequencies), but not the other 3 GRK4 gene variants (V247I, A253T, and G562D), were associated with low-renin hypertension in our hypertensive Japanese patients. ADD G460W was also associated with low-renin essential hypertension, in agreement with other reports (24–26, 48, 49). However, the single best genetic model for low-renin hypertension was GRK4 A142V, with 78.4% prediction accuracy in hypertensive Japanese, a result that differed from that for salt sensitivity. A 2-locus model including both GRK4 A142V and CYP11B2 was also highly statistically significant ($P < 0.001$) with a prediction success of 77.8%, which was indistinguishable from the GRK4 A142V model. These analyses support the conclusion that salt sensitivity and low-renin hypertension are not of identical in genetic makeup or physiologic origins.

Gene–gene interactions are important in the development of hypertension (50). We have reported that in Ghanaian hypertensive patients, GRK4 variants (termed FF) were in nonrandom association with the variants of ACE, AGT, and AT, R in hypertensive but not in normotensive individuals and that ACE and GRK4 2-locus genotypes were significantly predictive of essential hypertension (not characterized for salt sensitivity or PRA status) (22, 51, 52). SS hypertensive Italians also have increased frequency of GRK4 A486V compared with nor-

![Urine Periods](image)

Fig. 5. Effect of oral docarpamine on ratio of urinary sodium to urine creatinine (UNa/Ucr) in normotensive participants without any GRK4 variants (C) or with 3 GRK4 variants (●). Docarpamine was administered at the end of 0 (baseline). Each period represents a 1-h urine collection. #, $P < 0.05$ vs other groups, with or without GRK4 variants (factorial or repeated measures ANOVA, Newman–Keuls test); *, $P < 0.05$ vs participants with 3 GRK4 variants (F-test). Data are the mean (SE; error bars). There are no error bars in instances where the symbol is larger than the SE.
motensive Italians (21), and the haplotype combination of GRK4 R65, 142V, and 486V was associated with essential hypertension in another group of Caucasians (53). The latter study also demonstrated a gene dosing effect (i.e., a positive correlation between the number of GRK4 alleles and blood pressure) in hypertensive patients, similar to our findings. Mice overexpressing human GRK4 Y486V on a C57BL/6 background developed hypertension only after sodium chloride intake was increased from 0.4% to 0.8% (54). In contrast, GRK4 Y142V transgenic mice on a C57BL/6 background were hypertensive regardless of salt intake (16, 55). Thus, different variants in the same gene can cause SS or salt-independent hypertension, depending on the gene variant, and may be influenced by the genetic background.

We found that a few of the studied genes were out of Hardy–Weinberg equilibrium, such as AT1R genotypes, which had deficiencies of homozygotes in both SS and SR patients, GRK4 A486V, which had deficiencies in the number of homozygotes in the SS group, and ACE, which had deficiencies in the number of heterozygotes in the SR group. Deviations from Hardy–Weinberg equilibrium may be caused by several factors, including systematic genotyping error, random chance, and true biological causes. These are not easy to differentiate, but if genotyping errors are systematic, deviations should appear in both cases and controls. Because this was not the case for all deviations, excepting AT1R, we draw no strong conclusions regarding the causation of deviations from Hardy–Weinberg equilibrium among the other variants. Because AT1R did not appear to associate significantly with disease in any analysis, this does not pose a problem in our final interpretations. That GRK4 A486V in the SS group is out of Hardy–Weinberg equilibrium may be of interest because this is one of the variants that strongly associates with the SS phenotype. The deficiency in the number of wild-type GRK4 A486 homozygotes is what would be expected if variation at this site is functional. In addition, ACE I/D, although not associated in the present study with the phenotype, has been previously reported to be associated with essential hypertension in an epistatic model with GRK4 (51). Our current findings may reflect this previously described interaction. Additional testing for Hardy–Weinberg equilibrium in the samples for low-renin hypertension reinforces the conclusion that deviations from Hardy–Weinberg equilibrium are not attributable to genotyping error, because the deviating markers are different from those for SS and SR patients. This difference is unexpected if the deviations were attributable to experimental error. The positive association between certain variants (GRK4 and ADD) for at least one of the phenotypes of interest (salt sensitivity or low renin) indicates that our sample size had enough power to detect certain genes associated with SS and low-renin hypertension.

In summary, the genotype and allele frequencies of variants of GRK4 (R65L, A142V, and A486V) are higher in SS than in SR patients, and variations at all 3 sites predict salt sensitivity in hypertensive Japanese. We did not detect any differences in allele frequencies of variants of ACE, AGT, AT1R, PAH, CYP11B2, D1R, GNB3, and ADD genes between SS and SR patients. In contrast, low-renin hypertension was associated not only with GRK4 variants but also with ADD variants, and the genetic model for low-renin hypertension is different and does not include all of the sites detected in single-site association analyses. Therefore, salt sensitivity and low-renin hypertension do not have the same underlying genetic architecture. Moreover, the presence of 3 GRK4 gene variants can impair renal dopamine-induced natriuresis, even in the absence of hypertension. Determining the SNP genotype for selected loci might be considered a model molecular diagnostic screening test for salt sensitivity and hypertension.
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