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

Jason A. Bonomo,[*†] Maggie C.Y. Ng,[†‡] Nicholette D. Palmer,[†‡] Jacob M. Keaton,[†] Chris P. Larsen,[§] Pamela J. Hicks,[†] The T2D-GENES Consortium, Carl D. Langefeld,[†‖] Barry I. Freedman,[¶] and Donald W. Bowden[†‡]*

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.

Clin J Am Soc Nephrol 9: 1434–1440, 2014. doi: 10.2215/CJN.00290114

Departments of *Molecular Medicine and Translational Science, [‡]Biochemistry, [‖]Biostatistical Sciences, and [¶]Internal Medicine-Nephrology and [†]Center for Human Genomics and Personalized Medicine, Wake Forest School of Medicine, Winston-Salem, North Carolina; and [§]Nephropathology, Nephropath, Little Rock, Arkansas

Correspondence: Dr. Donald W. Bowden, 1 Medical Center Boulevard, Winston Salem, NC 27157. Email: dbowden@wakehealth.edu

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

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 m^2 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 (https://t2d-genes.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).

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 SNPGWA 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 1.73^2 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
N	529	535	1305	760	1705	503
Women, %	61.2	57.3	60.7	57.9	43.7	58.7
Age, yr	61.6±10.5	49.0±11.9	61.3±10.8[a]	48.4±12.7[b]	54.6±14.6[c]	55.9±9.5
Age at T2D, yr	41.6±12.4	—	41.3±12.4	—	—	46.2±10.3
Duration of T2D before ESRD, yr	17.6±10.2	—	17.1±10.7	—	—	—
Duration of ESRD, yr	3.77±3.8	—	3.66±3.9	—	2.2±1.6	—
Blood glucose, mg/dl	—	88.8±13.1	—	89.2±13.6	88.6±8.7	—
BMI (at recruitment), kg/m^2	29.7±7.0	30.0±7.0	30.3±7.2	29.2±7.4	27.2±7.0	36.5±18.4
BUN, mg/dl	—	13.3±5.4	—	13.3±4.5	—	—
Serum creatinine, mg/dl	—	0.99±0.25	—	1.03±0.46	—	0.95±0.2

Categoric data expressed as percentage; continuous data described as mean±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 *APOL1* 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.
[a]Additive model.
[b]Dominant 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 α=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 $<1.2\times10^{-6}$) 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.

Acknowledgments

The authors thank the patients for their participation in this research.

This work was supported by National Institutes of Health (NIH) National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Grants F30-DK098836 (to J.A.B.), R01-DK070941 (to B.I.F.), R01-DK071891 (to B.I.F.), and R01-DK53591 (to D.W.B.). The T2D-GENES Consortium is supported by NIDDK Grants U01-DK085501, U01-DK085524, U01-DK085526, U01-DK085545, and U01-DK085584.

Disclosures

None.

References

1. USRDS: USRDS Annual Data Report: Volume Two, Chapter 11, 367–382, 2010. Available at: http://www.usrds.org/2010/pdf/v2_11.pdf. Accessed May 29, 2012
2. Spray BJ, Atassi NG, Tuttle AB, Freedman BI: Familial risk, age at onset, and cause of end-stage renal disease in white Americans. *J Am Soc Nephrol* 5: 1806–1810, 1995
3. Freedman BI, Tuttle AB, Spray BJ: Familial predisposition to nephropathy in African-Americans with non-insulin-dependent diabetes mellitus. *Am J Kidney Dis* 25: 710–713, 1995
4. Genovese G, Friedman DJ, Ross MD, Lecordier L, Uzureau P, Freedman BI, Bowden DW, Langefeld CD, Oleksyk TK, Uscinski Knob AL, Bernhardy AJ, Hicks PJ, Nelson GW, Vanhollebeke B, Winkler CA, Kopp JB, Pays E, Pollak MR: Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science* 329: 841–845, 2010
5. Kopp JB, Nelson GW, Sampath K, Johnson RC, Genovese G, An P, Friedman D, Briggs W, Dart R, Korbet S, Mokrzycki MH, Kimmel PL, Limou S, Ahuja TS, Berns JS, Fryc J, Simon EE, Smith MC, Trachtman H, Michel DM, Schelling JR, Vlahov D, Pollak M, Winkler CA: *APOL1* genetic variants in focal segmental glomerulosclerosis and HIV-associated nephropathy. *J Am Soc Nephrol* 22: 2129–2137, 2011
6. McDonough CW, Palmer ND, Hicks PJ, Roh BH, An SS, Cooke JN, Hester JM, Wing MR, Bostrom MA, Rudock ME, Lewis JP, Talbert ME, Blevins RA, Lu L, Ng MC, Sale MM, Divers J, Langefeld CD, Freedman BI, Bowden DW: A genome-wide association study for diabetic nephropathy genes in African Americans. *Kidney Int* 79: 563–572, 2011
7. Parsa A, Kao WH, Xie D, Astor BC, Li M, Hsu CY, Feldman HI, Parekh RS, Kusek JW, Greene TH, Fink JC, Anderson AH, Choi MJ, Wright JT Jr., Lash JP, Freedman BI, Ojo A, Winkler CA, Raj DS, Kopp JB, He J, Jensvold NG, Tao K, Lipkowitz MS, Appel LJ; AASK Study Investigators; CRIC Study Investigators: *APOL1* risk variants, race, and progression of chronic kidney disease. *N Engl J Med* 369: 2183–2196, 2013
8. Palmer ND, Freedman BI: Insights into the genetic architecture of diabetic nephropathy. *Curr Diab Rep* 12: 423–431, 2012
9. Kestilä M, Lenkkeri U, Männikkö M, Lamerdin J, McCready P, Putaala H, Ruotsalainen V, Morita T, Nissinen M, Herva R, Kashtan CE, Peltonen L, Holmberg C, Olsen A, Tryggvason K: Positionally cloned gene for a novel glomerular protein—nephrin—is mutated in congenital nephrotic syndrome. *Mol Cell* 1: 575–582, 1998
10. Lenkkeri U, Männikkö M, McCready P, Lamerdin J, Gribouval O, Niaudet PM, Antignac C K, Kashtan CE, Homberg C, Olsen A, Kestilä M, Tryggvason K: Structure of the gene for congenital nephrotic syndrome of the finnish type (NPHS1) and characterization of mutations. *Am J Hum Genet* 64: 51–61, 1999
11. Lahdenkari AT, Kestilä M, Holmberg C, Koskimies O, Jalanko H: Nephrin gene (NPHS1) in patients with minimal change nephrotic syndrome (MCNS). *Kidney Int* 65: 1856–1863, 2004
12. Philippe A, Nevo F, Esquivel EL, Reklaityte D, Gribouval O, Tête MJ, Loirat C, Dantal J, Fischbach M, Pouteil-Noble C, Decramer S, Hoehne M, Benzing T, Charbit M, Niaudet P, Antignac C: Nephrin mutations can cause childhood-onset steroid-resistant nephrotic syndrome. *J Am Soc Nephrol* 19: 1871–1878, 2008
13. Santín S, García-Maset R, Ruíz P, Giménez I, Zamora I, Peña A, Madrid A, Camacho JA, Fraga G, Sánchez-Moreno A, Cobo MA, Bernis C, Ortiz A, de Pablos AL, Pintos G, Justa ML, Hidalgo-Barquero E, Fernández-Llama P, Ballarín J, Ars E, Torra R; FSGS Spanish Study Group: Nephrin mutations cause childhood- and adult-onset focal segmental glomerulosclerosis. *Kidney Int* 76: 1268–1276, 2009
14. Cooke JN, Bostrom MA, Hicks PJ, Ng MC, Hellwege JN, Comeau ME, Divers J, Langefeld CD, Freedman BI, Bowden DW: Polymorphisms in MYH9 are associated with diabetic nephropathy in European Americans. *Nephrol Dial Transplant* 27: 1505–1511, 2012
15. Divers J, Wagenknecht LE, Bowden DW, Carr JJ, Hightower RC, Xu J, Langefeld CD, Freedman BI: Ethnic differences in the relationship between albuminuria and calcified atherosclerotic plaque: The African American-diabetes heart study. *Diabetes Care* 33: 131–138, 2010
16. Keene KL, Mychaleckyj JC, Leak TS, Smith SG, Perlegas PS, Divers J, Langefeld CD, Freedman BI, Bowden DW, Sale MM: Exploration of the utility of ancestry informative markers for genetic association studies of African Americans with type 2 diabetes and end stage renal disease. *Hum Genet* 124: 147–154, 2008
17. Wu MC, Lee S, Cai T, Li Y, Boehnke M, Lin X: Rare-variant association testing for sequencing data with the sequence kernel association test. *Am J Hum Genet* 89: 82–93, 2011
18. Milliken J: *Analysis of Messy Data. Volume I: Designed Experiments*, Van Nostrand Reinhold Publishing, Boca Raton, FL 1984, p 33
19. Kraft P, Wacholder S, Cornelis MC, Hu FB, Hayes RB, Thomas G, Hoover R, Hunter DJ, Chanock S: Beyond odds

ratios—communicating disease risk based on genetic profiles. *Nat Rev Genet* 10: 264–269, 2009
20. Beltcheva O, Martin P, Lenkkeri U, Tryggvason K: Mutation spectrum in the nephrin gene (NPHS1) in congenital nephrotic syndrome. *Hum Mutat* 17: 368–373, 2001
21. Freedman BI, Langefeld CD, Lu L, Divers J, Comeau ME, Kopp JB, Winkler CA, Nelson GW, Johnson RC, Palmer ND, Hicks PJ, Bostrom MA, Cooke JN, McDonough CW, Bowden DW: Differential effects of MYH9 and *APOL1* risk variants on FRMD3 association with diabetic ESRD in African Americans. *PLoS Genet* 7: e1002150, 2011
22. Welsh GI, Saleem MA: Nephrin-signature molecule of the glomerular podocyte? *J Pathol* 220: 328–337, 2010
23. Fornoni A, Jeon J, Varona Santos J, Cobianchi L, Jauregui A, Inverardi L, Mandic SA, Bark C, Johnson K, McNamara G, Pileggi A, Molano RD, Reiser J, Tryggvason K, Kerjaschki D, Berggren PO, Mundel P, Ricordi C: Nephrin is expressed on the surface of insulin vesicles and facilitates glucose-stimulated insulin release. *Diabetes* 59: 190–199, 2010
24. Divers J, Palmer ND, Lu L, Langefeld CD, Rocco MV, Hicks PJ, Murea M, Ma L, Bowden DW, Freedman BI: Gene-gene interactions in APOL1-associated nephropathy. *Nephrol Dial Transplant* 29: 587–594, 2014

Received: January 9, 2014 **Accepted:** May 1, 2014

Published online ahead of print. Publication date available at www.cjasn.org.

This article contains supplemental material online at http://cjasn.asnjournals.org/lookup/suppl/doi:10.2215/CJN.00290114/-/DCSupplemental.

Supplementary Data

Supplementary Table 1: *NPHS1* exome sequencing results (missense variants, dominant model, admixture adjusted)

SNP	CHR	Position (hg19)	Amino Acid Change	Protein Position	OR	CI - L95	CI - U95	P Value	MAF**	Genotype Count Cases[†]	Genotype Count Controls[†]
rs35238405	19	36340467	THR,ALA	233/1241	2.89	1.041	8.028	0.04165	0.0096	0/15/506	0/5/513
var_19_36336287	19	36336287	TYR,CYS	638/1241	5.05	0.5858	43.54	0.1407	0.0029	0/5/516	0/1/517
rs4806213	19	36322601	ASN, SER	1077/1241	1.26	0.9256	1.72	0.1413	0.14	10/105/301	6/95/333
var_19_36340212	19	36340212	ARG,TRP	256/1241	4.27	0.4741	38.47	0.1955	0.0024	0/4/515	0/1/515
rs113825926	19	36340009	THR,ILE	294/1241	0.39	0.07452	2.006	0.2579	0.0034	0/2/519	0/5/513
var_19_36322018	19	36322018	ARG,CYS	1140/1241	1.62	0.7009	3.74	0.2593	0.012	0/15/506	0/9/509
rs116700257	19	36321778	ALA, THR	1188/1241	0.53	0.1764	1.596	0.2594	0.0067	0/5/516	0/9/509
rs35240811	19	36317544	PRO,SER	1200/1241	1.53	0.708	3.3	0.2799	0.013	0/17/504	0/11/507
rs33950747	19	36339247	ARG,GLN	408/1241	1.36	0.6431	2.882	0.4201	0.014	0/16/505	0/13/505
rs3814995	19	36342212	GLU,LYS	117/1241	1.15	0.8128	1.625	0.4312	0.077	2/78/441	5/67/445
var_19_36341302	19	36341302	SER,TYR	191/1241	0.48	0.04369	5.379	0.5554	0.0014	0/1/520	0/2/516
rs34736717	19	36330277	VAL,LEU	991/1241	0.92	0.6985	1.221	0.5771	0.14	9/121/391	9/127/382
rs34320609	19	36339295	LEU,PRO	392/1241	0.91	0.5864	1.401	0.6588	0.045	4/39/478	1/45/472
rs140626538	19	36342505	VAL,ALA	43/1241	1.19	0.5074	2.773	0.6935	0.011	0/12/509	0/10/507
rs114112112	19	36339558	MET,THR	382/1241	1.4	0.2315	8.399	0.7166	0.0024	0/3/518	0/2/516
rs34982899	19	36340187	PRO,ARG	264/1241	0.95	0.5292	1.71	0.8671	0.023	0/23/497	1/23/493
var_19_36341311	19	36341311	ASN, ILE	188/1241	1.14	0.1592	8.213	0.8939	0.0019	0/2/519	0/2/516
var_19_36326622	19	36326622	THR,ALA	1051/1241	1.17	0.07268	18.9	0.9109	0.00096	0/1/520	0/1/517
rs115489112	19	36321820	HIS,TYR	1174/1241	0.96	0.1916	4.779	0.9572	0.0029	0/3/518	0/3/515
var_19_36336581	19	36336581	GLU,LYS	583/1241	0.93	0.05797	14.93	0.9594	0.00097	0/1/517	0/1/517
var_19_36335305	19	36335305	GLU,LYS	663/1241	1	0.06242	16.09	0.9987	0.001	0/1/488	0/1/507
var_19_36336918	19	36336918	ALA,GLU	540/1241	6.46E-10	0	Inf	0.999	0.0016	0/0/307	0/2/325
var_19_36326643	19	36326643	GLU,LYS	1044/1241	7.80E-10	0	Inf	0.9993	0.00048	0/0/521	0/1/517
var_19_36332686	19	36332686	ALA,SER	916/1241	7.35E-10	0	Inf	0.9993	0.00048	0/0/521	0/1/517
var_19_36334400	19	36334400	PRO,THR	770/1241	1.40E+09	0	Inf	0.9993	0.00048	0/1/520	0/0/518

var_19_36335035	19	36335035	ALA,SER	728/1241	6.05E-10	0	Inf	0.9993	0.00048	0/0/521	0/1/517
var_19_36335107	19	36335107	LEU,VAL	704/1241	1.85E+09	0	Inf	0.9993	0.00048	0/1/520	0/0/518
var_19_36335326	19	36335326	VAL,LEU	656/1241	1.61E+09	0	Inf	0.9993	0.00056	0/1/433	0/0/460
var_19_36336411	19	36336411	ALA,PRO	597/1241	1.99E+09	0	Inf	0.9993	0.00048	0/1/517	0/0/517
var_19_36338980	19	36338980	ILE,THR	468/1241	5.77E-10	0	Inf	0.9993	0.00048	0/0/521	0/1/517
var_19_36339202	19	36339202	ALA,GLY	423/1241	6.10E-10	0	Inf	0.9993	0.00048	0/0/521	0/1/517
var_19_36339233	19	36339233	LEU,VAL	413/1241	6.40E-10	0	Inf	0.9993	0.00048	0/0/521	0/1/517
var_19_36339298	19	36339298	GLY,GLU	391/1241	1.45E+09	0	Inf	0.9993	0.00048	0/1/520	0/0/518
var_19_36339634	19	36339634	LEU,VAL	359/1241	1.53E+09	0	Inf	0.9993	0.00048	0/1/520	0/0/518
var_19_36339636	19	36339636	THR,ILE	358/1241	5.20E-10	0	Inf	0.9993	0.00048	0/0/521	0/1/517
var_19_36339923	19	36339923	VAL,MET	323/1241	1.64E+09	0	Inf	0.9993	0.00048	0/1/520	0/0/518
rs115308424	19	36340175	ARG,GLN	268/1241	1.51E+09	0	Inf	0.9993	0.00048	0/1/520	0/0/518
var_19_36340545	19	36340545	ARG,TRP	207/1241	2.00E+09	0	Inf	0.9993	0.00048	0/1/520	0/0/518
var_19_36342518	19	36342518	GLU,LYS	39/1241	7.80E-10	0	Inf	0.9993	0.00048	0/0/521	0/1/517
var_19_36342539	19	36342539	ARG,TRP	32/1241	7.28E-10	0	Inf	0.9993	0.00048	0/0/521	0/1/516

*Dominant model; **reflects combined case and control MAF

ᵗreflects counts (number of observations) of “homozygous minor / heterozygotes / homozygous major” alleles, respectively.

Supplementary Table 2. *NPHS1* Locus-Wide Analysis

SNP	Chr	Position	PolyPhen2	Amino Acid Δ	Protein Pos.	Alleles
rs35238405	19	36340467	probably-damaging	ALA,THR	233/1241	C/T
rs4806213	19	36322601	probably-damaging	SER,ASN	1077/1241	C/T
rs141141839	19	36336287	probably-damaging	CYS,TYR	638/1241	C/T
C19:36340212	19	36340212	probably-damaging	TRP,ARG	256/1241	A/G
rs148104086	19	36342539	probably-damaging	TRP,ARG	32/1241	A/G
rs138173172	19	36332686	possibly-damaging	SER,ALA	916/1241	A/C
rs33950747	19	36339247	probably-damaging	GLN,ARG	408/1241	T/C
rs3814995	19	36342212	possibly-damaging	LYS,GLU	117/1241	T/C
rs115308424	19	36340175	possibly-damaging	GLN,ARG	268/1241	T/C
rs115489112	19	36321820	possibly-damaging	TYR,HIS	1174/1241	A/G
rs140673499	19	36326622	possibly-damaging	ALA,THR	1051/1241	C/T
rs147641617	19	36336581	probably-damaging	LYS,GLU	583/1241	T/C
rs112624813	19	36340499	probably-damaging	LEU,PRO	222/1241	A/G
rs115333628	19	36340506	possibly-damaging	ALA,SER	220/1241	C/A
rs139472106	19	36341989	possibly-damaging	ALA,PRO	134/1241	C/G
rs143649022	19	36332704	probably-damaging	PRO,SER	910/1241	G/A
rs143986233	19	36333098	probably-damaging	HIS,ARG	864/1241	T/C
rs144203682	19	36322556	probably-damaging	HIS,ARG	1092/1241	T/C
rs146400394	19	36333388	probably-damaging	HIS,ARG	800/1241	T/C
rs146858871	19	36322629	possibly-damaging	THR,ALA	1068/1241	T/C
rs149649169	19	36340211	probably-damaging	GLN,ARG	256/1241	T/C
rs150623032	19	36333180	possibly-damaging	SER,ALA	837/1241	A/C
rs151121915	19	36317420	probably-damaging	ALA,VAL	1241/1241	G/A
C19:36337051	19	36337051	probably-damaging	SER,ARG	496/1241	T/G
C19:36339181	19	36339181	possibly-damaging	ARG,LYS	430/1241	C/T
C19:36339287	19	36339287	probably-damaging	SER,GLY	395/1241	T/C
C19:36340454	19	36340454	probably-damaging	GLN,LEU	237/1241	T/A
C19:36342559	19	36342559	possibly-damaging	VAL,ALA	25/1241	A/G
rs34982899	19	36340187	possibly-damaging	PRO,ARG	264/1241	G/C

rs116620503	19	36342451	N/A	ALA,VAL	61/1241	A/G
rs73928330	19	36342697	benign	GLY,ARG	15/1241	C/G
rs34320609	19	36339295	benign	LEU,PRO	392/1241	A/G

Supplementary Table 3. T233A characteristics within all ESKD cases.

	African Ancestry	Sex (%)	Age	*APOL1* G1/G2 (2 risk alleles)	BMI	Family History of ESKD (%)	Age of ESKD
T233A (+) ESKD Cases	0.83 ± 0.11	62.4 (F)	55.9 ± 14.4	0.33 ± 0.47	29.62 ± 7.4	38.6	51.8 ± 14.8
T233A (-) ESKD Cases	0.80 ± 0.12	51.3 (F)	56.1 ± 14.3	0.25 ± 0.43	28.93 ± 7.2	28.9	53.5 ± 14.3
P-Value*	0.020	0.043	0.89	0.090	0.40	0.16	0.30

Categoric data are expressed as percentage; continuous data as mean ± SD.

*P-values were computed using a two tailed T-test at a significance threshold of α=0.05

Supplementary Table 4.

Reported rs35238405 (T233A) minor allele frequencies

Source		MAF*
dbsnp		0.006
EVS (Af. American)		0.012
1000 Genomes		
	All	0.006
	AFR	0.024
	LWK	0.041
	YRI	0.017
	ASW	0.008
	EUR	0
	AMR	0
	CLM	0.008

*MAF: minor allele frequency

Supplementary Table 5. Rs35238405 (T233A) Single-SNP association testing (Dominant Model) in T2D-only, non-nephropathy cases, adjusted for admixture only.

Study	N Case / Control	MAF Case / Control	P	OR	95% CI
T2D-Only (vs. controls)	502 / 1280	0.0070 / 0.0043	0.28	1.7	0.65, 4.41

Supplementary Table 6. Breakdown of variants identified in T2D-GENES exome sequencing "Discovery" study (genome wide)

Effect	Frequency (N)	Proportion
Nonsynonymous Coding	237,989	0.34
$p < 0.0001$	4	
$0.0001 \leq p < 0.001$	23	
$0.001 \leq p < 0.01$	358	
$0.01 \leq p < 0.05$	1968	
Start Gained	2,166	0.0031
Stop Gained	4,254	0.0060
Stop Lost	263	0.00040

Supplementary Table 7: *NPHS1* variants included in SKAT analysis

Chr	SNP	BP (hg19)	Notes	Source
19	rs151121915	36317420	-	Exome Variant Server
19	rs115489112	36321820	-	Exome Variant Server
19	rs144203682	36322556	monomorphic	Exome Variant Server
19	rs4806213	36322601	-	Exome Variant Server
19	rs146858871	36322629	-	Exome Variant Server
19	rs140673499	36326622	-	Exome Variant Server
19	rs138173172	36332686	-	Exome Variant Server
19	rs143649022	36332704	-	Exome Variant Server
19	rs150623032	36333180	monomorphic	Exome Variant Server
19	rs146400394	36333388	-	Exome Variant Server
19	rs147641617	36336581	-	Exome Variant Server
19	19:36337051	36337051	-	1000 Genomes
19	19:36339181	36339181	monomorphic	1000 Genomes
19	19:36339287	36339287	-	1000 Genomes
19	rs34320609	36339295	-	Exome Variant Server
19	rs149649169	36340211	monomorphic	Exome Variant Server
19	rs35238405	36340467	-	T2D-GENES
19	rs112624813	36340499	monomorphic	Exome Variant Server
19	rs139472106	36341989	monomorphic	Exome Variant Server
19	rs3814995	36342212	-	Exome Variant Server
19	rs116620503	36342451	monomorphic	Exome Variant Server
19	rs148104086	36342539	monomorphic	Exome Variant Server
19	19:36342559	36342559	-	1000 Genomes

Supplementary Table 8: Missense variants identified in *NPHS2* in T2D-GENES exome-sequencing Discovery study (Dominant Model, Admixture adjusted).

SNP	Function	Gene	Chr	BP (HG19)	MAF*	P Value**	OR	L95 CI	U95 CI
1:179523626	MISSENSE	NPHS2	1	179523626	0.0009625	0.9909	0.984	0.06138	15.78
rs61747727	MISSENSE	NPHS2	1	179526175	0.07811	0.31	1.194	0.848	1.681
rs61747728	MISSENSE	NPHS2	1	179526214	0.006737	0.7222	0.8231	0.2815	2.407
rs116512679	MISSENSE	NPHS2	1	179544818	0.01359	0.3154	1.491	0.6837	3.251
1:179544824	MISSENSE	NPHS2	1	179544824	0.000499	0.9993	Inf	0	Inf

*Combined case/control MAF; **Dominant model