ORIGINAL ARTICLE

Angiotensin-related genetic determinants of cardiovascular disease in patients undergoing hemodialysis

Sharon M. Moe^{1,2}, Jin Long³, Tae-Hwi Linus Schwantes-An⁴, Brian S. Decker¹, Leah Wetherill⁴, Howard J. Edenberg^{4,5}, Xiaoling Xuei⁴, Matteo Vatta⁴, Tatiana M. Foroud⁴ and Glenn M. Chertow³

¹Division of Nephrology, Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, USA, ²Department of Medicine, Roudebush Veterans Administration Medical Center, Indianapolis, IN, USA, ³Division of Nephrology, Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA, ⁴Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA and ⁵Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN, USA

Correspondence and offprint requests to: Sharon M. Moe; E-mail: smoe@iu.edu

ABSTRACT

Background. Cardiovascular mortality in patients receiving dialysis remains unacceptably high, with unexplained ancestry differences suggesting a genetic component.

Methods. We analyzed DNA samples from 37% of subjects enrolled in the Evaluation Of Cinacalcet Hydrochloride (HCl) Therapy to Lower CardioVascular Events (EVOLVE) trial, a randomized trial conducted in patients receiving hemodialysis with secondary hyperparathyroidism, comparing cinacalcet to placebo on a background of usual care. DNA was analyzed for single-nucleotide polymorphisms (SNPs) in the genes encoding the angiotensin-converting enzyme receptor type I (*AGTR1*) and angiotensin-converting enzyme (*ACE*). Survival analyses were conducted separately in European Ancestry (EA) and African Ancestry (AfAn) due to known differences in cardiovascular events, minor alleles for the same variant and the frequency of minor alleles. Our primary determination was a meta-analysis across both races.

Results. Meta-analysis showed significant associations between rs5186 in *AGTR1* and increased rates by 25–34% for the primary endpoint (composite of death or nonfatal myocardial infarction, hospitalization for unstable angina, heart failure or peripheral vascular event), all-cause mortality, cardiovascular mortality and heart failure; all $P < 0.001$. Three correlated SNPs in *ACE* were associated with lower rates of sudden cardiac death (SCD) in EA samples. One *ACE* SNP, rs4318, only found in the AfAn samples, was associated with a lower rate of SCD in the AfAn samples.

Conclusions. The C allele in rs5186 in *AGTR1* was associated with higher rates of death and major cardiovascular events in a meta-analysis of EA and AfAn patients with end-stage kidney disease. SNPs in *ACE* were associated with SCD.

Keywords: angiotensin, cardiovascular mortality, genetics, hemodialysis, renin–angiotensin–aldosterone system

INTRODUCTION

Cardiovascular disease is the leading cause of death in patients with end-stage kidney disease (ESKD) receiving dialysis. In the Evaluation Of Cinacalcet Hydrochloride (HCl) Therapy to Lower CardioVascular Events (EVOLVE) trial [1, 2], 3883 patients with ESKD receiving hemodialysis with moderate-to-severe secondary hyperparathyroidism (sHPT) were randomized to either cinacalcet or placebo in addition to standard of care treatments that typically included phosphate binders and calcitriol (1,25-dihydroxy vitamin D) or other active vitamin D analogs. An independent Clinical Events Committee adjudicated all primary and secondary endpoints [1]. The primary composite endpoint of the EVOLVE trial was a composite of all-cause mortality, nonfatal myocardial infarction, hospitalization for unstable angina, heart failure and peripheral vascular event. A *post hoc* analysis [3] found that 54% of all deaths in EVOLVE were due to cardiovascular disease, and of those, nearly one in four were due to sudden cardiac death (SCD). In contrast to the general population, only 4% of deaths were due to acute myocardial infarction. The high preponderance of SCD compared with atherosclerotic events suggests a different pathology than that seen in the general population or in earlier stages of chronic kidney disease (CKD) [4, 5]. Moreover, better cardiovascular survival among patients of African ancestry undergoing dialysis has been found in the USA [6], Canada [7] and the UK [8] even after adjustment for younger age of onset of ESKD and other conventional risk factors, suggesting that genetic variants in African descendants might contribute to better survival.

Single-nucleotide polymorphisms (SNPs) in candidate genes can alter the expression and/or function of the translated protein, and have been investigated to determine their association with cardiovascular disease in patients with ESKD [9], including common genetic variants in the renin–angiotensin–aldosterone system (RAAS). Association results between these genes

and phenotypes in patients receiving dialysis are inconsistent [9–14], likely due to small sample size, lack of adjudicated endpoints, or lack of genetic diversity in previously published studies. To overcome these limitations, we investigated the association between SNPs in angiotensin receptor type I (*AGTR1*) and angiotensin-converting enzyme (*ACE*) and adjudicated cardiovascular endpoints using the large biospecimen and clinical data of the EVOLVE trial.

MATERIALS AND METHODS

Genotyping

DNA was collected at baseline from those patients in the EVOLVE trial who signed a separate consent ($n = 1919$). Each participating center in EVOLVE was asked to participate, but differences in policies and laws surrounding DNA collection for research in the 22 countries limited the number of sites participating in this substudy. Blood samples were processed by the central study laboratory (Covance, Princeton, NJ, USA) and de-identified samples sent to Indiana University and deemed not subject to the Common Rule by the Indiana University Institutional Review Board. After DNA extraction, 1852 samples were genotyped using the Sequenom MassArray system. DNA and genotyping quality assessment have been described previously [2]. Ancestry group was categorized by self-reported race. For this report, we only included patients of European Ancestry (EA; $n = 1067$) and African Ancestry (AfAn, $n = 405$) with nonmissing covariates (final total = 1472) due to relatively small numbers and genetic heterogeneity in other groups (e.g. Asians, Aboriginal and other Native persons and persons of mixed race). Based on previously published literature and reported linkage disequilibrium [9–14] in the targeted genes, we genotyped rs5186 in *AGTR1* and rs4318, rs4335, rs4343, rs4353, rs4359 and rs4363 in *ACE*. There was no significant deviation from Hardy–Weinberg equilibrium for any SNP in either ancestry group (all $P > 0.07$). We estimated linkage disequilibrium separately in each ancestry group (Figure 1A and B).

Statistical methods

Descriptive statistics to characterize the groups, with categorical characteristics summarized as counts/percentages and continuous characteristics summarized as mean with standard deviation or median with 10th and 90th percentiles, are provided in Table 1. The proportion of endpoints in the DNA cohort compared with the larger EVOLVE cohort was similar and is shown in Table 1. We used survival analyses to test for SNP association with the primary composite endpoint from EVOLVE, as well as with all-cause mortality, cardiovascular mortality, SCD and heart failure. Age, sex, vintage (years since starting dialysis), smoking history, treatment (cinacalcet/placebo group assignment and SNP $\times$ treatment interaction were included as covariates in each model [1]. We calculated hazard ratios (HRs) and 95% confidence intervals (95% CIs) using proportional hazards (Cox) regression accounting for the study randomization strata of region in which the study was conducted and presence or absence of diabetes known. We tested

the proportional hazards assumptions using Schoenfeld residuals, and none were violated. We considered death as a competing risk in the evaluation of nonfatal events, where appropriate. For the analyses including competing risks, cumulative incidence rather than survival plots were produced for each genotype, and subdistribution hazards calculated.

Due to known differences in cardiovascular risk in the EA and AfAn populations as well as differences in minor allele frequencies (proportion of the population with a specific nucleotide alteration), we performed the analyses separately within each ancestry group as secondary analyses and then performed a meta-analysis across the two ancestry groups as our primary analysis of interest. Analyses employed an additive genetic model, which assumed a similar increase (or decrease) in phenotype value for each copy of the reference allele. In order to compare results across groups in the meta-analysis, we assigned the minor alleles of the EA group as reference alleles. We employed the R package ‘rmeta’ (<https://cran.r-project.org/web/packages/rmeta/index.html>, 2.16) to assess genetic associations common to both ancestry groups using summary statistics. The inverse standard errors were implemented as weights. We accounted for sample heterogeneity by using a random effects model. One SNP, rs4318, was monomorphic in the EA subgroup (i.e. all EA individuals had the same genotype) and therefore could only be analyzed in the AfAn subgroup. Given that the SNPs in *ACE* were highly correlated (in strong linkage disequilibrium) and the phenotypes are interrelated, we did not correct for multiple testing and considered two-tailed P -values < 0.05 statistically significant. We performed all analyses using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and R.

RESULTS

Baseline demographic and laboratory data, and the number of endpoint events are provided in Table 1. A total of 1472 patients were analyzed (1067 EA and 405 AfAn samples). In general, characteristics of patients who were analyzed were similar to the overall EVOLVE cohort with multiple risk factors for cardiovascular disease and similar proportion of events (Table 1). Alleles and minor allele frequency (MAF) for SNPs in the *AGTR1* and *ACE* genes are shown in Table 2. The SNP rs4318 was monomorphic and therefore not analyzed in the EA sample; Figure 1 shows (linkage disequilibrium) patterns among SNPs in *ACE*.

Association of SNPs with patient outcomes

All meta-analysis heterogeneity P -values were > 0.20 , suggesting there was no significant heterogeneity between the two population groups, and meta-analysis of the EA and AfAn summary statistics was performed. The SNP in *AGTR1* (Supplementary data, Table S1), rs5186, showed significant association with the primary composite endpoint [HR 1.31, 95% CI (1.15–1.49), $P = 4.3 \times 10^{-5}$], all-cause mortality [1.28 (1.03–1.51), $P = 0.001$], cardiovascular mortality [1.25 (1.03–1.51), $P = 0.024$] and heart failure [1.34 (1.03–1.75), $P = 0.03$], but not SCD ($P = 0.68$). The C allele of rs5186 was associated with a significantly increased risk (25–34%) for the four endpoints. In the EA and AfAn subgroups, the magnitude and direction of the

FIGURE 1: Linkage disequilibrium plots for *ACE*: linkage disequilibrium plots for the *ACE* SNPs examined in the EA population (A = EA D' , B = EA r^2), and the AfAn population (C = AfAn D' , D = AfAn r^2). SNPs in higher linkage disequilibrium are represented by darker squares. Higher measures of linkage disequilibrium indicate higher correlation between SNP genotypes, and should therefore result in similar P-values.

HRs were similar (Supplementary data, Table S1). Figure 2 shows Kaplan–Meier curves for these endpoints by the rs5186 genotypes.

We observed no significant associations among SNPs in *ACE* and the five endpoints in meta-analysis and no significant SNP $\times$ treatment interactions. However, in EA samples, three correlated SNPs in *ACE* were associated with a 26–27% reduction in risk of SCD: rs4335 [0.74 (0.56–0.99), $P = 0.004$], rs4343 [0.74 (0.55–0.99), $P = 0.04$] and rs4353 [0.73 (0.54–0.98), $P = 0.036$] (Supplementary data, Table S1). In addition, the SNP rs4318 in *ACE* was associated with a 70% reduction in SCD for each copy of the G allele in the AfAn sample [0.30 (0.10–0.85), $P = 0.03$; Supplementary data, Table S1].

Association of RAAS SNPs with mineral metabolism

Recent studies have demonstrated that the RAAS system has a role in the regulation of vitamin D, fibroblast growth factor 23 (FGF23) and klotho [11, 12] and that elevated FGF23 is associated with cardiovascular disease [13]. Therefore, as a secondary analysis, we explored whether baseline serum concentrations of parathyroid hormone (PTH), FGF23 and 25-hydroxyvitamin D in patients were associated with the SNPs in *ACE* and *AGT1R* and found no significant associations (Supplementary data, Table S2). In addition, no SNP $\times$ treatment interactions were significant for these biochemical measures (Supplementary data, Table S3).

Table 1. Demographic and baseline laboratory values for DNA cohort compared with the full EVOLVE trial

	EA <i>n</i> = 1067	AfAn <i>n</i> = 405	EVOLVE cohort (<i>n</i> = 3883) ^a
Cinacalcet use, <i>n</i> (%)	551 (51.6)	203 (50.1)	1948 (50.2)
Demographics			
Age, years, mean (SD)	56.4 (14.0)	52.2 (13.4)	54.4 (14.4)
Sex (female), <i>n</i> (%)	406 (38.1)	171 (42.2)	1578 (40.6)
Ethnicity, <i>n</i> (%)			
EA	1067 (58.9)		2241 (57.7)
AfAn		405 (22.3)	837 (21.6)
Other			805 (20.7)
Dialysis vintage (months)			45.0 (9.0, 146.0)
<2 years, <i>n</i> (%)	315 (29.5)	138 (34.1)	1098 (28.3)
2 to <5 years, <i>n</i> (%)	340 (31.9)	141 (34.8)	1285 (33.1)
≥5 years, <i>n</i> (%)	412 (38.6)	126 (31.1)	1499 (38.6)
Current or previous smoker, <i>n</i> (%)	476 (44.6)	170 (42.0)	1696 (43.8)
Abnormal baseline ECG, <i>n</i> (%)	617 (59.8)	284 (72.4)	2263 (58.3)
Diabetes (Type 1 or Type 2), <i>n</i> (%)	295 (27.6)	181 (44.7)	1302 (33.5)
Chronic atrial fibrillation, <i>n</i> (%)	76 (7.1)	18 (4.4)	227 (5.8)
Arrhythmia of any type, <i>n</i> (%)	184 (17.2)	53 (13.1)	554 (14.3)
Hypertension, <i>n</i> (%)	954 (89.4)	402 (99.3)	3577 (92.1)
Coronary artery bypass graft <i>n</i> (%)	83 (7.8)	19 (4.7)	289 (7.4)
Myocardial infarction, <i>n</i> (%)	154 (14.4)	42 (10.6)	483 (12.4)
Heart failure, <i>n</i> (%)	239 (22.4)	134 (33.1)	906 (23.3)
Stroke, <i>n</i> (%)	108 (10.1)	43 (10.6)	355 (9.1)
Peripheral arterial disease, <i>n</i> (%)	185 (17.3)	55 (13.6)	635 (16.4)
Amputation, <i>n</i> (%)	53 (5.1)	31 (7.7)	250 (6.4)
Baseline drug use			
Calcium-based phosphate binders, <i>n</i> (%)	669 (62.7)	150 (37.0)	2062 (53.1)
Baseline vitamin D, <i>n</i> (%)	650 (60.9)	314 (77.5)	2310 (59.5)
Baseline laboratory values			
Corrected Ca, mean (SD), mg/dL	9.8 (0.72)	9.8 (0.59)	9.8 (0.7)
Phosphorus, mean (SD), mg/dL	6.6 (1.5)	6.3 (1.4)	6.5 (1.4)
Intact PTH, median pg/mL (p10, p90)	720 (371, 1698)	611 (338, 1406)	693.0 (363, 1694)
FGF23, median pg/mL (p10, p90)	5970 (680, 19 948)	5080 (540, 19 128)	5590 (580, 19 540)
Potassium, mean (SD) (mg/dL)	5.3 (0.76)	4.6 (0.69)	5.1 (0.78)
Hemoglobin, mean (SD) (mg/dL)	11.8 (1.5)	11.8 (1.4)	11.8 (1.5)
Total cholesterol, mean (SD) (md/dL)	174.0 (46.4)	160.2 (40.5)	166.8 (43.4)
HDL cholesterol, mean (SD) (mg/dL)	41.6 (13.6)	47.6 (17.2)	43.4 (15.0)
Triglycerides, mean (SD) (mg/dL)	186 (134)	144 (99)	169 (120)
Number of events (% of patients)			
EVOLVE composite endpoint	524 (49.2)	231 (52.6)	938 (48.2)
All-cause mortality	399 (37.4)	133 (32.8)	1421 (36.6)
Cardiovascular mortality	221 (20.1)	73 (18.1)	768 (19.8)
SCD	89 (8.3)	37 (9.1)	572 (14.7)
Heart failure	99 (9.3)	81 (20.0)	442 (11.4)

^aFrom references 1 and 2.

ECG, electrocardiogram; HDL, high-density lipoprotein.

Table 2. Summary of SNPs and MAF

CHR	SNP	Gene	EA			AfAn			Reference allele ^a
			Minor allele	Major allele	MAF	Minor allele	Major allele	MAF	
3	rs5186	<i>AGTRI</i>	C	A	0.28	C	A	0.05	C
17	rs4318	<i>ACE</i>	G	A	0.00	G	A	0.16	G
17	rs4335	<i>ACE</i>	A	G	0.47	G	A	0.23	A
17	rs4343	<i>ACE</i>	A	G	0.47	G	A	0.22	A
17	rs4353	<i>ACE</i>	G	A	0.47	G	A	0.41	G
17	rs4359	<i>ACE</i>	C	T	0.46	T	C	0.42	C
17	rs4363	<i>ACE</i>	A	G	0.46	G	A	0.42	A

SNPs with different minor alleles in the two racial groups are given in bold.

^aThe reference allele indicates the allele used to interpret the direction of effect in analyses. SNPs with different minor alleles in the two racial groups are bolded.

CHR, chromosome.

FIGURE 2: Probability of endpoint events and SNP rs5186 of the *AGT1R* gene: unadjusted Kaplan–Meier Curve for survival, or being free from the endpoints in [Supplementary Table 1](#): primary composite endpoints from the EVOLVE study (death or first non-fatal myocardial infarction, unstable angina, heart failure or peripheral vascular event), in the EA population (**A**) and AfAn population (**B**); all-cause mortality (EA, **C**; AfAn, **D**); cardiovascular mortality (EA, **E**; AfAn, **F**); and heart failure (EA, **G**; AfAn, **H**). Each genotype group is represented by a different colored line as shown in the legend in each panel, which denotes whether there is one or two adenine (A) or cytosine (C) alleles at position 1166 of the gene. The color-shaded area represents the 95% CI.

DISCUSSION

The results of the present study include a novel association between the SNP rs5186 in *AGTR1* and the EVOLVE primary composite endpoint (sum of all-cause mortality, nonfatal myocardial infarction, hospitalization for unstable angina, heart failure and peripheral vascular event), as well as all-cause mortality, cardiovascular mortality and heart failure individually. To our knowledge, this association has not been previously reported in patients undergoing dialysis. A much smaller study, of 80 patients with diabetes mellitus receiving dialysis in Spain, suggested an association with the same SNP and mortality; however, the association in that study did not reach statistical significance, likely due to a small sample size [14]. Buraczynska *et al.* found a high frequency of the combined AC and CC genotypes in rs5186 in patients receiving dialysis compared with healthy controls; these alleles were also associated with shorter time from diagnosis of kidney disease to the need for dialysis suggesting faster progression [15]. Raizada *et al.* found the CC genotype in rs5186 was associated with a prolonged QTc interval in patients receiving hemodialysis; the finding was more pronounced in patients with the *ACE* two deletion alleles (DD) genotype. This would suggest increased risk of arrhythmias; however, we did not find an association between rs5186 and SCD in the current population [16]. In patients with at least one copy of the C allele in *AGTR1*, there is a more pronounced decrease in blood pressure in response to angiotensin receptor blockers [17, 18] but not angiotensin-converting enzyme inhibitors [19]. Our results demonstrate a higher likelihood of adverse events with the C allele, which at first seems counterintuitive. However, if patients have lower blood pressure, it is possible that they have more difficulty with hemodialysis.

The importance of the RAAS in cardiovascular health is well known. The *AGTR1* receptor is widely expressed in tissue known to regulate metabolism and cardiovascular disease including heart, kidney, adrenal gland, brain, vascular and adipose tissue. Activation of the *AGTR1* receptor leads to multiple cell signaling pathways leading to diverse effects on vascular and cardiac remodeling, hypertension, fibrosis, inflammatory response and endothelial function. The rs5186 SNP is located in the 3' untranslated region and is a binding site for micro-RNA-155 (miR-155). *AGTR1* and miR-155 are co-expressed in most tissues and miR-155 translationally represses the expression of *AGTR1* in the presence of the A allele but not the C allele [20]. Chen *et al.* have previously demonstrated decreased circulating levels of miR-155 in patients with CKD and those undergoing dialysis [21]. Further, expression of miR-155 was decreased and *AGTR1* was increased in the aorta of rats with CKD compared with controls and lower levels of miR-155 were associated with increased arterial calcification in rats with CKD [21]. Thus, it is plausible that the combination of the abnormal binding site of miR-155 on the *AGTR1* in the presence of the rs5186 risk allele and low levels of locally expressed and circulating miR-155 in kidney disease act together to induce multiple adverse cardiovascular effects. These results require confirmation in other cohorts that include event adjudication, especially for heart failure and SCD.

Three correlated SNPs in the *ACE* gene, rs4335, rs4343 and rs4353, were associated with SCD reducing the risk by 26% in EA samples. A nearby SNP, which was polymorphic only in the AfAn sample, was associated with a 70% reduction in risk for the AfAn subgroup. To our knowledge, this is the first report of an association of SNPs in the *ACE* gene and SCD. SCD is the leading cause of cardiovascular death in patients undergoing dialysis accounting for one-fourth of the adjudicated cardiovascular deaths in the EVOLVE trial [1]. The rapid shifts in fluid and electrolytes with dialysis may predispose to SCD in patients with ESKD, but the risk of SCD increases with progressive worsening of kidney disease even prior to the need for dialysis, suggesting additional underlying causes. Our data suggest that genetics may play a prominent role.

The majority of the literature focusing on the *ACE* gene and cardiovascular disease in patients receiving dialysis has examined the 287 base pair insertion deletion (InDel) in intron 16 of the *ACE* gene. Early studies in EA and Asian populations, as well as in the Pima Indians, indicated that the InDel accounted for up to 47% of the variance in circulating *ACE* levels, with highest levels in those with two deletion alleles (DD) (reviewed in [22]). In contrast, there was no association between the InDel and circulating *ACE* levels in African Americans [23]. A meta analyses of 52 studies with 3663 case-patients and 8953 controls found the DD genotype to be the risk genotype associated with a 59% (95% CI 21–92%) increased risk of left ventricular hypertrophy (LVH) in East Asians [24]. No patients of AfAn were included.

Associations with the InDel and cardiovascular events in patients undergoing dialysis have been contradictory: the InDel was not associated with LVH in populations from Turkey or China, but was associated with LVH and cardiovascular events in Japanese patients [25, 26]. In the Netherlands, the DD genotype was associated with mortality in patients receiving dialysis but it was not in similarly sized studies in Italy and Brazil [27]. This InDel is reported to be in high linkage disequilibrium with rs4343 in EA populations, such that the A allele of rs4343 tags the Insertion allele, which acted in a protective fashion, and the G allele tags the Deletion allele, the risk allele as reported above [28, 29]. In our EA samples, the A allele of rs4343 was associated with a 25% reduced risk of SCD. A different SNP in *ACE*, rs4318, was associated with a 70% reduction in SCD in AfAn samples; however, that allele is rare in EA. Thus, ancestry may explain some of the seemingly disparate associations previously reported in the literature for the *ACE* gene.

The rationale to separately analyze the EA and AfAn populations, and then meta-analyze the population-specific results, was based on known ancestry differences in cardiovascular risk, as well as substantial differences in the allele frequencies. Taking a different approach, Wetmore *et al.* determined the presence of seven different polymorphisms in the *ACE* gene among 229 racially diverse patients with ESKD from San Francisco [30]. The authors found the haplotypes of rs4343, rs4359 and rs4363 in *ACE* were associated with survival regardless of race [30]. However, this study analyzed three racial groups (EA, AfAn and Asian) together, adjusting for race in the model. Given the differences in cardiovascular risk and

variation in allele frequencies, the ‘combined approach’ of this ESKD study may not have been sufficiently robust to permit population stratification. Our meta-analysis of a larger sample did not detect evidence of an association of *ACE* SNPs with overall mortality, although there was some evidence for an association with SCD.

This study has several strengths. First, it is the largest genetic study in patients on dialysis to date. Second, patients were followed for up to 64 months. Third, cardiovascular endpoints were adjudicated. Fourth, we evaluated two large racial groups. Fifth, we maximized the power of the study by utilizing meta-analysis as our primary analysis. However, there were several limitations. Patients in EVOLVE were enrolled because of moderate-to-severe sHPT despite therapy with phosphate binders and calcitriol or active vitamin D analogs; thus, these findings may not be generalizable to all patients receiving hemodialysis or to other patients with ESKD. While EVOLVE was the largest (in size) and longest (in duration) clinical trial ever conducted in patients with ESKD, the sample size may not be sufficient to identify clinically relevant associations between analyzed SNPs and cardiovascular events, particularly in the AfAn populations or to identify other significant effect modifiers.

In conclusion, utilizing stored samples and adjudicated endpoint data from EVOLVE, we found evidence of a novel association between the SNP rs5186 in the *AGTRI* gene and multiple endpoints in EVOLVE, including the primary composite endpoint, all-cause mortality, cardiovascular mortality and heart failure. Further, SCD, the leading cause of cardiovascular death in patients undergoing dialysis, was associated with SNPs in *ACE* in each EA and AfAn samples separately but we did not confirm previously published studies demonstrating an association of *ACE* with mortality. These findings indicate that genotypic variation in *AGTRI* in EA and AfAn and *ACE* in AfAn accounts for higher and lower death rates, respectively, in patients with ESKD.

SUPPLEMENTARY DATA

Supplementary data are available at [ndt](http://ndt.oxfordjournals.org/) online.

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AUTHORS’ CONTRIBUTIONS

S.M.M., B.S.D., H.J.E., M.V., T.M.F. and G.M.C. contributed to the study design. Experimental and statistical analyses was conducted by S.M.M., J.L., T.-H.L.S.-A., L.W., H.J.E., X.X., T.M.F. and G.M.C. All authors contributed to writing and review of manuscript.

CONFLICT OF INTEREST STATEMENT

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