The ras responsive transcription factor *RREBI* is a novel candidate gene for type 2 diabetes associated end-stage kidney disease

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Abstract

Familial clustering and presumed genetic risk for type 2 diabetic (T2D) and non-diabetic end-stage kidney disease (ESKD) appear strong in African Americans. Examination of exome sequencing data in African American T2D-ESKD cases and non-diabetic non-nephropathy controls identified two low frequency variants in the \textit{RREB1} gene, a repressor of the angiotensinogen (\textit{AGT}) gene previously associated with kidney function, as being associated with T2D-ESKD: rs9379084 (p=0.00087, OR=0.26; D1171N) and rs41302867 (p=0.00078, OR=0.21; splice site variant). Rs41302867 replicated association in an independent sample of African Americans with T2D-ESKD (rs41302867 p=0.033 [OR=0.50]), and a trend towards rs9379084 association was observed (p=0.070). In European Americans with T2D-ESKD compared to European American population based controls, both \textit{RREB1} variants replicated association (rs9379084 p=1.67x10^{-4} [OR=0.54] and rs41302867 p=0.013 [OR=0.69]). Rs9379084 was not associated with non-T2D ESKD or T2D in African Americans (p=0.55 and p=0.37, respectively), but was associated with T2D in European Americans (p=0.014, OR=0.65). In African Americans, rs41302867 was associated with non-T2D ESKD (p=0.036 [OR=0.54]) and hypertension attributed ESKD (H-ESKD, p=0.029 [OR=0.50]). A meta-analysis combining African American and European American T2D-ESKD data revealed p=3.52x10^{-7} and 3.70x10^{-5} for rs9379084 and rs41302867 association, respectfully. A locus-wide analysis evaluating putatively functional SNPs revealed several nominal associations with T2D-ESKD, non-T2D ESKD, and T2D in African and European Americans. \textit{RREB1} is a large, complex gene which codes a multidomain zinc finger binding protein and transcription factor. We posit that variants in \textit{RREB1} modulate seemingly disparate phenotypes (\textit{ie}, T2D, T2D-ESKD, and non-T2D ESKD) through altered activity resulting from splice site and missense variants.
Introduction

Ample evidence supports genetic influences on susceptibility to complex diseases such as end-stage kidney disease (ESKD). This is especially true in African Americans where incidence rates of ESKD are 3.5 fold higher than in European Americans and twice that of Native Americans and Hispanic Americans (1). Following adjustment for socioeconomic status and environmental influences, incidence rates and familial aggregation of ESKD remain markedly higher among African Americans compared to other ethnic groups (2, 3). The apolipoprotein L1 gene (*APOL1*) G1 and G2 alleles, present in those of West African descent, explain a substantial portion of the ethnic disparity in HIV-associated collapsing glomerulopathy, idiopathic focal segmental glomerulosclerosis (FSGS), and hypertension-attributed ESKD (4, 5). While these *APOL1* variants account for roughly 70% of the ethnic differences in risk for non-diabetic forms of ESKD (non-T2D ESKD), they fail to account for the excess risk of type 2 diabetic ESKD (T2D-ESKD) in African Americans (6). It is likely that other genetic loci contribute to T2D-ESKD (and non-T2D ESKD) risk in the African American population (7).

Next-generation exome sequencing (NGES) is a powerful technology which facilitates detailed exploration of previously untested genetic regions, aiding in the identification of genetic variations in coding sequences of genes for disease association. We utilized NGES data to survey the *RREB1* gene (ras-responsive element binding protein-1), an upstream regulator of the Renin-Angiotensin System (RAS). Multiple splice variants of *RREB1*, also known as Finb, have been shown to act as sequence-specific transcriptional repressors of the angiotensinogen (*AGT*) gene (8). A genome-wide association study (GWAS) published by the CHARGE consortium identified an association between an *RREB1* variant and estimated glomerular filtration rate (eGFR), and an intronic variant near the *RREB1* locus has evidence of interaction with the *APOL1* gene in African Americans with non-T2D ESKD (9, 10). Given the *a priori* functional and genomics data, we hypothesized that genetic variations in *RREB1* may contribute to nephropathy susceptibility in the African American population.
Results

The impact of genetic variations in \textit{RREB1} modulating T2D-ESKD, non-T2D ESKD, and T2D (in the absence of nephropathy) susceptibility in the general African and European American population was investigated using a multistage study design (Figure 1). Initially, \textit{RREB1} was assessed in exome sequence data from 529 African American T2D-ESKD cases and 535 non-diabetic non-nephropathy controls (Discovery). The two signals from \textit{RREB1}, in high linkage disequilibrium (LD; $r^2=0.63$), were amongst the top 58 hits across the entire exome with the strongest biological plausibility for association. This Discovery sample provides a unique resource, albeit with limited power, which results in nominal evidence of association. Thus, based on the signal from the Discovery study coupled with \textit{a priori} genetic associations (\textit{APOL1} interaction, prior association with eGFR) and biological plausibility, we pursued further investigation of \textit{RREB1} in additional DNA samples.

The Discovery study was followed by testing in an independent African American T2D-ESKD case-control sample (Replication), followed by analysis in African American non-T2D-ESKD cases, and African American T2D-only cases (\textit{i.e.} T2D, without nephropathy). \textit{RREB1} variants were also tested in European American case-control samples with T2D-ESKD and T2D lacking nephropathy.

Characteristics of the African American and European American samples are detailed in Tables 1 and 2, respectively. African American Discovery T2D-ESKD cases were broadly similar to those in the Replication T2D-ESKD study for all characteristics. The population-based controls for the Discovery and Replication studies are, on average, younger than the cases in the Discovery and Replication studies; however, the age of T2D diagnosis in the cases is younger than the mean age of the population-based controls at enrollment. All cohorts except African American non-T2D ESKD cases had a larger percentage of females. The distributions of body mass index (BMI) were similar across cohorts, with the non-T2D ESKD cohort having the lowest mean BMI. As with African American cases, European American T2D-ESKD and T2D-only cases tended to be older than controls, but the age at T2D diagnosis...
was younger than the mean age for controls. BMIs across European American cohorts were in the overweight to obese range, with the European American T2D-only cases having the highest BMI.

NGES data of 529 African American T2D-ESKD cases and 535 African American non-diabetic non-nephropathy controls (Discovery) included in the T2D-GENES Consortium (t2d-genes.sph.umich.edu) was evaluated, focusing on the RREB1 gene. The initial T2D-GENES exome sequencing study identified two protective variants that were associated with T2D-ESKD under an additive model following adjustment for admixture, age, gender, APOL1 G1/G2 allele status, and body mass index (BMI) (“Model 2”): rs9379084, a D1771N missense variant (p=0.00087; OR=0.26 [0.12, 0.55]), and rs41302867, a splice site variant 9kbp away from rs9379084 and 35bp from the nearest exon (p=0.00078; OR=0.21 [0.090, 0.49]), (“Model 2”) (Table 3). Rs9379084 and rs41302867 are low frequency variants with minor allele frequencies (MAF) of 1.1% and 0.77% in cases, and 3.90% and 3.30% in controls, respectively, with an r² of 0.63 (Table 3). A conditional analysis of this Discovery data showed that the two associations were not independent (Supplementary Table 1).

The rs9379084 and rs41302867 variants were next genotyped in 1,305 independent African American T2D-ESKD cases and 760 population-based African American non-T2D non-nephropathy controls (Replication) (Table 3). Rs41302867 replicated association after multiple testing correction with p=0.022 and OR=0.049 [0.29, 0.85] under Model 2. A trend for association with rs9379084 was observed (p=0.070 after multiple testing correction). Consistent MAF and ORs were observed for both variants (Table 3).

With consistent evidence of association of rs41302867 and rs9379084 with T2D-ESKD in African Americans, we investigated whether these two RREB1 variants were associated with non-diabetic etiologies of ESKD in African Americans (non-T2D ESKD). These variants were genotyped in a sample of 1,705 African American non-T2D ESKD cases. Rs41302867 was associated with non-T2D ESKD and “hypertension attributed” ESKD (H-ESKD) with (p=0.036 and p=0.029, respectively), remaining protective (OR=0.54 [0.34, 0.90] and 0.50 [0.28, 0.87], respectively), and with similar MAF (MAF=0.015 cases and 0.031 controls; Table 3). The rs9379084 variant was not associated with non-T2D ESKD or H-
ESKD (p=0.87 and p=0.61, respectively; Table 3, Supplementary Table 2) and a conditional analysis in those with H-ESKD demonstrated that the two SNPs were independent of one another in these samples (Supplementary Table 3). Based on consistent association of rs41302867 with ESKD in African Americans, we performed an African American “all-cause” ESKD analysis by pooling samples from the Discovery T2D-ESKD cases and controls, Replication T2D-ESKD cases and controls, and non-T2D ESKD cases. The African American all-cause ESKD analysis revealed p=1.10x10^{-4} for association with rs41302867 (OR 0.48 [0.34, 0.68]) under Model 2 after correcting for multiple comparisons (Table 3). A trend toward T2D association was observed in African Americans for rs41302867 (Table 3).

We next genotyped RREB1 variants rs9379084 and rs41302867 in 637 European Americans with T2D-ESKD and 1020 European American population-based controls (non-T2D, non-nephropathy). After correction for multiple testing both variants replicated T2D-ESKD association: rs41302867 p=0.013 (OR=0.69 [0.52, 0.90]) and rs9379084 p=0.00017 (OR=0.54 [0.40, 0.73]), following age, gender, and BMI adjustment (Table 3). These protective variants were found at greater MAF in European Americans, and consistent ORs were observed between African Americans and European Americans. In European Americans with T2D in the absence of nephropathy, rs9379084 was modestly associated (p=0.014, OR=0.65 [0.48, 0.89]) and a trend for association with rs41302867 was observed (Table 3).

A meta-analysis combing European American and African American data was performed for T2D-ESKD (all T2D-ESKD samples) and “all cause” ESKD (all T2D-ESKD samples + African American non-T2D ESKD cases) using METAL. After correction for multiple comparisons, p-values of 3.52x10^{-7} and 3.70x10^{-5} were observed for rs9379084 and rs41302867, respectively, under Model 2 for T2D-ESKD association (Table 4A). In the “all cause” ESKD meta-analysis, which includes all T2D-ESKD samples in addition to the 1,705 African Americans with non-T2D ESKD, p-values of 8.74x10^{-7} and 6.58x10^{-6} were observed for rs9379084 and rs41302867, respectively, under Model 2 (Table 4B).

Finally, in an effort to determine whether other putatively functional RREB1 SNPs were associated with T2D, T2D-ESKD, or non-T2D ESKD in African or European Americans, an additional 16 variants not found in the Discovery NGES Study were genotyped (Supplementary Table 4). These
variants were selected based on their amino acid change, location in transcription factor binding sites, and MAF discrepancies between African and European Americans. Modest associations were observed for several variants after correction for multiple testing (Supplementary Table 6).

Discussion

We tested whether genetic variations in *RREB1*, one of the top signals in a T2D-ESKD NGES Discovery study with the strongest *a priori* biological and genomic plausibility as a candidate gene, were associated with susceptibility to common forms of ESKD in general African and European American populations. Results in these groups with different population ancestry were consistent, but complex. Results consistently revealed that rs9379084 and rs41302867 were associated with protection from T2D-ESKD in African and European Americans. These two variants are in high LD ($r^2=0.63$ and 0.62 for African and European Americans, respectively) and conditional analysis demonstrated that they reflected the same signal for African and European Americans with T2D-ESKD. Based on the meta-analysis p-values, we hypothesize that rs9379084 (D1171N) is more likely to reflect true association with T2D-ESKD ($p=3.52 \times 10^{-7}$ versus $p=3.70 \times 10^{-5}$).

Interestingly, rs41302867, but not rs9379084, was associated with non-T2D ESKD in African Americans ($p=0.036$); we do not have a corresponding DNA collection from European Americans with non-T2D-ESKD since etiologies of ESKD are more heterogeneous than in African Americans. A conditional analysis demonstrated that these two SNPs were independent of one another for the non-T2D ESKD phenotype. We assessed whether rs41302867 was associated with H-ESKD by removing diagnoses of FSGS and HIVAN from the non-T2D ESKD samples and also removing hypertensive controls (African Americans with H-ESKD vs normotensive, population-based controls); rs41302867 remained associated ($p=0.029$, OR=0.50 [0.28, 0.87]) under Model 2 (Supplementary Table 2). We extended this analysis to include all samples with hypertension (H-ESKD + hypertensive controls) compared to normotensive controls. Again, rs41302867, but not rs9379084, was associated ($p=0.033,$
OR=0.53 [0.31, 0.89]). This data suggests rs41302867 may be specific to the hypertensive phenotype and may play a role in protection from hypertension and H-ESKD (i.e., APOL1 associated nephropathies).

Finally, we performed targeted genotyping of 16 additional, putatively functional RREB1 variants in African Americans with T2D-only, T2D-ESKD, and non-T2D ESKD, European Americans with T2D-ESKD and T2D-only, as well as population based controls. Here data reveal nominal evidence of association with other RREB1 variants for T2D, T2D-ESKD, and non-T2D ESKD compared to population based controls in African Americans and European Americans (Supplementary Tables 5 and 6). Together, these observations appear consistent given the complex structure and function of the RREB1 gene.

RREB1 is a large gene spanning 144 kB with at least 12 isoforms. Amino acid (AA) lengths for isoforms range from 4 AAs with 1 coding exon (RREB1-12) to 1742 AAs and 10 coding exons resulting in a protein product with 15 C2H2 zinc finger domains (RREB1-1). These longer isoforms of RREB1, previously termed “Finb” and including RREB1-1 ("Finb 188"), have been shown to repress expression of the AGT gene (8). Relevant to nephropathy susceptibility, RREB1 polymorphisms reportedly interact with APOL1 and associate with kidney function (9, 10). Recent GWAS implicated RREB1 with fat distribution and fasting glucose, effects potentially related to the observed T2D associations (11, 12). A T2D locus was recently identified in the RREB1-SSR1 gene region (13).

The majority of studies evaluating RREB1 have focused on its role in oncology, as this transcription factor induces expression of p53 and represses p16 (14, 15). The roles RREB1 plays in non-oncogenic pathologies are less well studied, as are isoform-specific actions of RREB1. Aside from a study which documented larger transcripts of RREB1 repressing AGT expression, only one other study examined isoform-specific actions of RREB1 in urologic cancer (8, 16). A large consortia recently published a meta-analysis with >80,000 individuals that located an association between a SNP in SSR1 (rs9505118) and T2D (13). The authors reported that this SNP, although in SSR1, was part of the RREB1-SSR1 gene region. Finally, the association of an RREB1 SNP with eGFR in a CHARGE consortium GWAS was reported in supplementary data (9).
In addition to actively repressing AGT, a precursor component of the RAS, \textit{RREB1} has been shown to repress ZIP3/SLC39A3, a gene involved in zinc transport whose homolog SLC30A8 has been associated with type 1 and type 2 diabetes (18-21). Moreover, \textit{RREB1} induces the expression of MT-IIA, a metallothioein produced extensively in the liver and kidneys which protects against oxidative stress (22), and secretin, which promotes osmoregularity, triggers insulin release, and promotes the growth and maintenance of the pancreas (23, 24). \textit{RREB1} also potentiates NeuroD1/β2 activation, a gene implicated in Mature Onset Diabetes of the Young (MODY), and \textit{RREB1} activates the nuclear androgen receptor (AR); a known target of AR activation is insulin-like growth factor-1 (IGF-1) (24-26). Finally, GWAS have associated \textit{RREB1} with fat distribution and fasting glucose levels (11, 12). Thus, variations in \textit{RREB1} may impact disparate phenotypes including predisposition to T2D and ESKD related to both diabetic and non-diabetic etiologies in multiple population ancestral groups.

In addition to establishing genetic association for \textit{RREB1} in T2D-ESKD and non-T2D ESKD, and nominal evidence of association in related phenotypes, this study provides genetic insights into the disproportionate burden of T2D and ESKD borne by the African American community. Our data suggests that after centuries of admixture between European and African Americans, common protective variants (rs41302867 and rs9379084) were transferred from European Americans to African Americans. These differences in protective MAF may contribute to the disproportionate burden of T2D-ESKD and non-T2D ESKD borne by the African American community compared to other population ancestries. Results from this study approach, but do not meet genome-wide significance for T2D-ESKD. This is likely a limitation of our power reflected in the sample size and MAFs in African Americans.

This study demonstrates how a single gene with numerous splice variants may influence seemingly unrelated pathologies including T2D, T2D-ESKD, ESKD, hypertension, and malignancy. The ability of one gene to modulate a range of clinically relevant phenotypes warrants further investigation into pharmacologic targets which can exploit these pleiotropic properties. Finally, this study validates the effectiveness of NGES coupled with pragmatic, biological data and \textit{a priori} knowledge of the pathology
(eg. prioritizing variants based on allelic discrepancies between disproportionately affected populations) to aid in the discovery of clinically relevant genetic variants.

Materials & Methods

Study Participants

Recruitment and sample collection procedures have been reported (6, 27). The study was approved by the Institutional Review Board at Wake Forest School of Medicine (WFSM). All participants were unrelated, born in North Carolina, South Carolina, Georgia, Tennessee, or Virginia and provided written informed consent. The PureGene system was used for DNA extraction (Gentra Systems, Minneapolis, MN, USA). African Americans with ESKD were recruited from dialysis facilities. T2D was diagnosed in those developing diabetes after age 25 years, without historical evidence of diabetic ketoacidosis or receiving solely insulin therapy since diagnosis. T2D-ESKD was diagnosed after >5 year diabetes duration prior to renal replacement therapy, or with diabetic retinopathy or ≥ 100 mg/dl proteinuria on urinalysis (when available), in the absence of other causes of nephropathy. Unrelated African American controls without diabetes or renal disease (based on a serum creatinine concentration <1.5 [men] or <1.3 mg/dl [women]) were recruited from the community and internal medicine clinics at WFSM. Ethnicity was self-reported and confirmed by genotyping with ancestry informative markers. The mean African ancestry of the samples was 79.85% ± 11.54 for cases and 77.7% ± 10.96 for controls. Recruitment and sample collection for European Americans with T2D-ESKD was performed as above controls were recruited from medicine clinics at WFSM (non-nephropathy, non-T2D population based controls).

African American non-diabetic (non-T2D) ESKD cases

African American non-T2D ESKD cases lacked diabetes (or diabetes developed after initiating renal replacement therapy). ESKD was attributed to chronic glomerular disease (e.g., FSGS), HIV-associated nephropathy, “hypertension-attributed”, or unknown cause. Patients with ESKD due to
urologic/surgical cause, polycystic kidney disease or IgA nephropathy were excluded. The mean African ancestry of the non-T2D ESKD samples was 80.01% ± 10.96.

**African American T2D (non-nephropathy) samples**

African Americans with T2D but lacking nephropathy were recruited from a previously published African American T2D GWAS study (27), as well as internal medicine clinics at WFSM. Diabetic controls were receiving insulin or oral agents, had a HbA1c >6.5% or a fasting plasma glucose >126 mg/dl, and serum creatinine concentration <1.5 mg/dl (men) or 1.3 mg/dl (women). All T2D-only non-nephropathy controls in this study had an eGFR >60 ml/min/1.73m² and a urine albumin:creatinine ratio (UACR) <30 mg/g.

**European American T2D (non-nephropathy) samples**

European Americans with T2D but lacking nephropathy were recruited from the Diabetes Heart Study (DHS) at WFSM. These diabetic controls were receiving insulin or oral agents, had a HbA1c >6.5% and a fasting plasma glucose >126 mg/dl, and serum creatinine concentration <1.5 mg/dl (men) or 1.3 mg/dl (women). All T2D-only non-nephropathy controls in this study had an eGFR >60 ml/min/1.73m² and a UACR <60 mg/g.

**Sample Preparation, Genotyping, and Quality Control**

**African American T2D-ESKD T2D-GENES Discovery cases and controls**

Exome sequencing data was provided by the T2D-GENES consortium for the 529 African American T2D-ESKD cases and 535 African American population based controls from WFSM.

**Targeted Genotyping**

Targeted genotyping of RREB1 (RefSeq NM_001003699.3) variants rs9379084 and rs41302867 were performed across all cohorts utilizing the Sequenom MassArray system (Sequenom, San Diego, CA) in the Center for Genomics and Personalized Medicine Research at WFSM. SNPs were PCR-amplified using primers designed in MassARRAY Assay Design 3.1 (Sequenom, San Diego, CA) and genotypes were analyzed using MassARRAY Typer (Sequenom). Call rates >97% were achieved with the exception...
of rs77510523 which achieved >97% call rates in cases but 95% call rate in controls; quality control was ensured using blind duplicates within each cohort of samples (100% concordance rate).

Locus-wide analysis

The 1000 Genomes Project (ASW, YRI, LWK), Exome Variant Server (NHLBI), and RegulomeDB (28; http://regulome.stanford.edu/) were mined for additional rare (<0.5%), low (MAF 0.5-5%), and common (MAF >5%) frequency RREB1 (RefSeq NM_001003699.3) missense and functional intronic variants located in DNase1 hypersensitivity clusters and transcription factor binding sites (TFBS) (based on Regulome-DB score) beyond those found in T2D-GENES. Variants for analysis in this study were chosen based on allele enrichment in African versus European ancestral populations, type of mutation (missense and functional intronic), and PolyPhen2 prediction (http://genetics.bwh.harvard.edu/pph/data/). In total, an additional 15 RREB1 variants were selected for targeted genotyping in African American T2D-ESKD, non-T2D ESKD, and population-based control cohorts. Of the 15 variants, 3 failed quality control.

Statistical Analysis

Single SNP Association Testing

Each SNP was tested for departure from Hardy-Weinberg Equilibrium (HWE) expectations through Fisher’s exact test (HWE p > 0.01 cases and HWE p > 0.05 controls). The overall genotypic test of association and the two genetic models (dominant and additive) were computed to test for association between each SNP and each phenotype. Data for all tests of association were adjusted for admixture (29), APOL1 G1/G2 risk allele status (assuming a recessive model of disease risk) (4), age, and gender (Model 1) or Model 1 + BMI (Model 2), unless noted (ie, in the T2D-only, non-nephropathy analysis or in European Americans, where admixture and APOL1 are not applicable). These tests were computed using the SNPGWA program (http://www.phs.wfubmc.edu/public_bios/sec_gene/downloads.cfm).

Conditional Analyses

Conditional analyses were run on PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/) v1.07.

Meta-Analysis
The METAL program (http://www.sph.umich.edu/csg/abecasis/metal/index.html) was used to combine data from African and European Americans.

*Multiple Comparisons*

For each analysis with rs9379084 and rs41302867, a multiple comparison adjustment for two SNPs was implemented. In the locus wide analysis a multiple comparison adjustment for 12 SNPs was used in African Americans (three failed QC) and 5 SNPs for European Americans (four failed QC and five were unable to be tested in single-SNP association testing due to low MAF).

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CDK2A/B, IGF2BP2, and FTO in Type 2 Diabetes and Obesity in 6,719 Asians. Diabetes. 57, 2226-2233.


Legends to Figures

Figure 1. Workflow of *RREBI* study.

Caption: Abbreviations: T2D, type-2 diabetes mellitus; ESKD, end-stage kidney disease
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<th>Replication T2D-ESKD</th>
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<th>Non-T2D ESKD</th>
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<td>0.95 ± 0.2</td>
</tr>
</tbody>
</table>

Categoric data expressed as percentage; continuous data as mean ± SD. Abbreviations: BMI, body mass index; BUN, blood urea nitrogen.
Table 2. Clinical Characteristics of European American Study Cohorts

<table>
<thead>
<tr>
<th></th>
<th>European American T2D-ESKD cases</th>
<th>European American Controls</th>
<th>T2D-only (UACR &lt; 60mg/g and eGFR &gt; 60ml/min/1.73m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>637</td>
<td>1020</td>
<td>560</td>
</tr>
<tr>
<td>Female (%)</td>
<td>49.5</td>
<td>63.5</td>
<td>54.4</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.3 ± 10.4</td>
<td>53.9 ± 15.1</td>
<td>63.1 ± 9.0</td>
</tr>
<tr>
<td>Age at T2D (years)</td>
<td>45.4 ± 13.6</td>
<td>-</td>
<td>51.2 ± 10.4</td>
</tr>
<tr>
<td>Duration T2D prior to ESKD (years)</td>
<td>20.1 ± 10.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Duration of ESKD (years)</td>
<td>2.60 ± 3.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Blood Glucose (mg/dl)</td>
<td>-</td>
<td>93.1 ± 16.9</td>
<td>-</td>
</tr>
<tr>
<td>BMI (at recruitment; kg/m²)</td>
<td>29.6 ± 7.1</td>
<td>28.4 ± 5.7</td>
<td>32.6 ± 7.1</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>-</td>
<td>15.1 ± 5.1</td>
<td>-</td>
</tr>
<tr>
<td>Serum Creatinine (mg/dl)</td>
<td>-</td>
<td>0.96 ± 0.50</td>
<td>1.03± 0.29</td>
</tr>
</tbody>
</table>

Categoric data expressed as percentage; continuous data as mean ± SD. Abbreviations: BMI, body mass index; BUN, blood urea nitrogen.
Table 3. *RREB1* variants identified through African American T2D-ESKD Exome Sequencing Study and African American Replication Analyses (Additive Model, correction for multiple tests [n=2] applied)

<table>
<thead>
<tr>
<th>Study</th>
<th>SNP</th>
<th>N Case / Control</th>
<th>MAF Case / Control</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Discovery T2D-ESKD</strong></td>
<td>rs9379084</td>
<td>525 / 510</td>
<td>0.011 / 0.039</td>
<td>0.0010</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>rs41302867</td>
<td>521 / 507</td>
<td>0.0077 / 0.033</td>
<td>0.00092</td>
<td>0.21</td>
</tr>
<tr>
<td><strong>Replication T2D-ESKD</strong></td>
<td>rs9379084</td>
<td>1220 / 605</td>
<td>0.019 / 0.029</td>
<td>0.12</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>rs41302867</td>
<td>1238 / 611</td>
<td>0.016 / 0.031</td>
<td>0.032</td>
<td>0.52</td>
</tr>
<tr>
<td><strong>non-T2D, ESKD Cases</strong></td>
<td>rs9379084</td>
<td>1424 / 605</td>
<td>0.020 / 0.028</td>
<td>0.62</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>rs41302867</td>
<td>1427 / 611</td>
<td>0.015 / 0.031</td>
<td>0.038</td>
<td>0.56</td>
</tr>
<tr>
<td><strong>Combined, “all cause” ESKD</strong></td>
<td>rs9379084</td>
<td>3166 / 1115</td>
<td>0.018 / 0.033</td>
<td>0.0026</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>rs41302867</td>
<td>3186 / 1118</td>
<td>0.014 / 0.032</td>
<td>1.46x10^-4</td>
<td>0.49</td>
</tr>
<tr>
<td><strong>T2D-only vs. population based controls</strong></td>
<td>rs9379084</td>
<td>809 / 662</td>
<td>0.024 / 0.029</td>
<td>0.32</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>rs41302867*</td>
<td>828 / 675</td>
<td>0.021 / 0.031</td>
<td>0.064</td>
<td>0.57</td>
</tr>
<tr>
<td><strong>European Americans</strong></td>
<td>rs9379084</td>
<td>572 / 693</td>
<td>0.097 / 0.14</td>
<td>1.54x10^-4</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>rs41302867</td>
<td>589 / 697</td>
<td>0.11 / 0.13</td>
<td>0.015</td>
<td>0.70</td>
</tr>
<tr>
<td><strong>T2D-only vs. population based controls</strong></td>
<td>rs9379084</td>
<td>535 / 693</td>
<td>0.098 / 0.14</td>
<td>0.0046</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>rs41302867</td>
<td>541 / 697</td>
<td>0.11 / 0.13</td>
<td>0.060</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Sample sizes reflect those with complete clinical and genotypic data used in each analysis.

*Dominant model (lack of fit to additivity < 0.05); No *APOL1* G1/G2 adjustment in T2D-only analysis

**Model 1 covariates:** age, gender, *APOL1* G1/G2 status, admixture [no admixture/*APOL1* adjustment in European Americans]

**Model 2 covariates:** Model 1 + BMI [body mass index]
Table 4A. *RREB1* T2D-ESKD African & European American meta-analysis

<table>
<thead>
<tr>
<th>SNP</th>
<th>N Case / Control</th>
<th>P Model 1</th>
<th>Effect [SE]</th>
<th>P Model 2</th>
<th>Effect [SE]</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs41302867</td>
<td>2348 / 1815</td>
<td>4.36x10⁻⁵</td>
<td>-0.49 [0.12]</td>
<td>3.69x10⁻⁵</td>
<td>-0.51 [0.12]</td>
</tr>
<tr>
<td>rs9379084</td>
<td>2317 / 1808</td>
<td>6.06x10⁻⁷</td>
<td>-0.59 [0.12]</td>
<td>3.5x10⁻⁷</td>
<td>-0.60 [0.12]</td>
</tr>
</tbody>
</table>

Model 1: adjusted for admixture, age, sex, *APOL1* G1/G2
Model 2: Model 1 + BMI

Table 4B. *RREB1* all-cause ESKD African & European American meta-analysis

<table>
<thead>
<tr>
<th>SNP</th>
<th>N Case / Control</th>
<th>P Model 1</th>
<th>Effect [SE]</th>
<th>P Model 2</th>
<th>Effect [SE]</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs41302867</td>
<td>3775 / 1815</td>
<td>9.10x10⁻⁶</td>
<td>-0.49 [0.11]</td>
<td>6.58x10⁻⁶</td>
<td>-0.51 [0.11]</td>
</tr>
<tr>
<td>rs9379084</td>
<td>3738 / 1808</td>
<td>8.46x10⁻⁷</td>
<td>-0.53 [0.10]</td>
<td>8.74x10⁻⁷</td>
<td>-0.54 [0.11]</td>
</tr>
</tbody>
</table>

Model 1: adjusted for admixture, age, sex, *APOL1* G1/G2
Model 2: Model 1 + BMI
Abbreviations

AGT  angiotensinogen
BMI  body mass index
bp   base pair
kbp  kilo base pair
NGES next-generation exome sequencing
RREB1 ras-responsive element binding protein 1
T2D  type 2 diabetes
T2D-ESKD type 2 diabetes associated end-stage kidney disease
non-T2D ESKD non-type 2 diabetes associated end-stage kidney disease
Stage 1: Analyze Next-Generation Exome Sequencing data
529 African American T2D-ESKD vs 535 population based controls

Stage 2: Replicate *RREB1* findings in independent samples with targeted genotypes
1305 African Americans with T2D-ESKD vs 760 population based controls

Stage 3a: *RREB1* variants associated with T2D in the absence of ESKD?
850 African Americans with T2D (lacking nephropathy) vs 760 population based controls

Stage 3b: *RREB1* variants associated with ESKD in the absence of T2D?
1705 African Americans with ESKD (lacking T2D) vs 760 population based controls

Stage 4: Are findings able to be replicated in European Americans with T2D-ESKD?
637 European Americans with T2D-ESKD vs 1020 population based controls

Stage 5: *RREB1* variants associated with T2D in the absence of ESKD in European Americans?
560 European Americans with T2D (lacking nephropathy) vs 1020 population based controls

Figure 1: Workflow of *RREB1* study