FGF23 Concentration and *APOL1* Genotype Are Novel Predictors of Mortality in African Americans With Type 2 Diabetes

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OBJECTIVE

Cardiovascular and renal complications contribute to higher mortality in patients with diabetes. We assessed novel and conventional predictors of mortality in African American–Diabetes Heart Study (AA-DHS) participants.

RESEARCH DESIGN AND METHODS

Associations between mortality and subclinical atherosclerosis, urine albumin–to–creatinine ratio (UACR), estimated glomerular filtration rate (eGFR), plasma fibroblast growth factor 23 (FGF23) concentration, African ancestry proportion, and apolipoprotein L1 genotypes (*APOL1*) were assessed in 513 African Americans with type 2 diabetes; analyses were performed using Cox proportional hazards models.

RESULTS

At baseline, participants were 55.6% female with median (25th, 75th percentile) age 55 years (49.0, 62.0), diabetes duration 8 years (5.0, 13.0), glycosylated hemoglobin 60.7 mmol/mol (48.6, 76.0), eGFR 91.3 mL/min/1.73 m^2 (76.4, 111.3), UACR 12.5 mg/mmol (4.2, 51.2), and coronary artery calcium 28.5 mg Ca^{2+} (1.0, 348.6); 11.5% had two *APOL1* renal-risk variants. After 6.6-year follow-up (5.8, 7.5), 54 deaths were recorded. Higher levels of coronary artery calcified plaque, carotid artery calcified plaque, albuminuria, and FGF23 were associated with higher mortality after adjustment for age, sex, and African ancestry proportion. A penalized Cox regression that included all covariates and predictors associated with mortality identified male sex (hazard ratio [HR] 4.17 [95% CI 1.96–9.09]), higher FGF23 (HR 2.10 [95% CI 1.59–2.78]), and absence of *APOL1* renal-risk genotypes (HR 0.07 [95% CI 0.01–0.69]) as the strongest predictors of mortality.

CONCLUSIONS

Accounting for conventional risk factors, higher FGF23 concentrations and *APOL1* non–renal-risk genotypes associated with higher mortality in African Americans with diabetes. These data add to growing evidence supporting FGF23 association with mortality; mechanisms whereby these novel predictors impact survival remain to be determined.

It is estimated that 439 million people worldwide will develop type 2 diabetes by the year 2030 (1). Diabetes and its associated complications contribute to escalating social and economic burdens on patients, families, and health care systems. A major challenge in caring for patients with diabetes is managing risk factors associated with the

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development of cardiovascular disease (CVD) and chronic kidney disease (CKD). These complications are important contributors to the higher mortality rates seen in individuals with diabetes.

Identifying factors that predict mortality in patients with type 2 diabetes is important to improve outcomes and prolong life. The African American population is relatively understudied, and it is critical to perform analyses in this racial group because susceptibility to type 2 diabetes and its CVD and CKD complications appear to differ from those in European-derived cohorts (2). The African American–Diabetes Heart Study (AA-DHS) was initiated to identify the environmental and inherited factors contributing to mortality, subclinical CVD, and bone and kidney disease in African Americans with type 2 diabetes (3). Based on rates of treatment and control of hypertension, hyperlipidemia, and hyperglycemia, AA-DHS participants appear to have similar access to health care as European Americans in contemporary reports. The cohort lacked advanced nephropathy at enrollment and was genotyped for apolipoprotein L1 gene (*APOL1*) renal-risk variants (reportedly associated with subclinical CVD) and the ancestry-informative markers available on the OMNI 5 (San Diego, CA) genome-wide association study data (4). Measures of fibroblast growth factor 23 (FGF23) were available; recent studies support an important role for this biomarker on survival (5,6). The present analyses were performed to assess novel and conventional risk factors associated with mortality in this African American cohort with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Study Population

Self-reported African Americans with type 2 diabetes were recruited in the Wake Forest School of Medicine (WFSM) AA-DHS from May 2007 through August 2010 (3). Type 2 diabetes was clinically diagnosed based on disease onset after the age of 30 years in the absence of diabetic ketoacidosis and with active glucose-lowering treatment (insulin and/or oral agents), fasting glucose $\geq$7.0 mmol/L, nonfasting glucose $\geq$11.1 mmol/L, or glycosylated hemoglobin (GHb) $>$6.5% (48 mmol/mol). Individuals with a known prior serum creatinine concentration $\geq$176.8 μmol/L were not recruited. The study was approved by the WFSM Institutional Review Board, and all participants provided written informed consent. Baseline assessments consisted of interviews for medical history, anthropometric measurements, blood pressures, and fasting blood measurements (glucose, serum creatinine, GHb, lipid panels, and a spot urine for urine albumin–to–creatinine ratio [UACR]). As reported, FGF23 concentrations were measured in all participants using plasma samples stored at $-$80°C since recruitment (7). Estimated glomerular filtration rate (eGFR) was computed using the creatinine-based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (8). Hypertension was defined based on physician diagnosis, study blood pressure $>$140/90 mmHg, and/or receipt of antihypertensive medications.

Genotyping

Two single nucleotide polymorphisms in the *APOL1* G1 nephropathy risk allele (rs73885319 and rs60910145) and an indel for the G2 risk allele (rs71785313) were genotyped on the Sequenom platform using custom arrays designed at WFSM Center for Genomics and Personalized Medicine (3). African ancestry proportion was computed based on the ancestry-informative markers available on the Illumina OMNI 5 chip genome-wide association study data (4).

Vascular Imaging

Subclinical atherosclerosis was assessed as calcified atherosclerotic plaque in the coronary arteries, carotid arteries, and infra-renal abdominal aorta using single and multidetector computed tomography systems. A standardized scanning protocol based on the National Heart, Lung, and Blood Institute Multi-Ethnic Study of Atherosclerosis (MESA) was used (9). Scoring parameters included a 90–Hounsfield unit (HU) threshold and two adjacent pixels to define the maximum calcified lesion size. Because traditional Agatston (calcium) scores add noise to the computed tomography measurement of calcified plaque compared with volume-based measures, we used the calcium mass score (milligrams of calcium) derived from the volume score and accounting for density of calcified plaque on a pixel by pixel basis (10). We used log $(x+1)$ as the covariate in analyses for calcified plaque, where x represents the coronary, carotid, or aorta calcium score depending on the analysis. The addition of 1 to the calcium score made it possible to include individuals without evidence of calcification in the analyses. Quantitative coronary artery calcium mass scores were excluded from analyses in participants who had previously undergone coronary artery bypass grafting, stenting, or angioplasty; carotid scores were excluded in those with prior carotid endarterectomy.

Vital Status

Vital status was assessed through 31 December 2015 using the National Death Index. Cause of death was classified based on the primary factor reported on death certificates: 16 (23.5%) of the 54 deaths were attributed to CVD, 27.9% to cancer, 7.4% to infection, 7.4% to type 2 diabetes, and 33.8% to other causes.

Statistical Analyses

Demographics and laboratory characteristics of AA-DHS study participants were contrasted by survival status using Wilcoxon two-sample tests for continuous variables and χ^2 and Fisher exact tests for binary variables. The main outcome was time to death, determined by the interval between the dates of study enrollment and death. Study participants who were known to be alive as of 31 December 2015 were censored. Cox proportional hazards models were subsequently fitted (11). Covariates were selected to limit confounding effects and ensure that reported effects were not due to other measured variables not accounted for in the model. Association results are presented for a minimally adjusted model that accounted for age, sex, and African ancestry proportion. Additional covariates were included in a subsequent model; the list of covariates varied with the predictor. For calcified atherosclerotic plaque, subsequent models were further adjusted for diabetes duration, GHb, eGFR, and UACR. Association models with kidney function and albuminuria as predictors were further adjusted for GHb, presence of hypertension, and angiotensin converting enzyme inhibitor (ACEi)/angiotensin receptor blocker (ARB). The eGFR was included as a covariate when testing for association between UACR and time from study enrollment to death, and UACR was included when testing for association of eGFR with time to death. The model for association between African ancestry

proportion and survival additionally adjusted for age, sex, BMI, diabetes duration, smoking, coronary artery calcified atherosclerotic plaque (CAC), eGFR, and UACR; the model for *APOL1* renal-risk variant association included all those covariates plus African ancestry proportion; the model for FGF23 association accounted for age, sex, BMI, diabetes duration, smoking, eGFR, UACR, hormone replacement therapy, and use of steroids. Hazard ratios (HRs) were computed for presence of the risk factor for binary predictors (e.g., presence of calcified plaque, CKD, two *APOL1* renal-risk variants, etc.) and for specific amount of change in continuous predictors (e.g., increase of 100 mg Ca^{2+} in calcified atherosclerotic plaque, 10% in African ancestry proportion, 100 mg/g in UACR, 10 mL/min/1.73 m^2 in eGFR, and 237.8 RU/mL [one SD change]).

Spearman rank correlation was used to estimate the pairwise correlation between all the predictors and covariates considered in these analyses. The absolute values of the Spearman rank correlation between the predictors considered in these models were moderate, varying from a minimum of 0.01 (between age and sex) to a maximum of 0.4 (between eGFR and age). Given the correlation between these predictors, a penalized Cox regression, assuming the L1 penalty (LASSO), was then fitted with all of the predictors considered to identify the main contribution of these variables to mortality, including calcified plaque mass score in coronary and carotid arteries, UACR, eGFR, African ancestry proportion, plasma FGF23, and *APOL1* renal-risk genotypes. The LASSO model was fitted using the Cox model implemented in glmnet Package in R (http://www.jstatsoft.org/v39/i05/) (12). The shrinkage parameter was determined using cross-validation. All variables that had a nonzero parameter estimate in the model corresponding to the optimum λ (shrinkage parameter) were selected and used to fit a Cox proportional hazards model.

Area under the curve (AUC) computed as the area under the receiver operating characteristic (ROC) was used to compare the prognostic accuracy of a model that included established mortality risk factors (i.e., age, sex, African ancestry proportion, BMI, diabetes duration, smoking status, UACR, and CAC) to models that further incorporated *APOL1* risk and FGF23 as predictors at 1, 3, 5, and 7-year follow-up. Inference was performed under the Kaplan-Meier estimator of the censoring distribution as implemented in the R package timeROC (13). ROC curves for intermediary models (i.e., base model + *APOL1* and base model + FGF23) are also shown for completeness.

RESULTS

The cohort included 513 unrelated African Americans with type 2 diabetes; median (25th, 75th percentile) follow-up through 31 December 2015 was 6.6 years (5.8, 7.5). During this period, 54 deaths were recorded. Table 1 displays baseline demographic and clinical characteristics in the full sample (living and deceased subsets also provided). Participants were 55.6% female with median age 55.0 years

Table 1—Baseline demographic and clinical characteristics of AA-DHS cohort

	AA-DHS cohort						
Variables	Full sample	*n*	Living	*n*	Deceased	*n*	*P* value
Age (years)	55.0 (49.0, 62.0)	513	55.0 (49.0, 62.0)	459	55.5 (49.2, 64.0)	54	0.42
Female (%)	55.6%	513	58.2%	459	33.3%	54	5.0×10^{-4}
Smoking (%)	59.3%	513	58.0%	459	70.4%	54	0.08
BMI (kg/m^2)	33.8 (29.1, 39.9)	512	34.0 (29.4, 39.9)	458	31.4 (27.7, 39.8)	54	0.05
Diabetes duration (years)	8.0 (5.0, 13.0)	513	8.0 (4.5, 13.0)	459	8.5 (5.0, 13.8)	54	0.38
Follow-up time (years)	6.6 (5.8, 7.5)	513	6.8 (6.1, 7.6)	459	3.3 (1.6, 4.3)	54	1.0×10^{-30}
Hypertension (%)	82.5%	513	81.0%	459	94.4%	54	0.01
Systolic blood pressure (mmHg)	132.0 (121.0, 145.0)	513	132.0 (121.0, 144.0)	459	132.0 (120.0, 148.8)	54	0.67
Diastolic blood pressure (mmHg)	78.0 (70.0, 85.0)	513	78.0 (70.0, 85.0)	459	78.0 (69.2, 84.0)	54	0.57
Angina (%)	14.3%	484	13.3%	435	22.4%	49	0.08
Heart attack (%)	9.7%	506	9.9%	454	7.7%	52	0.61
Stroke (%)	7.5%	507	7.0%	454	11.3%	53	0.26
Coronary artery bypass surgery (%)	3.5%	513	3.3%	459	5.6%	54	0.39
Coronary angioplasty (%)	12.5%	513	12.6%	459	11.1%	54	0.75
Carotid endarterectomy (%)	0.4%	513	0.2%	459	1.9%	54	0.07
Insulin use (%)	39.6%	513	37.3%	459	59.3%	54	0.002
Metformin use (%)	63.3%	510	64.1%	457	56.6%	53	0.28
Sulfonylurea use (%)	38.8%	510	39.8%	457	30.2%	53	0.17
Statin use (%)	49.0%	512	49.8%	458	42.6%	54	0.32
ACEi/ARB use (%)	48.7%	513	49.2%	459	44.4%	54	0.51
Steroid use (oral or inhaled) (%)	7.1%	509	7.0%	456	7.5%	53	0.89
Bisphosphonate use (%)	0.2%	510	0.0%	457	1.9%	53	0.003
Hormone replacement therapy (%)	25.4%	280	25.1%	263	29.4%	17	0.69
African ancestry proportion (%)	83.2 (76.4, 88.6)	513	83.3 (76.8, 88.8)	459	82.3 (74.0, 87.6)	54	0.28
Two *APOL1* renal-risk variants (%)	11.5%	513	12.6%	459	1.9%	54	0.02

Median (25th percentile, 75th percentile) for continuous variables.

(49.0, 62.0) at baseline, diabetes duration at enrollment 8.0 years (5.0, 13.0), and African ancestry proportion 83.2% (76.4, 88.6); 11.5% possessed two *APOL1* renal-risk variants. Medications included 63.3% of the cohort on metformin, 39.6% on insulin, and 38.8% on a sulfonylurea; none received DPP4 inhibitors, GLP-1 receptor agonists, or SGLT-2 inhibitors. In univariate analyses, male sex, lower BMI, hypertension, insulin use, and lower proportion of *APOL1* renal-risk genotypes were associated with higher mortality. Since metformin is likely more protective from CVD than sulfonylureas, we assessed differences in survival over time based on metformin use. Significant differences were not detected (HR 0.76; *P* value = 0.31).

Table 2 displays the baseline biochemical and radiographic measurements in the full sample (living and deceased subsets also provided). At baseline, median GHb was 60.7 mmol/mol (48.6, 76.0), CAC mass score 28.5 mg Ca^{2+} (1.0, 348.6), eGFR 91.3 mL/min/1.73 m^2 (76.4, 111.3), UACR 12.5 mg/mmol (4.2, 51.2), and FGF23 224.8 pg/mL (158.1, 331.6). A total of 68 vascular beds were excluded from CAC scoring (based on 50 with angioplasty, 4 with CABG, and 14 with both) and 3 were excluded from carotid calcified plaque measures due to endarterectomy. In univariate analyses, higher FGF23, higher UACR, lower LDL cholesterol, and calcified plaque in coronary and carotid vascular beds (both amount and presence) were associated with higher mortality. A second GHb was available in 347 participants after median follow-up of 3.8 years; the median difference in GHb was -0.1 ($q_1 = -0.9$, $q_3 = 0.9$), suggesting that glycemic control changed little over time.

Table 3 presents multivariate association analysis results for time from study enrollment to death based on presence and severity of calcified atherosclerotic plaque in three vascular beds, albuminuria, eGFR, presence of kidney disease, African ancestry proportion, *APOL1* genotype, and FGF23 concentration. In analyses assessing calcified plaque, a minimal model adjusted for age, sex, and African ancestry proportion and a subsequent model included all covariates in the minimal model plus BMI, diabetes duration, GHb, smoking, eGFR, and UACR. Presence (mass score $\geq$10 mg Ca^{2+}) of calcified plaque in the coronary arteries and carotid arteries and total number of affected vascular beds were associated with higher mortality in the minimal, but not the full, model. Higher UACR was associated with higher mortality in the minimal model adjusting for age, sex, BMI, and African ancestry proportion and remained significant in the subsequent model that also included GHb, hypertension, and renin-angiotensin system blockade.

Table 3 presents association results between mortality and the novel risk factors African ancestry proportion, *APOL1* genotypes, and FGF23. Higher plasma FGF23 concentration was strongly associated with higher mortality after adjusting for age, sex, and African ancestry (minimal model) and in the subsequent model that also included BMI, diabetes duration, smoking, eGFR, hormone replacement therapy, and use of steroids (HR 1.74 [95% CI 1.44–2.09]; $P = 6.9 \times 10^{-9}$). For each 237.8 RU/mL increase in FGF23 concentration, 74% higher mortality was seen. Presence of the *APOL1* renal-risk genotype (two *APOL1* renal-risk variants) was associated with lower mortality in the minimal model ($P = 0.05$), with a trend in the full model that also included age, sex, African ancestry, diabetes duration, BMI, smoking, eGFR, UACR, and CAC (HR 0.16 [95% CI 0.02–1.17]; $P = 0.07$). African ancestry proportion was not associated with mortality in either model.

Table 2—Baseline biochemical and radiological characteristics of AA-DHS cohort

	AA-DHS cohort						
Variables	Full sample	*n*	Living	*n*	Deceased	*n*	*P* value
Fasting glucose (mmol/L)	7.4 (5.9, 9.7)	508	7.3 (5.9, 9.3)	454	8.4 (6.4, 10.5)	54	0.06
GHb (%)	7.7 (6.6, 9.1)	499	7.7 (6.6, 9.0)	446	7.9 (6.8, 9.6)	53	0.44
GHb (mmol/mol)	60.7 (48.6, 76.0)	499	60.7 (48.6, 74.9)	446	62.8 (50.8, 81.4)	53	0.44
Serum creatinine (μmol/L)	79.6 (70.7, 97.2)	508	79.6 (70.7, 97.2)	454	88.4 (70.7, 97.2)	54	0.02
FGF23 (RU/mL)	112.4 (79.0, 165.8)	513	107.3 (79.0, 154.0)	459	162.3 (97.3, 362.0)	54	5.0×10^{-4}
FGF23 (pg/mL)	224.8 (158.1, 331.6)	513	214.6 (158.0, 308.1)	459	324.7 (194.7, 724.0)	54	5.0×10^{-5}
HDL cholesterol (mmol/L)	1.2 (1.0, 1.4)	508	1.2 (1.0, 1.4)	454	1.2 (1.1, 1.4)	54	0.46
LDL cholesterol (mmol/L)	2.7 (2.1, 3.4)	499	2.7 (2.2, 3.4)	446	2.3 (1.8, 3.1)	53	0.008
Triglycerides (mmol/L)	1.2 (0.9, 1.6)	508	1.2 (0.9, 1.6)	454	1.1 (0.8, 1.6)	54	0.66
Coronary artery CP mass (mg Ca^{+})	28.5 (1.0, 348.6)	438	19.0 (0.0, 282.5)	393	187.0 (16.5, 796.5)	45	9.0×10^{-4}
Coronary artery CP >10 mg Ca^{+} (%)	56.8%	438	54.7%	393	75.6%	45	0.008
Carotid artery CP mass (mg Ca^{+})	4.5 (0.0, 102.2)	503	3.5 (0.0, 92.2)	450	52.5 (0.0, 284.5)	53	0.008
Carotid artery CP >10 mg Ca^{+} (%)	44.9%	503	43.1%	450	60.4%	53	0.02
Aorta CP mass (mg Ca^{+})	1,033.0 (34.8, 6,465.8)	504	968 (22.0, 6,071.5)	451	1,564 (205.0, 10,759.0)	53	0.06
Aorta CP >10 mg Ca^{+} (%)	78.2%	504	77.6%	451	83.0%	53	0.37
Vascular beds with CP, *n*	2.0 (1.0, 3.0)	431	2.0 (1.0, 3.0)	387	2.0 (2.0, 3.0)	44	0.007
eGFR (mL/min/1.73 m^2)	91.3 (76.4, 111.3)	508	91.4 (76.5, 112.8)	454	89.7 (73.5, 104.6)	54	0.29
eGFR <60 mL/min/1.73 m^2 (%)	9.8%	508	9.3%	454	14.8%	54	0.19
UACR (mg/mmol)	12.5 (4.2, 51.2)	504	11.0 (4.2, 46.0)	451	40.0 (5.0, 152.0)	53	0.004
UACR >3.39 mg/mmol (%)	35.9%	504	33.5%	451	56.6%	53	9.0×10^{-4}

Median (25th percentile, 75th percentile) for continuous variables. CP, calcified plaque.

Table 3—Multivariate risk factor association analysis results for mortality

		Model 1					Model 2				
Predictor	Change/status	*n*	Deaths	HR	95% CI	*P* value	*n*	Deaths	HR	95% CI	*P* value
Coronary artery CP, mass[a]	100 (mg Ca^+)	438	45	1.01	(0.99, 1.03)	0.26	422	43	1.01	(0.99, 1.03)	0.38
Coronary artery CP, presence[a]	Yes	438	45	2.18	(1.04, 4.57)	0.04	422	43	1.7	(0.76, 3.81)	0.19
Carotid artery CP, mass[a]	100 (mg Ca^+)	503	53	1.02	(0.99, 1.05)	0.13	486	51	1.03	(0.99, 1.06)	0.10
Carotid artery CP, presence[a]	Yes	503	53	1.76	(0.99, 3.14)	0.05	486	51	1.72	(0.92, 3.22)	0.09
Abdominal aorta CP, mass[a]	1,000 (mg Ca^+)	504	53	1.02	(1.00, 1.04)	0.11	488	51	1.02	(0.99, 1.04)	0.19
Abdominal aorta CP, presence[a]	Yes	504	53	1.09	(0.49, 2.39)	0.83	488	51	0.69	(0.29, 1.65)	0.40
Number of affected vascular beds[a]	1	431	44	1.52	(1.07, 2.14)	0.02	416	42	1.32	(0.90, 1.94)	0.16
UACR[b]	100 (mg/g)	504	53	2.57	(1.32, 5.03)	0.006	495	52	2.4	(1.18, 4.92)	0.02
UACR $>$30 mg/g[b]	Yes	504	53	2.24	(1.31, 3.83)	0.003	495	52	2.26	(1.26, 4.04)	0.006
UACR $>$300 mg/g[b]	Yes	504	53	2.3	(1.03, 5.14)	0.04	495	52	1.89	(0.78, 4.54)	0.16
eGFR[b]	10 (mL/min/1.73 m^2)	508	54	0.9	(0.77, 1.06)	0.22	499	53	0.9	(0.76, 1.07)	0.24
eGFR $<$60 mL/min/1.73 m^{2b}	Yes	508	54	1.94	(0.76, 4.94)	0.17	499	53	2.12	(0.81, 5.51)	0.13
CKD[b]	Yes	504	53	1.85	(0.91, 3.73)	0.09	495	52	1.79	(0.84, 3.80)	0.13
African ancestry proportion[c]	10%	512	54	0.89	(0.68, 1.15)	0.37	429	44	0.83	(0.63, 1.11)	0.21
APOL1 renal-risk variants[d]	Two risk variants	512	54	0.13	(0.02, 1.01)	0.05	429	44	0.16	(0.02, 1.17)	0.07
Plasma FGF23[e]	237.8 RU/mL[f]	513	54	1.69	(1.43, 1.98)	3.3×10^{-10}	503	53	1.74	(1.44, 2.09)	6.9×10^{-9}

CP, calcified plaque. [a]Model 1 adjusts for age, sex, and ancestry proportion. Model 2 adjusts for model 1 + BMI, diabetes duration, GHb, smoking, eGFR, and UACR. [b]Model 1 adjusts for age, sex, BMI, and ancestry. Model 2 adjusts for model 1 + GHb, hypertension, and renin-angiotensin system blockade. CKD reflects eGFR $<$60 mL/min/1.73 m^2 and/or UACR $>$300 mg/g. [c]Model 1 adjusts for age, sex, BMI, and diabetes duration. Model 2 adjusts for model 1 + smoking, eGFR, UACR, and coronary artery CP. [d]Model 1 adjusts for age, sex, ancestry, and diabetes duration. Model 2 adjusts for model 1 + BMI, smoking, eGFR, UACR, and coronary artery CP. [e]Model 1 adjusts for age, sex, and ancestry. Model 2 adjusts for model 1 + BMI, diabetes duration, smoking, eGFR, hormone replacement therapy, and steroids (118.6 RU/mL value corresponds to a change of 0.5 $\times$ SD in FGF23 concentration). [f]237.8 RU/mL is equivalent to 473.2 pg/mL.

Results of the LASSO multivariate model are presented in Table 4. Predictors of mortality included sex (male risk, $P = 2.0 \times 10^{-4}$), *APOL1* risk genotypes (absence of two *APOL1* renal-risk variants risk, $P = 0.02$), and plasma FGF23 concentration (higher risk, 1.4×10^{-7}); trends were observed for effects of diabetes duration ($P = 0.07$), number of affected vascular beds ($P = 0.07$), and UACR ($P = 0.11$). CAC, eGFR, smoking, BMI, and GHb were not significant predictors of mortality in this analysis.

Figure 1 displays four ROC plots for predicted survival at 1, 3, 5, and 7 years in a model including age, sex, ancestry, BMI, diabetes duration, smoking, UACR, and CAC. *P* values compare the AUC of the models with these covariates to the AUC also including FGF23 and *APOL1* genotypes; they reveal significantly improved prognostic accuracy of the model at all time points after 1-year follow-up. For example, the AUC improved from 0.72 to 0.80 at 7 years ($P = 2.1 \times 10^{-4}$).

CONCLUSIONS

This study assessed factors associated with mortality in an African American cohort with type 2 diabetes that was intensively phenotyped for subclinical CVD, nephropathy, and associated risk factors. Considering the known effects of subclinical atherosclerosis, kidney disease, glycemic control, and smoking, novel relationships were identified between higher plasma FGF23 concentrations and absence of *APOL1* renal-risk genotypes with higher mortality. The AA-DHS is a unique cohort of African Americans with relatively preserved kidney function, access to health care based on achieved blood pressure, lipid levels, and blood sugars, and linkage to the National Death Index (3). Traditional CVD risk factors were associated with higher mortality in this cohort, including male sex, hypertension, presence of clinical and subclinical CVD, and kidney disease (14–16). The novel finding of association between increasing plasma FGF23 concentrations and higher mortality was robust, observed in the final multivariate model (Table 4). In addition, significant association between *APOL1* non–renal-risk genotypes and higher mortality was independent from severity of albuminuria and degree of kidney function in this model.

Table 4—Multivariate model with nonzero predictors of mortality in the LASSO model

Predictor	LASSO estimate	Change/ status	HR	95% CI	*P* value
Age	0	—	—	—	—
Sex (male)	−0.59	1	4.17	(1.96, 9.09)	0.0002
BMI	0	—	—	—	—
Diabetes duration	0.16	4	1.13	(0.99, 1.28)	0.07
Smoking status	0.01	1	1.31	(0.65, 2.64)	0.46
Coronary artery CP, mass	0	—	—	—	—
Carotid artery CP, mass	0	—	—	—	—
Abdominal aorta CP, mass	0	—	—	—	—
Number of affected vascular beds	0.24	1	1.42	(0.98, 2.06)	0.07
UACR	0.19	100	1.94	(0.86, 4.41)	0.11
eGFR	0	—	—	—	—
CKD	0	—	—	—	—
African ancestry proportion	−0.11	10	0.81	(0.60, 1.08)	0.15
APOL1 renal-risk variants	−0.34	1	0.07	(0.01, 0.69)	0.02
Plasma FGF23	0.39	238	2.1	(1.59, 2.78)	1.4×10^{-7}
GHb	0	—	—	—	—

The LASSO and the final Cox regressions were fit based on a sample size of 416 individuals and 42 deaths. CP, calcified plaque.

African Americans with and without diabetes are at higher risk than European Americans for major CVD events and higher mortality rates (17,18). However, many reports on African Americans included individuals without access to adequate health care and with suboptimal control of conventional CVD risk factors (19,20). Lack of access to health care likely confounds many outcome studies in minority populations. In support of this concept, multiethnic studies in populations with equivalent access to health care demonstrated marked reductions in risk of myocardial infarction and lower mortality in African Americans than European Americans (20–23). This contrasts with the increased risk of CVD and higher mortality in African Americans than in the general population. Dramatically lower rates of subclinical CVD, typically assessed as calcified atherosclerotic plaque, are seen in African Americans relative to European Americans (24–29). This is observed in populations with and without diabetes and despite the presence of more severe conventional CVD risk factors in African Americans. MESA and the AA-DHS reported that higher proportions of African ancestry were associated with lower levels of CAC (30,31). It is likely that the biologically protective effect of African ancestry on CVD can be overwhelmed by the environmental factor suboptimal access to health care.

A novel finding was the positive association between plasma FGF23 concentrations and mortality in this cohort lacking advanced kidney disease. FGF23 is an osteocyte-derived phosphaturic hormone that plays a key role in CKD–mineral and bone disorder. In addition to suppressing parathyroid hormone secretion, FGF23 downregulates proximal tubule sodium phosphate cotransporter Na/Pi IIa and IIc and inhibits renal calcitriol synthesis to achieve phosphate homeostasis in early stages of CKD (32). FGF23 is reproducibly associated with an elevated risk of CVD and death in cohorts with moderate to severe CKD. A prospective study of individuals from the Chronic Renal Insufficiency Cohort (CRIC) with moderate CKD found FGF23 to independently predict mortality (33). Likewise, elevated FGF23 levels have been associated with higher mortality in incident hemodialysis patients (34). Epidemiological studies reveal positive associations between FGF23 and CAC, thoracic, and abdominal calcified plaque including in a prior AA-DHS report (7), but this finding was not replicated in CRIC or MESA (35,36).

Levels of FGF23 rise early in the course of CKD and may be one of the earliest biomarkers of CKD–mineral and bone disorder. In cohorts with mild CKD, elevated circulating FGF23 levels have demonstrated adverse CVD outcomes in predominantly non–African American cohorts (37–39). The present results extend this observation to an African American sample with normal to mildly reduced kidney function. In addition, the association was robust to adjustment for eGFR and albuminuria, suggesting that FGF23 effects are independent from severity of kidney disease. Findings extend our prior work on FGF23 based on the addition of mortality data through calendar year 2015 and inclusion of CAC and *APOL1* as covariates in the model (7).

Participants who had two *APOL1* renal-risk variants (defining the renal-risk genotype) also had lower mortality rates than those with fewer than two risk variants. *APOL1* is not associated with kidney disease in patients with diabetes (3). Although *APOL1* renal-risk variants were reproducibly associated with lower levels of calcified plaque in the Jackson Heart Study and AA-DHS, as well as with less severe cerebral small vessel disease, associations with CVD remain controversial (40). The present results support *APOL1* renal-risk genotype association with lower mortality after accounting for the competing effects of age, sex, BMI, diabetes duration, smoking, African ancestry proportion, UACR, and FGF23.

The AA-DHS is focused on the understudied African American population; it has strengths and limitations. Strengths include the relatively large population-based cohort with type 2 diabetes, extensive CVD and renal phenotypes, and long-term follow-up. One limitation is that all causes of death were assessed in this report. This related to use of the National Death Index. Death certificates revealed that 23.5% of the 54 deaths were attributed to CVD; however, inaccuracies exist with death certificates and we were unable to adjudicate cause of death. Analyses used baseline GHb measures, which remained stable during 3.8-year median follow-up in the majority of participants. However, longer-term changes in glycemic control in the full cohort could not be assessed. A potential drawback of using a LASSO multivariate model is that it typically selects only one predictor from a group of correlated predictors. This could explain why the parameter estimate associated with CAC was shrunken to 0 in the presence of other, possibly stronger, predictors of death in this data

set. Few cohorts contain African Americans with type 2 diabetes who lack nephropathy and have measures of CAC and FGF23; as such, replication in a similar population may prove challenging. We note that smoking did not associate with mortality in this report. This paradoxical observation likely relates to limitations in the sample size and duration of follow-up. Finally, current findings are limited to African Americans with type 2 diabetes. Results cannot be extrapolated to other populations or those without diabetes. We could not assess effects of FGF23 in European American DHS participants because plasma samples were lost after a freezer malfunction; European populations also lack *APOL1* risk variants.

Analyses in a large cohort of African Americans with type 2 diabetes at high risk for CVD confirm that hypertension, male sex, lower BMI, subclinical atherosclerosis, and albuminuria were associated with higher mortality. In addition

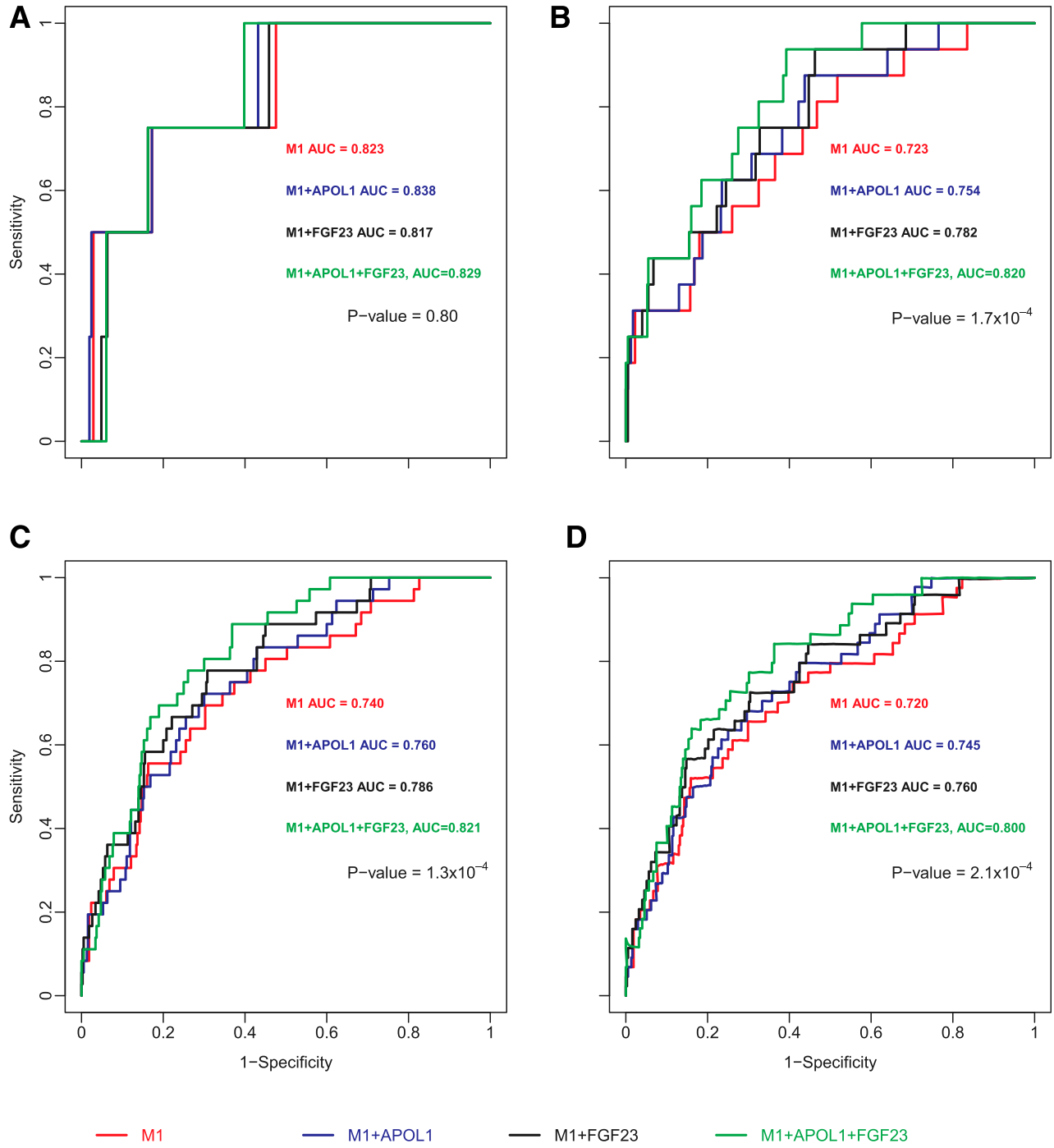

Figure 1—Comparison of ROC curves for models with and without *APOL1* renal-risk genotypes and FGF23. *A*: Predicted survival at 1 year. *B*: Predicted survival at 3 years. *C*: Predicted survival at 5 years. *D*: Predicted survival at 7 years. Model 1 (M1) includes age, sex, African ancestry proportion, BMI, diabetes duration, smoking status, UACR, and CAC. *P* values compare the AUC of the full model that includes all the covariates in model 1 plus *APOL1* risk and FGF23 to model 1.

and independent of kidney function, higher plasma FGF23 concentrations and absence of *APOL1* renal-risk genotypes were associated with higher mortality rates. Roles for measuring FGF23 levels and *APOL1* genotypes in clinical practice to aid in risk prediction remain unclear. The consistent association between FGF23 and mortality suggests that this biomarker is a risk factor that warrants measurement; herein, we extend this observation to the African American population with diabetes. Additional replication studies will be required to assess the association with *APOL1*, and this effect would only be relevant in populations with recent African ancestry, such as African Americans. Higher FGF23 levels and absence of *APOL1* risk genotypes had previously been associated with calcified atherosclerotic plaque (subclinical atherosclerosis). Although reports that show higher mortality rates and rates of myocardial infarction in African Americans relative to European Americans may have been confounded by suboptimal access to health care, the AA-DHS cohort appears to have had similar access to health care as European Americans in most contemporary reports. Mechanisms whereby FGF23 and *APOL1* renal-risk variants impact subclinical atherosclerosis and mortality remain to be identified.

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Duality of Interest. Wake Forest University Health Sciences and B.I.F. filed for a patent related to *APOL1* genetic testing. B.I.F. is a consultant for Ionis Pharmaceuticals and AstraZeneca. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. G.C.C. helped interpret the results and drafted the manuscript. J.D. helped interpret the results and performed the statistical analyses. G.B.R., C.D.L., L.E.W., and F.-C.H. helped interpret the results. J.X. managed the database. S.C.S. recruited participants. N.D.P. and P.J.H. performed the *APOL1* genotyping. D.W.B., T.C.R., L.M., and J.J.C. reviewed and approved the manuscript. B.I.F. designed the study, helped interpret the results, and contributed to data analyses, interpretation, and drafting the manuscript. B.I.F. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010;87:4–14
2. Freedman BI, Divers J, Palmer ND. Population ancestry and genetic risk for diabetes and kidney, cardiovascular, and bone disease: modifiable environmental factors may produce the cures. Am J Kidney Dis 2013;62:1165–1175
3. Freedman BI, Langefeld CD, Lu L, et al. APOL1 associations with nephropathy, atherosclerosis, and all-cause mortality in African Americans with type 2 diabetes. Kidney Int 2015;87:176–181
4. Palmer ND, Divers J, Lu L, et al. Admixture mapping of serum vitamin D and parathyroid hormone concentrations in the African American-Diabetes Heart Study. Bone 2016;87:71–77
5. Qin Z, Liu X, Song M, et al. Fibroblast growth factor 23 as a predictor of cardiovascular and all-cause mortality in prospective studies. Atherosclerosis 2017;261:1–11
6. Haring R, Enserro D, Xanthakis V, et al. Plasma fibroblast growth factor 23: clinical correlates and association with cardiovascular disease and mortality in the Framingham Heart Study. J Am Heart Assoc 2016;5:pii:e003486
7. Freedman BI, Divers J, Russell GB, et al. Plasma FGF23 and calcified atherosclerotic plaque in African Americans with type 2 diabetes mellitus. Am J Nephrol 2015;42:391–401
8. Levey AS, Stevens LA, Schmid CH, et al.; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604–612
9. Carr JJ, Nelson JC, Wong ND, et al. Calcified coronary artery plaque measurement with cardiac CT in population-based studies: standardized protocol of Multi-Ethnic Study of Atherosclerosis (MESA) and Coronary Artery Risk Development in Young Adults (CARDIA) study. Radiology 2005; 234:35–43
10. Wagenknecht LE, Divers J, Register TC, et al. Bone mineral density and progression of subclinical atherosclerosis in African-Americans with type 2 diabetes. J Clin Endocrinol Metab 2016; 101:4135–4141
11. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. Biometrics 1986;42:121–130
12. Simon N, Friedman J, Hastie T, Tibshirani R. Regularization paths for Cox's proportional hazards model via coordinate descent. J Stat Softw 2011;39:1–13
13. Blanche P, Dartigues JF, Jacqmin-Gadda H. Estimating and comparing time-dependent areas under receiver operating characteristic curves for censored event times with competing risks. Stat Med 2013;32:5381–5397
14. Almdal T, Scharling H, Jensen JS, Vestergaard H. The independent effect of type 2 diabetes mellitus on ischemic heart disease, stroke, and death: a population-based study of 13,000 men and women with 20 years of follow-up. Arch Intern Med 2004;164:1422–1426
15. Elkeles RS, Godsland IF, Feher MD, et al.; PREDICT Study Group. Coronary calcium measurement improves prediction of cardiovascular events in asymptomatic patients with type 2 diabetes: the PREDICT study. Eur Heart J 2008;29: 2244–2251
16. Lau KK, Wong YK, Chan YH, et al. Prognostic implications of surrogate markers of atherosclerosis in low to intermediate risk patients with type 2 diabetes. Cardiovasc Diabetol 2012;11:101
17. Clark LT, Ferdinand KC, Flack JM, et al. Coronary heart disease in African Americans. Heart Dis 2001;3:97–108
18. Xu J, Murphy SL, Kochanek KD, Bastian BA. Deaths: final data for 2013. Natl Vital Stat Rep 2016;64:1–119
19. Cook NL, Mensah GA. Eliminating health disparities: what can we learn from the Veterans Health Administration? Circulation 2015;132: 1519–1521
20. Kovesdy CP, Norris KC, Boulware LE, et al. Association of race with mortality and cardiovascular events in a large cohort of US veterans. Circulation 2015;132:1538–1548
21. Karter AJ, Ferrara A, Liu JY, Moffet HH, Ackerson LM, Selby JV. Ethnic disparities in diabetic complications in an insured population. JAMA 2002;287:2519–2527
22. Young BA, Maynard C, Boyko EJ. Racial differences in diabetic nephropathy, cardiovascular disease, and mortality in a national population of veterans. Diabetes Care 2003;26:2392–2399
23. Young BA, Rudser K, Kestenbaum B, Seliger SL, Andress D, Boyko EJ. Racial and ethnic differences in incident myocardial infarction in end-stage renal disease patients: the USRDS. Kidney Int 2006;69:1691–1698
24. Newman AB, Naydeck BL, Whittle J, Sutton-Tyrrell K, Edmundowicz D, Kuller LH. Racial differences in coronary artery calcification in older adults. Arterioscler Thromb Vasc Biol 2002;22:424–430
25. Bild DE, Detrano R, Peterson D, et al. Ethnic differences in coronary calcification: the Multi-Ethnic Study of Atherosclerosis (MESA). Circulation 2005;111:1313–1320
26. Lee TC, O'Malley PG, Feuerstein I, Taylor AJ. The prevalence and severity of coronary artery calcification on coronary artery computed tomography in black and white subjects. J Am Coll Cardiol 2003;41:39–44
27. Freedman BI, Hsu FC, Langefeld CD, et al. The impact of ethnicity and sex on subclinical cardiovascular disease: the Diabetes Heart Study. Diabetologia 2005;48:2511–2518
28. Carnethon MR, Bertoni AG, Shea S, et al. Racial/ethnic differences in subclinical atherosclerosis among adults with diabetes: the Multiethnic Study of Atherosclerosis. Diabetes Care 2005;28: 2768–2770
29. Budoff MJ, Nasir K, Mao S, et al. Ethnic differences of the presence and severity of coronary atherosclerosis. Atherosclerosis 2006;187:343–350
30. Wassel CL, Pankow JS, Peralta CA, Choudhry S, Seldin MF, Arnett DK. Genetic ancestry is associated with subclinical cardiovascular disease in African-Americans and Hispanics from the multiethnic study of atherosclerosis. Circ Cardiovasc Genet 2009;2:629–636
31. Divers J, Palmer ND, Lu L, et al. Admixture mapping of coronary artery calcified plaque in African Americans with type 2 diabetes mellitus. Circ Cardiovasc Genet 2013;6:97–105
32. Miyamoto K, Ito M, Tatsumi S, Kuwahata M, Segawa H. New aspect of renal phosphate reabsorption: the type IIc sodium-dependent phosphate transporter. Am J Nephrol 2007;27:503–515
33. Isakova T, Xie H, Yang W, et al.; Chronic Renal Insufficiency Cohort (CRIC) Study Group. Fibroblast growth factor 23 and risks of mortality and

end-stage renal disease in patients with chronic kidney disease. JAMA 2011;305:2432–2439
34. Gutiérrez OM, Mannstadt M, Isakova T, et al. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. N Engl J Med 2008;359:584–592
35. Scialla JJ, Lau WL, Reilly MP, et al.; Chronic Renal Insufficiency Cohort Study Investigators. Fibroblast growth factor 23 is not associated with and does not induce arterial calcification. Kidney Int 2013;83:1159–1168
36. Hsu JJ, Katz R, Ix JH, de Boer IH, Kestenbaum B, Shlipak MG. Association of fibroblast growth factor-23 with arterial stiffness in the Multi-Ethnic Study of Atherosclerosis. Nephrol Dial Transplant 2014;29:2099–2105
37. Tuñón J, Fernández-Fernández B, Carda R, et al. Circulating fibroblast growth factor-23 plasma levels predict adverse cardiovascular outcomes in patients with diabetes mellitus with coronary artery disease. Diabetes Metab Res Rev 2016;32:685–693
38. Shah NH, Dong C, Elkind MS, et al. Fibroblast growth factor 23 is associated with carotid plaque presence and area: the Northern Manhattan Study. Arterioscler Thromb Vasc Biol 2015;35:2048–2053
39. Panwar B, Jenny NS, Howard VJ, et al. Fibroblast growth factor 23 and risk of incident stroke in community-living adults. Stroke 2015;46:322–328
40. McLean NO, Robinson TW, Freedman BI. APOL1 gene kidney risk variants and cardiovascular disease: getting to the heart of the matter. Am J Kidney Dis 2017;70:281–289