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Original Paper

Apolipoprotein L1 Genetic Variants Are Associated with Chronic Kidney Disease but Not with Cardiovascular Disease in a Population Referred for Cardiac Catheterization

Hanghang Wang[a] Patrick H. Pun[b] Lydia Kwee[a] Damian Craig[a] Carol Haynes[a] Megan Chryst-Ladd[a] Laura P. Svetkey[b] Uptal D. Patel[b] Elizabeth R. Hauser[a] Martin R. Pollak[d] William E. Kraus[a,c] Svati H. Shah[a,c]

[a]Duke Molecular Physiology Institute, [b]Division of Nephrology, Department of Medicine, [c]Division of Cardiology, Department of Medicine, Duke University School of Medicine, Durham, NC, and [d]The Laboratory of Inherited Kidney Disease, Beth Israel Deaconess Medical Center, Boston, MA, USA

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Apolipoprotein L1 · Chronic kidney disease · Cardiovascular disease · Gene polymorphism · Population genetics

Abstract
Background: While the association between *APOL1* genetic variants and chronic kidney disease (CKD) has been established, their association with cardiovascular disease (CVD) is unclear. This study sought to understand CKD and cardiovascular risk conferred by *APOL1* variants in a secondary cardiovascular prevention population. ***Methods:*** Two risk variants in *APOL1* were genotyped in African-Americans (n = 1,641) enrolled in the CATHGEN biorepository, comprised of patients referred for cardiac catheterization at Duke University Hospital, Durham, NC, USA (2001–2010). Individuals were categorized as noncarriers (n = 722), heterozygote (n = 771), or homozygote carriers (n = 231) of *APOL1* risk alleles. Multivariable logistic regression and Cox proportional hazards models adjusted for CVD risk factors were used to assess the association between *APOL1* risk variants and prevalent and incident CKD, prevalent coronary artery disease (CAD), incident CVD events, and mortality. ***Results:*** The previously identified association between *APOL1* variants and prevalent CKD was confirmed (OR: 1.85, 95% CI: 1.33–2.57, p = 0.0002). No statistically significant associations were detected between *APOL1* variants and incident CKD or prevalent CAD, incident CVD events or mortality. Age, type 2 diabetes, and ejection fraction at baseline were significant clinical factors that predicted the risk of incident CKD in a subgroup analysis of *APOL1* homozygous individuals. ***Conclusion:*** *APOL1* genetic variants are not associated with CAD or incident CVD events in a cohort of in-

Svati H. Shah
300 North Duke Street
Durham NC 27701 (USA)
E-Mail svati.shah@duke.edu

dividuals with a high burden of cardiometabolic risk factors. In individuals with homozygous *APOL1* status, factors that predicted subsequent CKD included age, presence of type 2 diabetes, and ejection fraction at baseline.

Background

Racial disparities in the risk of developing cardiac and renal diseases have been attributed to environmental, socioeconomic, and genetic factors [1]. The presence of 2 genetic variants (G1 and G2) in the apolipoprotein L1 *(APOL1)* gene is strongly associated with increased susceptibility to developing chronic kidney disease (CKD) [2–6]. These variants are rare in individuals of European ancestry but common in African-Americans, with 50% of African-Americans harboring at least 1 risk allele and 10–15% carrying 2 risk alleles [2]. The *APOL1* gene encodes apolipoprotein L1, a protein involved in the lysis of the parasite *Trypanosoma brucei* that causes African sleeping sickness. The trypanolytic function may explain the increased allele frequency of G1 and G2 in individuals of African descent as a possible result of positive selection in recent evolutionary history [7].

Apart from the observed association between *APOL1* risk variants and CKD, recent results from the Jackson Heart Study (JHS) and Women's Health Initiative (WHI) also reported an increased risk of cardiovascular disease (CVD) in African-Americans with *APOL1* variants [6]. However, an increased CVD risk was not observed in other cohort studies of *APOL1* carriers [4, 5, 8], and no studies so far have examined this association in a secondary cardiovascular prevention population. Thus, to clarify and expand the understanding of *APOL1* variants, we assessed the association of *APOL1* variants with CKD, coronary artery disease (CAD), and incident CVD events in a secondary cardiovascular prevention population with a high incident CVD event rate, the CATHeterization GENetics (CATHGEN) study [9].

Methods

Study Cohort

The cardiac CATHGEN study is approved by the Duke University Institutional Review Board and consists of samples collected from 9,249 sequential consenting adult patients undergoing cardiac catheterization at Duke University Hospital, Durham, NC, USA, between 2001 and 2010 [9]. Consistent with previous studies [2, 5, 10], we restricted our analysis to 1,641 African-Americans in CATHGEN.

Baseline and subsequent estimated glomerular filtration rates (eGFR) were calculated using the CKD-EPI equation [11], with creatinine measurements obtained on the day of catheterization and during the follow-up visits. Prevalent CKD was defined as baseline eGFR <60 mL/min/1.73 m^2; incident CKD was defined as no CKD at enrollment with at least 2 subsequent follow-up eGFR measurements <60 mL/min/1.73 m^2 and obtained at least 3 months apart. Prevalent CAD was defined as the presence of at least 1 diseased vessel (>50% occlusion) by angiography performed within 1 year of enrollment. Additional characterization of prevalent CAD included the CAD index [12, 13], an ordinal measure of location, and severity of CAD. Incident CVD events were defined as the composite endpoint of incident MI or need for therapeutic surgical (coronary artery bypass grafting or CABG) or endovascular interventions (percutaneous coronary intervention or PCI) during follow-up.

Genotyping

Consistent with previous studies [4], the 2 SNPs in the *APOL1* G1 and the insertion/deletion polymorphism for the G2 risk allele were genotyped using Taqman genotyping assay (Life Technologies). Individuals' genotypes were categorized as noncarriers (0 or Ref/Ref), heterozygote carriers (1, G1/Ref, or G2/Ref), or homozygote carriers (2, G1/G1, G1/G2, or G2/G2). Details are described in the online supplementary material (www.karger.com/doi/10.1159/000453458).

Table 1. Baseline characteristics of African-Americans in the CATHGEN study by *APOL1* genotype, including renal disease and CVD

Clinical characteristics	Noncarriers (*n* = 684)	Heterozygote carriers (*n* = 726)	Homozygote carriers (*n* = 231)	*p* value*
Mean age ± SD, years	57.8±11.6	58.3±11.4	55.7±11.6	0.01
Male gender	364 (53.2)	357 (49.2)	130 (56.3)	0.11
Mean BMI ± SD	31.9±8.1	32.3±8.5	32.4±8.7	0.65
Type 2 diabetes	266 (38.9)	295 (40.6)	93 (40.3)	0.79
Hypertension	516 (75.4)	570 (78.5)	188 (81.4)	0.13
Smoking	303 (44.3)	293 (40.4)	104 (45.0)	0.24
Mean eGFR ± SD	77.7±28.9	76.9±28.6	67.9±35.9	<0.001
Prevalent CKD	158 (24.5)	164 (23.9)	75 (33.5)	0.01
On dialysis at baseline	22 (3.2)	27 (3.7)	28 (12.1)	<0.001
Incident CKD	134 (39.1)	139 (37.8)	44 (43.1)	0.61
Prevalent CAD	336 (50.2)	327 (45.8)	108 (47.4)	0.25
Median CAD index[a] (q1, q3)	23 (0, 45)	23 (0, 37)	23 (0, 37)	0.08
Number of diseased vessels				0.09
0	333 (49.8)	387 (54.2)	120 (52.6)	
1	119 (17.8)	141 (19.7)	52 (22.8)	
2	84 (12.6)	80 (11.2)	24 (10.5)	
3	133 (19.9)	106 (14.8)	32 (14.0)	
Median LVEF (q1, q3)	55 (45, 63)	55 (48, 64)	55 (44, 62)	0.16

Values are *n* (%) or means ± SD, unless otherwise specified. * *p* value by ANOVA or Kruskal-Wallis test.

[a] CAD index: an ordinal measure of location and severity of CAD (0: no vessels with >50% occlusion; 23: >1 vessel with 50–74% occlusion; 37: 2 diseased vessels).

Statistical Analyses

Genetic models were defined as additive (0, 1, or 2 risk allele carriers), dominant (0 vs. 1 or 2 allele-carriers), or recessive (0 and 1 vs. 2 allele carriers). Clinical variables were compared among genotype groups using ANOVA or the Kruskal-Wallis test and the χ^2 or the Fisher exact tests as appropriate. The clinical outcomes analyzed include: (1) prevalent CKD; (2) incident CKD; (3) prevalent CAD; (4) incident CVD events, and (5) overall survival or major adverse cardiovascular event-free (MACE-free) survival. Both basic (adjusted for age and sex) and full multivariable models [adjusted for age, sex, BMI, type 2 diabetes status, hypertension, smoking status, left ventricular ejection fraction (LVEF), and number of diseased vessels] were used. Associations between *APOL1* variants and (1) prevalent CKD and (2) incident CKD were assessed under a recessive genetic model as established in previous studies [2, 5, 10], while the association between *APOL1* variants and CAD was assessed using recessive, dominant, and additive models. The Cox proportional hazards model was used to assess the associations between *APOL1* variants and incident CVD events or mortality under an additive model. Overall and MACE-free survival was estimated using Kaplan-Meier methods. A subgroup analysis restricted to homozygote carriers was also conducted to examine potential clinical factors modifying the risk of incident CKD. All statistical analyses were conducted using the R software package version 3.2 (Vienna, Austria).

Results

The distribution of noncarriers, heterozygote carriers, and homozygote carriers of *APOL1* risk alleles was 41.7% (*n* = 722), 44.5% (*n* = 771), and 13.8% (*n* = 231), respectively.

Association between APOL1 Genotypes and Prevalent and Incident CKD

Table 1 demonstrates baseline clinical characteristics stratified by *APOL1* genotype. Previously reported associations [2, 5, 10] between the *APOL1* risk variants and prevalent

Table 2. Clinical factors associated with incident CKD in African-Americans homozygous for *APOL1* risk variants with normal eGFR at enrollment

Variable	Estimate	OR	95% CI	*p* value
Age (5-year increment)	0.29	1.34	1.06–1.71	0.02
Sex	0.32	1.38	0.51–3.71	0.52
BMI (5-point increment)	0.27	1.31	0.99–1.76	0.06
Type 2 diabetes	1.00	2.71	1.09–7.03	0.03
Hypertension	–0.44	0.64	0.17–2.26	0.49
Smoking status	–0.52	0.60	0.23–1.47	0.27
LVEF (5% increment)	–0.15	0.86	0.74–0.98	0.04
Number of diseased vessels	0.11	1.12	0.72–1.73	0.60
Baseline eGFR	–0.02	0.98	0.95–1.01	0.18

CKD were observed: the mean eGFR was significantly lower in homozygotes compared to heterozygotes and noncarriers (Table 1). *APOL1* genotype was associated with prevalent CKD in the basic [odds ratio (OR): 1.85, 95% CI: 1.34–2.53, $p = 0.0002$] and full multivariable model (OR: 1.85, 95% CI: 1.33–2.57, $p = 0.0002$).

Information on incident CKD during follow-up was available for 813 (49.5%) individuals of the study cohort. There was no significant association between *APOL1* risk variants and incident CKD using either the basic or full multivariable logistic regression model (basic OR: 1.36, 95% CI: 0.87–2.10, $p = 0.17$; full OR: 1.28, 95% CI: 0.80–2.03, $p = 0.30$).

Subgroup Analysis

Given the finding that 149 (66.5%) homozygote carriers of *APOL1* risk alleles had normal baseline eGFR, we explored analyses in this subgroup. Follow-up renal function was available in 102 (68.5%) individuals of this subgroup, and 44 (43.1%) subjects with normal baseline eGFR developed incident CKD during follow-up. Older age, presence of type 2 diabetes at baseline, and lower baseline LVEF were significant predictors of developing incident CKD (Table 2), with the presence of baseline type 2 diabetes being the strongest predictor, with an OR of 2.71 (95% CI: 1.09–7.03, $p = 0.03$). Further analysis to assess an interaction between *APOL1* risk variants and type 2 diabetes at baseline in both heterozygote and homozygote carriers did not reveal a statistically significant interaction ($p = 0.62$).

Association between APOL1 Genotypes, CAD, Time to CVD Events, and All-Cause Mortality

No associations were detected between *APOL1* variants and CAD (Table 3) or *APOL1* variants and time to incident CVD events (heterozygote HR: 0.86, 95% CI: 0.63–1.19, $p = 0.38$; homozygote HR: 0.88, 95% CI: 0.57–1.36, $p = 0.57$). Kaplan-Meier analysis revealed no significant difference in overall survival and MACE-free survival among noncarriers, heterozygote carriers, and homozygote carriers ($p = 0.98$ and $p = 0.48$, respectively). Similarly, no significant association was detected between *APOL1* risk variants and time to all-cause mortality (heterozygote HR: 1.1, 95% CI: 0.87–1.30, $p = 0.53$; homozygote HR: 0.89, 95% CI: 0.67–1.19, $p = 0.44$).

Discussion

While the role of *APOL1* risk variants in CKD has been established in previous studies [2, 4–6, 10, 14], our results expand the findings into a secondary cardiovascular prevention population for the first time using a large cohort of African-American individuals from a popu-

Table 3. Association tests for *APOL1* genotypes with prevalent CAD using additive, recessive, and dominant models

Variable	Genetic model	Estimate	OR	95% CI	*p* value
APOL1 heterozygote	Additive, basic[a]	–0.18	0.84	0.67–1.04	0.12
	Additive, multivariable[b]	–0.22	0.80	0.63–1.01	0.06
APOL1 homozygote	Additive, basic[a]	–0.05	0.95	0.70–1.31	0.77
	Additive, multivariable[b]	–0.98	0.91	0.65–1.26	0.56
	Recessive, basic[a]	0.04	1.05	0.78–1.40	0.77
	Recessive, multivariable[b]	0.016	1.02	0.75–1.38	0.92
APOL1 homozygote or heterozygote carriers	Dominant, basic[a]	–0.15	0.86	0.70–1.06	0.17
	Dominant, multivariable[b]	–0.19	0.82	0.66–1.03	0.08

[a] Adjusted for age and sex. [b] Adjusted for age, sex, BMI, type 2 diabetes status, hypertension status, smoking status, and baseline eGFR.

lation with high cardiometabolic risk. Besides renal disease, we assessed the association of *APOL1* risk variants with CVD and did not demonstrate a significant relationship. Interestingly, we found that 66.5% of African-Americans presumed at risk of CKD based on their *APOL1* homozygous background had normal eGFR at the time of cardiac catheterization, prompting analyses demonstrating that age, type 2 diabetes, and baseline LVEF are factors influencing the risk of subsequent CKD in this at-risk population.

Our results suggest that incident CKD is a heterogeneous and multifactorial disease, even in individuals at high risk due to an *APOL1* homozygous background. Previous studies have alluded to the presence of other genetic and environmental factors that could contribute to the risk for CKD by interacting with *APOL1* [7, 15–17]. In a recent analysis of the African-American Study of Kidney Disease and Hypertension (AASK) [16], many sociodemographic (age >55 years, sex, education, income, etc.) and common clinical risk factors (systolic blood pressure, BMI, smoking, etc.) were examined, but none were shown to alter *APOL1*-related CKD progression.

In our study, older age, presence of type 2 diabetes, and lower LVEF at baseline are 3 factors that predicted incident CKD development in an *APOL1* homozygous background. Although the association between older age and renal function decline has been reported previously [18, 19], few studies have reported the effect of age on incident CKD in an *APOL1* background. In the study by Garg et al. [18], the authors found that each 5-year increase in age was associated with an increase of 0.01–0.1 mg/dL in serum creatinine. In another study that examined mortality risk stratification in CKD [19], the prevalence of CKD increased from 3 to 49% as age increased from 18–44 to 85–100 years. In the recent study by Chen et al. [16], age was not a significant modifier of the association between *APOL1* risk alleles and CKD progression. Our study differed from the AASK study in that we examined age in 5-year increments, instead of a binary variable with the cutoff at 55 years, which may have contributed to the difference in results.

A lower LVEF at baseline was another factor that predicted incident CKD development in an *APOL1* homozygous background. Most previous studies on low EF and CKD have focused on worsening renal function in heart failure [20–22]. Lower EF has been shown to predict worsening renal function ($p = 0.002$) in some previous studies [20], but not others [21]. The mechanisms responsible for renal decline in the setting of low EF may include decreased renal perfusion secondary to low cardiac output and neurohormonal activation [20].

In this study, the presence of type 2 diabetes was the strongest and only modifiable factor that increased the risk of CKD in individuals with an *APOL1* homozygous background. Few previous studies on *APOL1* included patients with diabetes [15, 16]. In studies that did, a stronger association between *APOL1* risk variants and renal decline has been shown in this subgroup [4]. For example, the Chronic Renal Insufficiency Cohort (CRIC) study found that over a mean follow-up of 4.4 years, African-American individuals with homozygous *APOL1* without diabetes experienced renal events at a rate of 7.5 per 100 person-years, compared with an event rate of 13.7 per 100 person-years in individuals with diabetes [4].

Potential mechanisms for the increased risk conferred by diabetes in an *APOL1* high-risk background are poorly understood, partially because the underlying mechanisms by which *APOL1* risk alleles lead to renal disease are unclear. A recent in vitro study investigated the effects of overexpressing nonrisk and risk variants of *APOL1* in human podocytes through a lentivirus expression system and discovered that *APOL1* risk variants induced podocyte necrosis through compromising lysosomal membrane permeability [23]. The same study also identified hypoxia as an environmental modifier that enhanced the effects of *APOL1* risk variants. Hypoxia secondary to the mismatch of oxygen demand and delivery [24] is a common finding in type 2 diabetes, and may be the underlying mechanism explaining type 2 diabetes status as the strongest predictor of incident CKD in this subgroup. Other potential mechanisms could include the role of diabetes in renal damage itself, such as glycation end products causing mesangial expansion [25].

The lack of association between *APOL1* and CVD discovered in our study has been observed in other large trial-based studies, including the AASK and the Systolic Blood Pressure Intervention Trial (SPRINT) [4, 5]. However, these results are in contrast with the JHS and WHI study [6]. Possible explanations for these discrepancies include differences in patient selection, definition of clinical phenotypes, and censoring methods.

Our study has limitations. The follow-up for incident CKD was not available in some patients, which could introduce biases. Additionally, we were unable to determine the exact cause of incident CKD in this study. Other potential factors associated with risk of incident CKD, such as blood pressure control and medications, were not included in our analysis. However, these factors were not shown to affect *APOL1*-related CKD progression in the recent study of the AASK population [16]. Detailed cardiovascular follow-up also allowed us to use true time-to-event information, as opposed to using nested cases and controls, to assess the associations between *APOL1* risk variants and incident CVD events and survival.

Conclusions

We confirmed that *APOL1* G1 and G2 variants are associated with prevalent renal disease in African-Americans, and expanded these findings into a secondary prevention population. We found no association between *APOL1* risk variants and CAD or CVD events. Building a clinical model to identify factors increasing the risk of incident CKD in *APOL1* homozygous individuals, we demonstrated that the causes for incident CKD are multifactorial. Type 2 diabetes was the strongest and only modifiable factor that predicted incident CKD in this subgroup. These results highlight the need for further studies on the potential modifying effects of diabetes control on CKD development and studies to understand the potential interactive mechanisms between *APOL1* and diabetes on CKD.

Statement of Ethics

All subjects in the CATHGEN study have given their informed written consent. The study protocol was approved by the Institutional Review Board (IRB) of Duke University. Funding for this study was supported by the National Institutes of Health Grant HL095987 (S.H.S.); National Institute of Diabetes, Digestive and Kidney Diseases of the National Institutes of Health under Award No. P30DK096493 (S.H.S.), and T32HL007101 (H.W.).

Disclosure Statement

The authors have no conflicts of interest to disclose.

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