HK-2 Human Renal Proximal Tubule Cells as a Model for G Protein–Coupled Receptor Kinase Type 4–Mediated Dopamine 1 Receptor Uncoupling

John J. Gildea, Ishan Shah, Ryan Weiss, Nicholas D. Casscells, Helen E. McGrath, Jin Zhang, John E. Jones, Robin A. Felder

Abstract—HK-2 human renal proximal tubule cells (RPTC) are commonly used in the in vitro study of “normal” RPTCs. We discovered recently that HK-2 cells are uncoupled from dopamine 1 receptor (D1R) adenylyl cyclase (AC) stimulation. We hypothesized that G protein–coupled receptor kinase type 4 (GRK4) single nucleotide polymorphisms may be responsible for the D1R/AC uncoupling in HK-2. This hypothesis was tested by genotyping GRK4 single nucleotide polymorphisms, measuring D1-like receptor agonist (fenoldopam)-stimulated cAMP accumulation, quantifying D1R inhibition of sodium transport, and testing the ability of GRK4 small interfering RNA to reverse the D1R/AC uncoupling. We compared HK-2 with 2 normally coupled human RPTC cell lines and 2 uncoupled RPTC cell lines. The HK-2 cell line was found to have 4 of 6 potential GRK4 single nucleotide polymorphisms known to un couple the D1R from AC (namely, R65L, A142V, and A486V). AC response to fenoldopam stimulation was increased in the 2 normally coupled human RPTC cell lines (FEN: 2.02±0.05-fold and 2.33±0.19-fold over control; P<0.001; n=4) but not in the 2 uncoupled or HK-2 cell lines. GRK4 small interfering RNA rescued the fenoldopam-mediated AC stimulation in the uncoupled cells, including HK-2. The expected fenoldopam-mediated inhibition of sodium hydrogen exchanger type 3 was absent in HK-2 (n=6) and uncoupled RPTC cell lines (n=6) but was observed in the 2 normally coupled human RPTC cell lines (~25.41±4.7% and ~27.36±2.70%; P<0.001; n=6), which express wild-type GRK4. Despite the fact that HK-2 cells retain many functional characteristics of RPTCs, they are not normal from the perspective of dopaminergic function. (Hypertension. 2010;56:505-511.)

Key Words: HK-2 ▪ renal proximal tubule ▪ dopamine receptors ▪ GRK4 ▪ NHE3 ▪ FRET ▪ NaKATPase

HK-2 Human Renal Proximal Tubule Cell (RPTC) has been the subject of research interest since it was first isolated and cultured in 1984,1 with >170 publications using this cell line listed in PubMed. The relevance of using RPTCs of human origin to study human physiology and pathophysiology over using those derived from nonhuman sources, such as the MDCK (dog), LLC-PK (pig), LLC-RK1 (rabbit), and OK (opossum), cannot be overemphasized. Initially, primary RPTC lines were used to study renal cellular physiology because they retained many of their in vivo characteristics in vitro.2 However, primary cell lines cannot be sustained in long-term culture, which makes interassay comparisons difficult.1,3 Therefore, transformed cell lines were generated using immortalizing virus (eg, SV40, human papilloma virus), which extended their growth potential beyond the 8- to 15-passage limit of primary cells.4,5 Transformed RPTCs contain many of the functional and morphological characteristics of RPTCs in primary culture.5,6 There are instances where the HK-2 do not always accurately represent normal human RPTC physiology. For example, when cultured in a 3D matrix, HK-2 form aggregates or cysts similar to primary cultures of RPTCs from autosomal dominant polycystic kidney disease as compared with primary cultures of RPTCs from normal kidney, which form tubular structures.7

While using HK-2 cells as normal controls for human primary RPTCs, we discovered recently that HK-2 cells are uncoupled from dopaminergic stimulation of adenylyl cyclase (AC). Because we have reported previously that the uncoupling of the dopamine 1 receptor (D1R) from AC is because of variants of single nucleotide polymorphisms (SNPs) of G protein–coupled receptor kinase type 4 (GRK4), we hypothesized that the uncoupling of D1R to AC in HK-2 cells may also be caused by GRK4 SNPs.8 This hypothesis was tested in HK-2 cells by genotyping GRK4 SNPs, measuring D1-like receptor agonist (fenoldopam [FEN])-stimulated AC, quantifying D1R inhibition of sodium transport, and testing the ability of GRK4 small interfering RNA (siRNA) to rescue the D1R/AC coupling. We compared the HK-2 RPTCs...
with human RPTCs that are normally coupled (nRPTC) or uncoupled (uRPTC) from AC to determine whether HK-2 is suitable for the study of normal dopaminergic activity and cellular function.

### Materials and Methods

Please see http://hyper.ahajournals.org for Expanded Methods in the online Data Supplement.

#### Cell Lines

Using previously published methods, we cultured HK-2 cells (ATCC CRL-2190) and compared them with 4 immortalized human RPTC lines routinely used in our laboratory (2 normally D,R,A,C-coupled nRPTC lines i14 and i16 and 2 uncoupled uRPTC lines i2 and i25). Each cell line was obtained from the ATCC and compared with our stock cells originally obtained from the ATCC several years ago.

### Immunofluorescent Staining of HK-2 and Human RPTC for Proximal Tubule Characteristics

Markers of proximal tubules used were as follows: Lotus tetragonolobus agglutinin, γ-glutamyl transpeptidase, megalin, aminopeptidase A, aminopeptidase N (CD13), and villin. Levels of genes expressed in the proximal tubule, as well as other segments, include Na+/H+ exchanger (NHE) 3 (proximal tubule and thick ascending limb), caveolin 1 (proximal tubule, distal tubule, glomerulus, and cortical collecting duct). Markers of cells from other nephron segments that served as negative controls were Tamm-Horsfall protein and sodium chloride cotransporter. Controls for nonspecific secondary antibody binding were performed for each cell type. See the online Data Supplement and Table S1 for details about the antibodies and staining.

### Determination of cAMP Accumulation

cAMP was measured both by a commercial ELISA (Cayman Chemical) and an intracellular real-time kinetic fluorescence resonance energy transfer (FRET) cAMP sensor (ICU3), as described previously. Agonists used were FEN (1 μmol/L, D1-like receptor agonist) and isoproterenol (1 μmol/L, β-adrenergic receptor agonist) to compare alternative AC G protein-coupled receptor pathways.

### Rescue of cAMP Coupling After Transfection With GRK4 siRNA

GRK4 (100 nmol/L) siRNA (target sequence: 5′ AATACAAA-GAGAAAGTCAA 3′) was transfected for 24 hours into cells by electroporation along with the ICU3 cAMP biosensor plasmid. Details of transfection have been published previously.

### Immunofluorescent Staining for GRK4 After Transfection With GRK4 siRNA

The cell lines were stained for GRK4 after being transfected with GRK4 siRNA or SCR control as stated above (without the ICU3 biosensor). Immunofluorescent staining was performed as described above, using the Santa Cruz GRK4 antibody (SC-13079) at a 1:100 dilution. Identical exposures were taken at ×100 magnification and quantitated using Slidebook v.4.2 software.

### NHE3-Mediated Sodium Accumulation Assay

NHE3 activity as a function of sodium accumulation was measured with modifications of our previous method. Details of our current method are in the online Data Supplement. Briefly, cells were loaded with sodium ion indicator, sodium benzozenan sulfonate (SBFI), and treated for 30 minutes with combinations of the following drugs: ouabain (OUB; 100 μmol/L, NaKATPase inhibitor), FEN (1 μmol/L, D1-like receptor agonist), LE300 (10 μmol/L, D1-like receptor antagonist), EIPA (10 μmol/L, NHE inhibitor), S3226 (10 μmol/L, NHE3 inhibitor),22,23 and cariporide (HOE-642, 10 μmol/L, NHE1 inhibitor). The specificity of the assay was verified by the positive inhibition seen with S3226 (NHE3 selective) and negative inhibition seen with cariporide (NHE1 selective). Time-lapse ratiometric images were acquired every 3 minutes. Each intracellular sodium measurement (in millimoles per liter) is derived from the emission at 510 nm when excited at 340 or 380 nm. Internal calibration of sodium concentration was performed according to the manufacturer’s instructions in Slidebook version 4.2, using calibration buffers described previously.

### NaKATPase-Mediated Sodium Efflux Assay

The detailed method used to measure the rate of sodium efflux was preformed simultaneously on the 5 cell lines as published previously. In brief, cells were cultured in glass bottom collagen-coated Matrical plates and were serum starved overnight before loading with a sodium ion indicator, SBFI (5 μmol/L, Molecular Probes). After a 30-minute recovery, cells were washed and incubated in potassium-free HEPES medium (20 mmol/L of HEPES [pH 7.4], 130 mmol/L of NaCl, 1 mmol/L of CaCl2, and 1 mmol/L of MgCl2) to raise the internal sodium concentration. VEH, FEN, or OUB was added, then directly before imaging, EIPA (10 μmol/L of final concentration, a selective inhibitor of the NHE3) and KCl (2.7 mmol/L, final concentration) were added to all of the wells as 10X stock. Changes in intracellular sodium concentration were measured by live multiwell ratiometric fluorescence imaging of SBFI and internally calibrated using the ratio imaging module of Slidebook version 4.2.

### Statistical Analysis

The data are expressed as mean±SE. Comparisons within and among groups were made by repeated-measures or factorial ANOVA, respectively, followed by Holm-Sidak or Duncan test. The r test was used for 2-group comparisons. P values of <0.05 were considered significant.

### Results

Our RPTC lines, as well as the HK-2 line, were characterized for their renal proximal tubule origin using a series of selective stains for proximal tubular proteins and carbohydrates (Figure S1). All of the primary and immortalized cell lines showed positive staining for the proximal tubule markers and were definitively negative for the markers for other nephron sites.

The HK-2 cells had virtually nonmeasurable expression of a proximal tubule-specific marker, aminopeptidase N, a

### Table. Sequencing of Cell Lines for GRK4 SNPs

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>R65L (Exon 3)</th>
<th>A142 (Exon 5)</th>
<th>A486 (Exon 1-4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HK-2</td>
<td>HETERO</td>
<td>HOMO</td>
<td>HETERO</td>
</tr>
<tr>
<td>i2 (uRPTC)</td>
<td>HOMO</td>
<td>HOMO</td>
<td>WILD</td>
</tr>
<tr>
<td>i25 (uRPTC)</td>
<td>HETERO</td>
<td>HETERO</td>
<td>HOMO</td>
</tr>
<tr>
<td>i14 (nRPTC)</td>
<td>WILD</td>
<td>WILD</td>
<td>WILD</td>
</tr>
<tr>
<td>i16 (nRPTC)</td>
<td>WILD</td>
<td>WILD</td>
<td>WILD</td>
</tr>
</tbody>
</table>

HOMO indicates homozygous variant; HETERO, heterozygous variant; WILD, wild-type genotype.
markedly reduced expression of another proximal tubule-specific marker, lotus tetragonolobus agglutinin,26 and a slightly reduced expression of another proximal tubule-specific marker, aminopeptidase A,27 when compared with our cell lines i2, i25, i14, and i16 (Figure S1, top).

To determine whether the D1Rs were normally coupled with AC in HK-2 cells, we used 2 methods for measuring intracellular cAMP.10 An established ELISA method to measure cAMP extracted from the cell cytoplasm (Figure 1) was compared with the intracytoplasmic FRET-based sensor (Figure 2). We compared HK-2 with 2 nRPTC and 2 uRPTC lines, in their response to the dopamine D1-like receptor agonist FEN (1 μmol/L, 30 minutes) or DMSO vehicle (VEH) control. Figure 1 shows that there was no response to FEN stimulation in HK-2, which is similar to that seen in the uncoupled uRPTC lines (i2 and i25). However, there was a >2-fold increase in cAMP accumulation (ELISA) after FEN stimulation in nRPTCs (i14 and i16; 2.02±0.05 and 2.33±0.19-fold, respectively; P<0.001; n=4 versus VEH). This effect was blocked by the D1-like receptor antagonist LE300 (10 μmol/L), indicating that the stimulatory effect was via D1-like receptors. LE300 had no effect when added alone. The HK-2 cells showed a similar lack of AC stimulation by FEN when measured using a cAMP FRET biosensor, ICUE3. D1-like stimulation with FEN (1 μmol/L, 30 minutes) caused a significant rise in intracellular cAMP levels in nRPTCs (i14 and i16) but not in HK-2 cells or uRPTCs (i2 and i25; Figure 2).

Figure 1. Comparison of FEN-stimulated coupling efficiency to AC in HK-2 and immortalized human cell lines, uRPTC (i2 and i25) and nRPTC (i14 and i16). Innate cAMP accumulation was compared in HK-2, i2, i25, i14, and i16. Innate cAMP accumulation was similar in all of the cell lines (VEH-treated cells). The D1-like receptor agonist FEN (1 μmol/L, 30 minutes) stimulated cAMP accumulation in the coupled cell lines i14 and i16 (*P<0.001 vs others; n=6 per group) but not in the uncoupled HK-2, i2, or i25 cells. The D1-like receptor antagonist LE300 (10 μmol/L) had no effect alone but reversed the FEN stimulation observed in i14 and i16.

Figure 2. Failure of FEN-stimulated cAMP accumulation in HK-2 cells can be reversed by the silencing of GRK4. Intracellular cAMP accumulation was measured in real time using a cAMP FRET biosensor, ICUE3. Similar to cAMP accumulation demonstrated in Figure 1, FEN (1 μmol/L, 30 minutes) increased cAMP in i14 and i16 (*P<0.001 vs VEH; n=6 per group) but not in HK-2, i2, or i25. SCR RNA control had no effect on the FEN-stimulated cAMP accumulation in the coupled cell lines i14 and i16. GRK4 siRNA rescued FEN-stimulated cAMP accumulation in HK-2, i2, and i25 cell lines, although not to control levels (#P<0.05 vs FEN±SCR; n=4 per group).
This lack of response to FEN by the uncoupled cells was not because of an intrinsic defect in their G protein coupling, as is shown in Figure S2. The β-adrenergic receptor agonist isoproterenol (1 μmol/L, 30 minutes) significantly increased cAMP accumulation in each of the cell lines (*P<0.001 versus VEH; n=10). In addition, the response of the uRPTCs i2 and i25 was significantly higher than that seen in their normally coupled counterparts, i14 and i16, or the HK-2 (**P<0.01; n=10 versus the other 3 cell lines; P<0.001 from VEH).

We then investigated the role of GRK4 SNPs in the uncoupling of AC in the HK-2, i2, and i25 cell lines by treating the cells with either SCR or siRNA to GRK4 (Figure 3) in addition to FEN. siRNA to GRK4 rescued the uncoupling of AC in the HK-2, i2, and i25 cell lines (P<0.05; n=4 versus FEN+SCR), suggesting that GRK4 SNPs mediated the uncoupling of the D1-like receptor to AC. siRNA slightly decreased the FEN stimulation of AC in nRPTC lines i14 and i16, which is in agreement with our reports that wild-type GRK4 is needed for normal D1R function.28-29

Figure 3 demonstrates that basal levels of GRK4 were similar among the 5 cell lines. GRK4 expression was quantified, and equivalent immunoreactive staining was observed in all of the cell lines. Figure 3 also demonstrates the efficacy of the GRK4 siRNA used in Figure 2. The cell lines showed a 63% to 83% reduction in GRK4 expression level after they were transfected with 100 nmol/L of GRK4 siRNA for 24 hours, compared with the SCR-transfected cells. Equivalent expression levels of GRK4 were seen between the SCR control cells and mock-transfected cells without siRNA (data not shown).

Because a normal action of dopamine is to inhibit sodium transport via NHE3,30 we investigated whether D1R/AC uncoupling decreased intracellular sodium accumulation in these cells. Figure 4 shows the effect of OUB (100 μmol/L) in increasing intracellular sodium concentration by preventing sodium efflux via inhibition of NaKATPase activity. This effect was reduced by FEN (10 μmol/L) in nRPTC lines i14 and i16 (P<0.05 versus OUB+VEH; n=6). Additional controls for the sodium influx assay are provided in Figure S4. This figure depicts the change in intracellular sodium over the 30-minute time interval tested, in response to various pharmacological agents by themselves or in combination with OUB. FEN (1 μmol/L), cariporide (HOE; 10 μmol/L), and LE300 (10 μmol/L) by themselves had no effect on sodium influx over VEH alone. OUB (100 μmol/L) increased intracellular sodium, whereas the NHE3 inhibitors EIPA (10 μmol/L) and S2336 (10 μmol/L) by themselves decreased intracellular sodium concentrations relative to VEH. The combination of OUB+FEN inhibited sodium influx by 32.00±0.48% (P<0.05; n=6) compared with OUB alone. This effect of FEN was reversed by LE300 (OUB+FEN+LE300), confirming the specificity of the FEN effect via D1-like receptors. The addition of either NHE3 inhibitor EIPA or S3226 almost completely blocked the OUB-mediated increase in intracellular sodium concentration. The addition of the NHE1 inhibitor cariporide (HOE) did not reduce the OUB response, indicating that the increase in intracellular sodium concentration with OUB is not through NHE1 but rather through NHE3.

Because dopaminergic stimulation of RPTCs also causes an inhibition of NaKATPase activity,31 we compared these 5 cell lines in our NaKATPase-dependent sodium efflux assay.

**Figure 3.** GRK4 immunofluorescence in HK-2, uRPTC i2 and i25, and nRPTC i14 and i16 cells that were transfected with GRK4 siRNA or the SCR. Cells were transfected with 100 nmol/L of GRK4 siRNA or SCR and 24 hours later were stained for GRK4. GRK4 siRNA significantly knocked down GRK4 expression in each of the cell lines (P<0.001; n=6). Representative images of GRK4 expression with SCR or GRK4 siRNAs are shown above each bar in the graph.
FEN inhibited sodium efflux to a greater extent in normally coupled RPTCs compared with the uncoupled cell lines (Figure 5). The 2 AC-coupled cell lines i14 and i16 had a large decrease in sodium efflux on agonist stimulation (**P<0.01 versus VEH, ANOVA, Holm-Sidak test; n=6 per group), but this was not seen in the 2 uncoupled RPTCs (i2 and i25). HK-2 cells displayed an FEN-mediated reduction in sodium efflux, although it was significantly less than that seen in i14 and i16 (*P<0.05 versus VEH, i14 and i16, ANOVA, Holm-Sidak test; n=6 per group).

The 3 GRK4 SNPs R65L, A142V, and A486V are associated with human essential hypertension or salt-sensitive hypertension,8,14,32–35 and cause the uncoupling of the D1R to AC. Table shows that the HK-2 cell line is homozygous variant at exon 5 and heterozygous variant at exons 3 and 14, as compared with our control cell lines i14 and i16, which are wild type at all 3 of the GRK4 exons and have normal AC coupling.

**Discussion**

The HK-2 cell line has been relied on as a surrogate for “normal” renal proximal tubular physiology for over 2 decades. HK-2 cells have been reported to express parathyroid hormone receptor, which participates in the proliferative response after energy depletion,36 but other cellular parameters do not always show concordance with primary cultures of RPTCs (eg, low basal angiotensinogen, basal nuclear factor-κB, and signal transducer and activator of transcription 3 activities and total protein expression).37 In the current study, we found that HK-2 cells had virtually nonmeasurable expression of aminopeptidase N25 and a reduced expression of the proximal tubule-specific markers lotus tetragonolobus agglutinin27 and aminopeptidase A25 when compared with our human RPTC lines i2, i25, i14, and i16. The differential expression of cell surface markers led us to examine dopamine-stimulated AC activity and sodium transport in HK-2 cells.

In the current studies, we demonstrate the novel finding that the D1-like stimulation in the HK-2 is uncoupled from D1-like stimulation of AC as well as D1-like stimulation of sodium influx (NHE3 activity) and displayed a blunted D1-like stimulated reduction in sodium efflux (NaKATPase activity). The fact that β-adrenergic receptor stimulation of

**Figure 4.** FEN reduces intracellular sodium accumulation in i14 and i16 but not in HK-2, i2, or i25 cell lines. Intracellular sodium concentration was determined using the sodium sensitive dye SBFI. The OUB (100 μmol/L plus VEH)-increased intracellular sodium, by inhibition of sodium efflux via NaKATPase, was linear over a 30-minute time interval. OUB+FEN (1 μmol/L) reduced Na⁺ accumulation (**P<0.05; n=6) in only the i14 (□) and i16 (×)-coupled cell lines.

**Figure 5.** FEN-mediated reduction in NaKATPase-dependent sodium efflux is greater in nRPTCs than in uRPTCs. Cells labeled with the sodium-sensitive dye SBFI were sodium loaded by incubating the cells in potassium-free medium, and ratiometric fluorescence images were collected simultaneously on an automated confocal fluorescence microscope. The two AC normally coupled cell lines i14 and i16 had a large decrease in sodium influx after agonist stimulation (**P<0.01 vs VEH, ANOVA, Holm-Sidak test; n=6 per group), but the 2 uncoupled RPTCs (i2 and i25) had no decrease. HK-2 cells displayed a small but significant FEN-mediated reduction in sodium efflux, although it was less than i14 and i16 (*P<0.05 vs VEH, i14, and i16, ANOVA, Holm-Sidak test; n=6 per group).
AC remains intact suggests that the uncoupling phenomenon is unique to the D₃-like receptors. Similar to our previous findings, the siRNA silencing of GRK4 (which normally prevents the phosphorylation and inhibition of the D₃-like receptors) restores D₃-like coupling to AC in the uncoupled cells.

Zhang et al. demonstrated normal coupling between D₁R-like stimulation with FEN and inhibition of NaKATPase activity in HK-2 cells. Their results suggest the presence of functional D₁-like receptors in HK-2 cells, yet in our study HK-2 cells show a reduced inhibition of NaKATPase activity compared with normally coupled RPTC cells. Because GRK4 may not regulate D₃R, the other D₁-like receptor, it is possible that the cell culture conditions of Zhang et al. increased the expression of the D₃R, overcoming the impaired D₃R function, thus allowing the FEN to work through the D₃R. PMA (phorbol myristate acetate), a known stimulator of the protein kinase C pathway, has been shown to inhibit NaKATPase independent of cAMP. PTH has also been shown to inhibit NaKATPase independent of cAMP. However, the role of the D₃R in the FEN-mediated inhibition of NHE3 or NaKATPase in HK-2 cells remains to be further evaluated. One other possibility for our results differing from those of Zhang et al. is that they may be using an entirely different subclone of HK-2 cells.

The present findings demonstrate that GRK4 may be a potential therapeutic target and that uncoupled cells may serve as a tool for screening for compounds with therapeutic potential. Moreover, there are significant cost and efficiency benefits to using cells expressing the intracellular cAMP sensor ICUE3 for high-throughput screens. The D₁R expressed in the HK-2 cell was uncoupled from AC similar to our 2 D₁R uncoupled cell models. The presence of gene variants at 3 exons in GRK4 in the HK-2 cell line is in keeping with the known effects of GRK4 variants on uncoupling of the D₁R to AC. We have demonstrated previously that variants R65L and A142V independently reduce the coupling of the D₁R to AC by ~50%, whereas A486V is more potent at reducing D₁R/AC coupling. When the expression of GRK4 was decreased with antisense oligonucleotides, normal coupling was restored between the D₁R and AC. The fact that the uncoupling could also be reversed by the addition of siRNA to GRK4 in HK-2 cells suggests that both the HK-2 and our uncoupled cell lines possess similar mechanisms responsible for the uncoupling phenomenon.

We have reported that GRK4 gene variants are associated with an increased incidence of hypertension in several populations. These reports have been corroborated by others, in hypertensive Han Chinese and a white Australian cohort but not in an European cohort. However, in the same European population, polymorphisms in the GRK4 promoter were found to affect transcriptional activity.

The possibility that increased GRK4 expression may also be a cause of hypertension is supported by in vivo studies in rats: chronic renal silencing of GRK4 in spontaneously hypertensive rats (that overexpress GRK4 but do not have an altered GRK4 genotype) resulted in an ~30% reduction in blood pressure. Thus, there is a need for human cell lines with various GRK4 polymorphisms to test the impact of these gene variants on RPTC function. Future studies will focus on the association between GRK4 genotype and the hypertension/salt-sensitivity phenotype.

**Perspectives**

Renal cell culture models must be used with caution because they may contain genes that are not associated with normal cell function. Moreover, the disease phenotypes that are encoded in the genome and possibly expressed are usually not available for the various renal cell lines that are available to study. In these studies, we demonstrated that the widely used HK-2 cell line carries homozygous SNPs at 1 GRK4 allele and heterozygous SNPs at 2 other alleles that are associated with either hypertension and/or salt sensitivity. The presence of these variants was associated with uncoupling of the D₁R from AC, similar to that found in 2 human RPTC lines carrying >3 GRK4 variants. Two control cell lines that do not carry the GRK4 variants had normal D₁R/AC coupling. These results suggest that the HK-2 cell line may be a good model for cellular physiology associated with GRK4 variants only when used in conjunction with additional cell models.

**Sources of Funding**

This work was supported by National Institutes of Health grants HL074940, HL23081, DK39308, HL68886, and HL092196.

**Disclosures**

R.A.F. and Pedro A. Jose were awarded a US Patent (No. 6 660 474) on “GRK variants in essential hypertension,” which has been assigned to Hypogen, Inc.

**References**

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Hypertension. 2010;56:505-511; originally published online July 26, 2010;
doi: 10.1161/HYPERTENSIONAHA.110.152256

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