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Analysis of Coding Variants Identified from Exome Sequencing Resources for Association with Diabetic and Non-diabetic Nephropathy in African Americans

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Abstract

Prior studies have identified common genetic variants influencing diabetic and non-diabetic nephropathy, diseases which disproportionately affect African Americans. Recently, exome sequencing techniques have facilitated identification of coding variants on a genome-wide basis in large samples. Exonic variants in known or suspected end-stage kidney disease (ESKD) or nephropathy genes can be tested for their ability to identify association either singly or in combination with known associated common variants. Coding variants in genes with prior evidence for association with ESKD or nephropathy were identified in the NHLBI-ESP GO

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database and genotyped in 5045 African Americans (3324 cases with type 2 diabetes associated nephropathy [T2D-ESKD] or non-T2D ESKD, and 1721 controls) and 1465 European Americans (568 T2D-ESKD cases and 897 controls). Logistic regression analyses were performed to assess association, with admixture and *APOL1* risk status incorporated as covariates. Ten of 31 SNPs were associated in African Americans; four replicated in European Americans. In African Americans, SNPs in *OR2L8*, *OR2AK2*, *C6orf167* (*MMS22L*), *LIMK2*, *APOL3*, *APOL2*, and *APOL1* were nominally associated ($P=1.8\times 10^{-4}$ -0.044). Haplotype analysis of common and coding variants increased evidence of association at the *OR2L13* and *APOL1* loci ($P=6.2\times 10^{-5}$ and 4.6×10^{-5} , respectively). SNPs replicating in European Americans were in *OR2AK2*, *LIMK2*, and *APOL2* ($P=0.0010$ -0.037). Meta-analyses highlighted four SNPs associated in T2DESKD and all-cause ESKD. Results from this study suggest a role for coding variants in the development of diabetic, non-diabetic, and/or all-cause ESKD in African Americans and/or European Americans.

Keywords

African Americans; Association; European Americans; Exonic Variants; Type 2 Diabetes; Nephropathy

Introduction

Kidney disease affects approximately 40% of patients with type 2 diabetes (T2D) (de Boer et al. 2011) and 10-20% of individuals with diabetes ultimately die from renal complications (WHO 2011). Diabetes accounts for more than 40% of new cases of end-stage kidney disease (ESKD) (USRDS 2009). Despite increased use of diabetes-related medications over the last two decades, diabetes-associated kidney disease has increased and this trend is expected to continue (de Boer et al. 2011).

African Americans have the highest adjusted incidence and prevalence of ESKD in the U.S., with rates 3.5 times higher than in European Americans (USRDS 2011). ESKD incidence is higher among ethnic minorities regardless of uniform health care coverage, supporting the role of genetics and/or unknown environmental influences in kidney disease (Karter et al. 2002; Lipworth et al. 2012). Genetic variants with low minor allele frequencies (MAF) are more likely than common variants to be population-specific and are a possible source of the unaccounted-for risk for common diseases (Bustamante et al. 2011; Gravel et al. 2011; Manolio et al. 2009). It has been proposed that these coding variants will elucidate part of the “missing heritability” of diseases left undiscovered by genome-wide association studies (GWAS) (Manolio et al. 2009; Zeggini 2011). Exome sequencing technology has motivated the creation of data resources facilitating unbiased interrogation of genetic variation present in protein-coding regions (Teer and Mullikin 2010).

In this report we evaluated whether coding variants identified from large-scale exome sequencing resources were associated with nephropathy in African Americans and European Americans. Variants were chosen in genes with prior evidence of association with diabetic and non-diabetic forms of nephropathy, possible functional impact, and MAF in African Americans. We asked two specific questions: 1) can exonic variants alone demonstrate

evidence of association with ESKD and 2) can exonic variants contribute to evidence of association with ESRD in combination with known common variants?

Subjects and Methods

Subjects

All subjects were unrelated, born in North Carolina, South Carolina, Georgia, Virginia, or Tennessee, of self-reported ethnicity. Diagnostic criteria for individuals with T2D-ESKD were diabetes diagnosis at age >30 years (in the absence of diabetic ketoacidosis), with either renal histologic evidence of diabetic nephropathy or diabetes duration ≥ 5 years before renal replacement therapy initiation, in the presence of diabetic retinopathy or proteinuria ≥ 500mg/24h and absence of other causes of nephropathy (Freedman et al. 2009b; McDonough et al. 2011). Patients with non-T2D ESKD were also evaluated and included those undergoing renal replacement therapy with no history of diabetes and meeting the same criteria for nephropathy diagnosis. Individuals with cystic renal diseases, hereditary nephritis, or urologic causes of ESKD were excluded from this group. Non-diabetic, non-nephropathy controls were recruited based on low risk for nephropathy due to the lack of a personal or family history of kidney disease; therefore, renal function testing was not routinely performed. The African American subject group included 2172 T2D-ESKD cases, 1152 non-T2D ESKD cases and 1721 controls. Thus, a combined total of 3324 African Americans with nephropathy diagnosis in the presence or absence of diabetes, referred to as “all-cause” ESKD, were evaluated. The European American subject group included 568 cases with T2D-ESKD and 897 non-diabetic, nonnephropathy controls. All participants provided written informed consent and studies were approved by the Wake Forest School of Medicine Institutional Review Board and adhere to the tenets of the Declaration of Helsinki.

In African Americans, ancestry proportions were evaluated using 70 ancestry informative markers (AIMs; (Keene et al. 2008)). Ancestral proportions were generated for each subject based on individual ancestral allele frequencies estimated by genotyping the 70 AIMs in Yoruba Nigerians and European Americans; results were evaluated under a two-population model implemented in FRAPPE (Tang et al. 2005). African ancestry ($\pm$ standard deviation) was 0.80 ± 0.11 , 0.80 ± 0.11 , and 0.78 ± 0.11 , T2D-ESKD cases, non-T2D ESKD cases, and controls, respectively.

Variant Selection and Genotyping

The National Heart Lung and Blood Institute (NHLBI) ESP GO resource (<https://esp.gs.washington.edu/drupal/>, (Tennessen et al. 2012)) was used to identify single nucleotide polymorphisms (SNPs) in African Americans. SNPs were identified in genes with prior evidence for association with nephropathy (ACACB, AGER, APOL1, APOL2, APOL3, C6orf66, C6orf167 (*MMS22L*), C6orf191, CNDP1, CNDP2, LIMK2, MYH9, OR2AK2, OR2L8, OR2L13, SFI1, TMEM5, and *UNC5C*). Genotyping was carried out using the iPLEX™ Sequenom MassARRAY platform (Sequenom; San Diego, CA). The genotyping efficiency of each SNP exceeded 95.5%. Only individual DNA samples with genotyping efficiency ≥ 75% were retained for analysis. Blind duplicates were included in genotyping and were 100% concordant.

Statistical Analyses

Genotypic association was evaluated using the SNPGWA statistical package (www.phs.wfubmc.edu), which performs the two degrees of freedom global test of genotypic association in addition to individual genetic models including dominant, additive, recessive, and lack-of-fit to additivity. This is consistent with the Fisher's protected least significant difference multiple comparisons procedure. Haplotype analysis was evaluated using PLINK (Purcell et al. 2007) for haplotype-specific and omnibus tests of association. LD between variants was determined using the Haploview program (Barrett et al. 2005) by evaluating variants in the African American samples.

Variants in the *APOL1* gene have been shown to be powerfully associated with non-diabetic nephropathy in African Americans (Genovese et al. 2010) and their presence in both T2D-ESKD and non-diabetic nephropathy cases has the potential to confound evidence of association (Freedman et al. 2011). To minimize the potential influence of risk alleles at the *APOL1* G1 and G2 risk variants (Genovese et al. 2010), each African American sample was genotyped for the two *APOL1* G1 haplotype SNPs (rs73885319 [S342G] and rs60910145 [I384M]) and the G2 six base pair deletion (rs71785313) (Genovese et al. 2010); genotyping was performed using the iPLEX™ Sequenom MassARRAY platform (Sequenom; San Diego, CA). Data from this genotyping was used as a covariate in association analyses. Parenthetically we have shown that the *APOL1* G1 and G2 risk alleles are very rare in European Americans (<0.33%) (Cooke et al. 2012) and therefore were not taken into account in European American analyses.

African American and European American data were analyzed for association separately and, when appropriate, were combined in a meta-analysis implementing the inverse variance weighted method in METAL (<http://www.sph.umich.edu/csg/abecasis/metal/>) to determine the overall association of each SNP with T2D-ESKD and all-cause ESKD.

Results

Clinical characteristics of study samples are shown in Table 1. On average, there were more females in all groups, with the exception of the African American non-T2D ESKD cohort. T2D-ESKD and non-T2D ESKD cases were older than controls, although age at T2D diagnosis was lower than the average age in control groups. On average, all participants were overweight or obese at the time of enrollment.

Thirty-one SNPs from 19 genes met criteria for selection and were genotyped (Supplementary Table 1). Genes were selected based on prior evidence of association with ESKD or nephropathy; therefore, SNPs with P-values<0.05 were considered nominally associated. *APOL3*, *C6orf167* (*MMS22L*), *C6orf191*, *C12orf66*, *LIMK2*, *MYH9*, *OR2L13*, *OR2L8*, *OR2AK2*, *SASH1*, *SFI1*, *TMEM5*, and *UNC5C* were selected from a prior GWAS of diabetic nephropathy in African Americans (McDonough et al. 2011). *MYH9* was chosen based on its implication in non-diabetic nephropathy in various ethnic groups but most prominently in African Americans (Behar et al. ; Freedman et al. 2009b; Freedman et al. 2009c; Kao et al. 2008; Kopp et al. 2008; O'Seaghdha et al. 2011), prior to identification of the nearby *APOL1* ESKD risk gene (Genovese et al. 2010), which was also selected for

analysis. Advanced glycation end-products (AGEs), for which *AGER* is a receptor, have been implicated in diabetic complications (reviewed in (King and Loeken 2004)). *ACACB* was selected because a variant in this gene associated with diabetic nephropathy in Japanese and European Americans (Maeda et al. 2010). *CNDP1* was selected because variants in this gene have been reported to influence glucose metabolism (Sauerhofer et al. 2007) and were associated with T2D-ESKD and diabetic nephropathy in European Americans and African Americans (Freedman et al. 2007; Janssen et al. 2005; McDonough et al. 2009); variants in *CNDP2* have been implicated in T2D-ESKD in African Americans (McDonough et al. 2009).

Several analyses were performed given the various subjects groups: African American and European American, T2D-ESKD and non-T2D ESKD. In addition, in contrast to common non-coding variants which are conventionally thought to act through additive genetic effects, the impact of coding variants could be expressed in various ways, e.g. dominant, additive, or recessive models. Supplementary Figure 1 illustrates the progression of the study. The primary inferences were drawn from analysis of all African American samples together, all European American samples, and the combination of the two with subgroup analysis in African Americans, as appropriate to assess impact on etiology of ESKD, e.g. T2D-ESKD or non-T2D ESKD.

Table 2 lists all SNPs nominally associated ($P < 0.05$) with all-cause ESKD in African Americans with their analysis presented in all-cause ESKD, T2D-ESKD, and non-T2D-ESKD. The best genetic model is reported for each SNP except where a lack-of-fit to additivity was observed. MAFs differed greatly between European Americans and African Americans, as shown in Supplementary Table 1. African American all-cause ESKD analyses identified seven associated SNPs in seven genes including *OR2L8*, *OR2L13*, *C6orf167* (*MMS22L*), *LIMK2*, *APOL3*, *APOL2*, and *APOL1* (P -values = 5.4×10^{-4} –0.037, $OR = 0.65$ –1.22). In all but two cases (rs61098917 in *LIMK2* and rs7285167 in *APOL2*), the minor allele was associated with ESKD protection. Results for analysis in stratified T2D-ESKD and non-T2D-ESKD samples showed substantial differences in association suggesting preferential influence on one form of ESKD, e.g. rs4925583 in *OR2L8*. In addition, SNPs rs4478844 in *OR2AK2* and rs3747154 in *LIMK2* were nominally associated with T2D-ESKD and non-T2D-ESKD, respectively, but not with all-cause ESKD. Results for all SNPs in the African American populations are shown in Supplementary Tables 2–4.

To determine if the SNPs contributed to association across ethnicity, the 31 SNPs were genotyped in a cohort of European Americans including 568 T2D-ESKD cases and 897 controls (Table 3). This analysis did not include an ability to capture non-T2D ESKD variants in European Americans. Four SNPs in four genes, *OR2AK2* (rs4478844), *OR2L13* (rs74153072), *LIMK2* (rs3747154), and *APOL2* (rs7285167), had evidence of association in European Americans in the same direction as in African Americans. In addition, a SNP in *CNDP1* (rs73973908) was associated in European Americans but not in African Americans. Results for all SNPs in the European American population are shown in Supplementary Table 5.

Meta-analysis of African Americans and European Americans examined the overall association of each SNP with T2D-ESKD ($\text{Meta}P_{\text{T2D-ESKD}}$) and all-cause ESKD ($\text{Meta}P_{\text{N}}$) (Table 4). Four SNPs were associated in both T2D-ESKD and all-cause ESKD analyses; including rs4925583 ($\text{Meta}P_{\text{T2D-ESKD}}=0.038$, $\text{Meta}P_{\text{N}}=0.0051$) in *OR2L8*, rs116147257 ($\text{Meta}P_{\text{T2D-ESKD}}=0.0014$, $\text{Meta}P_{\text{N}}=7.2\times 10^{-4}$) in *APOL3*, rs7285167 ($\text{Meta}P_{\text{T2D-ESKD}}=0.0032$, $\text{Meta}P_{\text{N}}=8.5\times 10^{-4}$) in *APOL2*, and rs2239785 ($\text{Meta}P_{\text{T2D-ESKD}}=0.0025$, $\text{Meta}P_{\text{N}}=0.0023$) in *APOL1*. rs2269529 in *MYH9* was nominally associated in the T2D-ESKD meta-analysis ($\text{Meta}P_{\text{T2D-ESKD}}=0.048$) and trended toward association with all-cause ESKD ($\text{Meta}P_{\text{N}}=0.053$) but was not associated in individual cohorts. rs61098917 in *LIMK2* was associated in the all-cause ESKD meta-analysis ($\text{Meta}P_{\text{N}}=0.0069$) and also in African American non-T2D ESKD ($P=0.0030$) and all-cause ESKD ($P=0.019$).

To extend these findings, we evaluated whether combining data from these exonic variants with known common variants would increase evidence for association between a specific genetic locus and ESKD. Table 5 lists two haplotype P-values: the omnibus P-value compares the overall distribution of haplotypes between cases and controls, while the risk haplotype P-value compares the frequency of the risk haplotype (i.e. the haplotype combination made up of the risk alleles for each SNP) between cases and controls. Supplementary Table 6 summarizes risk alleles, haplotype frequencies, and odds ratios in addition to P-values. For example, a common SNP in *OR2L3* (rs10888287) was associated with T2D-ESKD ($P\text{-value}=2.7\times 10^{-4}$) with three coding variants (rs4925583, rs4478844, rs74153072) possessing individual P-values ranging from 6.9×10^{-4} to 0.79. In haplotype analysis of the most strongly associated coding variant (rs4925583) and rs10888287, the resulting omnibus P-value was 2.69×10^{-4} and comparable to either individual SNP and the risk haplotype was nominally more strongly associated, $P\text{-value}=6.2\times 10^{-5}$. Other analyses provide a range of results: addition of coding variants and haplotype analysis reduces the evidence of association (C6orf167; rs3822908/rs9481410) to stronger evidence for association (*APOL1*; rs16996381/rs2239785). In most cases, however, the combination of common and coding variant data does not appreciably improve evidence for association.

Discussion

The purpose of this study was to assess the value of coding variants identified in exome sequencing studies for detecting association with ESKD susceptibility. This was accomplished by performing a locus-wide analysis of coding variants in genes previously associated with nephropathy in cohorts with different etiologies of renal disease. Assessing known or suspected ESKD genes is a logical first step in evaluating coding variants. With prior evidence of association with ESKD, it is more likely that a coding variant in these genes would contribute to ESKD risk. This approach facilitated comparison testing to ascertain the potential effect of each of the 31 SNPs. Analyses revealed nominal evidence of association with multiple SNPs. Individual coding variants were associated with ESKD, but, in the sample tested, the strength of association alone would not provide compelling proof of association unless the prior evidence of association was taken into consideration. While the effect size of individual, nominally associated coding SNPs was in general greater than that observed for common variants, the odds ratios are not dramatically higher (or lower). When

combined with the low MAF of most coding variants, this results in, at best, nominal evidence of association. In addition, with the exception of a few loci, combining common and coding variant data added little to evidence of association.

Of the 31 SNPs in 19 genes, 10 showed nominal evidence ($P < 0.05$) of association African Americans. SNPs in genes on chromosome 22 were notably represented. The importance of chromosome 22 in African American nephropathy has been established by various reports in non-diabetic (Behar et al. 2010; Freedman et al. 2009b; Freedman et al. 2010; Freedman et al. 2009c; Freedman et al. 2012; Genovese et al. 2010; Kao et al. 2008; Kopp et al. 2008; O'Seaghdha et al. 2011; Pattaro et al. 2009), T2D-associated (Cooke et al. 2012; Freedman et al. 2009a; McDonough et al. 2011), and type 1 diabetic nephropathy (Wessman et al. 2011). With the established importance of chromosome 22, many of the genes targeted in this study, including *APOL3*, *APOL2*, *APOL1*, *LIMK2*, and *MYH9*, were from this chromosome. Analyses conditioned on *APOL1* G1 (rs73885319 and rs60910145) and G2 (rs71785313) variants (Genovese et al. 2010) minimized the influence of these alleles. This approach has been useful for identifying association signals in complex traits and disorders (Lango Allen et al. 2010; Ripke et al. 2011; Sklar et al. 2011; Yang et al. 2012). Accounting for *APOL1* G1/G2 variants, coding variants in *APOL3*, *APOL2*, and *APOL1* were associated with all-cause ESKD, T2D-ESKD, and non-T2D ESKD in African Americans with little correlation among variants ($r^2 < 0.28$ for all comparisons).

We previously reported a common SNP in *APOL3*, rs16996381, associated with protection in all-cause ESKD in a GWAS of T2D-ESKD in African Americans (McDonough et al. 2011). Here the *APOL3* coding SNP rs116147257, a missense (I220T) variant with $MAF \approx 4.5\%$ in African Americans, was also protective in African American and European American T2D-ESKD (Tables 2-3). This lends further support to a role for the *APOL3* gene in nephropathy and suggests a more complex combination of alleles leading to risk or protection from ESKD in this region. Parenthetically, rs16996381 and rs116147257 have no evidence of correlation ($r^2 = 0.03$). Further, a missense (C182R) SNP in *APOL2* (rs7285167) was associated with risk in African American T2D-ESKD, non-T2D ESKD, and all-cause ESKD (Table 2), and in European American T2D-ESKD (Table 3). It was also significant in the meta-analyses of African American and European American T2D-ESKD ($_{Meta}P = 0.0032$) and all-cause ESKD ($_{Meta}P = 8.5 \times 10^{-4}$) (Table 4), and therefore appears to be associated with ESKD in both African Americans and European Americans.

APOL1 was also notable in these analyses. A missense (K150E; rs2239785) SNP in *APOL1* was associated with protection in African American T2D-ESKD, non-T2D ESKD, and all-cause ESKD (Table 2), and in meta-analyses of T2D-ESKD and all-cause ESKD in African Americans and European Americans (Table 4). Based on observed P-values in the individual cohorts and the lack of association in European American T2D-ESKD, it appears that this SNP is an African American all-cause ESKD variant.

Two common SNPs in *LIMK2* on chromosome 22, rs2106294 and rs4820043, were associated with protection in a GWAS of T2D-ESKD in African Americans (McDonough et al. 2011). In the analysis reported here, two SNPs in *LIMK2*, rs61098917 (intronic) and rs3747154 (R684Q), were associated with risk in African American non-T2D ESKD (Table

2). Further, rs61098917 was also associated with risk in African American all-cause ESKD (Table 2) and in the African American and European American all-cause ESKD meta-analysis (Table 4), while rs3747154 was associated with risk in European American T2D-ESKD (Table 3). rs61098917 and rs3747154 were not correlated with one another or with previously implicated SNPs ($r^2 < 0.015$). rs61098917 and rs3747154 appeared to be African American non-T2D ESKD risk variants, and rs3747154 may also be a European American T2D-ESKD risk variant. Although a lower P-value and higher OR for risk were observed in European Americans (Table 4), risk allele frequencies were lower compared to African Americans (Table 2).

Several other SNPs had evidence of nominal association consistent with protection from nephropathy in African Americans. These included SNPs in the *OR2L13/OR2L8/OR2AK2* region and in *C6orf167* (*MMS22L*). SNPs in the olfactory receptor-encoding genes *OR2L13*, *OR2L8*, and *OR2AK2*, whose coding sequences overlap on chromosome 1, included rs4925583, rs4478844, and rs74153072, were associated with T2D-ESKD, non-T2D ESKD and/or all-cause ESKD in African Americans (Table 2). SNPs rs4478844 and rs74153072 were also associated and rs4925583 trended toward association ($P=0.066$) in European Americans (Table 3). There was moderate correlation between rs4925583 and rs4478844 ($r^2=0.37$), but no correlation between either of these SNPs and rs74153072 ($r^2=0.02$). A common intronic SNP in this region, rs10888287, was associated with protection in an African American GWAS of T2D-ESKD (McDonough et al. 2011). The LD between this non-coding SNP and the coding SNPs reported here was nominal to moderate ($r^2=0.60$). The olfactory receptor gene family is large and the chromosomal organization is complex (Malnic et al. 2004). The coding sequence of *OR2L13* overlaps *OR2L8* and *OR2AK2*. The role of this class of genes in nephropathy requires further elucidation, but it does appear to represent an independent nephropathy locus.

Two additional missense SNPs associated with protection were located in *C6orf167* (*MMS22L*) and *CNDP1*. rs9481410 (M564T) in *C6orf167* (*MMS22L*) was associated in African American T2D-ESKD and all-cause ESKD (Table 2). A SNP in this gene, rs3822908, was associated with protection in T2D-ESKD and all-cause ESKD in the prior African American GWAS (McDonough et al. 2011); however, the LD between these SNPs was low ($r^2=0.13$). In European American T2D-ESKD analyses, rs73973908 (N270H) in *CNDP1* was associated (Table 3) but was not associated in analyses of or including African Americans. *CNDP1* encodes secreted serum carnosinase that degrades carnosine (Teufel et al. 2003), a free oxygen radical scavenger with anti-glycating effects which inhibits the formation of AGEs (Boldyrev 2000; Hipkiss et al. 1998) and influences glucose metabolism (Sauerhofer et al. 2007). Variants in *CNDP1* have previously been associated with diabetic nephropathy in Europeans (Janssen et al. 2005), European Americans (Freedman et al. 2007), and African Americans (McDonough et al. 2009).

In addition, we assessed whether the coding SNPs tested in this study added to evidence for association with ESKD in combination with previously identified GWAS SNPs (McDonough et al. 2011). Haplotype analyses in a subset of African American individuals ($n=2488$ all-cause ESKD cases, $n=1561$ controls) resulted in moderately stronger evidence for association in particular cases. For example, evaluating SNPs in *LIMK2*, rs4820043

(McDonough et al. 2011) and rs61098917, yielded a two-marker haplotype $P=5.7\times 10^{-14}$, compared to individual P-values (rs4820043 $P=3.8\times 10^{-12}$, rs61098917 $P=4.2\times 10^{-6}$) in this subset of individuals. This suggests that the combination of common non-coding and coding SNPs will be useful, especially in a comprehensive genome-wide analysis.

A striking characteristic of the associated SNPs is the difference in MAF between African Americans and European Americans. Differences in allele frequencies are >16.9% for seven of 11 associated SNPs (difference in MAF for all associated SNPs 4.4-32.5%). This observation, in conjunction with the observation that many of the coding variants were protective, suggests the possibility that protective variants have arisen in African-origin populations to compensate for the ancillary health risks associated with the anti-trypanosomal *APOL1* G1 and G2 alleles. While this is speculative, it bares additional investigation.

We appreciate that there are limitations to the current study. The number of SNPs that were tested was small and odds ratios of associated SNPs were not striking and did not provide dramatic evidence of association. However, a test of whether coding variants were associated with nephropathy is most easily tested in genes with a prior history of nephropathy association. Additionally, it is noteworthy that seven of the associated variants were predicted to be probably or possibly damaging by PolyPhen (<http://genetics.bwh.harvard.edu/pph2/>). The power to detect significant effects, afforded by the relatively large sample size (for studies of nephropathy) examined in this study (>5000 African Americans and >1450 European Americans), is a strength. In the combined African American sample we estimate that we have 80% power to detect an OR of 1.30 for a 5% MAF variant (additive model).

Coding variants lack an intrinsic likelihood that a specific model (e.g. dominant, additive, recessive) is likely to be more appropriate. Power for recessive models was limited and moreover, power to assess association in European Americans was limited. Investigation of SNPs in a large sample of African Americans with multiple etiologies of nephropathy allowed for the detection of variants associated with T2D-ESKD, non-T2D ESKD, and/or all-cause ESKD. As we have noted (Bowden and Freedman 2012) it remains unclear whether individual nephropathy genes contribute to multiple etiologies of disease. This is complicated further by the high frequency of the *APOL1* G1 and G2 risk alleles in the African American population. Replication of four of the associated variants in a small European American cohort (in which the *APOL1* G1/G2 risk variants are virtually absent) supports the case that several SNPs were indeed associated with ESKD.

In summary, coding variants in previously implicated nephropathy genes can be useful additions to genetic analyses of ESKD. This was particularly true in analysis of the *APOL* gene cluster on chromosome 22. Based on these data, studies of coding variants complement common variant analyses. It is notable that odds ratios of associated SNPs were not striking and did not provide dramatic evidence of association. Thus, genome-wide searches for coding variants influencing nephropathy risk are likely to be productive, but will also likely require sample sizes comparable to GWAS using common variants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Clinical characteristics of study samples.

	African Americans			European Americans		
	T2D-ESRD	Non-T2D ESRD	Controls	T2D-ESRD	Controls	Controls
n	2172	1152	1721	568	897	
Female (%)	57.5	43.4	54.9	50.4	63.2	
Age (years)						
at enrollment	61.6 ± 10.7	54.6 ± 14.6	47.5 ± 12.4	66.0 ± 10.3	54.5 ± 15.2	
at T2D diagnosis	41.2 ± 13.0	---	---	45.5 ± 13.7	---	
Duration of diabetes (years)	20.2 ± 10.7	---	---	20.5 ± 10.1	---	
BMI (kg/m ²)	30.0 ± 0.1	27.2 ± 7.0	29.8 ± 7.23	29.5 ± 7.0	28.2 ± 5.5	

Categorical data are expressed as percentage; continuous data as mean ± SD

Table 2

Individual SNP analysis in diabetic, non-diabetic, and all-cause ESRD in African Americans.

Variant Info			All-Cause ESRD Cases (n= 3024) vs. Controls (n=1672)				T2D-ESRD Cases (n= 1896) vs. Controls (n=1672)				Non-T2D ESRD Cases n=1128 vs. Controls (n=1672)			
SNP Position (hg19)	Alleles (minor/ major)*	Gene	MAF (cases/ controls)	P	OR (95% CI)	Model	MAF (cases/ controls)	P	OR (95% CI)	Model	MAF (cases/ controls)	P	OR (95% CI)	Model
rs4925583 1:248112809	G/A	OR2L8	0.32/0.36	0.014	0.89 (0.81-0.98)	add	0.33/0.36	0.11	0.92 (0.83-1.02)	add	0.31/0.36	1.8E-04	0.57 (0.43-0.77)	rec
rs4478844 1:248129240	A/G	OR2AK2	0.16/0.18	0.15	0.92 (0.82-1.03)	add	0.16/0.18	0.044	0.86 (0.74-1.00)	dom	0.17/0.18	0.69	0.97 (0.83-1.13)	add
rs74153072 1:248263495	G/T	OR2L13	0.19/0.19	0.037	0.65 (0.43-0.98)	rec	0.20/0.19	0.57	1.04 (0.92-1.17)	add	0.19/0.19	0.86	0.99 (0.84-1.16)	add
rs9481410 6:97677118	G/A	MMS22L	0.48/0.50	0.019	0.84 (0.73-0.97)	dom	0.48/0.50	0.017	0.89 (0.81-0.98)	add	0.49/0.50	0.18	0.92 (0.82-1.04)	add
rs61098917 22:31668720	T/C	LIMK2	0.26/0.22	0.019	1.14 (1.02-1.26)	add	0.24/0.22	0.15	1.09 (0.97-1.22)	add	0.28/0.22	0.0030	1.29 (1.09-1.52)	dom
rs3747154 22:31673116	G/A	LIMK2	0.05/0.04	0.49	1.08 (0.88-1.32)	add	0.04/0.04	0.26	0.87 (0.69-1.10)	add	0.06/0.04	0.0036	1.48 (1.14-1.93)	dom
rs116147257 22:36537798	A/G	APOL3	0.04/0.05	9.4E-04	0.69 (0.56-0.86)	dom	0.04/0.05	0.0013	0.67 (0.53-0.86)	dom	0.04/0.05	0.063	0.77 (0.58-1.01)	add
rs7285167 22:36623920	A/G	APOL2	0.38/0.29	0.0023	1.22 (1.07-1.39)	dom	0.35/0.29	0.0066	1.21 (1.06-1.40)	dom	0.44/0.29	0.0092	1.26 (1.06-1.51)	dom
rs2239785 22:36661330	A/G	APOL1	0.28/0.38	5.4E-04	0.77 (0.67-0.90)	dom	0.31/0.38	5.0E-04	0.76 (0.65-0.89)	dom	0.23/0.38	0.025	0.80 (0.65-0.97)	dom

All analyses adjusted for percentage of African ancestry and *APOL1* risk; best model reported unless SNP not associated in analysis, then additive model reported (add);

* minor/major allele refers to African Americans

Table 3

Individual SNP analysis in European American T2D-ESRD.

Variant Info				Minor Allele Frequency		T2D-ESRD Cases (n=568) vs. Controls (n=897)		
SNP	Chr	Position (hg19)	Alleles (minor/major)*	Gene	T2D-ESRD	Controls	P	Best Model OR (95% CI) Model
rs4925583	1	248112809	G/A	OR2L8	0.94	0.95	0.066	0.74 (0.54-1.02) add
rs4478844	1	248129240	A/G	OR2AK2	0.65	0.65	0.0087	0.66 (0.48-0.90) dom
rs74153072	1	248263495	G/T	OR2L13	0.19	0.19	0.037	0.65 (0.43-0.98) rec
rs9481410	6	97677118	G/A	MMS22L	0.27	0.24	0.077	1.17 (0.98-1.39) add
rs73973908	18	72238472	A/C	CNDP1	0.13	0.16	0.025	0.76 (0.60-0.97) dom
rs61098917	22	31668720	T/C	LIMK2	0.0062	0.0022	0.11	2.23 (0.80-6.25) add
rs3747154	22	31673116	G/A	LIMK2	0.011	0.0011	0.0010	6.43 (1.81-22.88) dom
rs116147257	22	36537798	A/G	APOL3	0.00090	0.0017	0.89	0.90 (0.23-3.56) add
rs7285167	22	36623920	A/G	APOL2	0.091	0.064	0.0065	1.47 (1.11-1.94) add
rs2239785	22	36661330	A/G	APOL1	0.79	0.80	0.61	0.95 (0.79-1.15) add

Analyses unadjusted; best model reported unless SNP not associated in analysis, then additive model reported (add);

* minor/major allele refers to African Americans.

Table 4

Meta-analysis of SNPs in African Americans and European Americans.

Variant Details					T2D-ESRD only			All Nephropathy		
SNP	Chr	Location (hg19)	Alleles (minor/major)*	Gene	Meta P-value	Heterogeneity P-value	Odds Ratio	Meta P-value	Heterogeneity P-value	Odds Ratio
rs4925583	1	248112809	G/A	OR2L8	0.038	0.21	0.90	0.0051	0.28	0.88
rs74153218	1	248129195	T/C	OR2AK2	0.99	0.97	1.00	0.57	0.89	1.09
rs4478844	1	248129240	A/G	OR2AK2	0.10	1.00	0.94	0.15	1.00	0.95
rs74153072	1	248263495	G/T	OR2L13	0.47	0.44	1.04	0.82	0.41	1.01
rs34585936	4	96091414	T/C	UNC5C	0.15	0.49	1.35	0.14	0.52	1.35
rs77170610	6	32150047	T/C	AGER	0.75	0.48	1.06	0.62	0.50	1.08
rs74540092	6	97676810	C/T	MMS22L	0.38	0.89	1.27	0.23	0.96	1.35
rs9481410	6	97677118	G/A	MMS22L	0.23	0.007	0.95	0.24	0.010	0.95
rs9492393	6	130154667	C/T	C6orf191	0.88	0.28	0.99	0.88	0.28	0.99
rs61996289	6	148869636	T/G	SASH1	0.60	0.67	0.92	0.32	0.61	0.87
rs141536395	12	64202766	G/A	TMEM5	0.21	1.00	1.22	0.14	1.00	1.25
rs188114016	12	64587684	G/C	C12orf66	0.53	0.94	0.91	0.54	0.95	0.92
rs17848802	12	109634834	G/A	ACACB	0.74	0.71	0.94	0.81	0.85	0.96
rs113576948	12	109687886	C/G	ACACB	0.29	0.69	1.36	0.33	0.73	1.31
rs34916325	18	72178130	A/G	CNDP2	0.92	0.42	1.01	0.58	0.45	1.05
rs4263028	18	72228124	A/G	CNDP1	0.58	0.65	1.06	0.42	0.67	1.08
rs73973908	18	72238472	A/C	CNDP1	0.29	0.06	0.94	0.61	0.032	0.98
rs62621182	18	72244208	A/G	CNDP1	0.99	0.54	1.00	0.55	0.46	0.96
rs61098917	22	31668720	T/C	LIMK2	0.10	0.18	1.10	0.0069	0.20	1.15
rs3747154	22	31673116	G/A	LIMK2	0.55	0.0043	0.93	0.23	0.012	1.13
rs16989291	22	31971282	C/T	SFI1	0.66	0.32	1.04	0.75	0.38	1.03
var22_32007810	22	32007810	A/G	SFI1	0.64	0.29	0.88	0.29	0.20	0.76
rs116147257	22	36537798	A/G	APOL3	0.0014	0.69	0.69	7.2E-04	0.72	0.70
rs6000152	22	36538053	A/G	APOL3	0.47	0.34	0.93	0.82	0.39	0.98
rs79419411	22	36556729	C/T	APOL3	0.64	0.31	0.96	0.70	0.32	0.97
rs7285167	22	36623920	A/G	APOL2	0.0032	0.087	1.18	8.5E-04	0.091	1.17
rs2239785	22	36661330	A/G	APOL1	0.0025	0.23	0.86	0.0023	0.31	0.87

Variant Details				T2D-ESRD only				All Nephropathy			
SNP	Chr	Location (hg19)	Alleles (minor/major) *	Gene	Meta P-value	Heterogeneity P-value	Odds Ratio	Meta P-value	Heterogeneity P-value	Odds Ratio	
rs136175	22	36661566	G/A	APOLI	0.24	1.00	1.14	0.61	0.16	0.96	
rs16996616	22	36661891	A/G	APOLI	0.87	0.72	0.99	0.87	0.72	0.99	
rs2269529	22	36684354	C/T	MYH9	0.048	0.74	0.87	0.053	0.67	0.88	
rs139134727	22	36685292	A/G	MYH9	0.70	0.80	1.20	0.46	0.75	1.40	

Fixed effects meta-analysis performed using METAL (<http://www.sph.umich.edu/csg/abecasis/metal/>), SE output presented.

* minor/major allele refers to African Americans.

Table 5
Haplotype analysis of loci associated in diabetic, non-diabetic, and all-cause ESRD in African Americans.

Gene	Common Variant	Common Variant P-value	Coding Variant	Coding variant P-value	Omnibus P-value	Risk Haplotype P-value
<i>OR2L13</i>	rs10888287	2.7E-04	rs4925583	6.9E-04	2.69E-04	6.2E-05
			rs4478844	0.030	0.0011	0.0027
			rs74153072	0.79	0.0019	0.20
<i>C6orf167</i>	rs3822908	1.0E-04	rs9481410	0.049	0.013	0.13
<i>LIMK2</i>	rs2106294	2.9E-05	rs61098917	0.0041	1.0E-05	3.8E-03
	rs4820043	3.3E-05	rs3747157	0.15	9.0E-05	0.15
<i>APOL3</i>	rs16996381	0.0083	rs116147257	2.6E-04	2.3E-04	0.0047
<i>APOL2</i>	rs16996381	0.0083	rs7285167	0.015	0.0081	0.0024
<i>APOL1</i>	rs16996381	0.0083	rs2239785	7.5E-05	0.0081	4.6E-05
<i>MYH9</i>	rs735853	0.016	rs2269529	0.36	0.040	0.014