G Protein–Coupled Receptor Kinase 2 Expression and Activity Are Associated With Blood Pressure in Black Americans

Heather I. Cohn, Yihuan Xi, Stephanie Pesant, David M. Harris, Terry Hyslop, Bonita Falkner, Andrea D. Eckhart

Abstract—Hypertension occurs with higher prevalence and morbidity in black Americans compared with other groups. Alterations in the signal transduction pathways of 7-transmembrane spanning receptors are found in hypertensive patients. G protein–coupled receptor kinases (GRKs) play an important role in regulating this receptor signaling. The 2 most abundantly expressed GRKs in the cardiovascular system are GRK2 and GRK5, and each has unique substrates. Understanding changes in expression may give us insight into activated receptors in the pathophysiological progression of hypertension. In heart failure and white hypertensives, increased GRK2 expression arises because of neurohormonal stimulation of particular receptors. GRK2 subsequently desensitizes specific receptors, including β-adrenergic receptors. In blood pressure control, β-adrenergic receptor desensitization could lead to increased blood pressure. GRK2 and GRK5 mRNA were evaluated in lymphocytes of black Americans via quantitative real-time PCR. GRK2 mRNA expression directly correlated with systolic blood pressure and norepinephrine levels. GRK2 was elevated >30% among those with systolic blood pressure ≥130 mm Hg. No significant correlation between GRK5 mRNA expression and blood pressure or catecholamines was observed. Diabetic status, age, sex, and body mass index were also compared with GRK2 expression using univariate and multivariate analyses. GRK2 protein expression was elevated 2-fold in subjects with higher blood pressure, and GRK activity was increased >40%. Our data suggest that GRK2, but not GRK5, is correlated with increasing blood pressure in black Americans. Understanding the receptors stimulated by increased neurohormonal activation may give insight into the pathophysiology of hypertension in this at-risk population. (Hypertension. 2009;54: 71-76.)

Key Words: hypertension ■ β-adrenergic receptors ■ lymphocytes ■ β-adrenergic receptor kinase ■ G protein–coupled receptors ■ catecholamines

Nearly 45% of black Americans have high blood pressure (BP). The prevalence of hypertension and the severity of cardiovascular morbidity are greater for blacks than other racial groups, but the reasons for these differences are not well understood. The influence of the sympathetic nervous system on BP is mediated predominantly through catecholamines (epinephrine and norepinephrine) binding to adrenergic receptors. β-Adrenergic receptors (βARs) are critical for regulating peripheral resistance, and disruption in their signaling can lead to an attenuation of vasodilation and subsequent BP elevation. Although the pathophysiology of high BP is undeniably complex, increased vascular resistance, βAR derangement, and increased plasma norepinephrine are likely involved. In addition, norepinephrine levels increase early in the progression of hypertension and are inversely correlated with lymphocyte βAR density, suggesting that understanding the impact of neurohormonal activation is important to understanding the development and progression of hypertension.

Constant stimulation of βARs by catecholamines in heart failure and hypertension leads to selective βAR downregulation and an overall attenuation of βAR-mediated adenylyl cyclase activity. G protein–coupled receptor kinases (GRKs) are important serine/threonine kinase regulators of agonist-occupied 7 transmembrane-spanning receptors (7TMRs), including βARs. GRK2 and GRK5 are the most abundant GRKs in the cardiovascular system. In hypertensive rat models, an increase in lymphocyte GRK2 expression was reflected by a parallel increase in vascular smooth muscle (VSM) GRK2 expression. In humans, it was found that lymphocyte GRK2 expression directly correlated with GRK2 in biopsies from the right atria of the same patient. It has been suggested that human lymphocyte GRK2 expression and activity are increased, at least in a small cohort of young...
white subjects with borderline hypertension.\textsuperscript{18} Experimentally, we have shown in transgenic mouse models that increasing the expression of GRK2 in VSM was sufficient to increase BP.\textsuperscript{19} Our data suggested that attenuation in vasodilation, mediated by \textbeta ARs, is an important contributor to the high BP in these mice.\textsuperscript{19}

Alterations in adrenergic nervous system activity appear to play a role in development and maintenance of high BP,\textsuperscript{20} and racial variations in the interplay of adrenergic activity and BP have been described.\textsuperscript{21,22} The role of \textbeta ARs and their desensitization is not well delineated in humans, especially in blacks. The purpose of this study was to quantify lymphocyte GRK2 activity in a human sample of black Americans and to determine whether there is an association of GRK2 levels with plasma catecholamines and BP. An increase in GRK2 levels may indicate increased neurohormonal activity and give insight into the pathogenesis of high BP.

Methods

Study Sample

Data were obtained from predominantly black participants who were enrolled in an ongoing cohort study on biomarkers of hypertension and cardiovascular injury. BP measurements were obtained according to well-standardized procedures\textsuperscript{23} and fully described in the online supplementary Methods (please see http://hyper.ahajournals.org). On the basis of the average systolic and diastolic BPs, participants were stratified as having normal BP (average BP: \textless 85 mm Hg). The Adult Treatment Panel III criteria for BP control were designated as having high BP regardless of the diabetic status, and hypertensive treatment (Table). Only systolic BP correlated significantly with GRK2 mRNA levels as a continuous and categorical variable. The most parsimonious multivariable models of GRK2 and GRK5 were used to better understand their clinical implications in relation to high BP, diabetes mellitus, obesity, and sex. Analyses were completed via the appropriate contrasts from the mixed-effects linear regression model, allowing for variance differences among the groups. Contrasts were computed and tested using the appropriate estimates of effects and SEs as computed from the variance-covariance matrix of parameter estimates. When distributions were not appropriately symmetrical, even with transformation, then nonparametric regression methods were completed using, eg, median regression. We then completed bivariate and multivariate analyses of all of the parameters. Data are expressed as mean±SEM. Data were analyzed using 1-way ANOVA, 2-way ANOVA, or unpaired 2-tailed Student\textquoteright s t test, as indicated.

Results

Study Sample Population

The study sample included 133 subjects, ages 18 to 67 years (Tables S2 and S3). In this cohort, systolic BP ranged from 90 to 188 mm Hg. Using a cut point of 130 mm Hg as a threshold (systolic BP: \textgeq 130 mm Hg), 71% of the sample was below this threshold, and 29% of the sample had a systolic BP \textgeq 130 mm Hg. The distribution of men (47.5%) and women (52.5%) was equivalent; 132 subjects were black.

GRK2 mRNA and Systolic BP

Robustness of the quantitative real-time PCR analysis was verified (Figure S1). Lymphocytes from the first 99 samples were used to determine GRK2 and GRK5 mRNA expressions (Table S2). Figure 1 provides data on the relationship of mRNA expression with systolic BP. There is a statistically significant correlation of GRK2 mRNA expression with systolic BP (Figure 1A), and GRK2 mRNA expression was significantly higher in the group of subjects with a systolic BP \textgeq 130 mm Hg as compared with \textlt 130 mm Hg (Figure 1B). The increase in GRK2 mRNA expression was even more profound in men with systolic BP \textgeq 130 mm Hg (Figure 1C). There was no significant correlation between GRK5 mRNA expression and systolic BP level for the total sample (Figure 1D) or when the data were analyzed for men and women separately (data not shown).

Univariate analysis was performed for GRK2 and GRK5 mRNA levels with age, sex, systolic BP, body mass index, diabetic status, and hypertensive treatment (Table). Only systolic BP correlated significantly with GRK2 mRNA levels
There was no correlation between GRK5 mRNA and systolic BP in univariate analysis. Multivariate analysis was performed with GRK2 mRNA as the dependent variable with systolic BP, sex, body mass index, diabetic status, and antihypertensive treatment status as independent variables (Table). In this model, systolic BP is the major determinant of GRK2 mRNA expression, with some small additional contribution of sex and body mass index.

We also considered GRK2 mRNA expression as compared with diastolic BP using 80 mm Hg to group the population, because this is considered the cutoff value for normal diastolic BP. GRK2 mRNA was increased if diastolic BP was ≥80 mm Hg (P=0.0082; Figure S2).

**GRK2 Protein and Systolic BP**

In the next 10 subjects who were recruited, protein expression was determined by immunoblot analysis. There was a significant correlation of GRK2 protein expression with systolic BP, and GRK2 protein was higher in those with systolic BP ≥130 mm Hg compared with those with systolic BP <130 mm Hg (Figure 2).

**GRK Activity and Systolic BP**

GRK activity in the human lymphocyte extracts was measured using a rhodopsin phosphorylation assay in the next 24 subjects. All of the GRKs are able to phosphorylate rhodopsin; however, GRK2 and GRK5 are the most abundant GRKs in the lymphocyte (data not shown). Rhodopsin phosphorylation assay determines total GRK activity in a sample, and, therefore, we refer to this as “GRK activity.” Autoradiography of a 7-point standard curve produced with increasing doses of purified GRK2 and rhodopsin substrate in the presence of radiolabeled ATP demonstrates assay sensitivity (Figure 3). Verification of assay robustness is shown in

### Table. Univariate and Multivariate Analyses of GRK2 mRNA Levels (Quantitative Real-Time Reverse-Transcription PCR)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age, ≥35 vs &lt;35 y</td>
<td>1.073</td>
<td>0.82 to 1.41</td>
</tr>
<tr>
<td>Sex, female vs male</td>
<td>0.858</td>
<td>0.70 to 1.05</td>
</tr>
<tr>
<td>Systolic BP, ≥130 vs &lt;130 mm Hg</td>
<td>1.352</td>
<td>1.10 to 1.66</td>
</tr>
<tr>
<td>BMI, ≥25 vs &lt;25</td>
<td>0.827</td>
<td>0.64 to 0.94</td>
</tr>
<tr>
<td>Diabetic status, yes vs no</td>
<td>1.242</td>
<td>0.979 to 1.600</td>
</tr>
<tr>
<td>On antihypertensive mediation, yes vs no</td>
<td>1.123</td>
<td>0.77 to 1.64</td>
</tr>
</tbody>
</table>

Statistical analyses of association between studied variables and the presence of elevated GRK2 levels. Multivariate analyses are adjusted for systolic BP. BMI indicates body mass index.
Figure S3. GRK activity was increased in subjects with systolic BP ≥130 mm Hg compared with those with systolic BP <130 mm Hg (Figure 3). We did not detect GRK5 mRNA changes and, therefore, predict that the activity changes that we observed are because of GRK2 activity attributed to increased mRNA (Figure 1) and protein expression (Figure 2), although this remains to be fully determined.

Plasma Norepinephrine Levels Directly Correlate With Systolic BP
In our sample of black adults, there was a small but statistically significant correlation of plasma norepinephrine level with systolic BP (Figure 4A). No relationship of plasma epinephrine level with BP was detected (Figure 4B). A direct correlation was detected for plasma norepinephrine levels with GRK2 mRNA (Figure 4C).

Discussion
In the current study, we found that GRK2 but not GRK5 mRNA, protein, and activity directly correlated with systolic BP and plasma norepinephrine levels in black adults, a population at higher risk for hypertension and cardiovascular complications. An increase in 7TMR signaling leads to an increase in GRKs. Sympathetic nerves of the heart, kidneys, and skeletal muscle vasculature in hypertensive patients are activated, and sympathetic overactivity in the renal sympathetic outflow is a prominent pathophysiological feature in
hypertensive patients.27–29 Greater sympathetic nervous system activation in black American adults has been reported,29 and our data confirm an increase in plasma norepinephrine levels with increased systolic BP. An increase in sympathetic nervous system activity results in an increase in βAR signaling and subsequent GRK-mediated βAR desensitization. In our black subjects, we found that, as BP and norepinephrine levels increased, there was a corresponding increase in lymphocyte GRK2 but not GRK5 expression and activity. The increased norepinephrine and GRK2 that we observed may contribute to a decrease in βAR signaling. Hypertensive black Americans also tend to be at higher risk for increased circulating plasma endothelin levels,30 and endothelin receptors are preferentially desensitized by GRK2.31 Hypertension has been associated with increased plasma levels of other ligands, including the components of the renin-angiotensin system,32 although hypertension in black Americans tends to be low renin in nature.30 Like endothelin receptors, angiotensin II type 1 receptors are regulated by GRK2. Thus, the increase in GRK2 that we observed may be attributable not only to increased plasma norepinephrine but also to increases in plasma or local levels of endothelin, angiotensin II, and/or perhaps other endogenous ligands. Although no change in GRK5 mRNA expression was observed, we did not investigate whether the Q41L polymorphism exists, a nonsynonymous polymorphism of GRK5 that is associated with a cardioprotection phenotype in black Americans.35

Data are accumulating to provide support for a direct relationship between GRKs and BP. We found previously that VSM overexpression of either GRK2 or GRK5 alone was sufficient to increase basal BP in mice.19,34 Increased GRK2 is associated with increased BP in both lymphocytes and VSM cells of 2 different high-BP rat models, the spontaneously hypertensive rat and the Dahl salt-sensitive hypertensive rat.16 In addition to the animal studies, in a small cohort of young white hypertensive subjects (n = 8), lymphocyte GRK2 protein and activity levels were increased compared with normotensive, age-matched control subjects.35 GRK5 is increased in rat VSMs during both angiotensin II and norepinephrine-induced hypertension.36 The augmented GRK5 level was attenuated by concurrent hydralazine administration, suggesting that hemodynamic stress itself was responsible for the GRK5 increase.36 Data from our laboratory suggest that the role of GRK in BP regulation is variable. In previous studies, we found that mice with increased VSM cell GRK2 expression have high BP and also have both VSM and cardiac hypertrophy. In contrast, mice with increased expression of VSM cell GRK5 have high BP without concomitant hypertrophy. Whether increased expression of GRK2 is also a predictor of increased cardiovascular risk and propensity toward increased morbidity remains to be determined.

In the current study, there was a direct correlation of GRK2, but not GRK5, with BP. Although the correlation in this study is low, our sample population was mostly considered to have BP within “normal” limits or to be “prehypertensive.” It will be particularly important to further test the direct correlation between GRK2 and BP in a greater number of subjects with high BP.

GRK2 upregulation occurs with heart failure.37 The increase in GRK2 in heart failure acts to decrease βAR signaling, leading to ionotropic and chronotropic decreases. It would seem counterintuitive, at least from a cardiac perspective, that increased GRK2 is associated with an increase in BP. However, the cardiovascular system is a highly complex system with multiple inputs and sites of regulation, including the heart, vasculature, brain, and kidney, as well as a multitude of different receptors and signaling pathways. Previously, we found that an increase in cardiac GRK2 accompanies an increase in BP that precedes cardiac dysfunction in the spontaneously hypertensive heart failure rat model.36 We predict, and data in this article suggest, that GRK2 levels are at least somewhat reflective of catecholamine activity. It remains to be determined how signaling by other hormones and neurotransmitters affects GRK2 levels, but it could be that, depending on the etiology of the high BP, the ligand that results in an increase in GRK2 might have minimal cardiac effects.

**Perspectives**

In American blacks, we have found that norepinephrine levels correlate with systolic BP. In addition, both norepinephrine and systolic BP directly correlate with GRK2 expression and activity. GRK2 is upregulated with stimulation of specific 7TMRs, including βARs, and its increase can have a profound impact on 7TMR signaling and, therefore, BP control. Understanding GRK levels may give us insight into the 7TMRs stimulated during hypertension, thus potentially broadening our understanding of etiology and progression of the disease, and may contribute to improved therapies and more targeted treatment strategies to control hypertension and reduce cardiovascular morbidity in this at-risk population.

**Acknowledgment**

The Pennsylvania Department of Health specifically disclaims responsibility for any analyses, interpretations, or conclusions of this work.

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**Disclosures**

None.

**References**


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G protein-Coupled Receptor Kinase 2 (GRK2) Expression and Activity are Associated with Blood Pressure in Black Americans

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Supplemental Methods

Study Sample
Participants in the cohort study were all volunteers with normal BP or with hypertension but without other known chronic disease. Recruitment of participants was achieved through local advertising in communities within Philadelphia. This study was conducted on participants enrolled from 2006 to 2008, and included men and women between age 18 and 67 years. Written informed consent on an institutionally approved protocol was obtained on each participant at enrollment. Exclusion criteria included individuals with secondary hypertension, pregnancy, known, cardiac, renal or autoimmune disease, thyroid disorder, Sickle Cell Anemia, chronic anemia, other endocrine disorders, psychiatric conditions, and individuals who are taking certain medications including systemic steroids. Individuals on antihypertensive medications or oral contraceptives were not excluded. Personal and family health history was obtained from each participant. Height and weight were measured and body mass index (BMI) was calculated as kg/m². The BP was measured on the right arm in the seated position using a mercury column Baumanometer and appropriate cuff size. Measurements were taken after the subject rested at least 10, but less than 20, minutes. The average of three or more systolic and diastolic BP measurements was used as the BP for each case.

RNA Isolation and cDNA Construction and Quantitative RT-PCR
RNA was recovered from lymphocytes by extraction using Qiagen RNeasy. On-column DNase I digestion (DNase Kit, Qiagen) was performed. cDNA was synthesized from 0.5 μg lymphocyte RNA (BioRad iScript kit). Bovine GRK2 in pcDNA3.1 and human GRK5 in pcDNA3 vectors were linearized via restriction digestion (BamHI, Promega) and purified using Qiagen Qiaquick columns. RiboMax Large Scale RNA Production System T7 (Promega) was utilized to create pure GRK2 and GRK5 RNA stocks, which subsequently were treated with RNase-Free DNase (DNase Kit, Qiagen). RNA was purified with Qiagen RNeasy. RNA copy number = [(X grams / liter)*(6.02 x 10^{23} molecules / mole)] / [(X nucleotide base pairs)*(330 grams / mol)*(10^6)] = X GRK molecules / μl. GRK2 and GRK5 cDNA were manufactured (iScript, BioRad) with human sample processing. A nine-point standard curve was prepared by 1:10 serial dilution, run in duplicates. Real-time PCR reactions were set up using BioRad iQ SYBR Green Supermix and run on a BioRad iCycler real-time PCR machine. Primers used for the amplification of GRK2, GRK5, and a selected reference gene (ribosomal subunit 28S) are described (Table S1). Sequencing was used to confirm product. Comparable primer amplification efficiencies of target (GRK2/5) and housekeeping (28S) genes were verified via serial dilution threshold analysis. A melting curve with continuous fluorescence monitoring was performed following RT-PCR to verify that only one PCR amplicon was detectable, and the final product obtained from RT-PCR amplification was further analyzed on agarose gel to confirm size. Duplicate RT-PCR reactions were performed for each sample and replicate variation greater than 0.7 Ct required repetition (Figure S1).
Immunoblot Analysis
10% Tris-glycine gels (Invitrogen) were loaded with 35µg protein/well of sample or calibrator. A four-point calibration curve was performed for accurate GRK2 determination using recombinant GRK2 protein in RIPA buffer. Following electrophoresis and transfer, PDVF membranes were blocked with Odyssey blocking buffer (927-4000, Licor) and probed with GRK2 primary antibody (C15 diluted 1:5000, Santa Cruz) then secondary antibody (680 Goat Anti-Rabbit, Pierce). Fluorescent intensity was measured via Odyssey Infrared Imager. Band densities were measured with integrated intensity quantification to reduce background interference. Membrane stripping was performed (Reblot plus Strong, Chemicon), and reblotted for protein loading control, protein phosphatase 2A (PP2A Catalytic α, BD Biosciences).
Table S1: Forward and reverse oligonucleotide primer sequences used for analysis of expression of GRK2, GRK5 and 28S in human lymphocytes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRK2 (GRK2)</td>
<td>5' ATG CAT GGC TAC ATG TCC A 3'</td>
<td>5' ATC TCC TCC ATG GTC AGC AG 3'</td>
<td>95°C 30s 55°C 45s 72°C 30s</td>
</tr>
<tr>
<td>NM_000868</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GRK5 (GRK5)</td>
<td>5' ACC TGA GGG GAG AAC CAT TC 3'</td>
<td>5' TGG ACT CCC TCC TCT TT 3'</td>
<td>95°C 30s 55°C 45s 72°C 30s</td>
</tr>
<tr>
<td>NM_005308</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28S</td>
<td>5' TTG AAA ATC CGG GGG AGA G 3'</td>
<td>5' ACA TTG TTC CAA CAT GCC AG 3'</td>
<td>95°C 30s 62°C 45s 72°C 30s</td>
</tr>
</tbody>
</table>

Genes identified by common name, alternative nomenclature in parenthesis and GenBank accession number.
Table S2. Distribution of Subject Samples

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRK mRNA</td>
<td>99</td>
</tr>
<tr>
<td>GRK2 Protein</td>
<td>10</td>
</tr>
<tr>
<td>GRK Activity</td>
<td>24</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>133</strong></td>
</tr>
</tbody>
</table>

Subject samples were studied by one of three methods as described in Materials and Methods and processed for GRK mRNA, protein or activity in order of recruitment.
Table S3: Subject Sample Population

<table>
<thead>
<tr>
<th>Variable</th>
<th>% Population</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td>38 (18-67)</td>
</tr>
<tr>
<td>≤35</td>
<td>21%</td>
<td></td>
</tr>
<tr>
<td>&gt;35</td>
<td>79%</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black American</td>
<td>99.99%</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>0.01%</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>47.5%</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>52.5%</td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td></td>
<td>122 (90-188)</td>
</tr>
<tr>
<td>&lt;130mmHg</td>
<td>71%</td>
<td></td>
</tr>
<tr>
<td>≥130mmHg</td>
<td>29%</td>
<td></td>
</tr>
<tr>
<td>BMI (Body Mass Index)</td>
<td></td>
<td>31.7 (18.1-58.1)</td>
</tr>
<tr>
<td>&lt;25</td>
<td>21%</td>
<td></td>
</tr>
<tr>
<td>≥25</td>
<td>79%</td>
<td></td>
</tr>
<tr>
<td>Diabetic Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12%</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>88%</td>
<td></td>
</tr>
</tbody>
</table>

Data represented as percent of sample population (n=133) and median number (low – high range).
Figure S1

A

B

GRK2

GRK5

C

GRK2

GRK5
Figure S1. Linearity and sensitivity of real-time quantitative reverse transcription PCR (qRT-PCR). Stringent sample criteria were implemented for our quantitative reverse transcription PCR (qRT-PCR). Our total RNA samples demonstrate clear 28S and 18S bands of rRNA upon gel electrophoresis from subjects #21-29 (Figure S1A). Exclusion criteria for patient RNA samples included RNA 260:280 absorbance ratio above 1.8 determined by NanoDrop ND-1000 (Wilmington, DE) and RNA visualization. Specific SYBR Green amplification of our two target genes (GRK2 and GRK5) and housekeeping gene (28S) were confirmed. GRK2 and GRK5 standard curves were created using RNA constructed from bovine GRK2 and human GRK5 sequences. Linearity of the standard curves is seen at the exponential phase of PCR and optimal threshold positions were restricted to lower half of the fluorescence plots in the exponential phase (Figure S1B). Figure S1C illustrates Ct threshold values of both our standards and patient samples, which fall neatly within our standards.

A, Gel electrophoresis of RNA extraction from subjects #21-29. One microgram loading of samples demonstrated clear 28S and 18S bands of rRNA. Stringent exclusion criteria for patient RNA samples; RNA 260:280 absorbance ratio above 1.8 determined by NanoDrop ND-1000 (Wilmington, DE) and RNA quality (intactness) determine by on an Agilent 2100 Bioanalyzer (Foster City, CA). B, Linearity of the standard curves is seen at the exponential phase of PCR and optimal threshold positions were restricted to lower half of the fluorescence plots in the exponential phase. C, PCR amplification and Ct threshold graph representation of both our standards and patient samples; patient samples (light and dark purple) fall neatly within the standard curve.
Figure S2. GRK2 mRNA is significantly greater in subjects with diastolic BP ≥80mmHg. When the normal diastolic BP limit of 80mmHg was chosen, GRK2 mRNA was significantly greater in the group with diastolic BP ≥80mmHg (n=29) versus the group with a diastolic BP <80mmHg (n=70). p<0.05 vs. <80mmHg, two-tailed, unpaired Student’s t-test.
Figure S3. Representative autoradiograms of rhodopsin kinase activity assays. A, Rhodopsin kinase activity assays were performed with recombinant GRK2 in a concentration-dependent manner. B, Representative autoradiogram for 2 subjects. Subject 139 had a systolic BP of 116mmHg and Subject 140 had a systolic BP of 137mmHg. Activity was greater as represented by increased rhodopsin band intensity in Subject 140 with higher systolic BP.