HHS Public Access

Author manuscript

Cardiorenal Med. Author manuscript; available in PMC 2023 August 23.

Published in final edited form as:

Cardiorenal Med. 2022 ; 12(5-6): 229–235. doi:10.1159/000525448.

APOL1 G3 variant is associated with cardiovascular mortality and sudden cardiac death in patients receiving maintenance hemodialysis of European Ancestry

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Abstract

Introduction: The G1 and G2 variants in the *APOL1* gene convey high risk for the progression of chronic kidney disease (CKD) in African Americans. The G3 variant in *APOL1* is more common in patients of European Ancestry (EA); outcomes associated with this variant have not been explored previously in EA patients receiving dialysis.

Methods: DNA was collected from approximately half of the patients enrolled in the Evaluation of Cinacalcet HCl Therapy to Lower Cardiovascular Events (EVOLVE) trial and genotyped for the G3 variants. We utilized an additive genetic model to test associations of G3 with the EVOLVE adjudicated endpoints of all-cause mortality, cardiovascular mortality, sudden cardiac death (SCD), and heart failure (HF). EA and African Ancestry (AfAn) samples were analyzed

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Author Contributions: Sharon Moe, Glenn Chertow, Tatiana Foroud, Tae-Hwi Schwantes-An, Matteo Vatta, and Howard J. Edenberg designed the study. Sharon Moe and Glenn Chertow collected the data as part of their involvement with the EVOLVE study and study design. Tae-Hwi Schwantes-An, Sai Liu, Margaret Stedman, Leah Wetherill performed the genetic/statistical analyses, all authors participated in data interpretations. Tae-Hwi Schwantes-An and Sharon Moe drafted the manuscript and all authors approved the final version.

Statement of Ethics: The study was considered ‘not subject to the common rule’ by the Indiana University Institutional Review Board, as the samples were pre-existing and de-identified, with prior consent through the EVOLVE clinical trial and confirmed by Amgen prior to release of specimens to Indiana University.

separately. Validation was done in the Vanderbilt BioVU using ICD codes for cardiovascular events that parallel the adjudicated endpoints in EVOLVE.

Results: In EVOLVE, G3 in EA patients were associated with the adjudicated endpoints of cardiovascular mortality and sudden cardiac death. In a validation cohort from the Vanderbilt BioVU, cardiovascular events and cardiovascular mortality defined by ICD codes showed similar associations in EA participants who had been on dialysis for 2 to <5 years.

Discussion/Conclusions: G3 in *APOL1* variant was associated with cardiovascular events and cardiovascular mortality in the EA patients receiving dialysis. This suggests that variations in the *APOL1* gene that differ in populations of different ancestry may contribute to cardiovascular disease.

Keywords

genetics; cardiovascular disease; dialysis; sudden cardiac death

INTRODUCTION:

Studies of abnormalities in the apolipoprotein-L1 gene (*APOL1*) have dramatically increased our understanding of the burden of end-stage kidney disease (ESKD) in persons of African ancestry (AfAn). Three genetic variants within the exon 7 of the gene (two missense single nucleotide polymorphisms [SNPs] termed G1 and a 6 base pair deletion termed G2) confer independent risks for progressive kidney disease[1, 2]. G1 and G2 are rarely seen in persons of European ancestry (EA)[1, 2]. The high prevalence of G1 and G2 in AfAn individuals is thought to arise from genetic selection due to the protective effects of the variants against African sleeping sickness caused by *Trypanosoma brucei gambiense* in Western Africa[3]. The mechanism by which the G1 and G2 variations in *APOL1* accelerate loss of (or decline) kidney function remains elusive, as detailed in recent reviews[4, 5], with potential mechanisms including alteration of cytoskeletal abnormalities, endocytic trafficking due to impaired acidification[6], mitochondrial dysfunction[7] and ion transport[8] defects in the podocyte. These same pathways that damage kidney podocytes can also damage cardiac myocytes and lead to arrythmias and fibrosis, providing a rationale for studies examining the association of G1 and G2 variants and cardiovascular risk in AfAn patients with and without CKD, the results of which have been conflicting[2, 9]. A recent larger analysis failed to find an association of *APOL1* with cardiovascular events in AfAn patients[10].

Ko et al.[3] sequenced *APOL1* in samples from multiple areas of Africa and identified a group of 8 SNPs in the same exon of *APOL1* that were in high linkage disequilibrium (LD), and termed G3. G3 was common in those of non-Yoruban descent, including the Fulani (21%), Bakola (11%), Sengwer (11%) and Iraqw (13%), populations[3]. Palmer and colleagues tested these G3 SNPs in AfAn patients and found no association with diabetic or non-diabetic ESKD compared to controls[11]. The minor allele frequencies (MAFs) of G3 SNPs are higher in persons of European ancestry (EA) than in AfAn, but no studies to date had evaluated the association of G3 SNPs and cardiovascular risk in an EA population, t. That is the focus of the current study.

MATERIALS and METHODS:

Primary Cohort:

The study used DNA collected from 1,919 patients randomized in the international multi-center trial “Evaluation of Cinacalcet HCl Therapy to Lower Cardiovascular Events (EVOLVE),” as previously described[12]. The study was considered ‘not subject to the common rule’ by the Indiana University Institutional Review Board, as the samples were pre-existing and de-identified, with prior consent through the EVOLVE clinical trial and confirmed by Amgen prior to release of specimens to Indiana University. At the time of collection, blood samples were collected by Covance (Princeton NJ) and de-identified samples sent to Indiana University for DNA extraction. Using the Sequenom MassArray system, 1,852 DNA samples met quality assessment (determined by A260/280 and A260/230 ratios), and the detection of SNPs > 70% (7 samples excluded). Additional SNPs were genotyped on the X and Y chromosomes to confirm self-reported sex and X- and Y-linked SNPs and 33 samples were excluded. The remaining 1,812 samples were evaluated Self-reported ancestry was collected as White/Caucasian (58.7%), Black/African (22.5%), Hispanic (12.2%) and other (3.8%). Given the small numbers in Hispanic and other categories we focused on individuals from the EA (n=1,083: 53.4% Europe, 21.9% Russia, 16.4% USA, 5.2% Canada, 3.1% Latin America) and AfAn (n=411; 92.8% USA, 4.7% Europe, and 2.5% Canada) groups for the EVOLVE analyses.

Supplemental Figure 1 shows SNPs in the G1, G2 and G3[3]. In EA patients, all 8 G3 SNPs are in complete LD ($r^2 = 1$). In AfAn patients there is complete LD with all SNPs ($r^2 = 1$) except rs136177 ($r^2 = 0.4$). Therefore, we chose to genotype rs136177 due to the differences, and rs136175 and rs136176 as representative of the other 7 SNPs in complete LD. All three SNPs passed Hardy-Weinberg equilibrium (HWE) tests (among EA all $p>0.50$ and among AfAn all $p>0.32$).

An independent review panel adjudicated major cardiovascular end-points as defined by the EVOLVE trial[12]. The index date was study enrollment and participants were followed for up to 64 months. We evaluated the associations of SNPs with the following endpoints: all-cause mortality, cardiovascular mortality, sudden cardiac death (SCD), and heart failure (HF). We used a global test combining zero-slope tests on the scaled Schoenfeld residuals plotted against time to examine the proportional hazards assumption for each covariate in the model. For the all-cause mortality endpoint, we performed a standard Cox proportional hazards regression analysis. For the cardiovascular and sudden death and for the non-fatal heart failure endpoint, we applied the Fine-Gray modification of the Cox model to account for competing (non-cardiovascular and all-cause mortality, respectively) risks. We constructed Kaplan-Meier survival plots for each genotype, and calculated sub-distribution hazards. We stratified all models by site and presence of diabetes and sites. Covariates included age, sex, vintage (number of years since starting dialysis), history of smoking (current or past), and treatment assignment (placebo/cinacalcet)[12].

We utilized an additive genetic model, which assumed a similar increase (or decrease) in the hazard ratio for each copy of the effect allele. We analyzed EA and AfAn samples separately to account for the known differences in minor allele frequencies (Table 1) and

cardiovascular risks [14, 15]. To compare results across EA and AfAn subsets, we assigned the minor alleles of the EA group as the reference alleles in both EA and AfAn samples. For multiple testing correction, we used the Bonferroni method for the number of independent outcomes and SNPs that are not in complete LD. There were four outcomes total and in EA, all the SNPs were in complete LD, thus only one independent variant was tested. In AfAn, rs136175 and rs136176 were in complete LD so a total of two independent variants were tested. In total, we adjusted for 12 tests which led to a post correction p-value significance threshold of 0.004. We performed all analyses using SAS 9.4 (SAS Institution Inc, Cary, NC) and R (version 3.4.3) with survival, cmprsk, and survminer packages.

Validation Cohort:

Additional cohorts of patients receiving hemodialysis with adjudicated endpoints were not available. Therefore, we chose to validate with the BioVU dataset at Vanderbilt University, as previous studies had examined patients receiving hemodialysis and cardiovascular events looking at Apol1 G1/G2 alleles in AfAn patients [10]. BioVU as a resource, including its ethical, privacy and other protection, has been previously described[16, 17]. In brief, BioVU accrues DNA samples during routine clinical care from Vanderbilt University Medical Center (VUMC) patients who have not opted out of participation, using blood that would otherwise be discarded after clinical testing. The samples are de-identified and considered “non-human subjects” research. Samples and genetic data within BioVU can be linked via anonymous research unique identifiers to the Synthetic Derivative, a de-identified version of VUMC’s electronic health record (EHR), comprising records from approximately 3.2 million patients with detailed longitudinal clinical data dating back to the 1990s. The Synthetic Derivative database is research-enabled and includes diagnostic and procedure billing codes (“PheCodes”); basic demographics; text from clinical care notes; laboratory values; inpatient and outpatient medication data; and other diagnostic reports[18]. The index date was defined as 60 days after the initiation of either peritoneal or maintenance hemodialysis. Given differences in index date in the two cohorts, we stratified the BioVU data by vintage: <2, 2 to <5 years on dialysis, and >=5 years on dialysis such that the percentages of subjects in each vintage cohort was similar to that in the EVOLVE DNA dataset. Cardiovascular events were defined by ICD9/10 codes that were similar to events defined in the EVOLVE cohort (Supplemental Table 1). Cardiovascular mortality was defined as death within 30 days of a cardiovascular event diagnosis. Statistical analyses were identical to those applied to the participants in EVOLVE except no stratification for site (not applicable) and no adjustment for smoking (not available).

RESULTS:

Table 1 shows minor allele frequencies (MAFs), minor alleles, and population frequencies of each tested SNP. Baseline demographics and laboratory values for patients undergoing dialysis who were genotyped in both EVOLVE[12] and BioVU were similar. Of note, the percent of patients in each category of dialysis vintage in the BioVU cohort (34%, 32%, and 34% for < 2 years, 2 to 5years, and ≥ 5 years) was similar to that in EVOLVE (28, 33, 39%). In the EVOLVE EA subset, rs136175 in G3 showed significant associations with cardiovascular mortality (SHR=1.44, p-value=0.0006, risk allele=G) and sudden cardiac

death (SHR=1.69, p-value=0.0009, risk allele=G) (Table 2, Figure 1). We then conducted a sensitivity analysis to determine a potential finding related to population stratification. In EVOLVE, the EA population is primarily from North America (23%), Western Europe (53%), and Russia (21%), where the minor allele frequency of rs126175 is 17, 20, and 21% respectively. We performed additional analyses using the same model within these three groups for sudden cardiac death and observed similar directionality of hazard ratios in the North American population of 2.1 (25% event rate), European population of 1.4 (7% event rate) and Russian population 1.3 (6% event rate), supporting that our findings are not likely due to population substructure. There were no statistically significant associations between any SNP, all-cause mortality and heart failure in the EVOLVE EA subset (Supplemental Table 2). Table 3 shows the association of G3 alleles with cardiovascular events and cardiovascular mortality in the BioVU EA population. The rs136175 G3 allele showed significant associations with cardiovascular mortality, but only in patients with dialysis vintage of 2 to <5 years. A similar trend was observed for all cardiovascular events.

In the AfAn patients, there were no statistically significant associations between the G3 SNPs in the *APOL1* gene and any end point in the EVOLVE or BioVU cohort (Supplemental Tables 2 and 4).

DISCUSSION/CONCLUSION:

To our knowledge, this report represents the first observed association between G3 in the *APOL1* gene and cardiovascular mortality and sudden cardiac death, in individuals from the EVOLVE cohort undergoing dialysis and of European Ancestry. Sudden cardiac death, adjudicated in the EVOLVE cohort, accounted for 25% of all cardiovascular deaths, whereas acute myocardial infarction only accounted for 4%[19]. The contribution of sudden cardiac death to cardiovascular mortality among patients receiving maintenance hemodialysis may explain why sudden cardiac death and cardiovascular death, but not all-cause mortality, were associated with G3. To date, studies evaluating cardiovascular outcomes and *APOL1* variants have only examined the G1 and G2 variants, and these have yielded mixed results. The Jackson Heart study[20], Systolic Blood Pressure Intervention Trial (SPRINT)[21], and Cardiovascular Health Study (CHS[22], and the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study[23] all showed increased risk of composite cardiovascular events or incident myocardial infarction with the G1 or G2 versus low risk haplotypes in African-Americans. In contrast, the African American Study of Kidney Disease and Hypertension (AASK)[24], and Multi-Ethnic Study of Atherosclerosis Risk in Communities Study (MESA)[24] found no difference in incident CVD. A meta-analysis using individual patient data from multiple studies found that the APOL1 G1/G2 variants in 21,305 Black Americans were not associated with cardiovascular or all-cause mortality[25]. None of those studies evaluated G3 variants, but with such a low frequency of the G3 allele, our sample size is inadequate to test this variant.

In the current study, we found that G3 variants were associated with cardiovascular death and sudden cardiac death in EA patients in the EVOLVE cohort. In the BioVU cohort, a similar association was seen for cardiovascular death assessed by ICD codes, but only in those patients on dialysis for 2 to 5 years. The two cohorts differ in the ascertainment

of events-adjudicated in EVOLVE by an independent panel consisting of cardiologists and nephrologists, and in BioVU, defined as death within 30 days after a CV event. Thus, it is not surprising that some differences were observed. The cohorts also differ in the definition of index date- start of the study for the EVOLVE cohort, and the start of dialysis + 60 days for the BioVU cohort. The potential impact of dialysis vintage is not surprising, as there are multiple reasons for mortality in patients with end-stage kidney disease who are starting dialysis, including many related to insufficient vascular access (the majority of patients in the US start dialysis with a tunneled hemodialysis catheter) or complications of protein energy malnutrition, particularly among those patients who were marginal candidates for dialysis. Long-term survivors on dialysis are also different in that they have survived the cardiac insults of maintenance dialysis and in EVOLVE the cohort of long dialysis patients were also those with secondary hyperparathyroidism and thus a selective group. Further, in both cohorts those most susceptible for cardiovascular disease associated with G3 variants may had already experienced a major cardiovascular event or death (e.g., selection effects). It is also possible that rather than defining a genetic association that we are instead identifying a population. However, this seems unlikely given the analyses stratified by study site, the diverse origin of patients in the EA cohort, consistent direction of HR in our sensitivity analyses, and similar allele frequency throughout European Ancestry assessed in 1000 genome data. The reported (and graphed) association is a summarized estimate across all strata.

The potential mechanism(s) by which the G3 variants may lead to sudden cardiac death are unknown. The G1 and G2 variants alter potassium, chloride and calcium channel function/activity, cell lysis, and cytoskeleton function (Reviewed in[8, 5]; therefore, G3 variants may have a similar effect resulting in depolarization abnormalities or cell death that may lead to arrhythmia sudden cardiac death. In addition, *APOL1*G1/G2 gene expression can be induced by interferon gamma, and induced by some, but not all viruses. Thus, CKD progression may requires a ‘two hit’ model and inflammation is thought to be the second hit[5]. It is also therefore plausible that inflammation may also play a role in the G3 association with CV mortality. All three variants, (G1, G2, G3) occur in the region encoding the serum resistance associated (SRA)-interacting-domain, modifying the resistance to the common (and chronic) West African form of sleeping sickness caused by *Trypanosoma brucei gambiense*. It remains unknown if G3 protects against the less common forms of sleeping sickness seen in Eastern and Southern Africa[3] or other infectious diseases. Thus, although the variants are all from the SRA domain, differences in function remain possible.

In summary, we highlight a novel association between G3 *APOL1* variants and cardiovascular mortality and sudden cardiac death in patients of European Ancestry receiving maintenance hemodialysis, results validated but limited to patients with moderate (but not short or long) dialysis vintage in the BioVU cohort. The major limitation is that the samples size is underpowered in both cohorts, and thus, these findings, however provocative, need to be confirmed. Another limitation is the use of diagnosis codes in the BioVU dataset to define events. Unfortunately, large clinical trials in ESKD with adjudicated end points are very few in number, and even fewer collect DNA. If validated, these findings suggest that variations in the *APOL1* gene may contribute to cardiovascular disease, as well as to kidney disease, depending on ancestry.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

The authors wish to thank Deb Abbott for her administrative support in preparation of this manuscript.

Funding Sources:

This work was supported by NIH R01DK100306 to Sharon Moe and Glenn Chertow. The EVOLVE trial was conducted and supported by AMGEN.

Conflict of Interest Statement:

Dr. Moe reports grants from Chugai, Keryx, Allena grants from NIH, consulting fees from Amgen, Sanifit, and Ardelyx outside the submitted work. Dr. Chertow reports personal fees from Akebia, grants and personal fees from Amgen, personal fees and other from Ardelyx, AstraZeneca, Baxter, Gilead, Reata, Sanifit, Vertex, other from CloudCath, other from Durect, Miromatrix, Outset Medical, Unicycive, all outside the submitted work. Dr. Schwantes-An reports consulting fees from Target RWE. The other authors report no disclosures.

Data Availability Statement:

The data for this manuscript were generated from samples provided, with permission, by AMGEN. Data will be shared upon reasonable request to the corresponding author but will also require the requestor to obtain an MTA with AMGEN by contacting Dr. Sunfa Cheng (sunfac@amgen.com).

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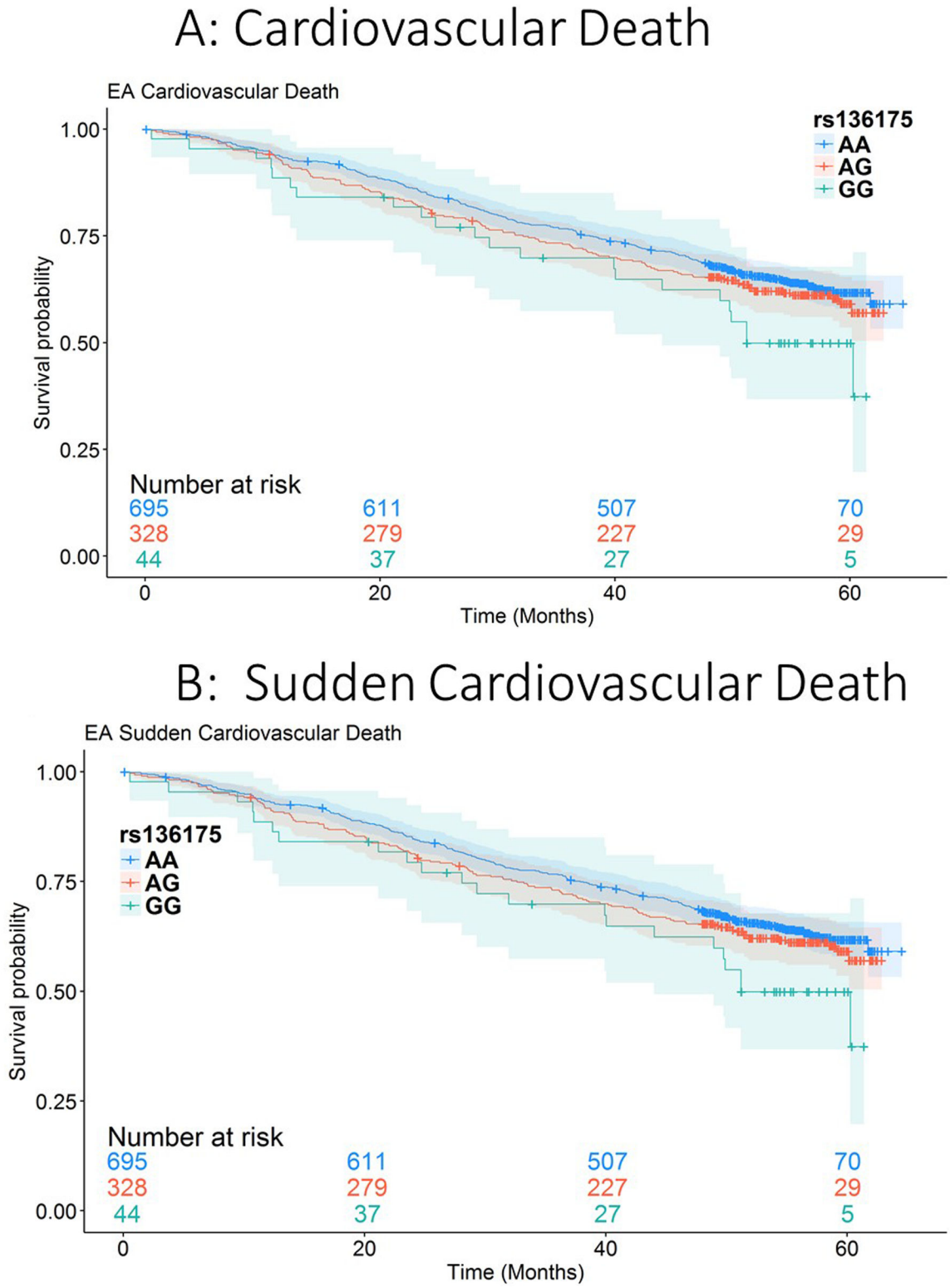

Figure 1: Probability of endpoint events by rs531675 genotype of the *APOL1* gene in the EA sample:

Unadjusted Kaplan–Meier Curve of cardiovascular-specific survival (1A) or sudden cardiac-specific survival (1B). 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 guanine (C) alleles. The color-shaded area represents the 95% confidence interval.

Table 1:

Summary of *APOL1* SNPs and Minor Allele Frequency (MAF):

SNP shows rsID of each genotype variant. Function is molecular function of each SNP. EA indicates European Ancestry samples from EVOLVE. AfAn indicates African Ancestry samples from EVOLVE. Eur indicates European ancestry samples from the 1000 Genomes Project. Afr indicates African ancestry samples from the 1000 Genomes Project. HWE P-value indicates Hardy-Weinberg Equilibrium test p-value for each genotyped variant in EVOLVE samples.

				EVOLVE COHORT		1000 GENOMES PROJECT		EVOLVE COHORT	
SNP	**Function**	**EA Minor Allele (Effect Allele)**	**AfAn Minor Allele**	**EA MAF**	**AfAn MAF**	**Eur MAF**	**Afr MAF**	**EA HWE P-value**	**AfAn HWE P-value**
rs136175	Missense	G	G	0.19	0.04	0.22	0.02	0.50	0.41
rs136176	Missense	G	G	0.20	0.03	0.22	0.02	0.56	0.32
rs136177	Missense	G	G	0.20	0.05	0.22	0.05	0.63	< 1.0

SNP = single nucleotide polymorphism

Table 2:

Association of *APOL1* G3 SNPs and outcomes in European Ancestry in the EVOLVE cohort

Cardiovascular Mortality	SHR [95%CI]	P value	Event	Censored	Competing
rs136175	1.44 [1.17–1.77]	**0.0006**	221	668	178
rs136176	1.44 [1.17–1.77]	**0.0006**	221	668	178
rs136177	1.42 [1.15–1.75]	**0.0009**	221	668	178
Sudden Cardiac Death					
rs136175	1.69 [1.24–2.31]	**0.0009**	89	668	310
rs136176	1.69 [1.24–2.30]	**0.001**	89	668	310
rs136177	1.65 [1.21–2.24]	**0.0015**	89	668	310

A total of 1,067 participants were included in these analyses. SHR [95%CI] shows Subdistribution Hazard Ratio and Lower and Upper 95% Confidence Interval values of the SHR. Event shows the number of participants having the defined event (Cardiovascular mortality or Sudden Cardiac Death). Censored shows the number of participants who were censored for the outcome. Competing shows the number of participants who had a competing event for the outcome. SHR = subdistribution hazard ratio. See supplemental Figure 1 for Chromosome and physical position.

Table 3:

Association of *APOL1* G3 SNPs and outcomes in European Ancestry in the BioVU cohort by dialysis vintage

	Effect allele	CV events / total N	SHR per copy of effect allele [95% CI]	p	CV death/ total N	SHR per copy of effect allele [95% CI]	p
EA overall							
rs136175	G	454/1080	1.14 (0.96,1.36)	0.1342	98/1080	1.32 (0.92,1.90)	0.1289
rs136176	G	454/1080	1.14 (0.96,1.36)	0.1342	98/1080	1.32 (0.92,1.90)	0.129
rs136177	G	454/1080	1.14 (0.96,1.36)	0.1342	98/1080	1.32 (0.92,1.90)	0.1289
EA <2 years dialysis vintage							
rs136175	G	187/399	1.28 (0.96,1.71)	0.0976	49/389	1.55 (0.90,2.68)	0.1169
rs136176	G	187/399	1.28 (0.96,1.71)	0.0978	49/389	1.55 (0.90,2.68)	0.1171
rs136177	G	187/399	1.28 (0.96,1.71)	0.0976	49/389	1.55 (0.90,2.68)	0.1169
EA 2–5 years dialysis vintage							
rs136175	G	122/303	1.62 (1.17,2.25)	**0.0036**	18/303	2.44 (1.11,5.34)	**0.0258**
rs136176	G	122/303	1.62 (1.17,2.25)	**0.0036**	18/303	2.44 (1.11,5.34)	**0.0258**
rs136177	G	122/303	1.62 (1.17,2.25)	**0.0036**	18/303	2.44 (1.11,5.34)	**0.0258**
EA >5 years dialysis vintage							
rs136175	G	145/378	1.13 (0.83,1.54)	0.4381	31/378	1.05 (0.54,2.06)	0.8778
rs136176	G	145/378	1.13 (0.83,1.54)	0.4381	31/378	1.05 (0.54,2.06)	0.8778
rs136177	G	145/378	1.13 (0.83,1.54)	0.438	31/378	1.05 (0.54,2.06)	0.8776

EA = European American, CV = cardiovascular, SHR = standardized hazard ratio