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Genetic Association and Gene-Gene Interaction Analyses in African American Dialysis Patients With Nondiabetic Nephropathy

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Abstract

Background—African Americans (AAs) have increased susceptibility to non-diabetic nephropathy relative to European Americans.

Study Design—Follow-up of a pooled genome-wide association study (GWAS) in AA dialysis patients with nondiabetic nephropathy; novel gene-gene interaction analyses.

Setting & Participants—Wake Forest sample: 962 AA nondiabetic nephropathy cases; 931 non-nephropathy controls. Replication sample: 668 Family Investigation of Nephropathy and Diabetes (FIND) AA nondiabetic nephropathy cases; 804 non-nephropathy controls.

Predictors—Individual genotyping of top 1420 pooled GWAS-associated single nucleotide polymorphisms (SNPs) and 54 SNPs in six nephropathy susceptibility genes.

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Outcomes—*APOL1* genetic association and additional candidate susceptibility loci interacting with, or independently from, *APOL1*.

Results—The strongest GWAS associations included two non-coding *APOL1* SNPs, rs2239785 (odds ratio [OR], 0.33; dominant; $p = 5.9 \times 10^{-24}$) and rs136148 (OR, 0.54; additive; $p = 1.1 \times 10^{-7}$) with replication in FIND ($p = 5.0 \times 10^{-21}$ and 1.9×10^{-05}, respectively). Rs2239785 remained significantly associated after controlling for the *APOL1* G1 and G2 coding variants. Additional top hits included a *CFH* SNP(OR from meta-analysis in above 3367 AA cases and controls, 0.81; additive; $p = 6.8 \times 10^{-4}$). The 1420 SNPs were tested for interaction with *APOL1* G1 and G2 variants. Several interactive SNPs were detected, the most significant was rs16854341 in the podocin gene (*NPHS2*) ($p = 0.0001$).

Limitations—Non-pooled GWAS have not been performed in AA nondiabetic nephropathy.

Conclusions—This follow-up of a pooled GWAS provides additional and independent evidence that *APOL1* variants contribute to nondiabetic nephropathy in AAs and identified additional associated and interactive non-diabetic nephropathy susceptibility genes.

Keywords

African American; *APOL1*; *CFH*; end-stage renal disease; FIND; FSGS; hypertension

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Relative to European Americans (EAs), African Americans (AAs) have increased susceptibility to nondiabetic forms of end-stage renal disease (ESRD), an effect that persists after controlling for hypertension and socioeconomic factors[1]. Although family studies in AAs confirm a strong genetic component to nondiabetic ESRD[2;3], few studies have focused on identifying susceptibility genes in AAs.

Recent studies implicated the chromosome 22q region as significantly associated with nondiabetic chronic kidney disease (CKD) in AAs [4–6]. Genovese et al. recently demonstrated highly significant association of mutations in the apolipoprotein L1 (*APOL1*) gene (the "G1" allele, consisting of reference single-nucleotide polymorphism [SNP] identification number 73885319 [rs73885319] and rs60910145 in perfect linkage disequilibrium [r^2=1.0 and tagged by either SNP] and the "G2" allele, corresponding to rs71785313) in AA cases with biopsy-proven focal segmental glomerulosclerosis (FSGS) and dialysis patients with nondiabetic nephropathy [7;8]. Evidence of association remained significant after controlling for other associated SNPs in this region, including in the non-muscle myosin heavy chain 9 gene (*MYH9*).

Investigators at the Wake Forest School of Medicine performed a genome-wide association study (GWAS) for nondiabetic nephropathy in AAs on dialysis using pooled DNA with follow-up genotyping of 62 SNPs in individual cases and controls [9]. The present study reports the admixture-adjusted results of additional individual genotyping of the top 1420 SNPs identified from this GWAS in AA nondiabetic nephropathy cases and non-nephropathy controls, with replication in a similar cohort of AAs with and without nondiabetic nephropathy. Study objectives were to confirm association identified in the *APOL1* gene region on chromosome 22 and identify additional candidate nondiabetic nephropathy susceptibility loci in AAs that may interact with, or act independently from, *APOL1*.

Methods

Samples

Wake Forest nondiabetic nephropathy cases were recruited from dialysis centers in North Carolina, South Carolina, Georgia, Virginia and Tennessee. Participants were self-described AA dialysis patients lacking diabetes mellitus at initiation of renal replacement therapy. Patients were classified as having nondiabetic nephropathy if they had hypertension or a primary or secondary chronic glomerular disease listed as their cause of nephropathy. Those classified as cases confirmed the onset of high blood pressure prior to dialysis initiation, in the absence of other kidney disease risk factors. Hypertension-attributed nephropathy was diagnosed in the presence of proteinuria ≤ 1.5 gm/day, urinalysis ≤ 100 mg/dl protein, or spot urine protein-creatinine ratio ≤ 1.5 gm/gm (when measures were available); typically with evidence of other hypertensive target organ damage. Chronic glomerulonephritis was diagnosed in those with kidney biopsy evidence or proteinuria ≥ 1.5 gm/day. Patients with polycystic kidney disease, Alport's syndrome, IgA nephropathy, urologic disease or surgical nephrectomy were excluded. Controls were self-described healthy AAs over the age of 18 years recruited from community sources and medical clinics in North Carolina who denied a history of kidney disease. Subjects provided blood for DNA extraction after providing written informed consent. Study procedures were in compliance with the Wake Forest School of Medicine Institutional Review Board and Declaration of Helsinki.

The replication DNA samples for the top 3 pooled GWAS hits came from the Family Investigation of Nephropathy and Diabetes (FIND) AA nondiabetic nephropathy Mapping by Admixture Linkage Disequilibrium (MALD) study (545 cases and 804 controls) and the CHOICE (Choices for Healthy Outcomes In Caring for ESRD) study, which is a national prospective cohort study of 1041 participants on dialysis (including 123 with nondiabetic nephropathy). FIND and CHOICE participant details have previously been published [5;10], Of FIND and CHOICE cases (hereafter referred to simply as FIND), 347 had hypertension-attributed nephropathy, 87 FSGS, 69 HIV-associated nephropathy, 126 other glomerular disorders and 39 other causes. AA FIND controls had estimated glomerular filtration rates > 60 ml/min/1.73 m^2 and spot urinary albumin-creatinine ratios < 30 mg/gm.

Genotyping

To identify genes which contribute to non-diabetic nephropathy, we performed a GWAS using pooled DNA [9]. DNA from 500 AA dialysis patients with nondiabetic nephropathy (cases) and 500 non-nephropathy controls were divided into 10 pools each and genotyped on the Illumina HumanHap 500 genotyping chip. The 65 most highly associated SNPs were genotyped on the same 1000 samples as individual DNAs. Thirty-six nephropathy cases and 22 controls were eliminated from this analysis due to failed genotyping or missing phenotype data. These 65 SNPs were replicated on 336 additional nondiabetic nephropathy cases and 363 non-nephropathy controls. Sixteen SNPs were associated with p-values < 7.7×10^{-4}, 12 of which were located on chromosome 22, in or around the *MYH9*/*APOL1* gene region. Several SNPs in *MYH9* were strongly associated with nondiabetic nephropathy (P values ranging from 0.027 to 2.601×10^{-14})[9]. SNPs from the pooled DNA analysis were selected as autosomal SNPs with association p-values < 0.001. The initially identified SNPs in the *MYH9* gene, which were highly associated with nondiabetic nephropathy and in strong linkage disequilibrium with *APOL1*, were excluded since they were evaluated in a prior report[6], as were those with minor allele frequencies < 0.05 in the HapMap sampling Yoruba in Ibadan (YRI), Nigeria (www.hapmap.org). SNPs associated with nondiabetic nephropathy after analysis of the Wake Forest pooled GWAS and SNPs associated with nephropathy in a subset of AASK (African American Study of Kidney Disease and Hypertension) participants undergoing a preliminary GWAS at the Mount Sinai School of

Medicine ($p < 0.01$) were included (courtesy of Drs. Iyengar, Lipkowitz and Bottinger). An additional 54 tag SNPs were evaluated in six genes known to be associated with FSGS or nephropathy: α actinin-4 (*ACTN4*) [11], nephrin (*NPHS1*)[12], podocin (*NPHS2*)[13], calcium-permeable canonical transient receptor potential 6 (*TRPC6*)[14], uromodulin (*UMOD*)[15], the amino acid transporter *SLC7A9*[16], and neurocalcin delta (*NCALD*)[17]. In total, 1536 SNPs were selected for genotyping (including 44 blind duplicates) on 988 Wake Forest nondiabetic nephropathy cases and 1036 controls (including the 500 Wake Forest cases and 500 controls from the original pooled GWAS) on the Illumina GoldenGate custom genotyping chip by the Center for Inherited Disease Research at Johns Hopkins University.

To adjust for African ancestry in Wake Forest samples, 70 ancestry-informative markers were also genotyped on all nondiabetic nephropathy cases and controls and the percentage of African ancestry was calculated, as described [18]. HapMap 3 (http://hapmap.ncbi.nlm.nih.gov/) genotypes from the YRI, CEU (Utah residents with Northern and Western European ancestry from the CEPH [Centre de'Etude du Polymorphism Humain] collection), and HAN (Han Chinese) were used to anchor these calculations.

The three most associated SNPs (two in *APOL1* and one in the complement factor H gene [*CFH*]) and 1 additional SNP in *MYH9* (rs4821480) were genotyped in a replication sample from the FIND African American MALD study. The top SNPs were chosen because they were located in the *APOL1* gene, as published previously; other top ranking SNPs were also located on chromosome 22 and are in linkage disequilibrium with the more strongly associated SNPs in *APOL1*. The SNP located in the *CFH* gene was the highest-ranking SNP not located on chromosome 22. In addition, *CFH* has been shown to have functional significance in kidney disease. The three candidate SNPs were genotyped using the ABI Taqman platform. Ancestry informative markers (N=1354) were genotyped in FIND using the Illumina GoldenGate custom genotyping chip [5].

Statistical Analysis

Comparison of demographic measures between Wake Forest cases and controls were calculated using a Wilcoxon rank sum test (SigmaStat 3.5, Systat Software, San Jose, CA). Tests for Hardy-Weinberg Equilibrium and genotypic association as well as associated summary statistics were computed using the analysis program SNPGWA [19;20]. Specifically, tests of association were computed adjusting for age, gender and percent African ancestry using a logistic regression model. Tests of association were computed for dominant, additive and recessive genetic models as well as a lack-of-fit to an additive genetic model. The primary inference is the additive genetic model unless the lack-of-fit test was significant ($p<0.05$). In this case, the minimum p-value of the dominant, additive, and recessive genetic models is reported. Local ancestry for the *MYH9* region of chromosome 22 was calculated using 14 SNPs from the *MYH9* gene which were identified as part of a genome-wide admixture scan for non-diabetic nephropathy. Local ancestry was calculated at each marker separately, and these 14 estimates were used to adjust the association analysis as in Kao et al [5]. To account for the number of hypotheses tested (i.e., multiple comparisons), a false discovery rate–adjusted p-value was computed for 904 comparisons. The latter number was obtained from a principal component analysis on the genotype data where 904 principal components explained >99% of the genetic variation in the SNPs [21;22]; thus, due to linkage disequilibrium there are approximately 904 independent dimensions in the SNP data and therefore 904 independent hypotheses.

The three SNPs tested for replication in the FIND were analyzed for association with nondiabetic nephropathy in unrelated AA cases and controls as described above while adjusting for age, gender, global and local ancestries, which were assessed as percent

European ancestry. Global ancestry was estimated with 1354 markers and local with 23 markers using the software ANCESTRYMAP[23].

To account for the recently described highly significant association of the *APOL1* gene (G1 [rs73885319 and rs60910145] and G2 [rs71785313] risk variants) with nondiabetic kidney disease in AAs [7], we completed two sets of analyses under a logistic regression model framework. Because of the linkage disequilibrium pattern where G1 and G2 risk variants are very rarely observed together, we constructed a binary variable representing the compound G1/G2 risk across these three markers, modeling *APOL1* risk as the response for all individuals with recessive haplotypes at either G1 or G2 or heterozygosity at both G1 and G2. To test for an interaction between the *APOL1* risk loci and each SNP we computed a logistic regression model with the G1/G2 compound risk variable and the individual SNP as covariates and tested the interaction as modeled using the standard centered cross-product of G1/G2 and the SNP. In addition, we computed the logistic regression model without the interaction term to test for the association of each SNP after accounting for the effects of G1/G2. These two sets of analyses continue to adjust for age, gender and percentage of African ancestry.

In Wake Forest samples (962 nondiabetic nephropathy cases; 931 non-nephropathy controls), adjusting for the number of comparisons and assuming an additive genetic model with a risk allele frequency of 0.20 and a type 1 error rate of 0.0001, the power to detect ORs of 1.35 and 1.45 was ~0.50 and ~0.80, respectively. Parallel power calculations for validating the three most associated SNPs in the FIND samples (668 nondiabetic nephropathy cases; 804 non-nephropathy controls), but for a type 1 error rate of 0.05, revealed the power to detect ORs of 1.19 and 1.29 was ~0.50 and ~0.80, respectively.

Results

Among Wake Forest participants, nondiabetic nephropathy cases were older, had higher BMI, fewer females, and greater African ancestry, compared to non-nephropathy controls (Table 1). In FIND participants, nondiabetic nephropathy cases were slightly older, had fewer females, and lower BMI compared to controls.

Of the 1536 SNPs that were selected for genotyping, 1420 were successful and used in these analyses. The mean SNP call rate was 0.999 (standard deviation 0.001). Of the 1980 DNA samples sent to the Center for Inherited Disease Research at Johns Hopkins University for genotyping, 1846 were retained for analysis after removal of failed or poorly genotyped samples, unexpected duplicates and unexpected relatedness. There was no evidence that individuals removed from the analysis based on quality control analysis differed from those analyzed for BMI, age at hypertension, age at dialysis, age at enrollment, admixture or gender ($p>0.20$). Phenotype data were missing in the other 87 excluded participants. Genotyping rates by individual ranged from 95.5% to 100%; less than 1% had genotyping rates below 98%.

Association analysis in Wake Forest participants genotyped on the GoldenGate chip resulted in 61 SNPs associated at p-values < 0.01 (Table 2). The two most associated SNPs, rs2239785 and rs136148, were in the *APOL1* gene, ($p = 5.91 \times 10^{-24}$ [dominant] and $p = 1.13 \times 10^{-7}$ [additive]), consistent with recently published results [7]. Two additional SNPs were associated with p-values $< 3.00 \times 10^{-5}$ (remaining significantly associated after Bonferroni correction), rs1573708 in an intergenic region and rs4820237 in the *FOXRED2* gene, both located on chromosome 22. Additional SNPs of interest were rs379489 in the complement factor H gene (*CFH*) ($p = 2.05 \times 10^{-4}$, additive; OR, 0.73) and rs16854341 in the podocin gene (*NPHS2;* $p = 0.004$, dominant; OR, 0.72), as both genes have previously

been associated with kidney disease [24;25]. In addition, the *APOL1* G1 and G2 variants were strongly associated with nephropathy in these Wake Forest cases ($p = 3.27 \times 10^{-32}$, recessive; OR, 5.21; 95% CI, 3.96–6.86).

To address the issue of multiple comparisons, we estimated the number of independent tests performed. A principal component analysis computed on the 1420 SNPs identified 904 principal components, suggesting 904 independent hypotheses tested. False discovery rate–adjusted p-values suggest that besides the chromosome 22 loci, the *CFH* rs379489 locus was significant (false discovery rate–adjusted p-value=0.03).

The top two *APOL1* SNPs from the association analysis, rs2239785 and rs136148, were tested for replication in 1474 FIND participants (668 nondiabetic nephropathy cases, 804 controls), FIND participants were not included in the initial reports of *APOL1* association [7;8]. Both SNPs replicated, with association p-values of 5.03×10^{-21} and 1.92×10^{-5}, respectively (Table 3). Adjustment for local ancestry on chromosome 22 did not significantly alter the significance of either *APOL1* SNP; rs2239785 and rs136148 remained strongly associated after adjustment for local admixture. Importantly, the *MYH9* E1 haplotype SNP rs4821480 ($p = 1.12 \times 10^{-10}$) remained strongly associated with nondiabetic nephropathy after adjusting for local ancestry and these *APOL1* SNPs.

We attempted to replicate the *CFH* SNP rs379489 association with nondiabetic nephropathy in the FIND participants. Although the association between this SNP was similar between the FIND and Wake Forest participants, the association was non-significant in FIND alone ($p = 0.345$; odds ratio, 0.92; additive). When FIND and Wake Forest samples were combined in a meta-analysis, rs379489 remained significantly associated ($p = 6.75 \times 10^{-4}$; odds ratio, 0.81; additive); although association was driven primarily by Wake Forest samples. The heterogeneity p-value in the meta-analysis was 0.0769 (additive).

As *APOL1* G1 and G2 variants are known to be strongly associated with nondiabetic nephropathy in AAs, we tested the 1420 SNPs that were genotyped for interaction with the G1 and G2 nephropathy risk variants (Table 4). The most significantly associated interactive SNP was rs16854341 on chromosome 1 in the podocin (*NPHS2*) gene ($p = 0.0001$). *NPHS2* is independently associated with susceptibility to glomerulosclerosis [13;25–27]. Six other SNPs were associated with p-values < 0.001; however, none were located in known kidney disease candidate genes.

In order to increase the statistical power for association, an exploratory analysis was computed using logistic regression models that tested for association with each of the 1420 SNPs adjusting for the *APOL1* G1/G2 risk loci (Table 5). Most loci provided comparable evidence with and without adjustment for the G1/G2 risk loci. However, five loci showed meaningful improvement in the association: rs1500474 on 2q37 (unadjusted OR, 0.94 [p=0.39]; adjusted OR, 0.61 [p=0.0018]); rs11191727 on 10q24 within the gene neuralized-like protein 1 (*NEURL1*; unadjusted OR, 0.73 [p=0.012]; adjusted OR, 0.59 [p=0.00033]); rs1355652 on 11q14 (unadjusted OR, 1.36 [p=0.0028]; adjusted OR, 1.58 [p=0.00014]); rs9318258 on 13q22 (unadjusted OR, 0.82 [p=0.0070]; adjusted OR, 0.75 [p=0.00070]) and rs10483956 on 14q31 (unadjusted OR, 0.76 [p=0.0056]; adjusted OR, 0.68 [p=0.00074]). The novel *APOL1* SNP, rs2239785, remained modestly associated (p=0.00363). This SNP also remained associated in the FIND population when adjusted for G1/G2 risk status (p=0.0000518; OR, 0.52) (Table 3).

Discussion

The present report confirmed and replicated the top SNP associations from a pooled GWAS for non-diabetic nephropathy in AAs [9] in a large sample of nondiabetic nephropathy cases

and non-nephropathy controls. It also reports the first *APOL1* G1/G2 gene-gene interaction analysis including all of the top GWAS SNPs, plus 54 additional tag SNPs in six glomerulosclerosis-associated genes. Two novel SNPs in *APOL1* were robustly associated with nondiabetic nephropathy in AAs, a result consistent with recently published identification of the G1 and G2 risk loci in these [7] and other populations[8]. We successfully replicated association of these new *APOL1* SNPs in another large sample of AA nondiabetic nephropathy cases and healthy non-CKD controls from FIND. When these SNPs were tested for association after adjustment for rs136148 in *APOL1* and an *MYH9* SNP in the E1 haplotype rs4821480[4;5], rs2239785 remained strongly associated ($p = 9.14 \times 10^{-13}$). Rs2239785 remained nominally associated with nondiabetic nephropathy after adjustment for the known coding G1 and G2 alleles in *APOL1* ($p = 3.63 \times 10^{-3}$ in Wake Forest samples; $p = 5.18 \times 10^{-5}$ in FIND samples). Adjustment for local ancestry on chromosome 22 did not significantly change association at this SNP ($p = 1.12 \times 10^{-10}$). In addition, rs136148 remained nominally associated with nondiabetic nephropathy in the FIND samples after adjustment for *APOL1* G1 and G2 coding variants ($p = 2.06 \times 10^{-2}$). Based on these analyses and the Genovese et al. [7] and Tzur et al. [8] reports, variants in *APOL1* are responsible for a significant portion of the increased susceptibility to nondiabetic nephropathy in AAs. Although the Genovese and Tzur reports identified *APOL1* using SNPs from the 1000 Genomes Project, we were able to independently identify strong association with *APOL1*, relative to *MYH9*, using a pooled GWAS on the Illumina HumanHap550-Duo BeadChip. We note that *APOL1* SNPs rs2239785 and rs136148 are not functional, whereas the G1 and G2 variants encode a non-conservative amino acid substitution and a 6–base pair deletion, respectively. G1 and G2 haplotypes are hypothesized to include causative kidney variants as well as protect from African sleeping sickness [28]. Interestingly, the rs2239785 SNP is not in high linkage disequilibrium with either G1 or G2, (r^2 values of 0.192 and 0.054 in AAs, respectively). These relatively low values, along with the results of the analyses conditional on the effects of G1 and G2, suggest that there may be additional functional variants in the region predisposing to kidney disease beyond the G1 and G2 variants.

There were several SNPs not on chromosome 22 that were modestly associated with nondiabetic nephropathy. The most associated was rs379489 in the *CFH* gene. *CFH* has reproducibly been associated with kidney disease, including IgA nephropathy [29], atypical hemolytic uremic syndrome (HUS) [30;31], glomerular C3 deposition with glomerulonephritis [24], and with rate of change in kidney function [32]. It is possible that some of these AA cases on dialysis had IgA nephropathy, HUS, or glomerular C3 deposition but our participants did not have protocol or indication kidney biopsies to determine disease etiology. Alternatively, *CFH* may independently be associated with glomerulosclerosis.

Finally, modest association of a podocin (*NPHS2*) SNP was detected. *NPHS2* is a podocyte-expressed gene associated with autosomal recessive glomerulosclerosis [13], microalbuminuria [27] and nephrotic syndrome [25]. The same SNP in the *NPHS2* gene was also the most strongly associated in the case-control interaction analysis with *APOL1* risk variants. The mechanism by which podocin interacts with *APOL1* to promote kidney disease is unknown at this time. The interaction between *NPHS2* and *APOL1*, both clearly replicated and powerful effect nephropathy genes, is likely to be clinically relevant and potentially useful for screening in high risk populations. Interactions exist between *Nphs2* and *HIVAN1*/*HIVAN2* in the HIV-1 transgenic mouse model of collapsing FSGS [33, 34].

In conclusion, the *APOL1* gene region is consistently and significantly associated with nondiabetic nephropathy among AAs, compared to controls without kidney disease. More modest association in the *CFH* gene appears relevant to nondiabetic kidney disease susceptibility in AAs, as well. Not only was the *APOL1* association with nondiabetic

nephropathy replicated in an almost equally large sample from FIND, we identified additional candidate genes associated with nondiabetic nephropathy and potentially interacting with *APOL1*, including *NPHS2*. Given the significance of the *APOL1* association and the high incidence rates of nondiabetic nephropathy in the AA population, this study highlights the need for further investigation of these genes. It is possible that survival bias or selection bias can impact results involving prevalent patients on dialysis. Future studies should include more detailed genetic analyses of the candidate genes identified here, as well as functional studies focusing on the role of *APOL1* in AAs with nondiabetic nephropathy and interactions between *NPHS2* and *APOL1*.

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Table 1

Demographic data for the Wake Forest non-diabetic nephropathy samples and the FIND replication samples

	No.	Age (y)	% Female	BMI (kg/m^2)	Age at dialysis (y)	% African Ancestry
Wake Forest						
nondiabetic nephropathy cases	939	54.3 ± 14.5*	43.3*	27.2 ± 7.1*	48.8 ± 15.5	79.6 ± 10.1*
non-nephropathy controls	907	47.2 ± 13.0	50.5	29.5 ± 7.4	NA	76.8 ± 10.9
FIND						
nondiabetic nephropathy cases	668	48.1 ± 12.9*	38.6*	26.9 ± 6.9 *a*	43.9 ± 12.4	83.0 ± 8.0
non-nephropathy controls	804	46.5 ± 11.9	61.1	29.6 ± 7.2*b*	NA	83.0 ± 8.2

Both Wake Forest and FIND samples comprised African American participants.

Note: Except where indicated, values shown are ??? ± ???.

BMI, body mass index; NA, not applicable; FIND, Family Investigation of Nephropathy in Diabetes

* denotes $p < 0.05$ between cases and controls

a n = 655

b n = 803

Table 2

The top 61 associated SNPs from genotyping of Wake Forest non-diabetic nephropathy cases and non-nephropathy controls

Marker	CHR	Nearest Gene	MAF cases	MAF controls	HWE controls	Lowest p-value	Model	OR (95% CI)	FDR best p-value
rs2239785	22q12.3	*APOL1*	0.209	0.376	0.753	5.91E-24	Dom	0.33 (0.27–0.41)	5.35E-21
rs136148	22q12.3	*APOL1*	0.093	0.148	0.660	1.13E-07	Add	0.54 (0.43–0.68)	5.13E-05
rs1573708	22q12.3	2.0 kb from *RBM9*	0.052	0.106	0.437	2.64E-06	Dom	0.49 (0.36–0.66)	7.95E-04
rs4820237	22q12.3	*FOXRED2*	0.169	0.241	0.190	6.94E-06	Add	0.66 (0.55–0.79)	0.002
rs6000197	22q12.3	2.416 kb from *APOL4*	0.370	0.430	0.598	7.98E-05	Dom	0.65 (0.53–0.81)	0.014
rs379489	1q31.3	*CFH*	0.243	0.295	0.790	2.05E-04	Add	0.73 (0.62–0.86)	0.031
rs5995255	22q12.3	*APOL4*	0.371	0.425	0.940	2.69E-04	Dom	0.67 (0.54–0.83)	0.035
rs713969	22q12.3	54.45 kb from *HMGXB4*	0.147	0.208	0.911	3.61E-04	Add	0.71 (0.58–0.86)	0.041
rs12487085	3p12.3	49.502 kb from *ROBO2*	0.492	0.421	0.363	5.48E-04	Add	1.29 (1.12–1.50)	0.055
rs738518	22q12.3	20.898 kb from *CACNG2*	0.417	0.462	0.054	7.92E-04	Rec	0.64 (0.50–0.83)	0.072
rs4907110	1p22.3	12.37 kb from *LPAR3*	0.463	0.516	0.089	0.001	Rec	0.68 (0.53–0.86)	0.093
rs3798696	6p24.3	*TFAP2A*	0.068	0.100	0.148	0.001	Add	0.65 (0.50–0.84)	0.090
rs6656763	1p31.3	*L1TD1*	0.265	0.314	0.732	0.001	Add	0.77 (0.65–0.90)	0.091
rs4412	22q12.1	480.62 kb from *MN1*	0.091	0.135	0.010	0.001	Add	0.69 (0.55–0.87)	0.087
rs5998876	22q12.3	*LARGE*	0.271	0.329	0.132	0.002	Add	0.78 (0.67–0.91)	0.107
rs11015914	10p12.1	50.13 kb from *MKX*	0.151	0.113	0.714	0.002	Dom	1.46 (1.15–1.85)	0.102
rs5756069	22q12.3	24.227 kb from *RBM9*	0.202	0.269	0.573	0.002	Add	0.76 (0.65–0.91)	0.108
rs178139	18q23	35.195 kb from *MBP*	0.171	0.138	0.877	0.002	Dom	1.42 (1.13–1.78)	0.113
rs9505192	6p25.3	99.875 kb from *EXOC2*	0.191	0.242	0.763	0.002	Add	0.76 (0.64–0.91)	0.111
rs551927	20p12.2	*LOC100131208*	0.405	0.460	0.603	0.002	Add	0.80 (0.69–0.92)	0.108
rs1549020	2q24.2	*KCNH7*	0.314	0.280	0.119	0.002	Dom	1.38 (1.12–1.69)	0.104
rs6007220	22q13.31	38.087 kb from *PRRS*	0.207	0.263	0.046	0.002	Add	0.77 (0.65–0.91)	0.102
rs7079133	10q21.1	PCDH15	0.294	0.339	0.680	0.003	Add	0.79 (0.68–0.92)	0.101
rs9626698	22q13.31	23.69 kb from *TBC1D22A*	0.359	0.400	0.000	0.003	Rec	0.66 (0.50–0.86)	0.101
rs1355652	10q21.1	None within 500 kb	0.312	0.278	0.043	0.003	Dom	1.36 (1.11–1.67)	0.102
rs9540221	10q21.1	None within 500 kb	0.222	0.275	0.711	0.003	Add	0.77 (0.65–0.91)	0.100
rs238973	21q21.1	C21orf34	0.275	0.326	0.401	0.003	Dom	0.73 (0.60–0.90)	0.098
rs6118591	20p12.2	*PLCB4*	0.383	0.358	0.054	0.003	Dom	1.37 (1.11–1.69)	0.097
rs727852	10q24.1	*BLNK*	0.181	0.235	0.918	0.003	Add	0.76 (0.64–0.91)	0.102

Marker	CHR	Nearest Gene	MAF cases	MAF controls	HWE controls	Lowest p-value	Model	OR (95% CI)	FDR best p-value
rs2826366	21q21.1	78.329 kb from *NCAM2*	0.368	0.329	0.209	0.003	Dom	1.36 (1.11–1.67)	0.100
rs17093	11p11.2	45.614 kb from *LOC221122*	0.227	0.185	1	0.004	Add	1.30 (1.09–1.56)	0.102
rs5998705	22q12.3	37.33 kb from *SYN3*	0.466	0.408	0.702	0.004	Add	1.24 (1.07–1.44)	0.099
rs930493	2p16.1	*FLJ42562*	0.215	0.267	0.706	0.004	Add	0.78 (0.65–0.92)	0.113
rs12651295	4q13.2	22.24 kb from *UGT2A3*	0.268	0.286	0.005	0.004	Dom	0.74 (0.60–0.91)	0.111
rs329134	3q22.3	2.06 kb from *CLDN18*	0.123	0.166	0.79	0.004	Add	0.74 (0.60–0.91)	0.108
rs16854341	1q25.2	*NPHS2*	0.149	0.172	0.603	0.004	Dom	0.72(0.58–0.90)	0.105
rs1532921	16p13.3	*A2BP1*	0.275	0.233	0.68	0.004	Dom	1.35(1.10–1.65)	0.108
rs852549	12p12.2	2.237 kb from *SLCO1B1*	0.287	0.238	0.308	0.005	Add	1.27(1.08–1.50)	0.111
rs10515944	2q33.2	*CD28*	0.152	0.118	0.724	0.005	Add	1.36(1.10–1.68)	0.110
rs1046889	17q25.3	*FN3KRP*	0.358	0.315	0.231	0.005	Dom	1.34(1.09–1.64)	0.119
rs11071959	15q23	*MAP2K5*	0.391	0.416	0.698	0.005	Dom	0.74(0.60–0.91)	0.119
rs3935962	15	302.8 kb from *RORA*	0.349	0.368	0.002	0.006	Rec	0.66(0.49–0.88)	0.119
rs715541	22q12.3	17.196 kb from *CACNG2*	0.33	0.383	0.389	0.006	Add	0.81(0.70–0.94)	0.117
rs11649200	15q22.2	*MLYCD*	0.373	0.413	0.939	0.006	Add	0.81(0.70–0.94)	0.115
rs401681	5p15.33	*CLPTM1L*	0.379	0.424	0.13	0.006	Dom	0.74(0.60–0.92)	0.113
rs10483956	14q31.1	None within 500 kb	0.144	0.179	1	0.006	Add	0.76(0.62–0.92)	0.111
rs6085252	20p12.3	35.988 kb from C20orf196	0.22	0.257	0.847	0.006	Add	0.79(0.66–0.93)	0.113
rs5003374	10q21.1	*PCDH15*	0.321	0.374	0.049	0.006	Add	0.81(0.70–0.94)	0.118
rs6602318	10p14	435.907 kb from *GATA3*	0.469	0.526	0.657	0.007	Add	0.82(0.71–0.95)	0.123
rs9318258	13q22.1	234.83 kb from *KLF12*	0.421	0.459	0.824	0.007	Add	0.82(0.71–0.95)	0.126
rs10062080	5q32	*AFAP1L1*	0.396	0.38	0.034	0.007	Rec	1.5(1.12–2.00)	0.125
rs9533534	13q14.11	*ENOX1*	0.429	0.375	0.813	0.007	Add	1.22(1.06–1.42)	0.123
rs335555	1q41	51.946 kb from *CENPF*	0.328	0.288	0.279	0.007	Dom	1.32(1.08–1.62)	0.127
rs268529	5p15.31	*SEMA5A*	0.416	0.45	0.03	0.008	Dom	0.74(0.60–0.92)	0.128
rs1517320	2q24.3	*STK39*	0.234	0.203	0.209	0.008	Dom	1.33(1.08–1.64)	0.133
rs243034	2p16.1	75.408 kb from *BCL11A*	0.41	0.451	0.823	0.008	Add	0.82(0.71–0.95)	0.136
rs10491542	9q21.32	130.013 kb from *RASEF*	0.315	0.278	0.168	0.009	Dom	1.31(1.07–1.61)	0.147
rs10121793	9q21.32	129.508 kb from *RASEF*	0.315	0.278	0.168	0.009	Dom	1.31(1.07–1.61)	0.144
rs7710366	5q22.3	*KCNN2*	0.168	0.205	1	0.009	Add	0.78(0.65–0.94)	0.144
rs2029099	2p24.3	375.892 kb from *FAM84A*	0.374	0.394	0.025	0.009	Dom	0.76(0.61–0.93)	0.142

Marker	CHR	Nearest Gene	MAF cases	MAF controls	HWE controls	Lowest p-value	Model	OR (95% CI)	FDR best p-value
rs7021144	9q34.13	*FLJ46082*	0.233	0.257	0.081	0.01	Dom	0.76(0.62–0.94)	0.148

Note: Associated SNPs are those with p <0.01; genotyping is by GoldenGate 1536-plex chip. The lowest p-value is reported with the most significant genetic model.

CHR = chromosomal location, MAF = Minor Allele Frequency, HWE = Hardy Weinberg Equilibrium, Dom = dominant model, Add = additive model, Rec = recessive model, OR = odds ratio, CI = confidence interval, FDR = false discovery rate; kb, kilobase

Table 3

Replication genotyping of selected SNPs in the FIND sample

Adjustment	SNP	CHR	Gene	A1	P-value	OR	Model	P (3SNP)[b]	OR (3SNP)[b]	P-1[a]	OR-1[a]
age, sex, and global admixture	rs136148	22q12.3	*APOL1*	C	1.92E-05	0.50	Dom	0.013	0.62	2.06E-02	0.62
	rs2239785	22q12.3	*APOL1*	A	5.03E-21	0.32	Dom	9.14E-13	0.33	5.18E-05	0.52
	rs4821480	22q12.3	*MYH 9*	C	2.91E-11	2.25	Rec	1.31E-05	1.91		
age, sex, and global and local admixture at *MYH9*	rs136148	22q12.3	*APOL1*	C	6.54E-04	0.57	Dom	0.021	0.64	4.10E-02	0.65
	rs2239785	22q12.3	*APOL1*	A	6.07E-17	0.34	Dom	1.12E-10	0.35	1.75E-04	0.53
	rs4821480	22q12.3	*MYH 9*	C	5.78E-07	1.97	Rec	3.40E-03	1.61		

Abbreviations and definitions: A1, ???; FIND, Family Investigation of Nephropathy in Diabetes; CHR = chromosomal location, Dom = dominant model, Rec = recessive model, OR = odds ratio; rs, reference single-nucleotide polymorphism identification number; SNP, single-nucleotide polymorphism

[a] P-1 and OR-1 are results from association analysis with adjustment for the *APOL1* G1/G2 risk alleles.

[b] OR (3SNP) and P (3SNP) are analyses adjusted for the other two SNPs

Table 4

The top 44 SNPs from the *APOL1* interaction analysis

Marker	CHR	Nearest Gene	Lowest p-value	Model	OR (95% CI)
rs16854341	1q25.2	*NPHS2*	0.0001	Add	0.39(0.24–0.64)
rs7986369	13q14.2	53.666 kb from *SUCLA2*	0.0002	Dom	0.27(0.14–0.55)
rs10191412	22p21	*PRKCE*	0.0005	Add	2.22(1.42–3.48)
rs9533534	13q14.11	*ENOX1*	0.0005	Rec	0.31(0.16–0.60)
rs12670380	7p22.3	9.03 kb from *SNX8*	0.0006	Add	0.5(0.33–0.74)
rs4273707	6q23.2	1.223 kb from *LOC285735*	0.0008	Add	2.09(1.36–3.20)
rs11138082	9q21.31	447.19 kb from *TLE4*	0.0009	Rec	0.12(0.04–0.42)
rs1012018	7q21.11	*CACNA2D1*	0.0012	Add	3.76(1.68–8.38)
rs7689126	4p15.1	None within 500 kb	0.0016	Dom	3.22(1.56–6.65)
rs2239785	22q12.3	*APOL1*	0.0018	Add	0.14(0.04–0.49)
rs1286005	6p25.1	61.376 kb from *RREB1*	0.0022	Dom	2.41(1.37–4.22)
rs10516481	4q21	52.771 kb from *BANK1*	0.0024	Add	0.43(0.25–0.74)
rs12988998	2p24.2	222.741 kb from *RAD51AP2*	0.0024	Rec	0.39(0.22–0.72)
rs7810220	7p12.1	380.915 kb from *POM121L12*	0.0025	Add	2.23(1.33–3.74)
rs7046901	9p21.3	98.072 kb from *MLLT3*	0.0031	Add	1.83(1.23–2.73)
rs11776207	8p12	188.145 kb from *DUSP26*	0.0034	Rec	0.37(0.19–0.72)
rs1668565	15q15.1	3.554 kb from *PLA2G4E*	0.0036	Rec	0.42(0.24–0.75)
rs8014363	14q22.2	8.022 kb from *BMP4*	0.0038	Dom	2.23(1.30–3.84)
rs8063251	16p13.3	437.089 kb from *A2BP1*	0.004	Dom	0.45(0.26–0.78)
rs392702	19q13.12	*NPHS1*	0.004	Dom	0.44(0.26–0.77)
rs6754952	2q37.1	2.196 kb from C2orf52	0.0041	Rec	0.42(0.23–0.76)
rs118501747	14q31.1	*NRXN3*	0.0044	Rec	0.17(0.05–0.58)
rs6494167	15q22.2	137.206 kb from *FOXB1*	0.0044	Rec	3.64(1.50–8.84)
rs4457349	8q24.13	43.674 kb from *TRIB1*	0.0045	Add	0.57(0.39–0.84)
rs437168	19q13.12	*NPHS1*	0.0048	Rec	0.39(0.21–0.75)
rs11193608	10q25.1	None within 500 kb	0.0049	Rec	0.35(0.17–0.73)
rs9510171	13q12.11	None within 500 kb	0.0055	Add	1.95(1.22–3.13)

Marker	CHR	Nearest Gene	Lowest p-value	Model	OR (95% CI)
rs1586171	8p21.2	174.243 kb from *STC1*	0.0058	Dom	2.28(1.27–4.09)
rs11206061	1p32.3	*SCP1*	0.0058	Dom	2.25(1.26–3.99)
rs11081516	18q23	24.12 kb from *SALL3*	0.0063	Add	2.05(1.23–3.43)
rs5998876	22q12.3	*LARGE*	0.0063	Rec	0.26(1.10–0.68)
rs7897598	10q26.13	164.946 kb from *GPR26*	0.0065	Add	1.81(1.18–2.78)
rs1344431	13q13.2	1.743 kb from *NBEA*	0.007	Add	0.59(0.41–0.87)
rs12587505	14q12	*LOC728755*	0.0071	Add	0.59(0.40–0.86)
rs11630183	15q26.3	*PCSK6*	0.0075	Add	0.59(0.41–0.87)
rs1877354	16q22.3	445.53 kb from *PSMD7*	0.0077	Dom	2.67(1.30–5.48)
rs2802723	1q43	*SDCCAG8*	0.0082	Add	2.03(1.20–3.42)
rs10253361	7q31.32	237.929 kb from *PTPRZ1*	0.0085	Dom	0.48(0.28–0.83)
rs4404222	2q24.1	11.134 kb from *ACVR1*	0.0085	Dom	2.07(1.20–3.57)
rs10517893	4q13.1	None within 500 kb	0.009	Dom	0.48(0.28–0.83)
rs1882646	2p25.1	20.273 kb from *KCNF1*	0.0091	Add	1.79(1.16–2.78)
rs436346	2q36.1	51.408 kb from *ACSL3*	0.0098	Add	2.07(1.19–3.59)
rs9804790	12p12.3	115.805 kb from *PLEKHA5*	0.0098	Add	0.6(0.41–0.88)
rs2317446	13q34	26.679 kb from C13orf35	0.0098	Rec	0.21(0.06–0.68)

All SNPs listed in this table are P < 0.01. The most associated genetic model is shown with the corresponding p-value

CHR = chromosomal location, OR = odds ratio, CI = confidence interval; kb, kilobase; Dom = dominant model, Rec = recessive model; Add = additive model; SNP, single-nucleotide polymorphism

Table 5

The top 46 SNPs from genotyping of Wake Forest non-diabetic nephropathy cases and non-nephropathy controls, adjusted for *APOL1* G1/G2 compound risk

Marker	CHR	Nearest Gene	MAF cases	MAF controls	HWE controls	Lowest p	Model	OR (95% CI)
rs1355652	**11q14.1**	**None**	**0.317**	**0.2631**	**0.022**	**1.35E-04**	**Dom**	1.58(1.25–2.00)
rs11191727	**10q24.33**	***NEURL***	**0.101**	**0.1396**	**0.723**	**3.29E-04**	**Dom**	0.59(0.45–0.79)
rs379489	1q31.3	*CFH*	0.244	0.3009	0.762	4.26E-04	Add	0.72(0.60–0.86)
rs9318258	**13q22.1**	**234.83 kb from *KLF12***	**0.419**	**0.4703**	**0.799**	**7.02E-04**	**Add**	0.75(0.63–0.89)
rs10483956	**14q31.1**	**None**	**0.138**	**0.1856**	**1**	**7.41E-04**	**Add**	0.68(0.54–0.85)
rs3798696	6p24.3	*TFAP2A*	0.067	0.0982	0.031	7.89E-04	Add	0.59(0.44–0.81)
rs9540221	13q21.31	None	0.22	0.2829	0.676	1.10E-03	Add	0.72(0.59–0.88)
rs4907110	1p22.3	12.37 kb from *LPAR3*	0.463	0.5288	0.05	1.13E-03	Rec	0.64(0.49–0.84)
rs852549	12p12.2	2.237 kb from *SLCO1B1*	0.291	0.2369	0.557	1.54E-03	Add	1.36(1.13–1.65)
rs335555	1q41	51.946 kb from *CENPF*	0.332	0.2919	0.081	1.63E-03	Dom	1.46(1.15–1.85)
rs1500474	**2q37.1**	***LOC339766***	**0.4**	**0.4368**	**0.167**	**1.75E-03**	**Rec**	0.61(0.45–0.83)
rs321601	17q12	*LOC100131387*	0.505	0.4649	0.006	1.92E-03	Dom	1.52(1.17–1.97)
rs329134	3q22.3	2.06 kb from *CLDN18*	0.119	0.1712	0.453	1.99E-03	Add	0.69(0.55–0.87)
rs7021144	9q34.13	*FLJ46082*	0.23	0.2613	0.227	2.14E-03	Dom	0.69(0.54–0.87)
rs1953232	14q23.3	*MAX*	0.184	0.2374	0.29	2.20E-03	Add	0.73(0.60–0.89)
rs738518	22q12.3	20.898 kb from *CACNG2*	0.419	0.4685	0.011	2.46E-03	Rec	0.64(0.48–0.85)
rs4899920	14q31.3	230.354 kb from *GALC*	0.203	0.1532	0.326	2.52E-03	Dom	1.48(1.15–1.90)
rs2239785	22q12.3	*APOL1*	0.205	0.3807	0.472	3.63E-03	Add	0.74(0.60–0.90)
rs6118591	20p12.2	*PLCB4*	0.379	0.3523	0.009	3.53E-03	Dom	1.43(1.12–1.81)
rs17444393	9q21.12	8.631 kb from *MAMDC2*	0.197	0.2283	0.629	4.02E-03	Add	0.74(0.61–0.91)
rs1916141	2p16.1	None	0.258	0.2189	0.025	4.67E-03	Dom	1.41(1.11–1.80)
rs719676	1p34.2	*LOC100129924*	0.08	0.1072	0.823	4.90E-03	Add	0.66(0.49–0.88)
rs16854341	1q25.2	*NPHS2*	0.148	0.182	0.776	5.01E-03	Add	0.72(0.58–0.91)
rs6756739	2p16.2	None	0.107	0.1459	0.398	5.16E-03	Dom	0.67(0.51–0.89)
rs10061706	5q21.2	25.39 kb from *RAB9BP1*	0.4	0.4603	0.608	5.34E-03	Add	0.79(0.67–0.93)
rs12651295	4q13.2	22.24 kb from *UGT2A3*	0.268	0.2807	0.008	5.42E-03	Dom	0.72(0.56–0.91)

Marker	CHR	Nearest Gene	MAF cases	MAF controls	HWE controls	Lowest p	Model	OR (95% CI)
rs1046889	17q25.3	*FN3KRP*	0.356	0.3207	0.08	5.63E-03	Dom	1.4(1.10–1.77)
rs1549020	2q24.2	*KCNH7*	0.31	0.2739	0.595	5.63E-03	Add	1.3(1.08–1.56)
rs12951391	17q24.3	205.577 kb from *SOX9*	0.221	0.2595	0.659	5.74E-03	Add	0.76(0.62–0.92)
rs4695355	4p12	*TEC*	0.3441	0.3396	0.002	6.43E-03	Rec	0.61(0.43–0.87)
rs6602318	10p14	435.907 kb from *GATA3*	0.466	0.5324	0.865	6.48E-03	Add	0.79(0.67–0.94)
rs930493	2p16.1	*FLJ42562*	0.2128	0.2685	1	6.75E-03	Add	0.76(0.63–0.93)
rs1532921	16p13.3	*A2BP1*	0.2737	0.2297	0.904	7.24E-03	Dom	1.38(1.09–1.75)
rs12530731	7q11.23	78.954 kb from *WBSCR28*	0.4694	0.5253	0.67	7.64E-03	Add	0.80(0.68–0.94
rs1638742	7q36.3	*PTPRN2*	0.1641	0.136	0.475	7.76E-03	Dom	1.43(1.10–1.86)
rs484019	6q14.1	184.043 kb from *BCKDHB*	0.4102	0.3649	0.647	7.88E-03	Add	1.26(1.06–1.50)
rs168664331	2q36.1	43.47 kb from *KCNE4*	0.0739	0.0892	0.11	7.95E-03	Dom	0.65(0.47–0.89)
rs6500291	16q12.1	*LOC100130390*	0.3171	0.2955	0.032	8.08E-03	Dom	1.38(1.09–1.75)
rs2826366	21q21.1	203.498 kb from C21orf131	0.3664	0.3279	0.0206	8.67E-03	Dom	1.37(1.08–1.74)
rs17093	11p11.2	45.614 kb from *LOC221122*	0.2212	0.1829	0.478	8.73E-03	Dom	1.39(1.09–1.78)
rs6547194	2p12	275.59 kb from *SNAR-H*	0.4297	0.4063	0.006	8.77E-03	Rec	1.54(1.11–2.12)
rs1547103	6p22.3	71.756 kb from *RBM24*	0.4103	0.4369	0.101	8.80E-03	Dom	0.71(0.55–0.92)
rs11071959	15q23	*MAP2KS*	0.393	0.4179	0.931	8.82E-03	Dom	0.72(0.56–0.92)
rs11193608	10q25.1	None	0.3939	1.3538	0.853	9.17E-03	Add	1.26(1.06–1.50)
rs7829952	8p22	155.564 kb from *MSR1*	0.3314	0.2812	0.092	9.76E-03	Add	1.26(1.06–1.51)
rs2422245	2p14	9.197 kb from *AFTPH*	0.1555	0.2054	0.152	9.92E-03	Add	0.75(0.60–0.93)

The top 46 SNPs (p <0.01) from the Golden Gate 1536 genotyping of Wake Forest non-diabetic nephropathy cases and non-nephropathy controls, adjusted for *APOL1* G1/G2 compound risk. (best p-value reported with most significant genetic model,

CHR = chromosomal location, MAF = Minor allele frequency. HWE = Hardy Weinberg Equilibrium, Dom = dominant model, Add = additive model, Rec = recessive model, OR = odds ratio, CI = confidence interval; kb, kilobase; SNP, single-nucleotide polymorphism