

Coding Variants in Nephrin (*NPHS1*) and Susceptibility to Nephropathy in African Americans

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Abstract

Background and objectives Presumed genetic risk for diabetic and nondiabetic end stage renal disease is strong in African Americans.

Design, setting, participants, & measurements Exome sequencing data from African Americans with type 2 diabetic end stage renal disease and nondiabetic, non-nephropathy controls in the T2D-GENES study (Discovery, $n=529$ patients and $n=535$ controls) were evaluated, focusing on missense variants in *NPHS1*. Associated variants were then evaluated in independent type 2 diabetic end stage renal disease (Replication, $n=1305$ patients and $n=760$ controls), nondiabetic end stage renal disease ($n=1705$), and type 2 diabetes-only, non-nephropathy samples ($n=503$). All participants were recruited from dialysis facilities and internal medicine clinics across the southeastern United States from 1991 to present. Additional *NPHS1* missense variants were identified from exome sequencing resources, genotyped, and sequence kernel association testing was then performed.

Results Initial analysis identified rs35238405 (T233A; minor allele frequency=0.0096) as associated with type 2 diabetic end stage renal disease (adjustment for admixture $P=0.042$; adjustment for admixture+*APOL1* $P=0.080$; odds ratio, 2.89 and 2.36, respectively); with replication in independent type 2 diabetic end stage renal disease samples ($P=0.018$; odds ratio, 4.30) and nondiabetic end stage renal disease samples ($P=0.016$; odds ratio, 4.48). In a combined analysis (all patients with end stage renal disease versus all controls), T233A was associated with all-cause end stage renal disease ($P=0.0038$; odds ratio, 2.82; $n=3270$ patients and $n=1187$ controls). A P -value of <0.001 was obtained after adjustment for admixture and *APOL1* in sequence kernel association testing. Two additional variants (H800R and Y1174H) were nominally associated with protection from end stage renal disease ($P=0.036$; odds ratio, 0.44; $P=0.0084$; odds ratio, 0.040, respectively) in the locus-wide single-variant association tests.

Conclusions Coding variants in *NPHS1* are associated with both risk for and protection from common forms of nephropathy in African Americans.

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Introduction

Multiple lines of evidence support genetic influences on susceptibility to end stage renal disease (ESRD). It is particularly true in African Americans, where ESRD incidence rates are 3.5-fold higher than in European Americans (1). After adjustment for socioeconomic status, incidence rates and familial aggregation of ESRD remain markedly higher among African Americans compared with European Americans and other ethnic minorities (2,3). The apolipoprotein L1 gene (*APOL1*) G1 and G2 alleles explain a substantial portion of the ethnic disparity in HIV-associated collapsing glomerulopathy, idiopathic FSGS, and hypertension-attributed ESRD (4,5). These variants account for a portion of the ethnic difference in risk for nondiabetic forms of ESRD (nontype 2 diabetes [T2D] ESRD); however, they fail to fully account for the excess risk of type 2 diabetic nephropathy (T2D-ESRD) in African Americans (6). Although *APOL1* contributes to nephropathy

progression in patients with and without hyperglycemia, it remains controversial as to whether it associates with classic histopathologic findings observed in diabetic nephropathy (7). Other genetic loci likely contribute to T2D-ESRD risk in the African-American population (8).

Next generation exome sequencing (NGES) allows one to comprehensively identify and test genetic variations in coding sequences of genes for disease association, facilitating the detailed exploration of previously untested genetic regions. We used NGES data to survey nephrin (*NPHS1*), an essential slit diaphragm protein, implicated in congenital nephrotic syndrome of the Finnish Type (CNS) (9,10). In the canonical CNS a 2-bp frameshift mutation (Fin_{major}) and a nonsense mutation in exon 26 (Fin_{minor}) are responsible for $>80\%$ of all CNS cases in Finland (9). In addition, variants in *NPHS1* have been linked to minimal change nephrotic syndrome and childhood-onset

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steroid-resistant nephrotic syndromes (11,12) as well as late-onset forms of steroid-resistant nephrotic syndrome (13). In contrast to these uncommon forms of nephropathy, we evaluated rare and low-frequency *NPHS1* variants for association with ESRD in a community-based sample of African Americans. We hypothesized that low-frequency (minor allele frequency [MAF]<5%) and rare (MAF<0.5%) coding variants in *NPHS1* modulated ESRD susceptibility in African Americans.

Materials and Methods

Study Participants

Detailed recruitment and sample collection have previously been described (6,14). The study was approved by the Institutional Review Board at Wake Forest School of Medicine (WFSOM). All Discovery and Replication participants were unrelated, were born in North Carolina, South Carolina, Georgia, Tennessee, or Virginia, provided informed consent, and were recruited from 1991 to present. DNA extraction was performed using the PureGene system (Gentra Systems). African Americans with T2D-ESRD were recruited from dialysis facilities. T2D was diagnosed in patients developing diabetes after age 25 years without historical evidence of diabetic ketoacidosis or receiving solely insulin therapy since diagnosis. T2D-ESRD was diagnosed after >5-years diabetes duration before RRT in the absence of other causes of nephropathy. Unrelated African-American controls without diabetes or renal disease (on the basis of a serum creatinine concentration<1.5 [men] or <1.3 mg/dl [women]) were recruited from the community and internal medicine clinics at WFSOM (both Discovery and Replication controls). Ethnicity was self-reported and confirmed by genotyping with ancestry informative markers. Mean African ancestry of the Replication samples was 79.85%±11.54% for patients and 77.7%±10.96% for controls.

African-American patients with non-T2D-ESRD did not have diabetes (or diabetes developed after initiating RRT). ESRD was attributed to chronic glomerular disease (*e.g.*, FSGS), HIV-associated nephropathy, hypertension, or unknown cause. Patients with ESRD caused by polycystic kidney disease or IgA nephropathy were excluded. The mean African ancestry of the non-T2D-ESRD samples was 80.01%±10.96%.

African Americans with T2D but lacking nephropathy were recruited from the African American-Diabetes Heart Study (15). These diabetic controls were receiving insulin or oral agents, had a hemoglobin A1C>6.5% or a fasting plasma glucose>126 mg/dl, and serum creatinine concentration<1.5 (men) or <1.3 mg/dl (women). All T2D-only non-nephropathy controls in this study had an eGFR>60 ml/min per 1.73 m² and urine albumin-to-creatinine ratio<60 mg/g. Although some diabetic controls had low-level microalbuminuria, a urine albumin-to-creatinine ratio≤60 mg/g was included to improve power, because many African Americans with T2D have mild albuminuria and normal eGFR.

Sample Preparation, Genotyping, and Quality Control

African-American T2D-ESRD T2D-GENES Discovery Patients and Controls. NGES was performed under the auspices of the T2D-GENES Consortium ([\[sph.umich.edu/\]\(http://sph.umich.edu/\)\) using an Agilent V2 capture array platform \(Agilent Technologies\) at the Broad Institute \(Cambridge, MA\). Data underwent multiple levels of quality control \(QC\) before release, including tests for cryptic relatedness, tests for heterozygosity, and confirmation of variant calling. Patients with T2D-ESRD and nondiabetic, non-nephropathy controls were selected from a previously published African-American T2D-ESRD genome-wide association study \(6\).](https://t2d-genes.</p>
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Targeted Genotyping. Genotyping of *NPHS1* variant rs35238405 was performed using the Sequenom MassArray system (Sequenom) in the Center for Genomics and Personalized Medicine Research at WFSOM. PCR primers designed in MassARRAY Assay Design 3.1 (Sequenom) and genotypes were analyzed using MassARRAY Typer (Sequenom). Call rates were >97%, and blind duplicates within each cohort were genotyped (100% concordance rate).

Locus-Wide Analyses. The 1000 Genomes Project and the Exome Variant Server (National Heart, Lung, and Blood Institute) were surveyed for additional *NPHS1* missense variants beyond the variants found in T2D-GENES. Variants were selected on the basis of allele enrichment in African versus European ancestral populations, missense mutation, and PolyPhen2 prediction (<http://genetics.bwh.harvard.edu/pph/data/>). An additional 32 *NPHS1* variants were selected for genotyping in T2D-ESRD, non-T2D-ESRD, and population-based control cohorts; four variants were unable to be genotyped because of their genomic context. Twenty-eight variants were genotyped as before, of which four variants failed QC.

Statistical Analyses

Each single-nucleotide polymorphism (SNP) was tested for departure from Hardy-Weinberg Equilibrium (HWE) expectations through a chi-squared goodness-of-fit test (HWE $P>0.001$ patients and HWE $P>0.01$ controls). Given the allele frequencies, only the dominant and additive genetic models were computed to test for association between each SNP and each phenotype. Data from T2D-GENES were adjusted for admixture only through a principal component analysis (Supplemental Table 1). All other tests for association were adjusted for admixture (16) and *APOL1* G1/G2 risk allele status (4). These tests were computed using the SNP-GWA program (http://www.phs.wfubmc.edu/public_bios/sec_gene/downloads.cfm).

Sequence kernel association testing (SKAT) was performed on all 16 nonmonomorphic *NPHS1* variants genotyped in patients with T2D-ESRD, patients with non-T2D-ESRD, and nondiabetic, non-nephropathy population-based controls (17). The SKAT Meta package was run on R 3.0.1 (<http://cran.r-project.org/web/views/Genetics.html>) using the default model weighted for rare variants termed c(1,25). An all-cause ESRD analysis was performed by combining genotype information from patients with T2D-ESRD and non-T2D-ESRD versus the controls. We used the Fisher protected least significant differences approach to evaluate significance of the variants (18). Specifically, we computed the overall gene-level test provided by SKAT Meta to determine if there was evidence of an effect within *NPHS1*. If the overall test was significant, we computed the individual SNP comparisons. The goal with the latter set of

comparisons is to identify the variants that are driving the gene-level test.

Results

The influence of *NPHS1* coding variants on susceptibility to ESRD was evaluated in the African-American population. Characteristics of the six sample groups are summarized in Table 1. Participants with T2D-ESRD in the T2D-GENES NGES cohort (Discovery patients) and patients in the Replication T2D-ESRD cohort were similar for all characteristics. Ages at enrollment for both T2D-ESRD case groups were older than ages for the two control groups (T2D non-nephropathy and nondiabetic, non-nephropathy controls); however, the ages at T2D onset among patients with T2D-ESRD were younger than the ages at enrollment for the controls. All cohorts except patients with non-T2D-ESRD had a larger percentage of women compared with men. The distribution of body mass index is similar across all samples; participants with non-T2D-ESRD had the lowest mean body mass index (Table 1).

Data from NGES on 529 African-American patients in the Discovery T2D-ESRD cohort and 535 African-American nondiabetic, non-nephropathy controls was evaluated. Forty coding *NPHS1* variants were identified (Supplemental Table 1). Because of the low power of this Discovery study, we focused on variants within *NPHS1* that were statistically associated with T2D-ESRD (Supplemental Tables 1 and 6). The *NPHS1* variant rs35238405 (T233A) was among the most common coding variants (MAF is approximately 1%) with PolyPhen2 prediction of affecting protein function. The T233A variant had an MAF of 1.4% in patients

in the T2D-ESRD Discovery group and 0.6% in Discovery controls (Table 2) ($P=0.042$ adjusted for admixture; odds ratio [OR], 2.89; 95% confidence interval [95% CI], 1.04 to 8.03 [Supplemental Table 1] and $P=0.080$ adjusted for admixture and *APOL1* [Table 2]). T233A was subsequently genotyped in 1305 independent African-American patients in the T2D-ESRD Replication group and 760 African-American Replication nondiabetic, non-nephropathy controls (T2D-ESRD Replication in Table 2). T233A was associated with T2D-ESRD ($P=0.018$; OR, 4.30; 95% CI, 1.29 to 14.34) in the Replication analysis after adjustment for admixture and *APOL1*; rs35238405 had similar MAFs in both samples (Table 2).

With consistent association between rs35238405 (T233A) and T2D-ESRD, we investigated the association of this variant with nondiabetic etiologies of ESRD; rs35238405 was genotyped in 1705 African Americans in the non-T2D-ESRD group, where it was significantly associated with non-T2D-ESRD (OR, 4.48; 95% CI, 1.33 to 15.11; $P=0.016$ compared with 671 controls used in the T2D-ESRD Replication analysis) (Table 2). The T233A variant was also tested for association with T2D in the absence of nephropathy; in 503 African-American T2D non-nephropathy samples (eGFR >60 ml/min per 1.73² and serum creatinine <1.5 [men] and <1.3 mg/dl [women]), no evidence for association with T2D was detected ($P=0.16$ and $P=0.28$ after adjustment for admixture and *APOL1* and admixture alone, respectively) (Supplemental Table 5). Finally, T233A was investigated in a combined all-cause ESRD analysis that pooled patients in the Discovery and Replication T2D-ESRD groups and the non-T2D-ESRD

Table 1. Clinical characteristics of African-American study cohorts

Variable	Discovery T2D-ESRD	Discovery Controls	Replication T2D-ESRD	Replication Controls	Non-T2D-ESRD	T2D Only (GFR ≥ 60 ml/min per 1.73 m ²)
Sample source	T2D-GENES patients	T2D-GENES controls	T2D-ESRD GWAS patients	T2D-ESRD GWAS controls	Non-T2D-ESRD patients	T2D non-nephropathy controls
<i>N</i>	529	535	1305	760	1705	503
Women, %	61.2	57.3	60.7	57.9	43.7	58.7
Age, yr	61.6 \pm 10.5	49.0 \pm 11.9	61.3 \pm 10.8 ^a	48.4 \pm 12.7 ^b	54.6 \pm 14.6 ^c	55.9 \pm 9.5
Age at T2D, yr	41.6 \pm 12.4	—	41.3 \pm 12.4	—	—	46.2 \pm 10.3
Duration of T2D before ESRD, yr	17.6 \pm 10.2	—	17.1 \pm 10.7	—	—	—
Duration of ESRD, yr	3.77 \pm 3.8	—	3.66 \pm 3.9	—	2.2 \pm 1.6	—
Blood glucose, mg/dl	—	88.8 \pm 13.1	—	89.2 \pm 13.6	88.6 \pm 8.7	—
BMI (at recruitment), kg/m ²	29.7 \pm 7.0	30.0 \pm 7.0	30.3 \pm 7.2	29.2 \pm 7.4	27.2 \pm 7.0	36.5 \pm 18.4
BUN, mg/dl	—	13.3 \pm 5.4	—	13.3 \pm 4.5	—	—
Serum creatinine, mg/dl	—	0.99 \pm 0.25	—	1.03 \pm 0.46	—	0.95 \pm 0.2

Categoric data expressed as percentage; continuous data described as mean \pm SD. T2D, type 2 diabetes mellitus; ESRD, end stage renal disease; GWAS, genome-wide association study; BMI, body mass index.

^a*n*=50 missing age data.

^b*n*=33 missing age data.

^c*n*=15 missing age data.

Table 2. rs35238405 (T233A) single single-nucleotide polymorphism association testing (dominant model) with adjustment for admixture and apolipoprotein L1 gene G1/G2 allele status

Study	N Patient/Control	MAF Patient/Control	P Value	OR	95% CI
T2D-GENES (exome sequence)	528/514	0.014/0.0056	0.080	2.36	0.90 to 6.16
T2D-ESRD Replication	1255/673	0.010/0.0022	0.018	4.30	1.29 to 14.34
Non-T2D ESRD	1487/673	0.012/0.0022	0.016	4.48	1.33 to 15.11
T2D-only (versus controls)	480/1187	0.0073/0.0038	0.16	2.04	0.75 to 5.53
All-cause ESRD	3270/1187	0.012/0.0038	0.0038	2.82	1.40 to 5.68

Sample sizes reflect those patients with complete clinical and genotypic data used in each analysis. MAF, minor allele frequency; OR, odds ratio; 95% CI, 95% confidence interval; T2D, type 2 diabetes mellitus; ESRD, end stage renal disease.

group as well as all population-based controls. Here, the T233A variant was associated with all-cause ESRD ($P=0.0038$) and had an OR of 2.82 (95% CI, 1.4 to 5.68) (Table 2). Adjusting for age and sex in addition to admixture and *APOL1* had no effect on the observed OR but decreased power because of reduced sample size (missing covariate data).

One third (33%) of T233A ESRD carriers had two *APOL1* risk alleles compared with 25% of patients with non-T233A ESRD, trending toward significance ($P=0.090$). It is possible that there is an additive interaction between T233A and *APOL1* G1/G2 risk alleles, although we may lack statistical power to detect this effect. Patients with ESRD carrying the T233A allele were more likely to be women (62.4% versus 51.3%; $P=0.043$), have a greater percentage of African ancestry (0.83 versus 0.80; $P=0.020$), and have a trend to family history of ESRD (38.6% versus 28.9%; $P=0.16$) (Supplemental Table 3).

We examined the histopathology of T233A in an independent sample of 542 African-American patients who underwent renal biopsy at Nephropath; 13 patients were carriers of T233A (MAF=2.4%). Findings on biopsy included diabetic glomerulosclerosis, membranous glomerulopathy, arterionephrosclerosis, and collapsing glomerulopathy (data not shown). There were no consistent changes associated with the T233A allele at the light microscopy or ultrastructural level.

To determine if other rare and low-frequency missense variants contribute to T2D-ESRD, non-T2D-ESRD, or all-cause ESRD, an additional 28 *NPHS1* variants chosen from exome sequence resources were genotyped. Four variants failed QC metrics, and eight variants proved to be monomorphic. MAFs of the remaining 16 observed variants in patients with T2D-ESRD ranged from 0.0004 to 0.13. The contributions of these *NPHS1* variations to genetic susceptibility to ESRD are reflected in an assessment of the cumulative effect of variants using SKAT (17), which weights variants on MAF to increase power. SKAT analysis combined genotype information from the T2D-ESRD Replication cohort, the non-T2D-ESRD cohort, and the Replication set of 760 non-T2D, non-nephropathy controls. All 16 nonmonomorphic *NPHS1* variants that passed QC were included in SKAT (Supplemental Table 7). Under a default model weighted for rare variants, P values <0.001, <0.001, and <0.001 were obtained in unadjusted, admixture, and admixture and *APOL1*-adjusted models, respectively, for all-cause ESRD (Table 3).

To assess which variants were driving the association observed in SKAT, we performed single SNP association testing. rs146400394 (H800R) was associated with protection from all-cause ESRD ($P=0.0084$; OR, 0.04; 95% CI, 0 to 0.44) after adjustment for admixture and *APOL1* (Table 4). rs115489112 (Y1174H) was nominally associated with protection from T2D-ESRD ($P=0.022$; OR, 0.29; 95% CI, 0.10 to 0.84) and all-cause ESRD ($P=0.036$; OR, 0.44; 95% CI, 0.2 to 0.95) after adjustment for admixture and *APOL1* but not non-T2D-ESRD (Table 4).

Finally, we calculated the population attribute risk of *NPHS1* variants for T2D-ESRD using formulas published by Kraft *et al.* (19). We obtained T2D-ESRD population attribute risks of 1.1%, 2.43%, and 4.56% for the T233A, Y1174H, and H800R variants, respectively.

Discussion

To identify coding variants associated with nephropathy susceptibility, we evaluated exome sequence data from a unique cohort of African-Americans with T2D-ESRD and nondiabetic, non-nephropathy controls. The Discovery study identified a missense mutation (T233A) in the *NPHS1* gene, rs35238405, which was associated with a heightened risk of T2D-ESRD (OR, 2.89; 95% CI, 1.04 to 8.03; $P=0.042$). Results were replicated in additional patients with T2D-ESRD and non-T2D-ESRD, with similar MAFs in patients and controls and consistent ORs (Table 2). We further evaluated whether rs35238405 contributes solely to T2D-ESRD or other nondiabetic etiologies of

Table 3. Locus-wide nephrin gene sequence kernel association testing analysis weighted for rare variants

Model	P Value	Q	cMAF (%)	N SNPs
Unadjusted	2.95×10^{-8}	280,417	0.29	16
Admixture	5.64×10^{-7}	159,005	0.28	15
Admixture and <i>APOL1</i> G1/G2	1.18×10^{-6}	145,754	0.28	15

Q, test statistic (similar to chi-squared); cMAF, cumulative minor allele frequency for all SNPs analyzed; SNP, single-nucleotide polymorphism; *APOL1*, apolipoprotein L1 gene.

Table 4. Nephrin gene variants nominally associated with end stage renal disease in locus-wide analysis using single single-nucleotide polymorphism association testing after adjustment for admixture and apolipoprotein L1 gene G1/G2 allele status

Study	SNP	N Patient/ Control	MAF Patient/ Control	P Value	OR	95% CI
T2D-ESRD Replication	rs115489112 (Y1174H) ^a	1222/642	0.0025/0.0078	0.022	0.29	0.10 to 0.84
All-cause ESRD	rs115489112 (Y1174H) ^a	2673/642	0.0039/0.0078	0.036	0.44	0.20 to 0.95
All-cause ESRD	rs146400394 (H800R) ^b	2757/642	0.00020/0.0022	0.0084	0.04	0 to 0.44

Sample sizes reflect those patients with complete clinical and genotypic data used in each analysis. SNP, single-nucleotide polymorphism; MAF, minor allele frequency; OR, odds ratio; 95% CI, 95% confidence interval; T2D, type 2 diabetes mellitus; ESRD, end stage renal disease.

^aAdditive model.

^bDominant model.

ESRD. After adjustment for admixture and *APOL1* G1/G2 allele status, rs35238405 was independently associated with non-T2D-ESRD ($P=0.016$) in African Americans. Importantly, consistent ORs and MAFs were observed across patients with ESRD in this study, and MAFs are consistent with those from previous exome sequencing reports (Supplemental Table 4). A combined all-cause ESRD analysis pooling genotypes from the Discovery study, the Replication study, and non-T2D-ESRD samples ($n=3270$ patients) and comparing them with non-T2D, non-nephropathy controls ($n=1187$ controls) revealed that the T233A variant was associated with all-cause ESRD ($P=0.0038$) and conferred an OR of 2.82 (95% CI, 1.40 to 5.68] after covariate adjustment; rs35238405 was not associated with T2D ($P=0.16-0.28$) (Table 1, Supplemental Table 5). The T233A variant is predicted to be probably damaging by PolyPhen2 and has been observed solely in African ancestral populations. *NPHS1* has previously been associated with CNS and other uncommon etiologies of nephropathy (9–13,20). Genetic variants in *NPHS1* have not previously been implicated in nephropathy in general populations.

NPHS1 was initially evaluated in patients with T2D-ESRD, although it is associated with nondiabetic glomerulosclerosis, because T2D-GENES whole-exome sequence data were only available in these patients. Association could reflect that many African Americans with clinically diagnosed T2D-ESRD actually have nondiabetic nephropathy in the FSGS spectrum (21). Additionally, there is evidence supporting *NPHS1*-modulating insulin signaling in podocytes and pancreatic islet cells (22,23). Hence, *NPHS1* could play a direct role in T2D-ESRD. A potential limitation of our design was the use of nondiabetic controls in the initial comparisons. The ideal control group would consist of African Americans with T2D lacking nephropathy; however, this population is difficult to identify, because most of these individuals have microalbuminuria after long diabetes durations. Therefore, we tested for association between nephropathy variants in a relatively small sample of African Americans with T2D lacking nephropathy and nondiabetic controls, and no association was detected. This finding supports *NPHS1* as a nephropathy susceptibility gene. A limitation of the whole-exome sequence data is the low power to test low frequency and rare variants. Replication in multiple samples, which was

performed in this report, is critical to validate findings. An additional limitation was that we did not adjust for adiposity or blood glucose/hypertension control, because they fluctuate over time. It is likely that a small percentage of T2D controls may develop nephropathy, which would minimize association with T2D-ESRD (bias to the null).

An additional 24 low-frequency *NPHS1* variants were subsequently genotyped (Supplemental Table 2). To assess the cumulative effect of the *NPHS1* locus on ESRD susceptibility, we used the SKAT program (17), which weights variants on the basis of their MAF and incorporates direction of effect (risk, protection, or neutral) for each variant. P values 5.64×10^{-7} and 1.18×10^{-6} were obtained after adjustment for admixture and admixture and *APOL1* G1/G2 status, respectively, providing compelling evidence that the *NPHS1* locus plays a role in nephropathy susceptibility in African Americans. Two additional *NPHS1* missense SNPs (H800R and Y1174H) were nominally associated with protection from all-cause ESRD (Table 4). rs115489112 (Y1174H) may be a T2D-ESRD-specific variant, because its OR and P value (OR, 0.29; 95% CI, 0.10 to 0.84; $P=0.022$) were weaker in the all-cause ESRD analysis but remained significantly associated ($P=0.036$; OR, 0.44; 95% CI, 0.20 to 0.95).

Collectively, our data implicate rare and low-frequency functional variations in *NPHS1* in nephropathy susceptibility independent of previously implicated loci (*i.e.*, the *APOL1* G1 and G2 alleles) or phenotypes (*i.e.*, T2D). The fact that the T233A variant was found in our control populations suggests incomplete penetrance of the allele. We observed no homozygotes for T233A and thus, cannot infer if that genotype would be at higher risk for ESRD; cases of T233A homozygotes have not been reported in the literature.

T233A is immediately distal to the bridge between the second and third Ig-C2 domains of *NPHS1*. This extracellular region is pivotal for *NPHS1*–*NPHS1* and *NPHS1*–Neph1/2 interactions between opposite and adjacent podocytes forming the slit diaphragm. We used three-dimensional models to assess structural implications of this variant and hypothesized that this mutation alters the secondary structure of a β -pleated sheet, compromising a critical structural region bridging the Ig-C2[2] and Ig-C2[3] domains (data not shown). This T233A variant has a MAF

of 4.1% in East African populations, whereas the MAF is 1.7% in West African populations, raising the possibility of a heterozygote advantage under certain environmental conditions.

Multiple mechanisms may exist through which rs115489112 (Y1174H) and rs146400394 (H800R) confer protection. Y1174H is in the 1160–1241 domain that binds to podocin, potentially enhancing the integrity of this interaction. The *APOL1* and *NPHS2* genes reportedly interact, supporting this concept (24). H800R is located in the seventh Ig-C2-type domain of *NPHS1* and may increase binding stability to *NPHS1* homodimers and *NPHS1*–Neph1/Neph2 heterodimers, thereby increasing stability of the slit diaphragm microstructure.

This study shows the challenges of working with low-frequency variants. Even with a substantial OR in the 2.82 [1.40, 5.68] range for T233A, overwhelming significance of association is not observed in a fairly large sample (approximately 4500 patients and controls for the primary tests). We overcome the initial power limitation through replication in T2D-ESRD and non-T2D-ESRD samples, where we estimate 98% power to detect an association at an MAF of 0.8% assuming an OR of 2.75 with 3000 patients and 1150 controls at an $\alpha=0.005$ (CaTs Power Calculator, University of Michigan); it is, thus, unlikely that the T233A association is a false positive. Moreover, we have complemented the single association tests by performing an unbiased locus-wide test using SKAT, resulting in both greater power and genome-wide gene-level evidence of association (*i.e.*, P is approximately $<1.2 \times 10^{-6}$) and consistently implicating missense variations in *NPHS1* to common nephropathies in African Americans. *NPHS1* missense variants are associated with ESRD risk and protection in African Americans, regardless of primary phenotype.

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Disclosures

None.

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