Adrenergic beta-1 receptor genetic variation predicts longitudinal rate of GFR decline in hypertensive nephrosclerosis

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5Supported by a fellowship from the International Society of Nephrology.

Abstract

Background. End-stage renal disease (ESRD) due to hypertension is common and displays familial aggregation in African Americans, suggesting genetic risk factors, including adrenergic activity alterations which are noted in both hypertension and ESRD.

Methods. We analysed 554 hypertensive nephrosclerosis participants (without clinically significant proteinuria) from the longitudinal National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) African American Study of Kidney Disease and Hypertension (AASK) cohort to determine whether decline in glomerular filtration rate (GFR) over ∼3.8 years was predicted by common genetic variation within the adrenergic pathway, particularly at ADRB1 receptors as predictors of glomerular filtration rate (GFR) decline in hypertensive nephrosclerosis.

Results. The polymorphism at Ser49Gly (though not Arg389Gly, in only partial linkage disequilibrium at $r^2 = 0.18$) predicted the chronic rate of GFR decline, with minimal decline in Gly49/Gly49 (minor allele) homozygotes compared to Ser49 carriers; concordant results were observed for haplotypes and diploid haplotype pairs at the locus. An independent replication study in 1244 subjects from the San Diego Veterans Affairs Hypertension Cohort confirmed that Gly49/Gly49 homozygotes displayed the least rapid decline of eGFR over ∼3.6 years.

Conclusion. We conclude that GFR decline rate in hypertensive renal disease is controlled in part by genetic variation within the adrenergic pathway, particularly at ADRB1. The results suggest novel strategies to approach the role of the adrenergic system in the risk and treatment of progressive renal disease.

Keywords: AASK; African Americans; glomerular filtration rate; kidney disease; sympathetic nervous system

Introduction

End-stage renal disease (ESRD) resulting from hypertension aggregates in families [1,2] and is approximately six times more likely in African Americans than white subjects [3]. Recently, genetic studies have linked hypertensive ESRD to polymorphisms in myosin heavy chain-9 (MYH9) [4] and chromogranin A (CHGA) [5], which has a pivotal role in formation of catecholamine storage vesicles [6]. The adrenergic system, implicated in a number of cardiovascular disease processes [7], contributes to the progression of renal failure [8]. Though activation of the adrenergic receptors is vital for physiologic processes, prolonged stimulation likely contributes to cardiovascular disease states, and thus the sensitization and down-regulation of these receptors may play a role in such chronic diseases [9,10].

Many reports have noted genetic contributions from adrenergic beta-1 receptors (ADRB1) to hypertension [11,12], heart failure [13] and coronary artery disease [14]. Given the relationship between cardiovascular and kidney disease [15], we elected to study subjects from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) African American Study of Kidney Disease and the Hypertension (AASK) trial [16] to evaluate common genetic variants of ADRB1 receptors as predictors of glomerular filtration rate (GFR) decline in hypertensive nephrosclerosis. The AASK trial is ideally suited to study such genetic variants, since it is a large-scale longitudinal cohort evaluating the impact of blood pressure (BP) goals (lower versus usual) and antihypertensive medications (ramipril, metoprolol, or amlodipine) on the progression of hypertensive nephropathy [17,18]. Subjects were carefully monitored for progressive hypertensive nephropathy [19], with GFR...
Fig. 1. Pictoral depiction of human adrenergic beta-1 receptor (ADRB1) gene. The illustration shows the only two common (minor allele frequency >5%) non-synonymous polymorphisms found in humans: Ser49Gly and Arg389Gly. Based on <www.gpcr.org>.

Methods

Subjects

AASK study subjects. Subjects were from the AASK study, a 21-centre randomized, controlled prospective trial that has been previously described [16,17]. Briefly, participants were 18- to 70-year-old self-identified African Americans with hypertension (n = 1094) with a clinical diagnosis of hypertensive renal disease, documented by an initial GFR (by [125I]-iothalamate clearance or Cockroft-Gault equation) between 20 and 65 ml/min/1.73 m², with UPCR <2.5 g/g, and no other identifiable causes of renal insufficiency. In particular, subjects with diabetes mellitus were excluded. Based on a 3 × 2 factorial design, participants were randomized to one of two goal BP ranges (‘usual’ mean arterial pressure goal of 102–107 mmHg, or a lower mean arterial pressure goal of ≤92 mmHg), and to double-blinded treatment with one of three antihypertensive drug classes (40% to beta-blockade with metoprolol, 50–200 mg/day; 40% to ACE inhibition with ramipril, 2.5–10 mg/day; or 20% to calcium channel blockade with amlodipine, 5–10 mg/day). GFR was assessed by renal clearance of [125I]-iothalamate at baseline twice, at 3 and 6 months and then every 6 months thereafter, for a median of 3.8 years (range 3.0–6.4 years) [20]. The primary endpoint in this study was chronic GFR slope, defined as that beginning 3 months after randomization, in order to better reflect long-term disease progression, given that the medical interventions (particularly calcium channel blockade) were noted to have acute effects on GFR that may differ from their long-term effects on disease progression [18,22].

Genomic DNA from blood leucocytes was ascertained and prepared from 830 of the original 1094 participants in the AASK study who consented for genetic evaluation. Each subject gave informed, written consent measured regularly through iothalamate clearance for a median of ~3.8 years [17].

Previous reports from the AASK trial indicate that subjects with minimal proteinuria (defined as a urine protein-to-creatinine ratio (UPCR) <0.22 g/g, approximating the ~300 mg/day threshold defining clinically significant proteinuria) display the most dynamic acute changes in GFR in response to drugs [18,20]. Such subjects without elevated proteinuria also have a slower rate of decline of renal function (~1.35 mL/min/1.73 m²/year when compared to −4.09 mL/min/1.73 m²/year in subjects with overt proteinuria) [20]. Since accelerated decline in GFR in the overt proteinuria group may be further confounded by proteinuria as an independent risk factor for renal function decline [20], we evaluated AASK study subjects without clinically significant proteinuria to determine whether risk of accelerated loss of kidney function is conferred by common genetic variation at ADRB1, focusing on the only two common (MAF>5%) non-synonymous (amino acid replacement) variants at ADRB1 which have also been shown to affect functional down-regulation of the receptor, Ser49Gly and Arg389Gly [21], shown in Figure 1, assessed as single nucleotide polymorphisms (SNPs), haplotypes and diploid haplotypes.
to the local institutional review boards. Of those, the 554 that did not have clinically significant proteinuria, defined as a UPCR <0.22 and were genotyped at the ADRB1 locus, were included in this study and are described in Table 1. UPCR of <0.22 corresponds to a urine protein excretion of ~300 mg/day, and separates out the ~2/3 of patients with the lowest ('normal' range) proteinuria from the 1/3 with highest proteinuria (the UPCR parameter is skewed towards higher values and is inversely associated with initial GFR). The 328 males and 226 females had a mean age of 55.6 ± 0.4 years and a baseline GFR of 51.2 ± 0.5 mL/min/1.73 m². The genotyped AASK subset (n = 830) did not differ from the complete AASK cohort (n = 1094) [19,23] in age, sex, BMI, duration of hypertension, SBP, DBP, serum creatinine, baseline GFR, baseline proteinuria or overall chronic rate of the progression of renal disease (by normal and high proteinuria groups).

Replication study: San Diego Veterans Affairs Hypertension Cohort (VAHC). The San Diego VAHC is a multiethnic, non-interventional/observational study of veterans recruited from San Diego Veterans Affairs Healthcare System primary care internal medicine clinics in 2003–2004 [24]. Subjects had been diagnosed by their primary care physicians with essential hypertension or were on BP-lowering medications, excluding subjects with known secondary causes of hypertension. The cohort utilizes the comprehensive VA complete electronic medical record (EMR) known as VISTA (Veterans Health Information Systems and Technology Architecture; http://www.va.gov/VISTA_MONOGRAPH/) [25], which includes all vital signs, laboratory data, medical diagnoses, pharmacy records and procedure codes in digital format. EMR data were extracted in Microsoft SQL-Server tables for study subjects from October 2000 to November 2007. Subjects were excluded if they had prevalent or incident ESRD during this period (n = 25). eGFR was estimated by the Modification in Diet and Renal Disease (MDRD) equation [26].

A total of 1244 subjects were genotyped at the ADRB1 locus and included in this replication study as described in Table 2. Of those, 95.6% were men, reflecting the veteran population, with 68.2% self-identified as white and 15.5% as black. At entry, 15.7% had evidence of chronic kidney disease (CKD), defined by eGFR <60 mL/min/1.73 m² [27]. By the end of the study, 30.1% had a diagnosis of type-2 diabetes with <2% of them coded for renal complications of diabetes. The mean age at the start of the study was 61.9 ± 0.4 years (range 24.5–89.6) with a mean follow-up of 3.64 ± 0.50 years (range <1 to 6.9 years). The primary endpoint in this study was chronic eGFR slope (change over time) in order to better reflect long-term disease progression. For subjects who were determined to have an episode of acute kidney injury (AKI), defined as an acute increase in plasma creatinine >0.3 mg/dL [28], only the eGFR measurements prior to the AKI were used in longitudinal analysis.

Genomics

SNP genotyping and haplotypes at ADRB1. Two common polymorphisms, illustrated in Figure 1, at ADRB1 were genotyped (Ser49Gly and Arg389Gly) in the AASK study, giving rise to three common haplotypes and three diploid haplotype combinations, as listed in Table 3.

AASK subjects without significant proteinuria (urine protein: creatinine ratio <0.22 g/g) were included in this study. Mean values ±1 SEM are reported. For both genotypes, Hardy Weinberg Equilibrium was maintained: Ser49Gly (χ² = 0.34, P = 0.56) and Arg389Gly (χ² = 0.051, P = 0.82). GFR: glomerular filtration rate. ACEI: angiotensin-converting enzyme inhibitor (ramipril). β-blocker: beta-adrenergic antagonist (metoprolol). CCB: calcium channel blocker (amlodipine).

Statistical analyses

Progression of GFR loss in AASK. Statistical analyses for the AASK study were performed using Statistical Package for the Social Sciences (SPSS v11.0; Chicago, IL, USA). ANOVA was used to determine the significance of genotype and haplotype effects on chronic decline of GFR slope (mL/min/1.73 m²/year). Mixed-effects models were utilized to determine the slope and were estimated by restricted maximum likelihood in each treatment group and considered covariates including treatment centre, proteinuria, sex, age and mean arterial pressure [18]. Various models included covariates in the analysis. Initial P-value is unadjusted, without any covariates, Model 1 includes baseline age and sex, Model 2 includes baseline GFR and UPCR (urine protein: creatinine ratio) and Model 3 includes baseline GFR, baseline UPCR, age at the start of the study, sex and randomized drug group (ramipril, metoprolol or amlodipine) and BP goal (low or usual). A conservative Bonferroni correction was applied for the two ADRB1 SNPs studied in this analysis, for an adjusted significance level of P = 0.025 (0.05/2) despite evidence of linkage disequilibrium between the two variants [10], with r² = 0.18. All analyses were performed by the study investigators and not by the AASK data coordinating centre.

Population admixture. African Americans represent an admixed population with genetic contributions from both African and European biogeographic origins [31]. To confirm that AASK individuals with or without trait-associated genotypes were of comparable overall genetic background, and the observed associations are not simply an artefact of differential admixture between faster and slower GFR decline rates, generalized analysis of molecular variance (GAMOVA) [32] was used to test for and quantify the relationship between the overall genetic background of the subjects and quantitative phenotype GFR decline rate (chronic GFR slope), with an IBS (identity-by-state) distance matrix based on genotypes at 126 bi-allelic markers. The admixture analysis was done in this study cohort without clinically significant proteinuria (N = 554), as well as in the entire genomic AASK cohort (N = 830).
Table 2. Subject demographics of San Diego Veterans Affairs Hypertension Cohort replication study

<table>
<thead>
<tr>
<th>Mean (n = 1244)</th>
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<tbody>
<tr>
<td>Age (years)</td>
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<tr>
<td>Baseline serum creatinine (mg/dL)</td>
</tr>
<tr>
<td>Baseline eGFR (mL/min/1.73 m²)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
</tr>
<tr>
<td>% values</td>
</tr>
<tr>
<td>Ethnicity (self-reported)</td>
</tr>
<tr>
<td>ADRB1 Ser49Gly Alleles</td>
</tr>
<tr>
<td>Diploid genotypes</td>
</tr>
</tbody>
</table>

Mean values ± 1 SEM are reported.

eGFR: estimated glomerular filtration rate by the Modification of Diet in Renal Disease equation. Hardy–Weinberg Equilibrium was maintained for Ser49Gly.

Replication study in VAHC. Mixed-effects linear models (PROC MIXED) in SAS 9.2 (Cary, NC, USA) [33] were utilized to assess the influence of ADRB1 Ser49Gly polymorphism on longitudinal ambulatory eGFR considering sex, age at start of study and race [18,34]. Subject-specific intercept and slope parameters were estimated and regressed against the predictor of interest simultaneously using mixed-effects models [33,35,36]. This approach can be applied to unbalanced longitudinal data, particularly to the repeated measurements taken at unequal time intervals of the VAHC study, which utilizes observational information in the EMR and yields information on both genotype and genotype-by-time effects [37]. Because only one polymorphism was studied in this cohort, P = 0.05 was considered statistically significant in this replication study.

To illustrate the influence of the ADRB1 Ser49Gly variant on longitudinal GFR decline, chronic longitudinal GFR was plotted by genotype over time (Figure 3). The longitudinal GFR profile by genotype was generated using an adaptive regression cubic spline approach [38], with 95% confidence intervals for fitted spline function of the adjusted GFR values (from mixed model results). To address the potential confounding effects of loss to follow-up, joint modelling of longitudinal and time to event data was implemented [33]. This joint model combines longitudinal and time to event analysis, allowing the covariance structure to be adjusted for censoring (loss to follow-up) and event (death) data [33,39].

Table 3. Effects of adrenergic beta-1 receptor (ADRB1) genetic variants on chronic glomerular filtration rate (GFR) slope in NIDDK AASK subjects

<table>
<thead>
<tr>
<th>Adrenergic beta-1 receptor (ADRB1) variant</th>
<th>Position</th>
<th>RefSNP</th>
<th>Minor allele frequency</th>
<th>Model for effect on GFR slope (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Unadjusted</td>
</tr>
<tr>
<td>ADRB1 Ser49Gly</td>
<td></td>
<td>rs1801252</td>
<td>Gly (0.22)</td>
<td>0.003</td>
</tr>
<tr>
<td>ADRB1 Arg389Gly</td>
<td></td>
<td>rs1801253</td>
<td>Gly (0.39)</td>
<td>0.53</td>
</tr>
<tr>
<td>Haplotype</td>
<td>Allele combination</td>
<td></td>
<td></td>
<td>0.43</td>
</tr>
<tr>
<td>ADRB1</td>
<td></td>
<td></td>
<td></td>
<td>0.36</td>
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<tr>
<td>Haplotype</td>
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<td></td>
<td></td>
<td>0.21</td>
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<tr>
<td>Total:</td>
<td></td>
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<td>1.00</td>
</tr>
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</table>

Results were obtained in AASK subjects without clinically significant proteinuria (urine protein: creatinine (UPCR) <0.22 g/g). Effects of ADRB1 polymorphisms, haplotypes and diploid haplotypes are shown. Linkage disequilibrium between Ser49Gly and Arg389Gly was estimated at r² = 0.18. Bold P-values denote significance after Bonferroni correction. Model 1 includes sex and baseline age as covariates. Model 2 includes baseline GFR and UPCR as covariates. Model 3 includes baseline GFR and UPCR as well as sex, baseline age, randomized blood pressure goal and medication groups as covariates.
**ADRB1 loci predict GFR decline in AASK**

**ADRB1 Ser49Gly: Effects on GFR decline in AASK cohort**

![Graph A](image1.png)

**ADRB1 haplotype 3 (Gly Arg 389) copies**

![Graph B](image2.png)

**ADRB1 diploid haplotypes(Ser49Gly→Arg389Gly): Effects on rate of GFR decline in the AASK cohort**

![Graph C](image3.png)

**ADRB1 diploid haplotype variation**

![Graph D](image4.png)

**Fig. 2.** Adrenergic beta-1 receptor (ADRB1) genetic variants: Effects on chronic glomerular filtration (GFR) decline rate in the NIDDK AASK cohort. AASK subjects without clinically significant proteinuria (urine protein/creatinine ratio <0.22 g/g) were evaluated. Analyses were performed by univariate ANOVA. Model shown is unadjusted without covariates, but Table 3 notes that there were no significant differences in the models which include covariates. [Model 1: sex and age at start of study; Model 2: baseline GFR and urine protein: creatinine ratio (UPCR); Model 3: baseline GFR, baseline UPCR, age at start, sex, randomization blood pressure goal and drug]. (A) **ADRB1** polymorphism, Ser49Gly. Minor allele homozygosity (Gly49/Gly49) predicted a slower decline of GFR compared to wild-type (Ser49/Ser49) or heterozygous (Ser49/Gly49) genotype, unadjusted \( P=0.003 \).

(B) **ADRB1** haplotype across Ser49Gly→Arg389Gly. Subjects with two copies of haplotype 3 (Gly49Arg389) had a slower decline in GFR compared to those with zero or one copy, unadjusted \( P=0.002 \). (C) **ADRB1** diploid haplotype: haplotype-3 (Gly49Arg389) and haplotype-1 (Ser49Gly389). Homozygotes for haplotype-3 (Gly49Arg389) had a slower decline in GFR compared with those with one or two copies of haplotype-1 (Ser49Gly389), unadjusted \( P=0.001 \). (D) **ADRB1** diploid haplotype: haplotype-3 (Gly49Arg389) and haplotype-2 (Ser49Arg389). Homozygotes for haplotype-3 (Gly49Arg389) had a slower decline in GFR compared with those with one or two copies of haplotype-2 (Ser49Arg389), unadjusted \( P=0.014 \).

no gene-by-drug interactions (based upon trial randomization groups) were noted with the **ADRB1** variants. Nor did the Ser49Gly polymorphism associate with drug or BP-goal randomization groups, age, GFR, UPCR or BMI at the start of the study.

**Genetic admixture in NIDDK AASK.** One hundred and twenty-six bi-allelic markers were genotyped in the
genomic AASK cohort. Generalized analysis of molecular variance (GAMOVA) indicated that people with similar predictor variables (GFR slope, mL/min/1.73 m²/year) were not genetically more related to each other than expected by chance alone in this group of AASK subjects without clinically significant proteinuria ($P = 0.87$), nor in the entire genomic AASK cohort ($n = 830$, $P = 0.45$). Thus, the adrenergic allele and haplotype influences on this chronic GFR decline trait cannot be attributed to differential admixture between higher and lower GFR decline rate strata.

Replication study: San Diego Veterans Affairs Hypertension Cohort (VAHC). This replication study consisted of 1244 veterans genotyped at the $ADRB1$ Ser49Gly locus (minor allele frequency 14.8%), as described in Table 2. Of those, 95.6% were male and 68.2% were white. At the start of the study the mean age was 61.9 ± 0.35 years, serum creatinine was 1.04 ± 0.01 mg/dL, eGFR (by MDRD equation) was 85.6 ± 0.7 mL/min/1.73 m² and body mass index was 30.1 ± 0.16 kg/m².

As noted in the AASK subjects, $ADRB1$ Ser49Gly predicted decline in eGFR over a mean follow-up of 3.6 ± 0.05 years in the VAHC cohort, adjusted by sex, age at start of study and race, shown in Figure 3. $ADRB1$ Ser49Gly displayed a significant genotype-by-time interaction on eGFR, at $P = 0.043$. Subjects homozygous for the minor allele (Gly49/Gly49) had slower decline in the slope of eGFR decline compared to heterozygotes (Ser49/Gly49) or wild-type homozygotes (Ser49/Ser49). A joint model, which combines longitudinal and time to event analysis adjusting for censoring (loss to follow-up) and death, yielded consistent results (data not shown, $P = 0.045$).

When restricting the analysis only to subjects self-identified as African American ($n = 191$, minor allele frequency = 14.8%), statistical significance was maintained in this model with sex and age at start of study as covariates ($P = 0.037$), again with Gly49/Gly49 homozygotes having slower decline in eGFR. The findings in other ethnic groups, including whites and Asian/Pacific Islanders, showed a similar trend (with Gly49/Gly49 variants having slower eGFR decline), but the difference did not reach statistical significance. Similarly, when evaluating subjects with CKD (eGFR at the start of the study ≤60 mL/min/1.73 m², $n = 195$), a significant genotype-by-time interaction on eGFR was noted ($P = 0.047$) when considering sex, race and age at the start of the study as covariates. A similar trend was noted for the gene-by-time interaction in subjects that started the study without CKD (eGFR > 60 mL/min/1.73 m²), but did not reach statistical significance ($P = 0.19$).

The Ser49Gly variant was not associated with sex, self-identified ethnicity, BMI, serum creatinine or eGFR at the start of the study (results not shown) in the VAHC study. The participants differed in age at baseline ($P = 0.025$), with Gly49/Gly49 homozygotes slightly younger (57.5 ± 2.3 years) than Ser49/Ser49 (62.3 ± 0.41 years) or Ser49/Gly49 (61.2 ± 0.71 years) subjects. Subjects with CKD at the start of the study were noted to be older (67.7 ± 0.78 years) compared with those without CKD (61.0 ± 0.37, $P < 0.001$).

Discussion

Overview

The autonomic nervous system is a key determinant of blood pressure and renal function. Previous studies showed that the adrenergic response to stress contributes to hypertension, proteinuria and renal pathologic changes in rats [42], and catecholamine infusion worsened GFR decline and proteinuria in humans [43]. African Americans, who are at an increased risk for hypertension and nephrosclerosis particularly within families, display exaggerated autonomic responses to environmental stressors [44]. Here, we found that common non-synonymous variation of $ADRB1$ predicted the rate of GFR decline in the NIDDK AASK cohort of hypertensive nephrosclerosis (Table 3) and then replicated the finding in an independent population of hypertensive veterans.

Adrenergic hereditary mechanisms

The adrenergic system, through adrenergic receptors, regulates multiple aspects of renal function [45] as well as systemic haemodynamics [46] and is under substantial hereditary control [47]. In this study, we found that $ADRB1$...
variants affected GFR slope (Table 3). ADRB1 receptors are expressed throughout the body in many regulatory pathways, but in the kidney, ADRB1 is expressed in renal arterial smooth muscle cells as well as in the mesangium [48]. The ADRB1 GPCR is typically stimulatory, coupling through $G_{i}$ to increase adenyl cyclase activity and hence cyclic AMP (cAMP). cAMP dilates renal arterioles and mesangial cells, thereby regulating not only renal blood flow but also filtration surface, consequently altering glomerular capillary hydrostatic pressure, which plays a role in progressive renal disease [49]. Adrenergic genetic variants have been found to be functional due to a number of mechanisms, such as altering functional receptor number, binding affinity, agonist coupling to effectors, or desensitization [50,51], each with the potential to affect GFR decline.

ADRB1 Ser49Gly lies within the extra-cellular amino terminus of the receptor, shown in Figure 1, and influences desensitization of the receptor after repeated agonist exposure [52]: the wild-type Gly$_{49}$ variant exhibits heightened desensitization (agonist-promoted down-regulation of signalling), particularly with prolonged agonist stimulation [52–54]. This mechanism appears to limit ADRB1 signalling in response to chronic catecholamine excess, and thus Gly$_{49}$ has been shown to be beneficial in subjects with heart failure [54].

The other ADRB1 variant included in this study, Arg389Gly, which was not independently correlated with decline in renal function in our study, has been evaluated extensively due to its location within the intra-cellular carboxy-terminal tail (Figure 1) that constitutes the $G_{i}$ coupling domain of the receptor. In recombinant systems, Arg$_{389}$ exhibited increased coupling to $G_{i}$ with higher adenyl cyclase activity but greater short-term desensitization than Gly$_{389}$ [21].

ADRB1 haplotypic variation (at Ser49Gly → Arg389Gly) also has functional consequences. Sandilands et al. [51] found that the β-adrenergic agonist isoprorenaline/isoproterenol increased cAMP production most prominently in the Gly$_{49}$Arg$_{389}$ haplotype, which also displayed the greatest isoprorenaline-induced desensitization. Our findings were consistent in that subjects with two copies of the Gly$_{49}$Arg$_{389}$ haplotype (Figure 2B) had a decreased rate of GFR decline. When evaluating diploid haplotypes, the subjects with diploid copies of the haplotype also had a decreased decline in their renal decline compared with subjects with one or two copies of either Ser$_{49}$/Arg$_{389}$ (Figure 2C) or Ser$_{49}$/Gly$_{389}$ (Figure 2D), suggesting a dominant action of this haplotype on the GFR decline trait.

These ADRB1 variants have been evaluated for roles in cardiac disease [55–57], hypertension [11,58], resting heart rate [58] and mortality [12], including pharmacogenetic studies [11,12], with mixed results. The studies with significant findings were consistent with our results in that those with the Gly$_{49}$ variant have improved cardiac outcomes [55,56], and also improved mortality [12], particularly when treated with a beta-blocker. The cardiac studies that were unable to determine associations with ADRB1 Ser49Gly had null findings and may have lacked statistical power [57]. This variant alone has not been shown to be independently associated with hypertension or response to beta-blocker therapy [11,59]. Thus, our findings here of improved renal decline are unlikely to be related simply to BP control or response to therapy, but whether they may be associated with improved cardiac function was not assessed here. The Arg389Gly polymorphism has been linked to hypertension [59], which likely contributes to ADRB1 haplotypes that have previously been associated with hypertension and response to beta-blocker therapy [11,60].

Given that ADRB1 stimulation increases renin release [61], further study is necessary to determine whether plasma renin activity is affected by ADRB1 genetic variants. If the Gly$_{49}$ allele results in desensitization and down-regulation, decreased renin may be released, as has been noted in beta-1/beta-2-adrenergic receptor-deficient mice [62].

**ADRB1 genetic associations with chronic GFR slope in the NIDDK AASK cohort**

ADRB1 genetic associations with GFR slope were found in AASK subjects without clinically significant proteinuria. Previous reports from the AASK trial indicate that subjects with minimal proteinuria display the greatest acute dynamic changes in GFR in response to drugs, while elevated proteinuria itself is a strong predictor of GFR decline [63]. Minimal proteinuria likely corresponds to less advanced pathological findings, such as fewer initial structural and fibrotic changes of nephrosclerosis; at this earlier stage, the regulation of GFR may be more sensitive to haemodynamic changes, with loss of such sensitivity as fibrosis progresses. The SNPs may also require active dynamic regulation of glomerular vessels, which may be absent when UPCR ≥0.22 g/g as previously seen in the AASK cohort acutely when BP meds were started [20]. A limitation to this study is that such conclusions may not be readily extended to subjects with more advanced proteinuria.

**Replication study**

An exact replication of the AASK trial is unlikely, given the scope of its duration, size, intensity, complexity and expense. Though randomized clinical trials are often considered the gold standard for clinical research, detailed comparison with observational studies via meta-analysis provides evidence that well-designed observational studies can produce valid and similar results [64,65]. The VAHC study as a replication study. Though the VISTA–EMR system provides a vast amount of medical information about the study subjects, one limitation is that accuracy and completeness are reliant upon the EMR. Advanced statistical methods (see the Method section) were employed to best utilize and transform the ambulatory setting data into models that may emulate a clinical trial.

These results are from veteran subjects, who are primarily male and with heterogeneous biogeographic ancestry.
This population was generally elderly (at 61.9 ± 0.35 years) with a corresponding modest decrease in eGFR (at 85.6 ± 0.7 mL/min/1.73 m²), though not so low as GFR in subjects with nephrosclerosis from the AASK study (Tables 1 and 2). Because of the relatively small number of African Americans (n = 191) in VAHC, we opted to study all of the subjects using ethnicity as a covariate. Nonetheless, even when confining the replication to only the African American subjects, the association of \textit{ADRB1} Ser49Gly genotype-by-time decline in eGFR maintained statistical significance (P = 0.037).

Because the VAHC cohort is based upon the EMR, iothalamate clearance results, which were utilized in the AASK study, were not available. Rather, eGFR was determined by the MDRD equation [26], using ambulatory serum creatinine measurements all performed at the same San Diego Veterans Affairs clinical laboratory. Such GFR values are most accurate in subjects with GFR < 60 mL/min/1.73 m², tending to underestimate GFR in healthy individuals [26]. Though our sample included healthy subjects as well as those with CKD stage 1–4 [27], imprecision of eGFR determination by underestimation in healthy individuals would be expected to bias the results towards the null, in contrast to the significant findings in our analyses (Figure 3). Despite the small subject number (n = 195) in the subset of subjects with CKD, statistical significance for an \textit{ADRB1} gene-by-time interaction with eGFR (P = 0.047) was maintained.

\textbf{Statistical confidence}

In a comprehensive study of a trait involving multiple genotypes, the possibility of false-positive (type 1) statistical conclusions must be considered. We approached this issue in four ways: reducing the target \(\alpha\) (P value) by Bonferroni correction based on the number of SNPs evaluated, by haplotyping (i.e., analysing multiple variants at a locus simultaneously), and by admixture analyses. These approaches continued to yield significant GFR predictions, with results that were congruent across approaches. Nonetheless, the ultimate guardian against false-positive errors is replication in an independent sample, which we tested in the VAHC cohort.

\textbf{Perspectives}

The adrenergic system, including its cardio renal effector the \textit{ADRB1} receptor, is implicated in the genesis and the progression of hypertension and cardiovascular disease. Our study utilized the large longitudinal AASK genomic cohort and found that the rate of GFR decline in nephrosclerosis is controlled in part by common genetic variation within the \textit{ADRB1} receptor, perhaps influencing renal function as a consequence of enhanced receptor desensitization. The results suggest novel pathophysiological links between the adrenergic system and chronic renal injury and suggest new strategies for probing the role and actions of the pathway in this setting. These findings may generate hypotheses for more rigorous testing of the role of adrenergic receptors in complex renal traits, which may assist in further understanding pathophysiological principles and designing potential therapeutic interventions that might be implemented after early identification of genetic risk.

\textbf{Acknowledgements.} We appreciate the support of the Department of Veterans Affairs, the NIH/NCMHD-sponsored (MD000220) EXPORT Minority Health Center, as well as the NIH/NCRR-sponsored (RR00827) General Clinical Research Center. NIS is supported in part by the Scripps Translational Sciences Institute Clinical Translational Science Award (U54 RR0252204-01).

\textbf{Conflict of interest statement.} VHB is employed by Roche Molecular Systems, Inc. that does not have a business interest in this field.


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A candidate gene approach to genetic prognostic factors of IgA nephropathy—a result of Polymorphism REsearch to DIstinguish genetic factors Contributing To progression of IgA Nephropathy (PREDICT-IgAN)

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Abstract

**Background.** Renal prognosis of IgA nephropathy (IgAN) is affected by environmental and genetic factors. Other studies demonstrated that some atherosclerotic disease-related genes were significantly associated with renal progression.

**Methods.** The Polymorphism REsearch to DIstinguish genetic factors Contributing To progression of IgAN (PREDICT-IgAN) was a multicentre retrospective observational study to investigate associations between progression of IgAN (a 50% increase of serum creatinine level and slope of eGFR) and a hundred atherosclerotic disease-related gene polymorphisms, mainly single nucleotide polymorphisms (SNPs) in 320 IgAN patients who had more than a normal range of urinary protein (≥0.25 g/day) at diagnosis.

**Results.** During 8.3 ± 4.2 years of a follow-up period, 83 patients (25.9%) developed progression. In log-rank tests, glycoprotein Ia GPIa C807T and G873A and intercellular adhesion molecule-1 ICAM-1 A1548G (K469E) were found to be significantly associated with progression even after adjustment for multiple comparisons by the method of Bonferroni (adjusted \( P = 0.0174, 0.0176 \) and 0.0430, respectively). In a multivariate Cox proportional-hazards model, GPIa 807TT (873CC) [versus 807TT, adjusted hazard ratio 2.05 (95% confidence interval 1.13–3.71)] and ICAM-1 1548GG [versus 1548AA, 2.55 (1.40–4.65)] were identified as independent genetic predictors of progression, along with conventional clinical prognostic factors such as eGFR, urinary protein and use of antihypertensives at diagnosis.

**Conclusions.** PREDICT-IgAN distinguished GPIa C807T/G873A and ICAM-1 A1548G from multiple atherosclerotic disease-related gene polymorphisms by their predictive indicator for progression of IgAN.

Keywords: genetic prognostic factor; glycoprotein Ia GPIa; IgA nephropathy; intercellular adhesion molecule-1 ICAM-1; single nucleotide polymorphism (SNP)

Introduction

IgA nephropathy was first described by J. Berger in 1969 and is now generally known to be the most common primary glomerulonephritis in the world [1–3]. Although long-term observational studies reported a very wide range of renal survival rate of IgAN, 15–25% of patients with IgA nephropathy advanced to end-stage renal disease within 10 years of diagnosis [4]. Impaired renal function, proteinuria,