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Genome-wide association studies suggest that *APOL1*-environment interactions more likely trigger kidney disease in African Americans with non-diabetic nephropathy than strong *APOL1*-second gene interactions

Carl D. Langefeld, PhD[1,2], **Mary E. Comeau, MA**[1,2], **Maggie C. Ng**[3,4], **Meijian Guan, PhD**[4], **Latchezar Dimitrov, MS**[4], **Poorva Mudgal, MS; PhD**[4], **Mitzie H. Spainhour, LPN**[5], **Bruce A. Julian, MD**[6], **Jeffrey C. Edberg, PhD**[7], **Jennifer A. Crocker, PhD**[7], **Jasmin Divers, PhD**[1,2], **Pamela J. Hicks, PhD**[3,4], **Donald W. Bowden, PhD**[3,4], **Gary C. Chan, MBBS**[5], **Lijun Ma, MD, PhD**[5], **Nicholette D. Palmer, PhD**[3,4], **Robert P. Kimberly, MD**[7], and **Barry I. Freedman, MD**[2,4,5]

[1]Division of Public Health Sciences, Department of Biostatistical Sciences; Wake Forest School of Medicine, Winston-Salem, North Carolina, USA

[2]Center for Public Health Genomics; Wake Forest School of Medicine, Winston-Salem, North Carolina, USA

[3]Department of Biochemistry; Wake Forest School of Medicine, Winston-Salem, North Carolina, USA

[4]Center for Diabetes Research; Wake Forest School of Medicine, Winston-Salem, North Carolina, USA

[5]Department of Internal Medicine, Section on Nephrology; Wake Forest School of Medicine, Winston-Salem, North Carolina, USA

[6]Department of Medicine, Division of Nephrology; University of Alabama at Birmingham School of Medicine, Birmingham, Alabama, USA

[7]Department of Medicine, Division of Clinical Immunology and Rheumatology; University of Alabama at Birmingham School of Medicine, Birmingham, Alabama, USA

Abstract

African Americans carrying two apolipoprotein L1 gene (*APOL1*) renal risk variants have a high risk for nephropathy. However, only a minority develops end-stage renal disease (ESRD). Hence, modifying factors likely contribute to initiation of kidney disease such as endogenous (HIV infection) or exogenous (interferon treatment) environmental modifiers. In this report, genome-

Correspondence: Barry I. Freedman, MD, Internal Medicine – Nephrology, Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, North Carolina 27157 USA, bfreedma@wakehealth.edu, Phone: 336-716-6461 ; Fax: 336-716-4318.

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wide association studies and a meta-analysis were performed to identify novel loci for non-diabetic ESRD in African Americans and to detect genetic modifiers in *APOL1*-associated nephropathy. Two African American cohorts were analyzed, 1749 non-diabetic ESRD cases and 1136 controls from Wake Forest and 901 lupus nephritis (LN)ESRD cases and 520 controls with systemic lupus erythematosus but lacking nephropathy from the LNESRD Consortium. Association analyses adjusting for *APOL1* G1/G2 renal-risk variants were completed and stratified by *APOL1* risk genotype status. Individual genome-wide association studies and meta-analysis results of all 2650 ESRD cases and 1656 controls did not detect significant genome-wide associations with ESRD beyond *APOL1*. Similarly, no single nucleotide polymorphism showed significant genome-wide evidence of an interaction with *APOL1* risk variants. Thus, although variants with small individual effects cannot be ruled out and are likely to exist, our results suggest that *APOL1*-environment interactions may be of greater clinical importance in triggering nephropathy in African Americans than *APOL1* interactions with other single nucleotide polymorphisms.

Keywords

African Americans; *APOL1*; chronic kidney disease; FSGS; gene-gene interaction; GWAS

Introduction

Apolipoprotein L1 gene (*APOL1*)-associated nephropathy is the result of an inherited predisposition coupled with exposure to modifying factors that induce a spectrum of progressive chronic kidney diseases (CKD).[1;2;3;] Infection with HIV and exogenous administration of interferon are environmental factors or “second hits” that trigger nephropathy in individuals possessing two *APOL1* renal-risk variants.[4;5] These gene-environment interactions cause HIV-associated nephropathy (HIVAN) and focal segmental glomerulosclerosis (FSGS), collapsing variant, respectively. Lupus nephritis (LN) is also characterized by high interferon levels and severe nephropathy or end-stage renal disease (ESRD) and is strongly associated with *APOL1*.[6;7] Other environmental factors likely modify nephropathy risk; inflammatory factors are logical triggers because they stimulate increased expression of *APOL1* messenger RNA.[8] Odds ratios (ORs) for *APOL1* risk genotype association in LN-ESRD and glomerulosclerosis range from ~3 to 7.3.[1;7] Although these are large effects for a complex disease, they are at the lower end of the *APOL1* disease spectrum (relative to ORs of 17 in FSGS and 29–89 in HIVAN). Based on the relatively lower ORs, we hypothesized that LN-ESRD and hypertension-attributed ESRD would more likely reveal second hits, because effects of the *APOL1* genotype were less strong than in FSGS and HIVAN.

The dominating effect of the *APOL1* G1 and G2 renal-risk alleles may mask or modify the effects of other CKD risk loci.[9] For example, a null variant in the nearby apolipoprotein L3 gene (*APOL3*) on chromosome 22q reproducibly interacts with *APOL1*-mediated risk for CKD in targeted analyses.[10] Roles for non-*APOL* genes in ESRD have been sought in a genome-wide association study (GWAS) using pooled DNA, a combined Wake Forest School of Medicine (WFSM)/National Institute of Diabetes and Digestive and Kidney

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Disease "Family Investigation of Nephropathy and Diabetes" (FIND) report and in the African American Study of Kidney Disease and Hypertension (AASK).[11;12] Initial results suggested that variation in or near the podocin (*NPHS2*), serologically defined colon cancer antigen 8 (*SDCCAG8*), bone morphogenetic protein 4 (*BMP4*), and glutathione s-transferase mu 1 genes (*GSTM1*) might interact with *APOL1* to alter risk of CKD.[12;13] These potential interactions require additional assessment and replication.

The present analyses sought to identify additional ESRD risk loci or loci whose effect is modified by the *APOL1* renal-risk genotype in an expanded set of samples from African Americans including cases with non-diabetic ESRD and controls without nephropathy. This study formally adjusted and stratified by *APOL1* G1/G2 risk status to increase power to detect novel ESRD risk-loci and tested for an interaction between *APOL1* G1/G2 risk status and single nucleotide polymorphisms (SNPs) across the genome. Explicitly incorporating *APOL1* risk genotype status into these analyses might help identify novel pathways. If strong associations do not emerge, results may suggest that the roles of secondary loci and SNP-by-*APOL1* interactions are individually less clinically relevant in initiation of renal injury and that environmental triggers interacting with *APOL1* are of greater clinical importance.

Results

Sample characteristics

The WFSM non-diabetic ESRD cohort and LN-ESRD cohort had comparable African ancestral proportions and *APOL1* genotype frequencies; however, several clinical differences were present (Table 1). Cases in the LN-ESRD cohort exhibited an earlier age at onset of dialysis than did cases in the non-diabetic ESRD cohort (34 years vs. 49 years) and a stronger female bias (88% vs. 41%), both expected clinical findings. Within the WFSM non-diabetic ESRD cohort, cases and controls differed in African ancestral proportions, percent female, and age at ESRD onset in cases and at recruitment in controls (Table 1). Within the LN-ESRD cohort, African ancestral proportions were comparable; controls with systemic lupus erythematosus (SLE) lacking nephropathy had a modest increase in percentage female relative to cases. LN-ESRD cases had a markedly earlier age of SLE onset and fewer American College of Rheumatology (ACR) SLE criteria, relative to the SLE controls without nephropathy. A power analysis for the case-control meta-analysis (2650 cases and 1656 controls) of the association with SNPs with ESRD showed that for a range of minor allele frequencies (MAF=0.1, 0.2, 0.3 and 0.4) and under an additive genetic model, the study was powered to detect odds ratios (ORs) of 1.28–1.46, and 1.25–1.41 for type 1 error rates of $\alpha=5\times10^{-8}$ and $\alpha=1\times10^{-6}$, respectively (Supplementary Figure S1). A parallel analysis for the SNP-by-*APOL1* risk variant interaction indicates the study is powered to detect interaction effects of OR=1.36–1.60, and 1.32–1.53 for type 1 error rates of $\alpha=5\times10^{-8}$ and $\alpha=1\times10^{-6}$, respectively (Supplementary Figure S1).

Meta-analysis of ESRD

Beyond the *APOL1* region, the meta-analysis across the two cohorts for SNP association with ESRD did not show evidence of association that met genome-wide significance

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($p<5\times10^{-8}$ or a significant Benjamini-Hochberg false discovery rate adjusted p-value (P_{FDR}) <0.05) (Supplementary Figure S2). Table 2 provides the five regions with the strongest evidence of association beyond *APOL1* (*i.e.*, smallest p-values and linkage disequilibrium [LD] support in genotyped SNPs). As expected, under a recessive genetic model the *APOL1* G1/G2 risk variants were most strongly associated with ESRD ($p=5.98\times10^{-76}$). The OR was more than twice as large in the non-diabetic cohort (OR=7.00) compared to the SLE cohort (OR=2.72). The non-*APOL1* regions exhibiting the strongest evidence of association and having at least two genotyped SNPs in LD support of the association were on chromosomes 8p23 and 14q24 (Table 2). The association at 8p23 (rs2404298, $p=1.4\times10^{-6}$) was observed in the WFSM non-diabetic ESRD cohort and is near disks large-associated protein 2 gene (*DLGAP2*), which is expressed in the brain and kidney. Although rs2404298 is within an enhancer histone mark, neither it nor a SNP in LD with it is a known expression quantitative trait locus (eQTL) for any gene and the functional link with *DLGAP2* is unclear. A second association within the 8p23 region (rs4292733, $p=2.1\times10^{-6}$) is intronic to an eQTL for Rho guanine nucleotide exchange factor 10 gene (*ARHGEF10*). *ARHGEF10* may play a role in neural morphogenesis but is not known to be expressed in the kidney. The association on 14q24 (rs7142086, $p=2.2\times10^{-6}$) is within promoter and enhancer histone marks in multiple tissues and is in proximity to DDB1- and CUL4-associated factor 4 gene (*DCAF4*), which has variants associated with shorting of telomere length in leukocytes. This association is potentially interesting, given the recent evidence of a link between telomere length and CKD.[14;15;16]

Adjusting for the *APOL1* G1/G2 risk genotype as a binary covariate in the logistic regression model accounted for the association within the *APOL1* region but had little effect on the evidence of association across the remainder of the genome (Figure 1). The strongest evidence of association, after adjusting for the *APOL1* risk genotype, was with rs13084795 at 3q25 near muscleblind-like splicing regulator 1 gene (*MBNL1*, $p=6.08\times10^{-7}$). Haploreg reports that rs13084795 is in LD ($r^2=0.84$) with SNPs within promoter and enhancer histone marks an eQTL for *RP11-788A4.1*, but little is known about this gene. Thus, neither meta-analysis (adjusting and not adjusting for the *APOL1* G1/G2 risk genotype) provided strong (statistically significant) evidence of a locus associated with ESRD in the two cohorts and the regions that provided evidence suggestive of association did not have clear relationships to kidney function.

The ESRD association analysis was repeated stratifying by *APOL1* G1/G2 risk genotype status. Again, little evidence was found for significant SNP-ESRD association within either stratum from the meta-analysis or in the individual cohorts (Supplementary Figures S3 and S4). In individuals without the *APOL1* G1/G2 risk genotype, the rs2404298 association noted above was the strongest association ($p=4.9\times10^{-7}$, non-diabetic ESRD OR=1.76). The most intriguing association in individuals without the *APOL1* risk genotype was with the intronic SNP rs17112571 on 10q23 ($p=3.4\times10^{-6}$, non-diabetic ESRD OR=1.45 and LN-ESRD OR=1.42) within the renalase gene (*RNLS*), coding for a flavoprotein that is secreted by the kidney into the blood and modulates cardiac function and systemic blood pressure (Supplementary Figure S5).[17;18] This SNP is in high LD in African ancestral populations with numerous *RNLS* intronic SNPs that appear conserved and are within promoter and enhancer histone marks in multiple tissues and are eQTL for *RNLS*.[19] Given the potential

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role of *RNLS* in cardiovascular disease and blood pressure control and that circulating renalase is reduced in individuals with CKD,[20] this association requires further study.

The GWAS Catalog is a joint effort of the National Human Genome Research Institute (NHGRI) and the European Bioinformatics Institute (EMBL-EBI) that curates GWAS to generate a dataset of SNP associations with p-values $<1.0 \times 10^{-5}$. Ainsworth *et al.*, queried the GWAS Catalog for CKD and related phenotypes (ESRD, estimated glomerular filtration rate, diabetic kidney disease, IgA nephropathy) and identified 132 SNP associations from 18 studies.[21] Supplementary Table S1 reports the evidence of association with ESRD in the WFSM non-diabetic ESRD and LN-ESRD cohorts, adjusting for age, gender, admixture, and *APOL1* G1/G2 risk genotype. Outside the chromosome 22q12 region that contains *APOL1*, none of these SNPs shows evidence of an association with ESRD. In consideration of the potential impact of the weighting strategy in GWAS, we repeated the analyses weighting by the inverse of the variance. The two GWAS methods yielded comparable results (Supplementary Table S3; Supplementary Figures S12 and S13).

GWAS for APOL1-SNP interaction

Given the magnitude of the effect of the *APOL1* genotype on ESRD risk, it is possible that other variants might predispose to ESRD with effects influenced by the presence or absence of the *APOL1* risk genotype. The genome-wide ESRD case-only meta-analysis for identifying SNP-by-*APOL1* risk genotype interactions identified three regions with suggestive evidence of an interaction (Table 3, Figure 2). However, none met genome-wide significance ($P<5\times10^{-8}$) or had a significant Benjamini-Hochberg false discovery rate-adjusted p-value (P_{FDR}) <0.05. The region with the strongest evidence of an interaction with *APOL1* risk genotypes was on 6q26 (rs79741405, case-only interaction OR=2.97, $p=7.7\times10^{-8}$), an intergenic region 6.9kb 5' of *LPA* (Supplementary Figure 6). The evidence of an interaction in ESRD cases vs. non-ESRD controls for this SNP was less significant (OR=4.44, p=0.0078). The interaction manifests as increased risk in those with *APOL1* G1/G2 risk genotypes and is slightly stronger in the LN-ESRD cohort (Table 3). At ~10kb from rs79741405, rs2115868 (r^2=0.95 with rs79741405) leads a group of SNPs with both promoter and enhancer histone marks in several tissues and the region appears to bind to the protein CTCF, a protein whose published effects are tied to various cancers and DNA loop structure. LD for the most strongly associated SNPs does not appear to extend to the most proximal gene, *LPA*, a gene that codes for a protein that comprises a substantial portion of lipoprotein(a) and has been repeatedly associated with cardiovascular disease.[22] Results were suggestive, but interesting given the mixed evidence for a role of *APOL1* in lipid biology and cardiovascular disease.[23;24;25]

The second strongest evidence of an interaction was on 4p15 (rs7660268, case-only interaction OR=1.63, $p=2.1\times10^{-7}$). As expected, the ESRD case vs. non-ESRD control analysis provided less significant evidence of an interaction for this SNP (OR=1.64, p=0.0148). This is an intergenic region with limited evidence of histone markers or transcription factor binding sites. The region with the third strongest evidence of an interaction was on 12q21 (rs1551122, case-only OR=1.49, $p=8.6\times10^{-7}$; ESRD case vs. non-ESRD control analysis OR=1.61, p=0.0016). This SNP is a missense variant and intronic

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within the otogelin-like gene (*OTOGL*) which is expressed in the inner ear of vertebrates. Variants in this gene are linked to various forms of deafness. Thus, the latter two potential *APOL1*-SNP interaction regions provide modest evidence of an interaction and have little supporting evidence for roles in renal dysfunction or related traits.

Previously, SNPs within genomic regions near *NPHS2* (SNP rs16854341), *SDCCAG8* (rs2802723) and *BMP4* (rs8014363) provided evidence suggestive of an interaction with *APOL1*.[12;13] Given the overlap of these samples in the WFSM cohort, none of these SNP-by-*APOL1* interactions was corroborated by the present results (rs16854341 in *NPHS2* OR=0.90, p=0.0523; rs2802723 in *SDCCAG8* OR=1.17, p=0.4878; rs8014363 near *BMP4* OR=1.29, p=0.0197). The locus zoom plots provide evidence for *APOL1*-by-SNP interactions across these regions (Supplementary Figures S7, S8 and S9), highlighting *a priori* SNP-interactions and the most significant association within the region. None of these SNPs provide evidence of an interaction with *APOL1* risk genotypes. The hemoglobin S variant was genotyped and the *GSTM1* gene region amplified (to measure its product size on an agarose gel to detect the putative null allele associated with kidney disease) in cases and controls from both cohorts. No evidence of a main effect of hemoglobin S or *GSTM1* (with or without adjusting for *APOL1* risk) was seen for association with ESRD, nor was there an interaction between these variants and *APOL1* risk variant status (Supplementary Table S2, Supplementary Figures S10 and S11). In addition, the effect of hemoglobin S alleles were assessed using LAMP (Local Ancestry in adMixed Populations)[26] and results were comparable (data not shown).

Discussion

Several disorders in the *APOL1*-associated nephropathy spectrum have clear mediating factors; these include HIVAN and interferon-associated FSGS, collapsing variant. Others lack obvious modifying factors, such as idiopathic forms of FSGS and focal global glomerulosclerosis (FGGS). Remaining disorders in this spectrum such as severe lupus nephritis and sickle cell nephropathy currently lack known modifying second hits. It is unlikely that anti-nuclear antibodies or sickled red blood cells *per se* are sufficient to initiate CKD because the majority of patients with SLE and sickle cell disease with two *APOL1* renal-risk variants do not develop nephropathy.[7;27;28] Results of these analyses in 4 306 African Americans, including 2 649 cases with ESRD on renal replacement therapy, failed to detect genome-wide significant (or FDR-adjusted p<0.05) evidence of polymorphisms associated with ESRD beyond *APOL1* (adjusting and not adjusting for the *APOL1* risk genotype). Proximal genes in 8p23 and 14q24, two regions with statistical evidence suggestive of association with ESRD, were linked to renal expression, DNA looping or shortened telomere length, respectively. Telomere length has been associated with CKD.[14] This study also failed to identify genome-wide significant SNP-by-*APOL1* risk genotype interactions. The most intriguing SNP-by-*APOL1* risk genotype interaction was on 10q23 linked to *renalase*, a gene whose protein has been implicated in cardiovascular disease and blood pressure regulation and whose levels are reduced in CKD. These results require further evaluation in well-powered studies.

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Modifying factors causing the approximately 20% of genetically susceptible individuals to develop *APOL1*-associated non-diabetic kidney disease remain critical to identify. Advantages to categorizing environmental and genetic factors interacting with *APOL1* to initiate CKD include the potential to develop novel treatments directed at disease modifiers and an improved ability to screen populations who possess recent African ancestry for risk of kidney disease. Individuals with the two-renal-risk *APOL1* genotype have an approximate 4% lifetime risk to develop FSGS with nephrotic syndrome and 50% of HIV-infected individuals will develop HIVAN absent anti-retroviral therapy;[5] approximately 20% are expected to develop sub-nephrotic FSGS or FGGS with low level proteinuria (Martin Pollak personal communication; May 2017). FSGS with low level proteinuria and FGGS are often misclassified as hypertension-attributed nephropathy, despite data that treating mild-to-moderate hypertension does not halt nephropathy progression in African Americans or members of other ethnic groups.[29;30] Although other genetic loci of more modest effect size cannot be excluded and are likely to exist, the present results lead us to hypothesize that environmental modifiers are more likely clinically impactful mediators in *APOL1*-associated nephropathy. This hypothesis is based on existing data that reveal inflammation-driven up-regulation of the *APOL1* gene, effects of interferons, and roles of interacting risk (HIV) and protective (JC polyoma) viruses in *APOL1*-associated nephropathy.[5;8;31] In addition, the sickle variant of the hemoglobin gene did not interact with *APOL1*-mediated risk of nephropathy in two prior reports, suggesting the hemoglobin S allele and *APOL1* variants have independent effects on CKD.[27;28] In the cohorts analyzed herein, *GSTM1* null alleles and hemoglobin S variants were not independent risk factors for ESRD, nor did they interact with *APOL1* to modify nephropathy risk.

This study has limitations. Although it is the largest report of its type to date, the sample size was relatively modest for a GWAS meta-analysis. Here, the genome-wide case-control meta-analysis was powered to detect ORs of 1.25–1.46. Thus, true associations of more modest effect sizes, as seen in many large studies of complex genetic traits, may reside in the loci with evidence suggestive of association. A similar statement holds for the test of SNP-by-*APOL1* risk genotype interactions, where the study is genome-wide powered to detect interactions ORs of 1.32 to 1.60. However, the effects of *APOL1* on non-diabetic ESRD are powerful (*e.g.*, ORs range from 3 to 89 in patients with lupus-ESRD and HIVAN, respectively) and adjusting for these effects should increase the power to detect associations.[4;5;7] Imputation here is a useful tool to improve the statistical power to detect associations and interactions ultimately require genotyping of any key associations, as was done. Given this study found only suggestive evidence, independent cohorts will be required for focused assessment of the potential effects of these loci. The study was powered to detect main effects and non-*APOL1* SNP-by-*APOL1* interactions common to both LN-ESRD and non-diabetic ESRD. If the specific SNP association or interaction is not shared across the disorders, the study is powered only for larger effects and focused recruitment for these diseases would be required. Potential associations with the renalase gene need to be further assessed in other cohorts of African Americans with ESRD. Epigenetic factors, copy number variants, untagged indels, SNPs with small individual effects and rare SNPs remain potential second hits in *APOL1*-associated nephropathy. Modifying factors in the nephropathies associated with LN and sickle cell trait may differ in *APOL1*-associated and non-*APOL1*

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associated forms of these diseases. This is important because *APOL1* appears to be a progression factor from CKD to ESRD; hence, second hits underlying the initiation and renal histology in LN and sickle cell nephropathy may differ from those that are *APOL1*-associated and likely to cause progression to ESRD.

In conclusion, this relatively large GWAS failed to identify genes or genetic variants that met genome-wide (or FDR-adjusted $p<0.05$) significance for association with non-diabetic ESRD in African Americans, beyond the known effect of *APOL1*. Furthermore, no SNP met the genome-wide criteria for interaction with *APOL1* risk genotype status to modify the risk of severe nephropathy. Although this finding does not exclude a role for interactive genes with weaker effects (lower ORs) and additional replication studies are required, the present results suggest that interactive SNPs are likely to individually have minor effects and will require larger sample sizes for detection. We conclude that interactive environmental factors are likely to play more clinically prominent roles in modifying nephropathy risk in individuals who are genetically susceptible to *APOL1*–associated nephropathy. As with use of highly active anti-retroviral therapy and rapidly falling rates of HIVAN, this interpretation provides hope that other modifiable environmental risk factors can be treated, or exposure prevented, to reduce the development of *APOL1*-associated kidney disease in genetically susceptible individuals.

Methods

Samples

DNA samples in this report came from non-diabetic WFSM participants who were self-described African Americans with ESRD (cases) and population-based individuals lacking nephropathy (controls).[13] Causes of CKD in these non-diabetic cases included hypertension-attributed ESRD, non-specific forms of glomerulosclerosis, FSGS, HIVAN, and “unknown cause” in the absence of a kidney biopsy. Individuals with ESRD attributed to diabetes mellitus, Alport’s syndrome, renal cystic disorders, surgical nephrectomy, obstructive nephropathy, or other glomerular diseases (IgA nephropathy, membranous or membranoproliferative glomerulonephritis) were excluded.

As reported, samples collected by the LN-ESRD Consortium from 18 academic referral centers included cases with ESRD due to LN and controls with systemic lupus erythematosus (SLE) who lacked nephropathy.[7] Cases met World Health Organization class III, IV or V disease in the native kidney and were documented by biopsy or physician report. Analyses in the LN-ESRD Consortium were limited to African American participants.

Genotyping

WFSM case and control DNA samples were genotyped on the Illumina Multi-Ethnic Genotyping Array (MEGA; www.illumina.com) at WFSM. LN-ESRD Consortium samples were genotyped on at least one of the following Illumina arrays as part of a large African American systemic lupus erythematosus (SLE) GWAS: Illumina HumanOmni1-Quad BeadChip, Omni1S, and Omni 2.5; a large number of samples were typed on both the Omni1 and Omni1S.

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WFSM and LN-ESRD cases and controls were genotyped on the Sequenom platform for the two SNPs in the *APOL1* G1 nephropathy risk locus (rs73885319 and rs60910145) and the indel for the G2 risk locus (rs71785313) using custom assays designed at WFSM. G1 and G2 calls were visually inspected for quality control. For 318 LN-ESRD Consortium samples without direct *APOL1* genotyping, imputed values were used for G1 and G2 where the imputation confidence and quality was high (see below for imputation methods). Patients were determined to be in the *APOL1* risk group if they possessed two *APOL1* risk alleles (heterozygous for G1 and G2, or homozygous for the G1 or G2 risk alleles).

Statistical Analysis

In both cohorts, samples were excluded if their call rates were <96% across high quality SNPs (described below), had excess autosomal heterozygosity, or had self-reported sex inconsistent with genetically inferred sex. Additionally, duplicates and first- and second-degree relatives were removed retaining a case over a control, or the sample with the highest call rate in pairs with the same affection status. Any sample with missing covariate data or *APOL1* G1/G2 genotyping was also removed.

Admixture estimates were computed using the program ADMIXTURE[32] on a linkage disequilibrium (LD)-pruned (r^2<0.2) set of autosomal, high quality SNPs. Individuals from three HapMap Phase 3 populations (CEU: Utah residents with ancestry from northern and western Europe; CHB: Han Chinese in Beijing, China; YRI: Yoruba in Ibadan, Nigeria) were included as anchoring populations. Resulting admixture proportions were used to trim out any genetic outliers.

SNPs were deemed high quality if they had call rates >95%, no evidence of differential missingness between cases and controls ($p<0.05$), and no evidence of departure from Hardy-Weinberg Expectation proportions (controls $p<0.01$, ESRD cases $p<0.000001$).

To facilitate a meta-analysis across cohorts and because cohorts were genotyped on different arrays, both cohorts were imputed to the 1000 Genomes Phase 1 v3 integrated reference panel using IMPUTE2 software.[33] Because LN-ESRD samples were genotyped across three distinct chips, imputation was performed separately by chip. For both the WFSM and LN-ESRD sets, the imputed genotyped data for SNPs with confidence scores >0.90 and information scores >0.50 were retained. Subsequent association analyses were computed using SNPTEST, which accounts for imputation uncertainty in the statistical analysis, as outlined below.[33]

Validation genotyping for significant imputed variants in the case-only analysis (8 SNPs: rs7660268, rs11942293, rs2327773, rs75167652, rs4348293, rs11114411, rs10862100, rs2053894) was completed for the ESRD cases on the Sequenom platform at WFSM with an efficiency of 95.2% (94.6–95.8%). Sixty-five blind duplicates were included to ensure genotyping accuracy and were >99% concordant.

Statistical association analyses

Logistic regression models were computed to test for SNP associations with ESRD within each cohort using SNPGWA and SNPTEST adjusting for age, gender, and admixture

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proportions; repeating the analyses adjusting only for admixture proportions yielded comparable results. Dominant, additive and recessive genetic models were computed for SNPs conditional on at least 10 or 30 homozygotes for the minor allele for the additive and recessive models, respectively. To combine results across the two cohorts, a weighted inverse normal meta-analysis was computed by genetic model, where the weights were the cohort sample size. Benjamini-Hochberg false discovery rate (FDR) adjusted p-values were computed within the meta-analysis to adjust for the number of tests computed.

To account for the dominating effect of the *APOL1* G1/G2 risk genotype, an *APOL1*-adjusted and an *APOL1* binary risk genotype-stratified analyses were computed. Specifically, in the first analysis *APOL1* G1/G2 risk genotype status was included in the model as a binary covariate with age, gender, and admixture proportions, and a weighted meta-analysis was computed as above. In the stratified analyses, the two cohorts were stratified by the binary *APOL1* G1/G2 risk genotype and the case-control analysis was computed within each stratum for each cohort (Wake Forest; LN-ESRD Consortium) and combined across cohorts via the weighted inverse normal meta-analysis. As in the overall analysis, FDR-adjusted p-values were computed within each of the three analyses (*i.e.*, *APOL1* G1/G2 included as covariate, *APOL1* G1/G2 non-risk stratum, and *APOL1* G1/G2 risk stratum).

Given the powerful effect of *APOL1* genotypes on ESRD risk it is possible that the effects of other loci are dependent on the *APOL1* genotype status of an individual. To test for an interaction between an individual SNP and *APOL1* risk genotype status, a case-only logistic regression analysis was computed.[34] Here, *APOL1* risk genotype status is the binary outcome in the logistic regression and the SNP is the independent variable of interest, adjusting for admixture, age, and gender as covariates. The case-only analysis is statistically more powerful under independence between the SNP and the *APOL1* genotype[34], an assumption valid on non-chromosome 22 loci due to Mendel's Law of Independent Assortment and valid outside the chromosome 22 *APOL1* region when there is no evidence of LD. In addition to the case-only analysis, the case-control analysis was computed to confirm the evidence of an interaction and to estimate the effect size (odds ratio [OR]) of the interaction.

The RegulomeDB database was used to explore what might be the most likely functional polymorphisms in LD with the top associated SNP in a region. RegulomeDB annotates SNPs with known and predicted regulatory elements (eQTLs, DNAase hypersensitivity, and binding sites of transcription factors) in the intergenic regions of the human genome.[35] It includes high-throughput, experimental data sets from GEO, the ENCODE project, published literature, as well as computational predictions and manual annotations to identify putative regulatory potential and identify functional variants. HaploReg v2 is a tool for exploring annotations of the noncoding genome at variants on haplotype blocks and uses LD information from the 1,000 Genomes Project Phase 1 individuals.[36] It analyzes sets of SNPs for an enrichment of cell-type specific enhancers, and includes all dbSNP build 137 SNPs, predicted chromatin state in nine cell types, conservation across mammals, motif instances from ENCODE experiments, enhancer annotations on 90 cell types from the Roadmap Epigenome Mapping Consortium and eQTLs from the GTEx eQTL browser. The query was

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performed using default settings, including LD calculations based on the 1,000 Genomes Phase 1 YRI individuals, and epigenome data from both the ENCODE and Roadmap Epigenome Mapping Consortium projects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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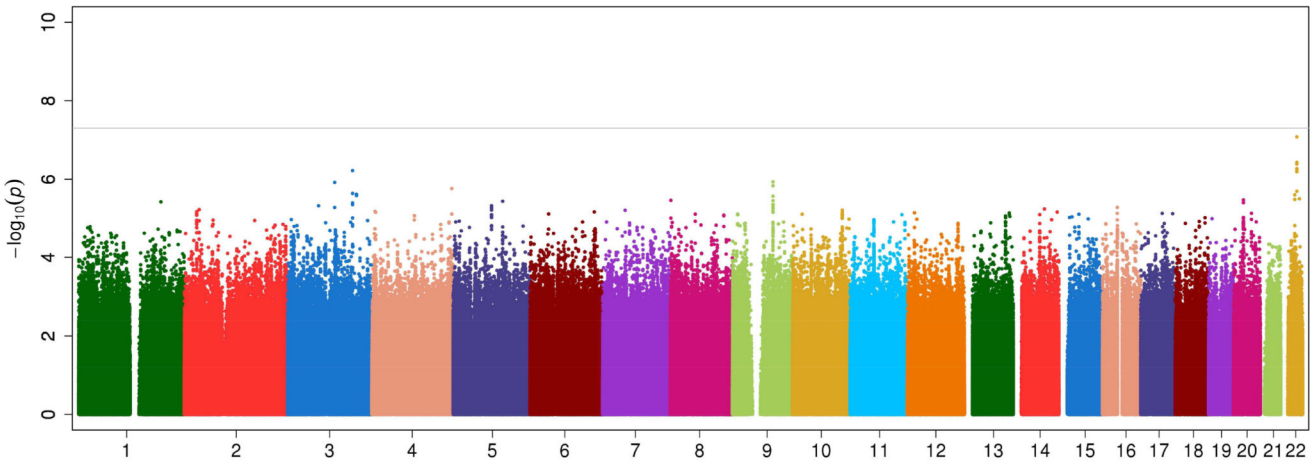

Figure 1.

Genome-wide association meta-analysis for ESRD adjusting for *APOL1* renal-risk genotype status (admixture proportion, age, and gender as covariates)

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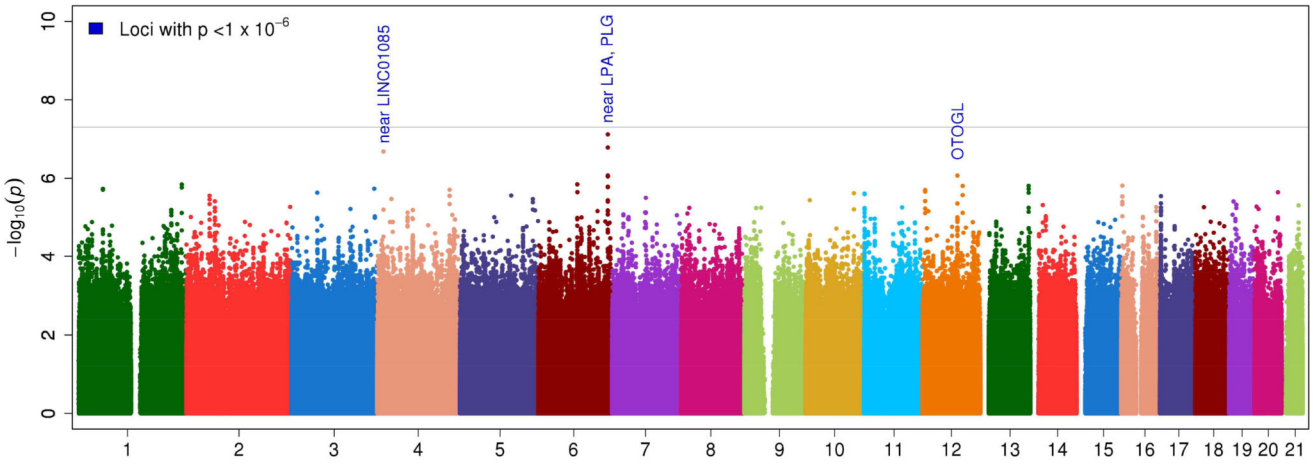

Figure 2.

Genome-wide ESRD case-only *APOL1* × SNP interaction analysis

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Table 1

Demographic and clinical characteristics of African American study cohorts[†]

	WF non-diabetic ESRD cases	WF non-nephropathy controls	P-value	LN-ESRD cases	SLE non-nephropathy controls	P-value
N	1749[*]	1136		901	520	
0/1 copies *APOL1*, n	945	988		678	465	
2 copies *APOL1*, n	803	148		223	55	
Female, n (%)	713 (40.8%)	563 (49.6%)	3.38×10^{-6}	789 (87.6%)	482 (92.7%)	0.0025
Age at recruitment, years	55.5 ± 14.3 (55)	50.6 ± 10.9 (50)	1.87×10^{-22}	41.5 ± 11.7 (42)	41.7 ± 11.8 (42.4)	0.6836
Age at ESRD, years	49.4 ± 15.5 (49)	NA	NA	33.9 ± 11.6 (33)	NA	NA
African ancestry, proportion	0.84 ± 0.08 (0.86)	0.83 ± 0.09 (0.84)	2.48×10^{-7}	0.83 ± 0.09 (0.85)	0.82 ± 0.09 (0.84)	0.1645
Hypertension, n (%)				780 (96.3%)	NA	NA
History of ESRD in first-degree relative		NA	NA	59 (8.4%)	NA	NA
Age at SLE onset, years	NA	NA	NA	27.2 ± 10.8 (25)	39.0 ± 12.3 (40)	5.28×10^{-46}
Time from SLE onset to ESRD, years	NA	NA	NA	7.51 ± 7.38 (5)	NA	NA
No. of ACR SLE criteria met (excluding renal criteria)	NA	NA	NA	4.32 ± 1.77 (4)	4.97 ± 1.13 (5)	1.23×10^{-12}
Cytotoxic therapy, n (%)	NA	NA	NA	567 (85.6%)	NA	NA

[†]Continuous variables reported as mean ± standard deviation (median)

[*]One case did not have *APOL1* typing, so only 1748 cases in case-only or *APOL1* adjusted analyses

Abbreviations: WF Wake Forest; LN lupus nephritis; ESRD end-stage renal disease; SLE systemic lupus erythematosus; ACR American College of Rheumatology

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Table 2

Genome-wide association study meta-analysis of ESRD case-control analysis

SNP	Chr	Position	Gene Region	Minor Allele	MAF WF non-diabetic ESRD cases (N=1749)	MAF WF non-nephropathy controls (N=1136)	MAF LN-ESRD cases (N=901)	MAF SLE non-nephropathy controls (N=520)	Meta-Analysis P-value	OR (95% CI)	OR Non-Diabetic ESRD	OR LN-ESRD
chr1:13891452 [d]	1q31.3	193891453		C	0.27	0.24	-	-	4.62×10^{-4}	1.32 (1.13–1.54)	1.32	
rs479618 [i,d]	1q31.3	193897885		A	0.27	0.24	0.26	0.22	3.42×10^{-6}	1.36 (1.19–1.55)	1.33	1.39
rs2404298 [i]	8p23.3	1369019	near *DLGAP2*	A	0.12	0.08	-	-	1.44×10^{-6}	1.55 (1.29–1.85)	1.55	-
rs4404941	8p23.3	1372399	near *DLGAP2*	C	0.12	0.08	-	-	4.26×10^{-6}	1.57 (1.29–1.90)	1.57	-
rs4292733 [i]	8p23.3	1839973	*ARHGEF10*	G	0.21	0.18	0.21	0.16	2.11×10^{-6}	1.33 (1.18–1.50)	1.27	1.42
rs4242520 [d]	8p23.3	1840697	*ARHGEF10*	G	0.15	0.13	0.15	0.12	1.87×10^{-5}	1.37 (1.18–1.58)	1.37	1.36
rs6420198 [i,d]	8p23.3	1840952	*ARHGEF10*	T	0.14	0.12	0.13	0.10	1.95×10^{-5}	1.37 (1.18–1.60)	1.38	1.36
rs112975886	9q22.31	95119255	*CENPP*	G	0.08	0.06	0.08	0.05	5.84×10^{-5}	1.44 (1.21–1.73)	1.41	1.50
rs73520599 [i]	9q22.31	95265685	*CENPP*	C	0.09	0.06	0.08	0.05	3.00×10^{-6}	1.51 (1.27–1.79)	1.49	1.54
rs7142086 [d]	14q24.2	73391593	*DCAF4*	T	0.36	0.40	-	-	2.20×10^{-6}	0.68 (0.58–0.80)	0.68	-
APOL1 G1/G2	22q12	36661784	*APOL1*		0.46	0.13	0.25	0.11	5.98×10^{-76}	4.74 (3.67–6.12)	7.00	2.72

[i] – imputed SNP in MegaChip cohort (all SNPs are imputed in the LN-ESRD cohort)

[d] or [r] – dominant, or recessive model; if not noted, additive model was used

Abbreviations: MAF minor allele frequency; WF Wake Forest; LN lupus nephritis; SLE systemic lupus erythematosus; OR odds ratio; CI confidence interval

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Table 3

Genome-wide association meta-analysis of ESRD case-only *APOL1* × SNP interaction analysis

					Meta-Analysis (ESRD cases only, *APOL1* risk is the outcome)								Non-diabetic ESRD case-control analysis		LN-ESRD case-SLE control Analysis	
SNP	**Chr**	**Position**	**Gene Region**	**Minor Allele**	**MAF WF non-diabetic ESRD cases *APOL1* risk (N=803)**	**MAF WF non-nephropathy controls *APOL1* non-risk (N=148)**	**MAF LN-ESRD cases *APOL1* risk (N=223)**	**MAF SLE non-nephropathy controls *APOL1* non-risk (N=55)**	**Meta P-value**	**OR (95% CI)**	**OR non-diabetic ESRD**	**OR LN-ESRD**	**OR *APOL1* Risk**	**OR *APOL1* Non-Risk**	**OR *APOL1* Risk**	**OR *APOL1* Non-Risk**
rs7660268 [d]	4p15	14349700	near *LINC01085*	A	0.15	0.10	0.14	0.12	2.10×10^{-7}	1.63 (1.32–2.00)	1.91	1.31	1.49	0.73	1.08	0.83
rs75167652 [d]	6q26	161091168	near *LPA*, *PLG*	G	0.04	0.02	0.05	0.02	1.70×10^{-6}	2.70 (1.81–4.04)	2.13	3.75	3.82	0.66	2.01	0.84
rs79741405 [i, d]	6q26	161094269	near *LPA*, *PLG*	T	0.05	0.02	0.05	0.02	7.70×10^{-8}	2.97 (2.01–4.39)	2.25	4.34	1.96	0.89	2.86	0.95
rs1551122[a]	12q21	80765800	*OTOGL*	G	0.19	0.15	0.21	0.17	8.61×10^{-7}	1.49 (1.27–1.76)	1.46	1.54	1.59	0.73	0.92	1.11

[i] – imputed SNP in MegaChip cohort (all SNPs are imputed in the LN-ESRD cohort)

[d] or [r] – dominant, or recessive model; if not noted, additive model was used

Abbreviations: MAF minor allele frequency; WF Wake Forest; LN lupus nephritis; SLE systemic lupus erythematosus; OR odds ratio; CI confidence interval