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Gene–gene interactions in *APOL1*-associated nephropathy

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ABSTRACT

Background. Two *APOL1* nephropathy variants confer substantial risk for non-diabetic end-stage kidney disease (ESKD) in African Americans (AAs). Since not all genetically high-risk individuals develop ESKD, modifying factors likely contribute. Forty-two potentially interactive single nucleotide polymorphisms (SNPs) from a genome-wide association study in non-diabetic ESKD were tested for interaction with *APOL1* to identify genes modifying risk for non-diabetic nephropathy.
Methods. SNPs were examined in an expanded sample of 1367 AA non-diabetic ESKD cases and 1504 AA non-nephropathy controls, with validation in an independent family-based cohort containing 608 first-degree relatives of index cases with non-diabetic ESKD. Logistic regression and mixed models were fitted to test for interaction effects with *APOL1* on ESKD, estimated kidney function and albuminuria.
Results. Among ESKD samples, 14 of 42 SNPs demonstrated suggestive *APOL1* interaction with P-values <0.05. After Bonferroni correction, significant interactions with *APOL1* were seen with SNPs in podocin (rs16854341; *NPHS2*, $P = 8.0 \times 10^{-4}$), in *SDCCAG8* (rs2802723; $P = 5.0 \times 10^{-4}$) and near *BMP4* (rs8014363; $P = 1.0 \times 10^{-3}$); with trends for *ENOX1* (rs9533534; $P = 2.2 \times 10^{-3}$) and near *TRIB1* (rs4457349; $P = 5.7 \times 10^{-3}$). The minor allele in *NPHS2* markedly changed the *APOL1*-ESKD association odds ratio (OR) from 7.03 to 1.76 (~50% reduction in effect per copy of the minor allele), rs2802723 changed the OR from 5.1 to 10.5, and rs8014363 increased the OR from 4.8 to 9.5. *NPHS2* (P = 0.05) and *SDCCAG8* (P = 0.03) SNPs demonstrated *APOL1* interaction with albuminuria in independent family-based samples.
Conclusions. Variants in *NPHS2*, *SDCCAG8* and near *BMP4* appear to interact with *APOL1* to modulate the risk for non-diabetic ESKD in AAs.

Keywords: African American, *APOL1*, bone morphogenetic protein 4 (*BMP4*), kidney disease, podocin (*NPHS2*), serologically defined colon cancer antigen 8 (*SDCCAG8*)

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INTRODUCTION

Mounting evidence supports the presence of modifiable environmental and inherited factors underlying the risk for apolipoprotein L1 gene (*APOL1*)-associated nephropathy in African Americans (AAs) [1–3]. Despite possession of two *APOL1* nephropathy risk variants with markedly increased risk for non-diabetic end-stage kidney disease (ESKD), all such individuals do not develop nephropathy [4–6]. Renal and urothelial viral infections, including human immunodeficiency (HIV) and JC polyoma virus, may modify inherited risk and serve as treatment targets for prevention of ESKD [1, 7]. In a similar manner, additional genes could interact with *APOL1* to alter the risk for the development of progressive nephropathy [8].

In contrast to many complex diseases, the impressive *APOL1* effect provides improved power for detection of interactive genetic variants; odds ratios (ORs) for nephropathy association of 29, 17 and 7.3 are reported in HIV-associated nephropathy (HIVAN), idiopathic focal segmental glomerulosclerosis (FSGS) and hypertension-attributed nephropathy [focal global glomerulosclerosis (FGGS), with interstitial fibrosis and arteriolar changes], respectively [4, 7]. These and additional *APOL1* variants have recently been implicated in CKD among African cohorts, as well [9, 10].

We previously reported a genome-wide association study (GWAS) aimed at identifying genes associated with non-diabetic ESKD in AAs, as well as an additional analysis testing for genes that might interact with *APOL1* to modify nephropathy risk [8]. The present report tested the top 42 *APOL1*-

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Table 1. Demographic characteristics of unrelated African American non-diabetic ESKD cases and non-nephropathy controls

Group	# APOL1 risk variants	*n*	Age at recruitment, years			African ancestry, %			Age at ESKD, years		
			Mean	SD	Median	Mean	SD	Median	Mean	SD	Median
Controls	0/1	1283	49.4	12.0	48.5	0.77	0.11	0.79	NA	NA	NA
	2	221	49.6	13.7	48.0	0.80	0.09	0.81	NA	NA	NA
	All	1504	49.4	12.3	48.0	0.77	0.11	0.79	NA	NA	NA
ESKD cases	0/1	726	57.5	14.1	57.0	0.80	0.11	0.81	53.9	38.3	52.0
	2	641	51.4	13.8	51.0	0.80	0.09	0.81	45.2	14.8	44.0
	All	1367	54.6	14.3	54.0	0.80	0.10	0.81	49.8	30.0	49.0
Combined	0/1	2009	52.6	13.5	51.0	0.78	0.11	0.80	53.9	38.3	52.0
	2	862	51.0	13.8	50.0	0.80	0.09	0.81	45.2	14.8	44.0
	All	2871	52.1	13.6	51.0	0.79	0.11	0.80	49.8	30.0	49.0

second gene interactive single nucleotide polymorphisms (SNPs) that demonstrated the initial interaction P-values <0.01 in the GWAS, in an expanded sample of unrelated AAs with and without non-diabetic ESKD. To confirm interaction, validation testing was performed in an independent sample of asymptomatic first-degree relatives of AAs with non-diabetic ESKD. These subjects lacked ESKD and had either normal kidney function or mild kidney disease manifested as reduced estimated glomerular filtration rate (eGFR) or albuminuria.

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MATERIALS AND METHODS

Participants

The ESKD case–control sample included subjects from the original GWAS [8], with an additional 503 unrelated AAs with non-diabetic etiologies of nephropathy and 892 non-nephropathy controls. New participants were recruited in the same fashion as original cases and controls. Cases with ESKD were recruited from dialysis facilities in North Carolina, South Carolina, Georgia, Virginia and Tennessee; all were self-described AAs and lacked diabetes mellitus at the initiation of renal replacement therapy. Non-diabetic ESKD was diagnosed in subjects with hypertension or a primary or secondary chronic glomerular disease listed as cause of ESKD on the Centers for Medicare and Medicaid Services 2728 form. Subjects confirmed the onset of hypertension prior to renal replacement therapy and the absence of other kidney disease risk factors. Hypertension-attributed ESKD was diagnosed with proteinuria ≤1.5 g/day, urinalysis ≤100 mg/dL protein or spot urine protein:creatinine ratio ≤1.5 g/g (when data were available), with evidence of other hypertensive target organ damage. Chronic glomerulonephritis was diagnosed in those with kidney biopsy evidence or proteinuria ≥1.5 g/day. Cases with polycystic kidney disease, Alport's syndrome, IgA nephropathy, urologic disease or surgical nephrectomy were excluded. AA controls over age 18 years were recruited from community sources and medical clinics in North Carolina. Controls denied a personal history of kidney disease.

The validation cohort included 608 related and unrelated AAs from 'Natural History of *APOL1*-associated Nephropathy' study families [1, 11]. A maximum of three relatives (siblings or children) were recruited per family for the measurement of albuminuria and kidney function, each subject had a first-degree relative with non-diabetic ESKD. Probands in families had ESKD attributed to hypertension, FSGS, HIVAN or unknown cause in the absence of diabetes. Parents of probands were not recruited, nor were relatives with ESKD. Siblings and offspring could not be younger than 15 years below the age at development of ESKD in the family proband. In this analysis, relatives without kidney disease >5 years younger than the age at ESKD in their family proband were excluded, since future development of nephropathy could not be excluded. All the participants provided blood for DNA extraction after giving written informed consent. Study procedures were performed in compliance with the Wake Forest School of Medicine Institutional Review Board and Declaration of Helsinki.

Genotyping

DNA extraction from whole blood was performed using the PureGene system (Gentra Systems, Minneapolis, MN, USA). Two SNPs in the *APOL1* G1 nephropathy risk variant (rs73885319; rs60910145) and an indel for the G2 risk variant (rs71785313) were genotyped using a custom assay designed and genotyped in the Wake Forest School of Medicine Center for Genomics and Personalized Medicine Research using the Sequenom platform (San Diego, CA, USA). In addition, 106 bi-allelic ancestry informative markers (AIMs) were genotyped to provide African-ancestry proportion estimates. The maximum likelihood approach of Tang *et al.* [12] as coded in the package FRAPPE was used to obtain the proportion of African and European ancestry for each individual. Genotype data at these markers were obtained from 44 HapMap Yoruba individuals (YRI) and 39 European American controls as 'anchors' and provided startup values for the Expectation–Maximization algorithm used in FRAPPE. The top interactive SNPs with interaction P-values <0.01 identified in the discovery ESKD GWAS [8] ($n = 42$) were genotyped in new unrelated ESKD cases and non-nephropathy controls, and in family-based samples on the Sequenom platform (San Diego, CA, USA).

Statistical analysis

Tests for Hardy–Weinberg equilibrium and genotypic association as well as associated summary statistics were computed using the analysis program SNPGWA (https://www.phs.wfubmc.edu/public/bios/gene/downloads.cfm). Tests of interaction in the ESKD data set were computed adjusting for age, gender and African-ancestry proportion using a logistic regression model where the log odds of ESKD served as the

Table 2. Demographic characteristics of African American family-based study samples

Kidney disease[a]	# *APOL1* risk variants	N	Age, years		African ancestry, %		BMI, kg/m^2		Urine ACR, mg/g		MDRD eGFR, mL/min/1.73 m^2	
			Mean (SD)	Median	Mean (SD)	Median	Mean (SD)	Median	Mean (SD)	Median	Mean (SD)	Median
No	0/1	340	49.5 (13.0)	50.0	0.79 (0.11)	0.81	33.0 (9.1)	32.0	6.7 (6.2)	4.1	99.2 (22.0)	98.3
	2	89	48.1 (14.6)	49.0	0.82 (0.10)	0.83	31.5 (8.6)	30.2	6.6 (5.1)	4.8	97.0 (20.6)	96.0
	All	429	49.2 (13.3)	50.0	0.80 (0.11)	0.82	32.7 (9.0)	31.5	6.7 (6.0)	4.3	98.8 (21.8)	98.0
Yes	0/1	136	53.6 (12.7)	55.0	0.81 (0.10)	0.83	35.1 (10.9)	32.8	444.9 (1200.0)	65.7	77.2 (30.3)	76.1
	2	43	47.1 (13.6)	49.0	0.83 (0.08)	0.83	34.3 (10.3)	30.7	242.5 (287.0)	89.1	81.3 (33.8)	81.0
	All	179	52.0 (13.2)	52.0	0.82 (0.10)	0.83	34.9 (10.7)	32.3	396.3 (1058.0)	68.3	78.2 (31.1)	76.8
Total	0/1	476	50.7 (13.0)	52.0	0.80 (0.11)	0.82	33.6 (9.7)	32.1	131.9 (669.9)	6.5	92.9 (26.6)	93.4
	2	132	47.8 (14.2)	49.0	0.82 (0.09)	0.83	32.4 (9.2)	30.5	83.5 (196.8)	7.8	91.9 (26.6)	91.5
	All	608	50.0 (13.3)	51.0	0.80 (0.10)	0.82	33.3 (9.6)	31.9	121.4 (599.9)	6.9	92.7 (26.6)	93.1

[a]Kidney disease defined as eGFR < 60 mL/min/1.73 m^2 and/or urine ACR > 30 mg/g.
BMI, body mass index; ACR, albumin:creatinine ratio; eGFR, estimated glomerular filtration rate.

outcome in case–control analyses. The odds of carrying the *APOL1* risk alleles in the analyses were modeled where we stratified by the ESKD status. The homogeneity of the OR test was computed for the stratified analyses.

We considered two outcomes in the family-based cohort: urine albumin:creatinine ratio (UACR) and the four-variable Modification of Diet in Renal Disease (MDRD) eGFR [13]. The Box-Cox method [14] identified the logarithm transformation for UACR in the family-based cohort. We added 1 to all UACR values before taking the logarithm to avoid generating missing values in case a participant had a value of 0. Linear mixed models were fitted for the continuous outcomes and non-linear mixed models for the mild kidney disease outcome, which took the value of 1 if an individual had UACR >30 mg/g and/or MDRD eGFR <60 mL/min/1.73 m^2 (and 0 otherwise). The mixed model framework allowed us to account for the familial relationship using the expected kinship coefficient matrix. This framework has been shown to provide valid inference, when the observed data are a combination of unrelated and related individuals [15]. We could not compute the observed relationship matrix, since genotype data were available only on the *APOL1* markers and the 106 AIMs.

Based on the known linkage disequilibrium pattern, where G1 and G2 risk variants are very rarely observed on a single chromosome, we constructed a binary variable representing the compound G1/G2 risk across these two 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 of the 42 potentially interactive SNPs in the ESKD case–control sample, a logistic regression model was fitted with age, gender, African-ancestry proportion, the binary variable representing the G1/G2 compound risk and the individual SNP as covariates and the interaction was tested as modeled using the standard centered cross-product of G1/G2 and the SNP. We assume an additive mode of inheritance (MOI) for the SNP. In addition, we computed the logistic regression model without the interaction term to test for association between each SNP and ESKD after accounting for the effects of G1/G2 and all the other covariates in the model. We also conducted analyses stratified by the *APOL1* risk status. Controlling for ancestry and in accordance with Mendel's second law of inheritance, independence is guaranteed whenever the SNP being considered was not located on chromosome 22. In each stratified analysis, based on the *APOL1* genotype, comparison of the effects observed between strata was performed by testing for equality between the parameter estimates obtained in each stratum for each model of inheritance. This test was based on the inverse variance weighted difference between these two parameters, and is expected to be more powerful than the Cochran–Mantel–Haenszel test when the effects are in opposite direction [16]. Statistical significance in the ESKD case–control analysis was determined based on a straight application of the Bonferroni correction rule which places the significance threshold at $\alpha = 0.05/42 = 0.0012$; trends toward association had an overall $P < 0.05$ with consistent directions of effect when evaluated solely in the newly added cases versus controls as were present in the original analysis [8]. Statistical significance in the family-based validation cohort was considered with a $P < 0.05$, due to a priori association in the ESKD analysis.

Table 3. Top 14 SNPs showing an interaction effect with APOL1 in non-diabetic ESKD

Chromosome/nearest gene	Position	SNP	OR	P-value[a]
1/NPHS2	177795549	rs16854341	0.6	0.0008
1/SDCCAG8	241564934	rs2802723	1.8	0.0005
7/~381 kb to POM12IL12	52689925	rs7810220	1.4	0.03
7/~238 kb to PTPRZ1	121062464	rs10253361	0.7	0.0099
8/~174 kb to STC1	23942506	rs1586171	1.6	0.0108
8/~44 kb to TRIB1	126563498	rs4457349	0.7	0.0057
9/~447 kb to TLE4	80929505	rs11138082	0.7	0.0192
10/~175 kb to GPR26	125250912	rs7897598	1.3	0.0816
13/ENOX1	43050726	rs9533534	0.7	0.0022
13/~54 kb to SUCLA2	47361123	rs7986369	0.7	0.0209
14/LOC728755	27053341	rs12587505	0.7	0.0274
14/~8 kb to BMP4	53501324	rs8014363	1.6	0.0010
15/~137 kb to FOXB1	58222638	rs6494167	1.3	0.065
19/NPHS1	41030902	rs392702	0.7	0.0135

[a]Reflects the additive effect of each SNP with *APOL1* risk (recessive).
All models adjusted for age, gender and proportion of African ancestry.

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RESULTS

Table 1 contains age at recruitment, African-ancestry proportion and age at renal replacement therapy in the 1367 unrelated AA cases with non-diabetic ESKD and the 1504 unrelated non-nephropathy controls. Table 2 contains demographic and kidney disease parameters in the 608 first-degree relatives of AA index cases with non-diabetic ESKD. Most of these family members were asymptomatic and had a normal MDRD eGFR with relatively low-level albuminuria.

Table 3 displays *APOL1*-interactive genetic association results for the ESKD samples for the 14 (of 42) SNPs that were identified in the initial report and displayed the same direction of interaction as the initial analysis [8] with association P-values <0.05 in the full ESKD case and non-nephropathy control sample. We present the additive-by-recessive interaction models, where all SNPs are tested assuming an additive MOI and *APOL1* is modeled under a recessive MOI. The other 28 SNPs failed to meet these criteria and were felt likely to be false positives (data not shown); they were not evaluated further. Supplementary Table S1 contains minor allele frequencies and Hardy–Weinberg equilibrium results for all 42 SNPs. Podocin gene (*NPHS2*) SNP rs16854341 significantly interacted with *APOL1* G1/ G2 risk (interaction OR = 0.6; $P = 8.0 \times 10^{-4}$) in the non-diabetic ESKD cases versus controls, as did rs2802723 in the serologically defined colon cancer antigen 8 gene (*SDCCAG8*; interaction OR = 1.8; $P = 5 \times 10^{-4}$), and rs8014363 located near *BMP4* (interaction OR = 1.6; $P = 1 \times 10^{-3}$). All met the Bonferroni-adjusted level of significance. Several other SNPs trended toward interaction with *APOL1* in the ESKD analysis, including rs9533534 located on chromosome 13 in the ecto-NOX disulfide-thiol exchanger 1 gene (*ENOX1*; interaction OR = 0.7; $P = 2.2 \times 10^{-3}$) and rs4457349 near *TRIB1* (interaction OR = 0.7; $P = 5.7 \times 10^{-3}$). A SNP located at ~174 kb from the stanniocalcin-1 gene (*STC1*; rs1586171) was weakly interactive in the combined analysis; another *STC1* variant was associated with GFR in a prior GWAS [17].

Interaction effects were quantified for the top SNPs in or near the interactive genes demonstrating the lowest P-values in Table 3; *NPHS2*, *SDCCAG8* and rs8014363 located near *BMP4* were significantly interactive; *ENOX1* and near *TRIB1* trended toward significance. In addition to inheriting two *APOL1* risk variants, each copy of the rs16854341 (*NPHS2*) minor allele reduced the ESKD OR by ~50%; from 7.03 for individuals who are homozygotes at the major allele to 3.52 for heterozygote individuals and 1.76 for minor allele homozygotes. Inheriting two *APOL1* risk variants with zero, one and two rs2802723 (*SDCCAG8*) minor alleles changed the OR for ESKD from 5.1 to 7.3 to 10.5, respectively. Inheriting two *APOL1* risk variants with zero, one and two rs8014363 (near *BMP4*) minor alleles changed the OR for association from 4.8 to 6.8 to 9.6, respectively and inheriting two *APOL1* risk variants with zero, one and two rs9533534 (*ENOX1*) minor alleles changed the OR for association from 7.45 to 6.32 and to 4.4, respectively.

To confirm these findings, it remained critical to replicate genetic association results in independent samples. To validate ESKD results, potentially *APOL1*-interactive SNPs were tested for effects on milder kidney disease phenotypes in participants from the 'Natural History of *APOL1* Nephropathy' study [11]. Assessed phenotypes included MDRD eGFR <60 mL/min/1.73 m^2 and/or UACR >30 mg/g (defined as the presence of kidney disease); and the natural logarithm of the (UACR + 1). Although milder renal phenotypes display weaker association with *APOL1* relative to ESKD, several SNPs replicated interaction for albuminuria with the same directions of effect as ESKD (Table 4). Importantly, rs16854341 in *NPHS2* demonstrated an *APOL1* interaction with albuminuria (P = 0.05, dominant model), and a trend for interaction with the binary trait of kidney disease (P = 0.08, recessive model). Similar results were seen for rs2802723 in *SDCCAG8* with albuminuria (P = 0.03, dominant model) and a trend for interaction with kidney disease (P = 0.11; dominant model); as well as for rs10253361 near *PTPRZ1* (albuminuria; P = 0.02, recessive model and kidney disease; P = 0.01, recessive model). Although rs7897598 appeared to interact with *APOL1* for kidney disease and albuminuria in family-based samples, the direction of effect opposed that seen in the ESKD analyses and failed to validate the original finding.

Table 5 displays results of the top 14 interactive SNPs from the ESKD analysis, stratifying the data set composed of non-diabetic ESKD cases and non-nephropathy controls into those

Table 4. Interaction effect between the 14 SNPs and APOL1 in the family-based cohort (age, gender and African ancestry adjusted)

Chromosome (nearest gene)	Position	SNP	Outcome	Additive		Dominant		Recessive	
				Parameter	P-value	Parameter	P-value	Parameter	P-value
1 NPHS2	17779554	rs16854341	Kidney disease	−0.4	0.44	−0.4	0.44	−1.9	0.08
	17779554	rs16854341	Log(UACR + 1)	−0.5	0.06	−0.63	0.05	−0.28	0.73
1 SDCCAG8	24156493	rs2802723	Kidney disease	0.3	0.84	0.73	0.11	−0.47	0.38
	24156493	rs2802723	Log(UACR + 1)	0.46	0.07	0.63	0.03	−0.16	0.85
7 ~381 kb to POM121L12	52689925	rs7810220	Kidney disease	−1.5	0.24	−0.46	0.33	0.48	0.17
	52689925	rs7810220	Log(UACR + 1)	0	1.00	0.14	0.65	−0.62	0.34
7 ~238 kb to PTPRZ1	12106246	rs10253361	Kidney disease	−2.18	0.04	−0.44	0.34	1.04	0.01
	12106246	rs10253361	Log(UACR + 1)	−0.02	0.93	0.26	0.39	−1.49	0.02
8 ~174 kb to STC1	23942506	rs1586171	Kidney disease	0.29	0.80	−0.59	0.25	0.23	0.60
	23942506	rs1586171	Log(UACR + 1)	−0.2	0.46	−0.38	0.23	0.56	0.47
8 ~44 kb to TRIB1	12656349	rs4457349	Kidney disease	−0.04	0.94	−0.38	0.40	0.15	0.52
	12656349	rs4457349	Log(UACR + 1)	−0.04	0.85	−0.02	0.94	−0.09	0.80
9 ~447 kb to TLE4	80929505	rs11138082	Kidney disease	−0.25	0.87	−0.57	0.22	−1	0.13
	80929505	rs11138082	Log(UACR + 1)	−0.27	0.28	−0.46	0.13	0.37	0.59
10 ~175 kb to GPR26	12525091	rs7897598	Kidney disease	−1.18	0.33	−0.59	0.19	−0.83	0.03
	12525091	rs7897598	Log(UACR + 1)	−0.38	0.09	−0.58	0.04	−0.14	0.78
13 ENOX1	43050726	rs9533534	Kidney disease	0.29	0.65	0.17	0.71	−0.26	0.32
	43050726	rs9533534	Log(UACR + 1)	0.06	0.76	−0.13	0.65	0.44	0.26
13 ~54 kb to SUCLA2	47361123	rs7986369	Kidney disease	0.19	0.71	0	1.00	0.22	0.28
	47361123	rs7986369	Log(UACR + 1)	0.19	0.36	0.22	0.50	0.3	0.39
14 LOC728755	27053341	rs12587505	Kidney disease	−0.15	0.80	0.56	0.23	0.41	0.07
	27053341	rs12587505	Log(UACR + 1)	0	1.00	0.12	0.69	−0.18	0.64
14 ~8 kb to BMP4	53501324	rs8014363	Kidney disease	0.5	0.46	0.38	0.41	0.43	0.09
	53501324	rs8014363	Log(UACR + 1)	0.15	0.50	0.07	0.81	0.48	0.31
15 ~137 kb to FOXB1	58222638	rs6494167	Kidney disease	−1.27	0.09	0.11	0.82	−0.41	0.11
	58222638	rs6494167	Log(UACR + 1)	−0.12	0.56	0	0.99	−0.38	0.29
19 NPHS1	41030902	rs392702	Kidney disease	−0.3	0.76	0.05	0.90	0.38	0.32
	41030902	rs392702	Log(UACR + 1)	−0.11	0.62	−0.14	0.63	−0.13	0.82

Kidney disease - MDRD eGFR < 60 mL/min/1.73 m^2 and/or urine albumin:creatinine ratio (UACR) > 30 mg/g.

with 2 *APOL1* risk variants (carriers) and 0 or 1 risk variants (non-carriers). *NPHS2* SNP rs16854341 demonstrated significant interactive effects solely in those with 2 *APOL1* risk variants (OR = 0.6, $P = 2.0 \times 10^{-4}$; dominant model), with a statistically significant, different interactive effect in the 0/1 *APOL1* risk variant group (P = 0.02 between strata). Other potentially interactive SNPs often displayed differential effects in *APOL1* 2 risk variant carriers versus 0/1 risk variant non-carriers. For example, *SDCCAG8* SNP rs2802723 displayed an OR of 1.4 for *APOL1* interaction in carriers, with an OR opposite in direction (0.8) in non-carriers (P = 0.002 between strata). Significantly, different effects for the *APOL1*-second gene interaction based on the presence of 2 risk variants (versus <2) were seen for 13 of the 14 interactive genes.

DISCUSSION

This series of *APOL1* by second gene interaction analyses in AAs with non-diabetic nephropathy confirms a relationship between *APOL1* with the *NPHS2*, *SDCCAG8* and near *BMP4* genes in susceptibility to non-diabetic ESKD, as well as with albuminuria for *NPHS2* and *SDCCAG8*.

With detection of the impressive *APOL1* association with a range of non-diabetic kidney diseases in AAs, including HIVAN, FSGS, FGGS, severe lupus nephritis and sickle cell nephropathy came the realization that modifying genetic and environmental factors were likely present [3–5, 18, 19]. Two *APOL1* risk variants are necessary, but not sufficient, for development (or progression) of kidney disease. Environmental modifiers have been identified in the form of viral infections; both HIV and JC polyoma virus maintain renal/uroepithelial reservoirs of infection and appear to interact with *APOL1*-mediated genetic risk [1, 7]. The incidence rate of HIVAN has been steadily declining since widespread use of highly active anti-retroviral therapy (HAART) [20]. Environmentally acquired infections interact with *APOL1* genotypes to increase nephropathy risk; ∼50% of AAs with untreated HIV infection possessing 2 *APOL1* risk variants develop HIVAN. The present analyses include the first replication efforts for genes potentially interacting with *APOL1* in non-diabetic nephropathy.

An *APOL1*-interactive SNP was detected in the podocin gene (*NPHS2*) on chromosome 1q25.2. Podocin is exclusively expressed in the glomerular podocyte [21] and mutations are associated with autosomal recessive FSGS [22], as well as proteinuria, microalbuminuria and clinical nephropathy in diverse populations [23–27]. Podocin is a membrane anchored protein that interacts with other components of the glomerular slit diaphragm, including nephrin and CD2-associated protein [21, 22, 28–30]. Our group previously examined *NPHS2* for its role in AA non-diabetic ESKD and uncommon variants were independently associated [31]. Although the presence of two functional *NPHS2* mutations is a requirement for developing steroid-resistant nephritic syndrome (homozygosity or compound heterozygosity), we speculated that one *NPHS2* risk variant might interact with additional mutations in a regulatory region of the gene or with another gene to produce kidney disease. In this series of replication analyses, it appears that

Table 5. Association between the top 14 SNPs and ESKD stratified by APOL1 risk status

Chromosome (nearest gene)	Position	SNP	APOL1	Additive		
				OR	P-value*	P-value**
1 NPHS2	177795549	rs16854341	0/1	1	0.73	0.02
	177795549	rs16854341	2	0.6	0.0002	
1 SDCCAG8	241564934	rs2802723	0/1	0.8	0.02	0.002
	241564934	rs2802723	2	1.4	0.04	
7 ~381 kb to POM12IL12	52689925	rs7810220	0/1	1.1	0.26	0.02
	52689925	rs7810220	2	1.6	0.002	
7 ~238 kb to PTPRZ1	121062464	rs10253361	0/1	1.0	0.64	0.006
	121062464	rs10253361	2	0.6	0.0006	
8 ~174 kb to STC1	23942506	rs1586171	0/1	0.9	0.54	0.02
	23942506	rs1586171	2	1.5	0.01	
8 ~44 kb to TRIB1	126563498	rs4457349	0/1	1.2	0.02	0.0005
	126563498	rs4457349	2	0.7	0.008	
9 ~447 kb to TLE4	80929505	rs11138082	0/1	1.0	0.63	0.11
	80929505	rs11138082	2	0.8	0.07	
10 ~175 kb to GPR26	125250912	rs7897598	0/1	0.9	0.12	0.01
	125250912	rs7897598	2	1.3	0.0499	
13 ENOX1	43050726	rs9533534	0/1	1.2	0.003	0.002
	43050726	rs9533534	2	0.8	0.16	
13 ~54 kb to SUCLA2	47361123	rs7986369	0/1	1.2	0.005	0.0008
	47361123	rs7986369	2	0.8	0.05	
14 LOC728755	27053341	rs12587505	0/1	1.1	0.49	0.04
	27053341	rs12587505	2	0.8	0.02	
14 ~8 kb to BMP4	53501324	rs8014363	0/1	0.8	0.02	0.0004
	53501324	rs8014363	2	1.4	0.01	
15 ~137 kb to FOXB1	58222638	rs6494167	0/1	0.8	0.02	0.03
	58222638	rs6494167	2	1.1	0.44	
19 NPHS1	41030902	rs392702	0/1	1.2	0.02	0.003
	41030902	rs392702	2	0.8	0.06	

*P-value computed in each stratum.
**P-value of the equality of the effects between strata.

APOL1 and *NPHS2* interact to impact the risk for non-diabetic ESKD, as well as albuminuria. Until the mechanisms whereby *APOL1* variants leading to severe nephropathy and disease progression are clarified, it may prove difficult to determine the mechanisms whereby these genes interact to cause kidney disease.

In addition to *NPHS2*, a known glomerulosclerosis gene, SNPs in novel genes and genomic regions appear to interact with *APOL1* and impact nephropathy risk. The significant association with rs2802723 (*SDCCAG8*) was interesting since mutations in this gene play roles in retinal and nephronophthisis-related ciliopathies, and severe nephropathy in Bardet–Biedl syndrome [32, 33]. A trend toward *APOL1* interaction with rs8014363 near *BMP4* is also of potential interest due to the apparent role of *BMP* pathway genes in susceptibility to nephropathy [34, 35]. Finally, the *NPHS2* and *SDCCAG8* validation in family-based samples with mild kidney disease was striking, since *APOL1* associates more strongly with ESKD than mild nephropathy [11, 36]. Several of these genes (*BMP4*, *NPHS2* and *APOL1*) are expressed by podocytes [37, 38]. The renal diseases in which they are involved often result in FSGS and FSGS, collapsing variant. It is therefore possible that podocyte injury explains their combined involvement, with potential for podocyturia and subsequent loss of glomerular structure.

This report has strengths and some limitations. We evaluated a relatively large number of DNA samples from AAs with non-diabetic ESKD and confirmed statistical significance and directions of interactive association with *APOL1* based on an initial analysis after including additional ESKD cases and non-nephropathy controls. Initial and replication study samples were combined due to the paucity of available DNA samples in AAs with non-diabetic ESKD. Evaluation of independent family-based samples, first-degree relatives of AAs with non-diabetic ESKD, was a unique resource that allowed for extension of findings in asymptomatic individuals enriched for *APOL1* nephropathy risk variants and with milder kidney disease. Confirmation of *APOL1* interaction with *NPHS2* and *SDCCAG8* in both ESKD samples and close relatives of non-diabetic ESKD index cases strongly supports true effect. It is likely that the high ORs for *APOL1* association in ESKD made it possible to identify interactive gene variants; a situation different from that in many complex diseases. Potential limitations include the fact that causative variants that interact with *APOL1* have not been identified. We note that the present analysis results were performed slightly differently than those previously published [8], since 106 AIMs were employed for African-ancestry adjustment (relative to 70 AIMs previously). The number of markers used to estimate this proportion plays an important role in the ability to control for the type I error rate [39].

In conclusion, this series of gene–gene interaction analyses in AAs with non-diabetic forms of nephropathy identified evidence of significant interaction between *NPHS2*, *SDCCAG8* and near *BMP4* with *APOL1*-associated ESKD and with albuminuria for the first 2 genes; *ENOX1* and a variant near *TRIB1* trended toward interaction. Identification of interactive genes

for *APOL1*-associated nephropathy will improve our discriminatory ability when screening high-risk populations of African ancestry for risk of progressive nephropathy, possibly for screening potential deceased and living kidney donors prior to transplantation. These results may improve understanding of the pathogenetic mechanisms underlying the progression of this diverse spectrum of non-diabetic kidney diseases and assist in explaining why not all individuals possessing two *APOL1* risk variants ultimately develop nephropathy.

SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxfordjournals.org.

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CONFLICTS OF INTEREST STATEMENT

The authors report no conflicts of interest. The results presented in this paper have not been published previously in whole or part, except in abstract format.

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ORIGINAL ARTICLE

Dyslipidaemia in children on renal replacement therapy

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ABSTRACT

Background. Information on lipid abnormalities in end-stage renal disease (ESRD) mainly originates from adult patients and small paediatric studies. We describe the prevalence of dyslipidaemia, and potential determinants associated with lipid measures in a large cohort of paediatric ESRD patients.
Methods. In the ESPN/ERA-EDTA registry, lipid measurements were available for 976 patients aged 2–17 years from 19 different countries from the year 2000 onwards. Dyslipidaemia was defined as triglycerides >100 mg/dL (2–9 years) or >130 mg/dL (9–17 years), high-density lipoprotein (HDL) cholesterol <40 mg/dL or non-HDL cholesterol >145 mg/dL. Missing data were supplemented using multiple imputation.
Results. The prevalence of dyslipidaemia was 85.1% in peritoneal dialysis (PD) patients, 76.1% in haemodialysis (HD) patients and 55.5% among renal allograft recipients. Both low and high body mass index (BMI) were associated with a less favourable lipid profile. Younger age was associated with a worse lipid profile among PD patients. HDL levels significantly improved after transplantation, whereas no significant improvements were found for triglyceride and non-HDL levels. In transplant recipients, use of cyclosporin was associated with significantly higher non-HDL and HDL levels than tacrolimus usage ($P < 0.01$). In transplant patients with eGFR < 29 mL/min/1.73 m^2, the mean triglyceride level was 137 mg/dL (99% confidence interval (CI): 119–159) compared with 102 mg/dL among those with eGFR > 90 mL/min/1.73 m^2 ($P < 0.0001$).
Conclusions. Dyslipidaemia is common among paediatric ESRD patients in Europe. Young age and PD treatment are associated with worse lipid profiles. Although lipid levels generally improve after transplantation, dyslipidaemia may persist due to decreased graft function, high BMI or to the use of certain immunosuppressants.

Keywords: children, dialysis, dyslipidaemia, renal replacement therapy, transplantation

INTRODUCTION

Cardiovascular disease is a major cause of morbidity and mortality in children with end-stage renal disease (ESRD) [1]. Paediatric dialysis patients are estimated to have an up to 1000-fold increased cardiovascular mortality risk compared with age-

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