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Genome-Wide Association Scan for Survival on Dialysis in African-Americans with Type 2 Diabetes

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Key Words
African-Americans · Diabetes mellitus · Dialysis · Genome-wide association study · Survival

Abstract
Background: African-Americans (AAs) with diabetes have high incidence rates of end-stage renal disease (ESRD) with associated high mortality. Genetic factors modulating the risk of mortality on dialysis are poorly understood. ***Methods:*** A genome-wide association study was performed in 610 AAs with type 2 diabetes (T2D) and ESRD on dialysis, using the Affymetrix 6.0 platform (868,155 SNPs). Time to death was assessed using Cox proportional hazards model adjusting for ancestry and other confounding variables. Cases were censored at kidney transplant or (if living) at study conclusion. ***Results:*** Mean follow-up was 5.4 ± 3.5 years; 434 deaths were recorded. Five SNPs were associated with time to death at $p < 1.00 \times 10^{-6}$: rs2681019 (HR = 2.58, $P_{REC} = 8.00 \times 10^{-8}$), rs815815 in *CALM2* (HR = 1.51, $P_{ADD} = 6.50 \times 10^{-7}$), rs926392 (HR = 2.37, $P_{REC} = 4.80 \times 10^{-7}$), and rs926391 (HR = 2.30, $P_{REC} = 7.30 \times 10^{-7}$) near *DHX35*, and rs11128347 in *PDZRN3* (HR = 0.57, $P_{ADD} = 6.00 \times 10^{-7}$). Other SNPs had nominal associations with time to death ($p < 1.00 \times 10^{-5}$). ***Conclusion:*** Genetic variation may modify the risk of death on dialysis. SNPs in proximity to genes regulating vascular extracellular matrix, cardiac ventricular repolarization, and smoking cessation are associated with dialysis survival in AAs with T2D. These results warrant replication in other cohorts and races.

Introduction

Despite advances in technique and medications, alarmingly high mortality rates continue to be observed in the dialysis population, particularly in patients with diabetes (http://www.usrds.org; last accessed September, 2010). Factors associated with accelerated mortality in the dialysis population include traditional cardiovascular disease risk factors as well as non-traditional inflammatory markers [1]. Cardiovascular disease contributes to the largest proportion of deaths, related in part to pre-dialysis co-morbidity from diabetes, hypertension, and metabolic syndrome [2]. Derangements in mineral metabolism, anemia and hemoglobin variability, malnutrition, BMI and fat composition, residual renal function, dialysis access type, biocompatibility of dialysis membranes, intensity of pre-dialysis care, and frailty are independent determinants of life expectancy on dialysis [1].

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Striking racial differences exist in the incidence and prevalence rates of end-stage renal disease (ESRD) and in mortality rates on dialysis (http://www.usrds.org; last accessed September, 2010). Compared with Caucasians, African-Americans (AAs) have a markedly increased risk of ESRD, yet manifest a paradoxical lower risk of death on dialysis [3, 4]. The survival advantage in AAs with kidney failure stands in stark contrast to the higher prevalence of negative prognosticators of survival and the higher mortality rate observed in the general (non-renal disease) AA population [3, 5].

Genetic factors have been demonstrated to underlie part of the risk for ESRD in AAs [6], and may also play a role in survival after initiation of dialysis. Several studies have explored genetic determinants for racial differences in atherosclerosis and heart failure mortality [7–9]. However, no such analysis has been performed in dialysis patients. In this study, an unbiased genome-wide association scan was performed to detect genetic variants associated with survival on dialysis in AAs with type 2 diabetes (T2D). Using genome-wide data from a local AA cohort with T2D [10], coupled with the dialysis survival span, we sought to detect genetic variants associated with survival on dialysis that may provide novel insights into the molecular mechanisms that underlie death in patients on dialysis and set the stage for future functional studies.

Research Design and Methods

Study Population and Measurements

Self-reported AAs initiating renal replacement therapy between February 1985 and September 2009 in eight ESRD Network 6 facilities comprised the study cohort. Participants were enrolled in a study of genetic factors in AAs with type 2 diabetic ESRD, and had ESRD clinically attributed to diabetic nephropathy on the Center for Medicare and Medicare Services 2728 form [11]. T2D was diagnosed with age at diabetes onset >30 years and/or use of oral hypoglycemic agents (not insulin alone) 1 year after onset. T2D-associated ESRD was diagnosed in the presence of either: (1) T2D preceding ESRD by >5 years, in the absence of other causes of nephropathy, (2) diabetic retinopathy, or (3) dipstick proteinuria ≥100 mg/dl (or 500 mg/day). Exclusion criteria included non-AA race and evidence of recovery of renal function or renal transplant within 6 months of initiating dialysis. Baseline measures of serum albumin and serum hemoglobin were obtained at the start of dialysis and BMI was computed. Proportions, mean values, and standard deviations were calculated for baseline variables.

End Points and Data Collection

The primary end point was death from any cause. Survival time on dialysis was computed as the time between the start of dialysis and the date of death. Cases lost to follow-up or undergoing kidney transplantation were censored at their final follow-up date or transplant date, respectively. Follow-up in surviving participants ended on January 1, 2010. Data involving deaths were collected by means of primary cause of death documentation in the Death Notification Form (form 2746) required for every death occurring in the US ESRD population. Death events were categorized into 5 broad groups. Deaths coded as any of the following were defined as 'cardiovascular': atherosclerotic heart disease, cardiac arrest, cardiac arrhythmia, cardiomyopathy, congestive heart failure, acute myocardial infarction, valvular heart disease, ischemic stroke, or ischemic brain damage/anoxic encephalopathy due to cardiac event. Deaths coded as any of the following causes were classified as 'infectious': abdominal infection, perforated bowel, diverticulitis, gallbladder infection, endocarditis, pulmonary infection, or septicemia. Deaths ascribed to any of the following causes were grouped as 'other': accident related or unrelated to treatment, acidosis, cachexia, intracranial hemorrhage, chronic obstructive lung disease, cirrhosis, dementia (including dialysis dementia), Alzheimer's, gastrointestinal hemorrhage, hemorrhage from vascular access, hyperkalemia, malignant disease, viral hepatitis, pancreatitis, pulmonary embolus, or seizures. Withdrawal from dialysis and unknown cause of death constituted the 2 remaining groups.

Study approvals were obtained from the Wake Forest University Baptist Medical Center Institutional Review Board, and all subjects provided written informed consent.

Genotyping

DNA extraction from whole blood was performed using the PureGene system (Gentra Systems, Minneapolis, Minn., USA). Genotyping was performed at the Center for Inherited Disease Research using 1 μg of genomic DNA (diluted in 1× TE buffer and at 50 ng/μl) on Affymetrix Genome-Wide Human SNP array 6.0. DNA from cases was interleaved on 96-well master plates. To confirm sample identity, a SNP barcode (96 SNPs) was generated prior to genotyping on the Affymetrix array and confirmed on downstream released genotyping data. Genotypes were called using Birdseed version 2; APT 1.10.0 by grouping samples by DNA plate to determine the genotype cluster boundaries. All autosomal SNPs (n = 868,157) were included in the analysis, but classified on data quality with primary inference drawn from polymorphic SNPs (minor allele frequency > 0.05) with <5% missing data (Hardy-Weinberg Equilibrium p values >0.0001) resulting in a total of 832,357 SNPs for analysis. The average sample call rate was 99.16% for all autosomal SNPs. Forty-six blind duplicates were included in genotyping and had a concordance rate of 99.59%. In addition, one individual was removed whose self-reported gender was inconsistent with X chromosome genotype data. Relatedness was estimated using the identity-by-descent analysis implemented in the PLINK analysis software package (http://pngu.mgh.harvard.edu/purcell/plink/). One duplicate pair and 110 first-degree relative pairs were identified, and a single individual from each pair was retained for analysis based on the completeness of the phenotypic data.

Statistical Analysis

To account for admixture, ancestral allele frequencies were estimated from the results of the 70 ancestry informative markers genotyped in 44 Yoruba Nigerians and 39 European Americans. Individual ancestral proportions were generated for each subject

Table 1. Demographics at dialysis inception

	n	Value
Age, years	610	59 ± 11
Females	610	279 (45)
BMI	475	29.6 ± 6.8
Pre-dialysis diabetes duration, years	524	20 ± 10
Duration of follow-up, years	610	5.4 ± 3.5
Undergoing hemodialysis		567 (93)
Hemoglobin, g/dl	481	9.8 ± 5.4
Serum albumin, g/dl	451	3.1 ± 0.6
ESRD Network	610	
3		3 (0.5)
4		3 (0.5)
5		2 (0.3)
6		597 (98)
7		1 (0.1)
8		2 (0.3)
10		1 (0.1)
14		1 (0.1)

Data presented as n (%) or means ± SD. Conversion factor for hemoglobin and serum album (g/dl to g/l) was ×10.

Table 2. Events at follow-up

	n (%)
Deaths	434 (71)
Cardiovascular death	236 (54)
Infection	80 (18)
Withdrawal from dialysis	26 (6)
Other	39 (9)
Unknown	53 (12)
Transplants	44 (7)
Switched dialysis modality	63 (10)
Censored[1]	176 (29)

[1] Patients transplanted (n = 44), lost to follow-up (n = 2), or alive (n = 130).

using FRAPPE, an expectation maximization algorithm, under a two-population model [12].

To test for association between each SNP and survival, adjusting for covariates, a Cox proportional hazard semi-parametric regression model was computed. The proportional hazards assumption for each covariate was examined before allowing that covariate into the model. Time to all-cause death was tested for each SNP using dominant, recessive, and additive models. Primary inference was based on the additive genetic model unless significant departure from additivity was observed ($p < 0.05$). All genetic models were defined relative to the minor allele where the dominant model tests whether the presence of the minor allele influences survival, the additive model tests for a dose effect on the number of minor alleles (0, 1, or 2) and the recessive model tests whether 2 copies of the minor allele were associated with survival. The recessive and additive genetic models required at least 30 and 10 individuals homozygous for the minor allele, respectively. All analyses adjusted for population structure using the ancestral proportions noted above. Quantile-quantile (Q-Q) plots and test inflation factors were computed to assure that the experiment had appropriate family-wise type 1 error rates. Participants were censored when lost to follow-up due to transfer to another ESRD Network, receipt of a kidney transplant, or (if living) on January 1, 2010. Due to recent improvements in dialysis prescription and medications, factors with potential impact on dialysis survival introduced in approximately the year 2000, we adjusted the analysis using a binary variable denoting pre- and post-2000 initiation of dialysis. Model 1 adjusted for ancestry, age at start of dialysis, sex, BMI, pre-dialysis diabetes duration, and incident year of dialysis; model 2 adjusted for these factors plus serum albumin and hemoglobin at the initiation of dialysis. HR with 95% CIs are presented. We estimated study power using the Genetic Power Calculator; assuming dominant models with minor allele frequencies of 0.07, our sample size had 95% power to detect a minimal HR of 1.6 for death while on dialysis at $\alpha = 0.05$ [13].

Results

A total of 647 participants were evaluated for inclusion. Of these, 5 recovered renal function, 4 had temporary discontinuation of dialysis, 2 were transplanted within 6 months of dialysis onset, and 26 did not have the date of first dialysis recorded; these patients were withdrawn. The remaining 610 subjects were included in the final analysis. Table 1 summarizes the baseline characteristics of participants. Hemodialysis was the predominant initial mode of renal replacement therapy (93% of patients), and we observed an overall 10% rate of modality conversion from any initial to an alternative renal replacement modality during the period of follow-up. After mean follow-up of 5.4 ± 3.5 years, 434 deaths, 44 kidney transplants, and 2 cases lost to follow-up were recorded (table 2). The mean survival on dialysis for the 434 subjects who died was 5.2 ± 3.1 years. A total of 130 subjects remained alive at the last follow-up. Table 2 summarizes the major causes of death. Sex, BMI, incident albumin, incident hemoglobin, and admixture were not individually significantly associated with survival, while age at dialysis inception (5-year HR = 1.2; $p < 0.0001$) and incident year of dialysis (dialysis vintage, 5-year HR = 1.16; $p = 0.0008$) were significantly associated with mortality (data not shown).

A number of genomic regions showed evidence of association with survival at $p < 1.00 \times 10^{-5}$, with 5 of the

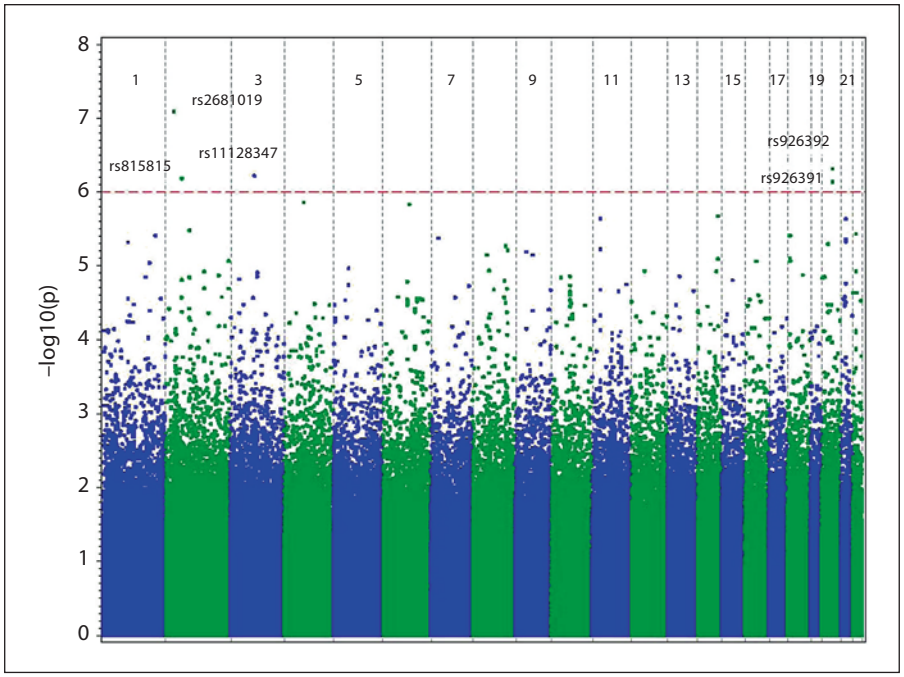

Fig. 1. GWAS results for death on dialysis. The genome-wide distribution of –log10 p values from the adjusted trend is shown across the chromosomes. The dotted line indicates the genome-wide significance threshold ($p < 1 \times 10^{-6}$). Annotated are the top five SNPs with genome-wide significance for dialysis survival at $p < 1 \times 10^{-6}$.

landmark SNPs associated at $p < 1.00 \times 10^{-6}$ (fig. 1). Overall, there was no evidence of inflation of the tests of association, and there was appropriate fit to the expected overall distribution of association (fig. 2).

After adjustment for ancestry, age at dialysis, sex, BMI, diabetes duration, and incident year factor (model 1), 30 SNPs provided evidence for association ($p < 1.00 \times 10^{-5}$), 12 were located in 10 genes (table 3), and 18 SNPs were located in intergenic regions (table 4). One of the top hits (rs2412980, chr22q12.2, HR = 1.65, $p = 3.67 \times 10^{-6}$) was located in *LOC729980*, a gene encoding a protein with unknown function. Two of the top hits (rs6546886, chr2p13.1, HR = 2.13, $p = 3.28 \times 10^{-6}$; rs9921518, chr16q12.2, HR = 2.11, $p = 8.62 \times 10^{-6}$) were located in regions of reported copy number variation. Of note, several SNPs clustered around A Disintegrin And Metalloproteinase with Thrombospondin Motifs (*ADAMTS*) and Iroquois (*IRX*) genes (rs6816344, chr4q13.3, HR = 1.7, p = 1.37x 10^{-6}; rs1452093, chr21q21.3, HR = 1.59, $p = 2.31 \times 10^{-6}$; rs9977499, chr21q21.3, HR = 1.57, $p = 4.36 \times 10^{-6}$; rs1817114, chr21q21.3, HR = 1.57, $p = 4.36 \times 10^{-6}$; rs2830881, chr6q22.31, HR = 1.57, $p = 4.67 \times 10^{-6}$; and rs9921518, chr16q12.2, HR = 2.11, $p = 8.62 \times 10^{-6}$).

Additional covariate adjustments in model 2 (incident serum albumin and hemoglobin) maintained comparable HRs, but diminished the p values modestly (data not shown).

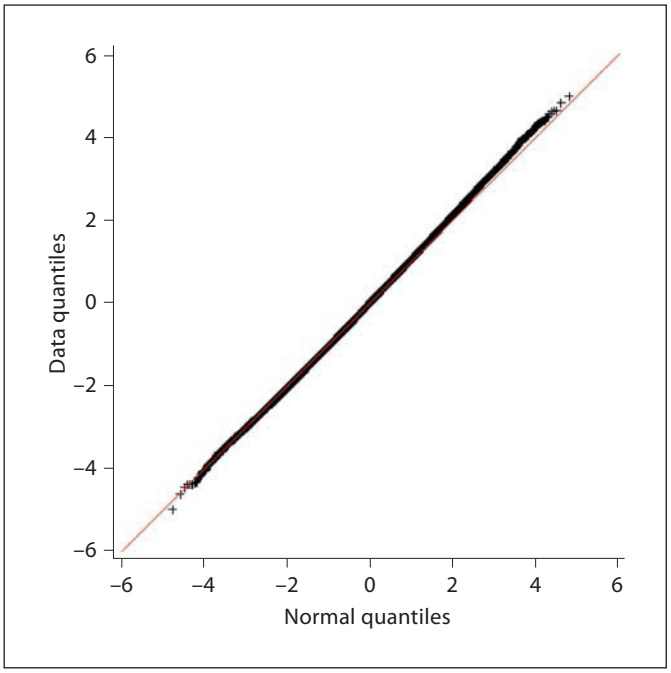

Fig. 2. Q-Q plot from the genome-wide association with dialysis survival analysis. The black bold line represents the observed p values; the red line is the expected line under the null distribution.

Table 3. Summary results for top gene SNPs associated with all-cause death on dialysis

Rank	SNP	Chromosome	Position	Gene	Name	Function	MAF	Best model	HR	CI	p value
3	rs11128347	3p13	73702252	*PDZRN3*	PDZ domain containing ring finger 3	intronic with no known function	11	additive	0.54	0.43–0.69	6.00×10^{-7}
4	rs815815	2p21	47252569	*CALM2*	calmodulin 2 (phosphorylase kinase, delta)	intronic enhancer	19	additive	1.51	1.28–1.77	6.50×10^{-7}
9	rs7111546	11p14.3	22786334	*GAS2*	growth arrest-specific 2	intronic enhancer	44	recessive	1.72	1.37–2.16	2.30×10^{-6}
12	rs2412980	22q12.2	28922070	*LOC729980*	hypothetical LOC729980	N/A	9	additive	1.65	1.33–2.04	3.67×10^{-6}
14	rs8098064	18p11.23	8199270	*PTPRM*	protein tyrosine phosphatase, receptor type, M	intronic with no known function	26	additive	1.42	1.22–1.65	3.87×10^{-6}
19	rs17110736	1p22.1	94235358	*ABCA4*	ATP-binding cassette, subfamily A (ABC1), member 4	intronic enhancer	35	recessive	2.08	1.52–2.86	4.80×10^{-6}
20	rs4814615	20p12.1	17305574	*PCSK2*	proprotein convertase subtilisin/kexin type 2	intronic enhancer	19	dominant	1.59	1.30–1.95	5.02×10^{-6}
21	rs712022	11p14.3	22799732	*SVIP*	small VCP/p97-interacting protein	downstream with no known function	47	dominant	0.62	0.51–0.76	5.96×10^{-6}
23	rs2439312	8p12	32531902	*NRG1*	neuregulin 1	intronic enhancer	24	recessive	2.36	1.62–3.45	7.08×10^{-6}
25	rs7243299	18p11.23	7745772	*PTPRM*	protein tyrosine phosphatase, receptor type, M	intronic enhancer	10	additive	1.63	1.31–2.03	8.01×10^{-6}
27	rs9953514	18p11.23	7821266	*PTPRM*	protein tyrosine phosphatase, receptor type, M	intronic with no known function	9	dominant	1.74	1.36–2.22	8.56×10^{-6}
28	rs11676855	2q37.2	235564911	*SH3BP4*	SH3-domain binding protein 4	promoter/regulatory region	33	additive	0.7	0.60–0.82	8.58×10^{-6}

Results ordered by p values and adjusted for ancestry, age at start of dialysis, gender, BMI, pre-dialysis diabetes duration, and incident year of dialysis. Chromosome positions are based on NCBI build 36_3. HR, CI, and p values are shown for the best-fit model. MAF = Minor allele frequency.

Discussion

This report represents the first genome-wide search for variation influencing dialysis survival in AAs with T2D. We found several alleles (30 SNPs) that had strong statistical associations ($p < 5.00 \times 10^{-6}$) with survival on dialysis, even after adjustment for important covariates known to impact dialysis survival (model 1). The lack of change in the HR with modest reduction of statistical significance (10-fold drop) in model 2 reflected a reduction in statistical power due to the reduced sample size resulting from a lack of measured covariates in some participants. Of the top 30 SNPs, some indicated a negative effect on survival by association with a higher propensity for death (HR > 1.0), while other SNPs indicated a protective effect by association with a lower rate of death (HR < 1.0). These associations tag potentially interesting genomic regions and the functional qualities of the top SNPs relating to death on dialysis are unknown, but may prove to be important based on their detection using unbiased methodologies. Replication, subsequent fine mapping, and functional studies are necessary to more accurately narrow down regions of interest and establish loci as influencing survival. Genome-wide association study (GWAS) results from the entire cohort have been entered in the Database of Genotypes and Phenotypes (dbGaP) [10].

The top SNPs correlating with dialysis survival were located primarily in or near genes with mechanistic roles that can be categorized in 3 major groups: regulation of extracellular matrix (ECM) composition and turnover, myocardial cell development and repolarization, and neurobiological regulation of smoking cessation. At this stage, these results are hypothesis-generating and require

Table 4. Summary results for top intergenic SNPs associated with all-cause death on dialysis

Rank	SNP	Chromosome	Position	Upstream gene and distance, kb	Downstream gene and distance, kb	MAF	Best model	HR	CI	p value
1	rs2681019	2p24.1	23041010	*KLHL29* (420)	none within 500 kb	28	rec.	2.58	1.82–3.66	8.00×10^{8}
2	rs926392	20q12	37123879	*LOC339568* (151)	*DHX35* (22)	29	rec.	2.37	1.69–3.33	4.80×10^{7}
5	rs926391	20q12	37123900	*LOC339568* (151)	*DHX35* (22)	30	rec.	2.3	1.65–3.20	7.30×10^{7}
6	rs6816344	4q13.3	74002269	*COX18* (137)	*ADAMTS3* (348)	49	rec.	1.7	1.37–2.12	1.37×10^{6}
7	rs594442	6q16.1	93904606	*EPHA7* (101)	none within 500 kb	5	add.	1.99	1.50–2.64	1.47×10^{6}
8	rs1009170	14q32.12	91706467	*SLC24A4* (152)	*CPSF2* (6)	43	rec.	0.5	0.38–0.67	2.10×10^{6}
10	rs1452093	21q21.3	27666228	*NCRNA00113* (350)	*ADAMTS5* (404)	23	dom.	1.59	1.31–1.94	2.31×10^{6}
11	rs6546886	2p13.1	74099286	*TET3* (27)	*DGUOK* (59)	29	rec.	2.13	1.55–2.93	3.28×10^{6}
13	rs1497828	1q41	215593648	*GPATCH2* (76)	*ESRRG* (215)	37	add.	0.71	0.61–0.82	3.85×10^{6}
15	rs17364464	7p15.3	22480579	*IL6* (252)	*RAPGEF5* (117)	8	add.	1.76	1.38–2.25	4.19×10^{6}
16	rs9977499	21q21.3	27656869	*NCRNA00113* (359)	*ADAMTS5* (3995)	24	dom.	1.57	1.29–1.91	4.36×10^{6}
17	rs1817114	21q21.3	27661425	*NCRNA00113* (355)	*ADAMTS5* (400)	24	dom.	1.57	1.29–1.91	4.36×10^{6}
18	rs2830881	21q21.3	27657679	*NCRNA00113* (358)	*ADAMTS5* (396)	24	dom.	1.57	1.29–1.91	4.67×10^{6}
22	rs17232789	8q24.13	122691682	*HAS2* (2)	none within 500 kb	37	dom.	0.63	0.52–0.77	6.09×10^{6}
24	rs6560517	9q21.13	78227991	*GCNT1* (18)	*RPSAP9* (23)	29	dom.	0.64	0.53–0.78	7.09×10^{6}
26	rs4904947	14q32.12	92041916	*RIN3* (7)	*SLC24A4* (9)	5	add.	1.9	1.43–2.52	8.03×10^{6}
29	rs9921518	16q12.12	53051926	*IRX5* (470)	*IRX3* (174)	28	rec.	2.11	1.52–2.93	8.62×10^{6}
30	rs16844716	1q32.1	197623140	none within 500 kb	none within 500 kb	9	add.	1.65	1.32–2.07	9.09×10^{6}

SNPs ordered by p values and ranked in relation to the intragenic SNPs. Results are adjusted for ancestry, age at start of dialysis, gender, BMI, pre-dialysis diabetes duration, and incident year of dialysis. Chromosome positions are based on NCBI build 36_3. MAF = Minor allele frequency. HR, CI, and p values are shown for the best-fit model.

replication in other cohorts and races. If replicated, they merit experimental analysis to define the molecular pathways that impact survival.

Putative Atherogenic SNPs

Accelerated atherosclerosis, a multi-factorial process involving inflammation, altered matrix turnover, and composition in vascular walls is often present in patients with diabetes and kidney disease [14]. Proteases from the ADAMTS family regulate ECM turnover in atherosclerotic plaque [15], and hyaluronic acid (encoded by *HAS2* gene) is a prominent constituent of the ECM in atherosclerotic vascular lesions [16]. Our analysis found several SNPs clustered near the *ADAMTS5* gene (rs6816344, rs1452093, rs9977499, rs1817114, rs2830881) and a single SNP located near the *HAS2* gene (rs17232789) to have significant correlations with survival. These SNPs may impact the rate of atherosclerosis, either pre-dialysis or on dialysis. Further studies are needed to replicate these findings and delineate their pathophysiology.

Putative Arrhythmogenic SNPs

Sudden cardiac death due to arrhythmia is the leading cause of death in patients on dialysis. Several genes are known to control myocardial development and repolarization. The protein tyrosine phosphatase receptor type M (*PTPRM*) gene and PDZ domain containing the ring finger 3 (*PDZRN3*) gene play important roles in myocardial development, myocardial cell resistance to ischemia-reperfusion injury, and electrolyte-induced depolarization [17, 18]. Calmodulin regulates intracellular calcium homeostasis in myocardial and vascular endothelial cells, and Ca^{2+}/calmodulin kinase complex plays a major role in ventricular repolarization [19]. Polymorphisms in the calmodulin 2 (*CALM2*) gene (rs815815), *PTPRM* gene (rs8098064, rs7243299, rs9953514), and *PDZRN3* gene (rs11128347) were among the top associations with dialysis survival, suggesting a role for differential myocardial cell response to triggers of cell depolarization. Experimental studies of the morphologic and functional impacts of genomic variation detected in this study could identify arrhythmogenic pathways in myocardial cells that contribute to survival on dialysis.

Smoking Cessation Genes

Association was detected between dialysis survival and polymorphisms in and near genes thought to predict successful smoking cessation: *PDZRN3, PTPRM*, neuregulin 1 *(NRG1)*, SH3-domain binding protein 4 *(SH3BP4)*, kelch-like 29 *(KLHL29)*, DEAH (Asp-Glu-Ala-His) box polypeptide 35 *(DHX35)*, ATP-binding cassette sub-family A member 4 (ABCA4), ephrins receptor A7 *(EPHA7)*,

proprotein convertase subtilisin/kexin type 2 *(PCSK2)*, tet oncogene family member 3 *(TET3)*, G patch domain-containing 2 *(GPATCH2)*, estrogen-related receptor gamma *(ESRRG)*, Rap guanine nucleotide exchange factor 5 *(RAPGEF5)*, and Ras and Rab interactor 3 *(RIN3)* [20, 21]. Unfortunately, our dataset lacks information pertaining to smoking history.

Unique strengths of this GWAS include the novel phenotype (dialysis survival) and use of an unbiased genetic approach in a population enriched for cardiovascular disease risk factors. Previous genetic studies of dialysis survival included mainly Caucasian and non-diabetic individuals, and applied a priori single gene analysis (e.g. Klotho, CCR5, Fetuin A) [22–24]. The current GWAS did not reveal polymorphisms in or near these genes, potentially due to race-dependent and environment-conducive genetic variation. These confounding factors were minimized in our analysis by virtue of homogenous ethnicity and cause of ESRD.

GWAS analyses have been applied in large study cohorts, including the Wellcome Trust Case Control Consortium (WTCCC) and Atherosclerosis Risk in Communities (ARIC) studies, to detect genetic variants associated with cardiovascular events. These analyses detected several SNPs associated with incident heart failure and coronary heart disease in African-ancestry populations, but there was no overlap with risk variants in our study [7, 9]. This is not surprising, since the mechanisms of cardiovascular death in dialysis patients are likely to differ from those in the general population. Since few studies with ESRD patients have undergone GWAS, it will be necessary to evaluate dialysis survival as a phenotype in other GWAS. Although the genetic mechanisms underlying various etiologies of death on dialysis may differ, we elected not to perform separate analyses by cause of death, since the sample sizes were small and inconsistencies might exist in the attributed cause of death. However, it is clear that the majority of deaths in our cohort were related to cardiovascular events – an expected observation.

Limitations of this study include (1) a relatively small cohort, (2) inability to adjust for being on hemodialysis versus peritoneal dialysis, (3) inability to assess the type of hemodialysis access used, (4) inability to ascertain that T2D was the actual cause of ESRD in clinically diagnosed subjects due to lack of kidney biopsies. An inherent limitation of the study represents the fact that the statistical association only tags regions of interest. Thus, replication and fine mapping studies will be necessary. Frequent changes in dialysis modality and access type are commonly observed, making adjustment for these factors difficult. The possibility of false-positive results relating to our relatively small sample size cannot be excluded; however, we detected suggestive evidence of genome-wide association. An unadjusted p value of $<1 \times 10^{-8}$ is regarded as significant in genome wide association studies. Several SNPs in this discovery cohort had p values $<1 \times 10^{-6}$; it is possible that these may play important roles in survival on dialysis or may be false-positive results. A replication cohort to validate these signals and exclude genomic noise is critical. The relatively large numbers of deaths was not unexpected in this high-risk sample of subjects with diabetes and ESRD. Known biological connections between the associated genes and their pathways suggest a plausible mechanism for effects on survival.

Conclusion

In summary, a GWAS employing 868,155 SNPs identified 30 genetic variants that appeared to influence the risk of death on dialysis in AAs with T2D and ESRD. These results highlight several important pathways and processes involved in cardiac and ECM physiology, and provide insights into the potential genetic architecture of dialysis survival as a polygenic trait. This is a hypothesis-generating GWAS that presents a preliminary set of SNPs associated with survival in AA ESRD patients with T2D. In the future, GWAS in other racial groups and dialysis cohorts, replication of these findings, fine-mapping and functional studies of these loci in myocardial, vascular, and/or neuronal cells will be necessary to validate these results and determine the role played by these polymorphisms in the pathogenesis of dialysis survival.

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Disclosure Statement

The authors report no conflicts of interest.

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