G Protein–Coupled Receptor Kinase 4
Role in Blood Pressure Regulation

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Nearly 30% of middle-aged Americans have hypertension, but the prevalence is higher in non-Hispanic blacks and individuals >60 years of age (65%). There is a direct and quantitative relationship between higher blood pressure values and mortality. Although 30% to 50% is thought to be heritable, the genetic cause(s) of essential hypertension has been difficult to identify. More than 1 gene is undoubtedly involved, because Mendelian dominant and recessive traits are not readily discernible in hypertensive subjects, except in those with monogenic forms of hypertension. Moreover, in any hypertensive individual, risk-predisposing genes are engaged in a complex network of gene-gene and gene-environment interactions.

The kidney plays a major role in the long-term regulation of blood pressure, and abnormal sodium chloride metabolism is frequently encountered in hypertension. Therefore, many studies have focused on the abnormal renal handling of sodium chloride in the pathogenesis of essential hypertension. Approximately 50% of subjects with essential hypertension are sodium chloride sensitive. Indeed, humans with salt-sensitive hypertension have increased sodium transport in the renal proximal tubule and medullary thick ascending limb, although distal tubular mechanisms may also be involved. The sodium retention in hypertension is because of enhanced sodium transport, per se, and/or a failure to respond appropriately to signals that decrease sodium transport. Sodium transport is regulated by natriuretic and antinatriuretic hormones and humoral agents, such as dopamine and angiotensin, which exert their effects via G protein–coupled receptors (GPCRs). Activation of certain postjunctional dopamine receptor subtypes (D1R, D3R, D4R, and D5R) and the angiotensin type 2 receptor inhibit, whereas activation of the postjunctional D1R and angiotensin type 1 receptor (AT1R) increase sodium transport.

GPCR Kinase Family
The GPCR kinases (GRKs) are a 7-member family of serine/threonine protein kinases characterized by their ability to phosphorylate or modify and desensitize agonist-occupied cell surface membrane GPCRs. GRKs 1 and 7 belong to the rhodopsin family; GRKs 2 and 3 belong to the β-adrenergic receptor kinase family, and GRKs 4, 5, and 6 belong to the GRK4 family (Table 1). GRKs 1 and 7 are expressed exclusively in rods and cones, respectively, in the retina. GRKs 2, 3, 5, and 6 are ubiquitously expressed, whereas GRK4 expression is limited (see below). Overexpression of GRK2, GRK3, and especially GRK5 in human embryonic kidney cells desensitizes the D1R. GRK activity and GRK2 expression are increased in lymphocytes of patients with essential hypertension and spontaneously hypertensive rats (SHRs). Overexpression of GRK2 in vascular smooth muscles in mice produces hypertension and impairs the vasodilatory action of β-adrenergic receptors. The vasoconstrictor response to angiotensin II is also impaired in these mice, which is at odds with the increased reactivity and sensitivity to angiotensin II in essential hypertension. Interestingly, GRK2 activates the epithelial sodium channel by phosphorylating the C terminus of its β subunit, making it insensitive to the ubiquitin protein ligases Nedd4 and Nedd2. GRK5 overexpression in vascular smooth muscle cells in mice also increases blood pressure. The hypertension in male GRK5 transgenic mice is, in part, because of decreased β-adrenergic receptor activity, whereas the high blood pressure in female mice is because of increased activity of AT1Rs. Dopamine D1R hypersensitivity occurs with disruption of the GRK6 gene in mice. Therefore, abnormalities of GRK6 gene can lead to D1R supersensitivity, which can result in a decrease in norepinephrine release, leading to decreased blood pressure or, alternatively, causing an increase in D1R-mediated sodium transport in renal proximal tubules, leading to an increased blood pressure, instead. Disruption of the D1R gene in mice, however, consistently leads to an increase in blood pressure, indicating a predominance of nonrenal mechanisms in the D1R-mediated regulation of blood pressure. GRK6 does not regulate the renal D1R, although it regulates intestinal D1Rs. The ubiquitous
expression of GRKs 2, 3, 5, and 6 contrasts with the centrality of the kidney in the pathogenesis of rodent and human essential hypertension. It is also not clear whether the increases in vascular GRK activity and GRK2 and GRK5 expression follow or precede the hypertensive process in the SHR. It also remains to be determined whether gene variants of GRK2 and GRK5 are associated with human essential hypertension, although in a limited study we found no difference in the sequence of the coding region of GRK2 between hypertensive and normotensive human subjects.

GRK4 Localization and Isoforms
GRK4, as with the other members of the GRK4 family, is predominantly localized at the plasma membrane, as a result of palmitoylation of its C-terminal cysteine residues. GRK4 has a relatively low sequence homology across species, suggesting that it is subject to lower evolutionary pressure for sequence conservation and has evolved more rapidly than the other members of its subfamily and the β-adrenergic receptor kinase subfamily. It is interesting that GRK4 also differs from the other GRKs in tissue distribution. As noted previously GRKs 2, 3, 5, and 6 are ubiquitously expressed, whereas GRK4 is abundantly expressed in the testes and human myometrium and expressed at much lower levels in other tissues, including the brain and the kidney.

GRK4 Splice Variants
GRK4 is the only GRK in which 4 splice variants (GRK4α, β, γ, and δ) have been identified in humans; the full-length isoform, is most homologous with the other GRKs. GRK4β lacks exon 2, resulting in a 32-residue deletion that encompasses the phosphatidylinositol bisphosphate binding domain near the N terminus. GRK4γ lacks exon 15, resulting in a 46-residue deletion near the C terminus, and GRK4δ, the shortest variant, lacks both exons 2 and 15. These human GRK4 splice variants are not found in other mammalian species. No alternative splicing has been reported in the mouse GRK4. Rat GRK4 undergoes alternative splicing, but in exons 6, 7, and 14, resulting in 5 Grk4 splice variants: Grk4A, B, C, D, and E. Rat Grk4A has 76%
identity with GRK4α, the longest of the human GRK4 splice variants. Rat Grk4B is characterized by a 31-amino acid deletion at the 5' end of the coding region, corresponding with exon 6 in the human GRK4α gene. Grk4C is characterized by a 157-amino acid deletion at the 5' end of the coding region, corresponding with exons 6 and 7 in the human GRK4α gene. Grk4C lacks the catalytic domain. Grk4D lacks the 138-bp sequence of the 3' end-coding region, corresponding with exon 14 of the human GRK4α gene. Grk4A mRNA is abundant in spermatogenic cells and renal outer medulla. Grk4B mRNA is poorly expressed in testis and most rat tissues and is expressed mainly in the outer and inner medulla of the kidney. Grk4C is also strongly expressed in the outer medulla. A fifth Grk4 isoform, Grk4E, which lacks exons 6 and 14, may be the functional isoform expressed in rat kidney cortex. The differential tissue distribution of Grk4A, Grk4B, and Grk4E suggests that individual GRK4 splice variants may serve distinct physiological functions.

**Physiological Role of GRK4**

Dopamine, produced by renal proximal tubules from circulating l-3,4-dihydroxyphenylalanine or filtered by the renal glomerulus, is responsible for >50% of sodium excretion during moderate sodium surfeit. Independent of innervation, renal proximal tubules synthesize dopamine that is not metabolized to norepinephrine.23 This is an important distinction between dopamine produced by neural tissue where dopamine is converted to norepinephrine and dopamine produced by nonneural tissue; otherwise an increase in dopamine production would also result in an increase in norepinephrine production, resulting in an increased ion transport because of the stimulation of α-adrenergic receptors. Such an action would then negate the decrease in ion transport caused by the stimulation of dopamine receptors.

Dopamine receptor function in renal proximal tubules is dysregulated in genetic hypertension. The importance of renal proximal tubule dopamine production in the pathogenesis of hypertension has recently been affirmed; selective disruption in the renal proximal tubule of aromatic amino acid decarboxylase, the enzyme that converts l-3,4-dihydroxyphenylalanine to dopamine, produces salt-sensitive hypertension in mice.23 Although renal dopamine production is not always decreased in genetic hypertension,24 the dopaminergic paracrine regulation of renal tubular sodium handling is consistently deficient in human essential hypertension and rodent models of genetic hypertension.2,7 The renal dopaminergic defect in hypertension has been attributed to a diminished D1-like receptor inhibition of sodium and chloride transport: NHE3, (SLC9A3) and Cl/HCO3- (SLC26A6) at the luminal membrane and the electronegative Na/HCO3- cotransporter, NBCe1A (SLC4A4), at the basolateral membrane of the renal proximal tubule and Na+-K+ ATPase at the basolateral membrane of the renal proximal tubule2 and thick ascending limb of Henle. The impairment of D1-like receptor stimulation of cAMP production in genetic hypertension is not caused by abnormalities in the primary structure of the D1-like receptors (D1R and D2R), adenylyl cyclases, and protein kinases A and C.2,7,24,25 The Gαs and adenylyl cyclase enzyme are not inactive, per se, because the ability of the parathyroid hormone, guanosine-5’-O-thiotriphosphatase (GTPγS), or forskolin to stimulate adenylyl cyclase is not impaired in renal proximal tubules of spontaneously hypertensive rats (SHRs)26 or in renal proximal tubule cells from hypertensive patients with impaired D1R function.29 Renal proximal tubule NHE3, Cl/HCO3- exchanger, NaHCO3 exchanger, and Na+-K+ ATPase activities can be directly inhibited in SHRs, indicating that there is no primary defect in these ion transporters or Na+-K+ ATPase, per se.23 Indeed, the natriuretic effect of parathyroid hormone is not impaired in humans with essential hypertension in or in SHRs.27 The phosphaturic response to parathyroid hormone may not also be impaired in SHRs.28

Regardless of the extent of renal D1-like receptor protein expression, the D1-like receptor is always uncoupled from its Gαq or Gβγ subunit. GRK4 activity is increased in kidneys of humans with essential hypertension, with increased renal cortical membrane Grk4 expression (unpublished data), has increased renal cortical membrane Grk4 expression and serine-phosphorylated D1R relative to Wistar-Kyoto rats. Selective renal cortical inhibition of Grk4 expression decreases D1R phosphorylation, decreases sodium excretion, and attenuates the increase in arterial blood pressure with age in SHRs, effects that are not observed in Wistar-Kyoto rats. These studies provide direct evidence of a
The crucial role of increased renal GRK4 expression in the D1R-mediated regulation of sodium excretion and blood pressure in the SHR.21

Increased activity of GRK4 because of constitutively active GRK4 variants causes the decrease in D1R function in renal proximal tubule cells from hypertensive humans. In these cells, knockdown of GRK4 gene expression decreases D1R phosphorylation and ameliorates the ability to produce cAMP in response to agonist stimulation,19 but the GRK4 splice variant regulating the D1R has not been determined. All of the GRK4 splice variants are expressed in human renal proximal tubule cells, GRK4γ gene variants (R65L, A142V, and A486V), heterologously expressed in Chinese hamster ovary cells, increase the serine phosphorylation and impair the function of D1Rs. In contrast, the constitutive phosphorylation of D1R in HEK293 cells is mediated exclusively by the GRK4α; the β, γ, and δ variants are ineffective. The cause of the differences in GRK4 splice variant effect on D1R between HEK293 and Chinese hamster ovary cells is not clear. It is possible that the lack of effect of human GRK4γ in HEK293 cells is because of differences in the cellular environment, indicating cell-specific function. Whether overexpression of human GRK4α gene variants in mice also impairs D1R function and increases blood pressure remains to be determined. We do know that human GRK4γ gene variants are important in the regulation of D1Rs in vivo. Mice overexpressing the human GRK4γ wild-type gene are normotensive and salt resistant19 depending on the genetic background,36 whereas mice overexpressing the human GRK4γ 142V are hypertensive even on a normal NaCl intake.19,37 In contrast, human GRK4γ 486V transgenic mice, depending on the genetic background, become hypertensive only after an increase in sodium intake.36 The increase in blood pressure in human GRK4γ 142V transgenic mice is not related to chromosomal integration, copy number, or renal human GRK4 mRNA level.37 Thus, it is the presence of the heterologously expressed human GRK4γ variant rather than the amount of human GRK4γ that impairs D1R function and produces hypertension in transgenic mice and probably in human essential hypertension.

The D1R also plays a role in sodium balance by inhibition of Na+-K+-ATPase activity in jejunal cells;38 dopamine fails to inhibit Na+-K+-ATPase activity in jejunal epithelial cells from SHR,38 whereas GRK6, rather than GRK4, regulates D1R function,39 which is different from that observed in the kidney.16,21

**GRK4 Polymorphisms and Hypertension**

The frequency of GRK4 polymorphisms (R65L, A142V, A486V, V247I, A253T, and G562D) varies according to ethnicity. There is limited haplotype diversity, with differing haplotype frequencies among the ethnic groups.40 GRK4 65L and 142V are more frequent among Ghanaians and blacks than the other ethnic groups studied (Chinese, Hispanic, Japanese, and whites),40 whereas GRK4 486V is more frequent among Chinese and Japanese subjects.40,41 (Table 2).

There is increasing evidence that GRK4 plays an important role in hypertension, especially in salt-sensitive hypertension. The GRK4 locus (4p16.3) is linked to hypertension.2 In Ghanaians, multilocus genotype combinations of angiotensin-
The 3 major substitutions in GRK4 (R65L, A142V, and A486V) have now been examined in 3 other, independent studies for essential hypertension. In 1 study, GRK4 486V is associated with hypertension (and systolic blood pressure elevation) in Australian white subjects. In another study, GRK4 486V is associated with hypertension in Italian subjects, reminiscent of the phenotype of the human GRK4 486V transgenic mice.

In a study of northern Han Chinese subjects, the GRK4 65L, 142V, and A486 haplotypes are associated with a 6-fold higher risk of both systolic and diastolic hypertension, regardless of salt-sensitivity status. The 3 major substitutions in GRK4 (R65L, A142V, and A486V) have now been examined in 3 other, independent studies for essential hypertension. In 1 study, GRK4 486V is associated with hypertension (and systolic blood pressure elevation) in Australian white subjects. In another study, GRK4 486V is associated with hypertension in Italian subjects, reminiscent of the phenotype of the human GRK4 486V transgenic mice. In a study of northern Han Chinese subjects, the GRK4 65L, 142V, and A486 haplotypes are associated with a 6-fold higher risk of systolic and diastolic hypertension (Table 2). In another report of the same Han Chinese data from the same group, the A486V polymorphism is also shown to be associated with hypertension, but the allele that increased the risk is 486V. To estimate the effects of the R65L, A142V, and A486V variants on hypertension, we performed a meta-analysis, using the data culled from the studies described above, except for the results for A486V in the Han Chinese subjects, because the data from the 2 articles are identical but the conclusions are opposite, preventing us from resolving the direction of association (Figure 3). The meta-analysis clearly shows a significant association of 486V with hypertension with an odds ratio of 1.5 (95% CI: 1.2 to 1.9). Neither of the other meta-analyses for the other variants indicates a significant association, but it should be noted that even our own previous results with a Ghanaian population suggest that single locus effects may not be detectable in such a complex phenotype. Therefore, it is important to perform a multilocus analysis to test for gene-gene interactions in hypertension.

The ability to excrete a sodium load in Japanese subjects with essential hypertension is inversely related to the number of GRK4 single nucleotide polymorphisms in the 2 alleles of the GRK4 gene. However, the presence of 3 GRK4 variants impairs the natriuretic effect of a dopaminergic drug, even in normotensive subjects. Thus, salt sensitivity may be imparted by GRK4 gene variants. Consistent with this model, in a study comparing genetic predisposition of salt-sensitive to salt-resistant hypertension in a Japanese cohort, each of the 3 variants has a significant association with salt sensitivity, and using all 3 together predicts salt sensitivity correctly in 94% of our samples. In the same Japanese study, the single best genetic model for low-renin hypertension includes only GRK4 A142V, by itself, or GRK4 A142V and CYP11B2, with an estimated predictive accuracy of 78%. These results show that the underlying genetic models of salt-sensitive,

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Cases n/N</th>
<th>Controls n/N</th>
<th>OR (random) 95% CI</th>
<th>Weight %</th>
<th>OR (random) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bengra et al</td>
<td>61/120</td>
<td>44/120</td>
<td>21.93</td>
<td>1.79 [1.07, 2.99]</td>
<td></td>
</tr>
<tr>
<td>Speirs et al</td>
<td>144/290</td>
<td>204/496</td>
<td>68.93</td>
<td>1.41 [1.05, 1.89]</td>
<td></td>
</tr>
<tr>
<td>Williams et al</td>
<td>29/248</td>
<td>9/102</td>
<td>9.44</td>
<td>1.37 [0.62, 3.00]</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>658</td>
<td>718</td>
<td>100.00</td>
<td>1.48 [1.16, 1.89]</td>
<td></td>
</tr>
<tr>
<td>Total events: 234 (Cases), 257 (Controls)</td>
<td></td>
<td></td>
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<tr>
<td>Test for heterogeneity: $\chi^2 = 0.65$, df = 2 ($P = .702$), $I^2 = 0%$</td>
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<tr>
<td>Test for overall effect: $Z = 3.19$ ($P = 0.001$)</td>
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Favors Controls Favors Cases

Figure 3. Meta-analyses of 3 GRK4 polymorphisms. A random-effect model used for meta-analysis (RevMan 4.2 software, http://www.cc-ims.net/RevMan) allows testing of the distribution of effects across different studies and provides wider confidence intervals when inconclusive data are present. Heterogeneity was tested using $\chi^2$ analysis, with $P < 0.1$ considered as significant. We also report the $I^2$ value, which estimates the proportion of total variation that is because of heterogeneity. An $I^2$ value >50% was considered significant heterogeneity. Meta-analyses for R65L and A142V indicate that these variants, by themselves, are not associated with essential hypertension (not shown); meta-analysis for A486V indicates that 486V associates with essential hypertension.
Table 3. GRK4 Isoform-Specific Regulation of GPCRs

<table>
<thead>
<tr>
<th>GRK4 Variant</th>
<th>GPCRs Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRK4α</td>
<td>Desensitization of metabotropic glutamate receptor 1, G protein-coupled calcium-sensing receptor, GABAA receptor, FSH receptor, rhodopsin, luteinizing hormone/chorionic gonadotropin receptor, wild-type and mutant (Y326A) β2 adrenergic receptor, and D2R (in NEK-293 cells)</td>
</tr>
<tr>
<td>GRK4β</td>
<td>Desensitization of the luteinizing hormone/chorionic gonadotropin receptor and maybe the V2 vasopressin receptor</td>
</tr>
<tr>
<td>GRK4γ</td>
<td>Desensitization of D2R and D2R(β3)</td>
</tr>
<tr>
<td></td>
<td>Sensitizes the AT1R(β2)</td>
</tr>
<tr>
<td>GRK4δ</td>
<td>Desensitization of the m2 muscarinic receptor (in the presence of GRK5 and GRK6)</td>
</tr>
</tbody>
</table>

low-renin, and possibly other subclasses of hypertension are different (Table 2). Zhu et al. determined the association of GRK4 variants (65L, 142V, and 486V) with blood pressure in black and white American twin subjects. Single-locus analyses revealed a significant interaction and a dose effect between 65L and age for systolic blood pressure. Individuals who are homozygous for the 65L-142V-A486 haplotype have a 1.05-mm Hg steeper increase in systolic blood pressure per year of increase in age compared with those homozygous for the most common R65-A142-A486 haplotype. In black adolescents with GRK4 65L, mental stress causes an increase in blood pressure and a decrease in sodium excretion. One study, however, did not find an association of GRK4 486V with the phenotype is unrelated to an intrinsic renal defect. J Clin Invest. 1992;89:1453–1459.


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Hypertension. 2008;51:1449-1455; originally published online March 17, 2008;
doi: 10.1161/HYPERTENSIONAHA.107.096487

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