APOL1 Gene Variants and Chronic Kidney Disease in Perinatally HIV-infected Youth

Murli U. Purswani, MBChB[1], Kunjal Patel, DSc, MPH[2], Brad Karalius, MPH[2], Cheryl A. Winkler, PhD[3], Stephen A. Spector, MD[4], Rohan Hazra, MD[5], George R. Seage III, DSc, MPH[2], George K. Siberry, MD[5], Lynne Mofenson, MD[5], Gwendolyn B. Scott, MD[6], Russell B. Van Dyke, MD[7], and Jeffrey B. Kopp, MD[8] for the Pediatric HIV/AIDS Cohort Study (PHACS).

[1]Bronx-Lebanon Hospital Center, Albert Einstein College of Medicine, Bronx, NY, [2]Harvard T. H. Chan School of Public Health, Boston, MA, [3]Center for Cancer Research, National Cancer Institute, Frederick, MD, [4]University of California San Diego, San Diego, CA, [5]Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD, [6]Miller School of Medicine, University of Miami, Miami, FL, [7]Tulane University School of Medicine, New Orleans, LA, and [8]National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD

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Name: Murli U. Purswani, MD
Mailing Address: 1685 Morris Avenue, Bronx, NY, 10457
Tel: 718-960-1010
Fax: 718-960-1011
Email: MPURSWAN@BRONXLEB.ORG

ABSTRACT*

Background: Two coding variants, G1 and G2, in the *APOL1* gene are present at high frequency in many sub-Saharan African populations. Studies in adults and children have repeatedly demonstrated strong associations between these alleles, operating in a recessive manner, and various glomerular diseases including HIV-associated nephropathy. We explored associations between *APOL1* variants and chronic kidney disease (CKD) in youth with perinatal HIV infection (P-HIV).
Methods: We carried out a nested case-control study within the PHACS Adolescent Master Protocol, a prospective study of children and youth with P-HIV. Using salivary DNA APOL1 risk alleles (RAs) were genotyped. Race was collected by self-report. Proportion of African ancestry was determined by genotyping 41 ancestry-informative markers and performing a principal components analysis. CKD was defined as ≥2 sequential urine protein/creatinine ratios ≥0.2 g/g (or dipstick protein ≥1+), or ≥2 sequential estimated GFRs <60 mL/min/1.73m2 (Schwartz equation) not followed by a normal value, or a history of kidney disease (nephropathy, nephrotic syndrome, chronic renal failure and focal segmental glomerulosclerosis). P-HIV controls had no evidence of kidney disease. Fisher's Exact test was used to compare RAs (2 versus 0/1 for G1 combined with G2) by case status using crude odds ratios (OR) and further adjusted for African ancestry.
Results: Of 448 P-HIV participants, 234 met case/control criteria yielding 27 CKD cases and 207 controls. 70% self- identified as black, 23% white; 41% were male. Mean (area under the curve estimate) HIV viral load was 4.07 logs higher in cases versus controls (95% confidence interval [CI]: –1.34 to 7.45). There was a trend for association between self-reported black race and CKD (OR: 3.0; 95% CI: 0.9-10.5). Among blacks, carriage of 2 *APOL1* RAs was 13%; among whites, none had 2 RAs, and 11% had 1 RA. Further, proportion of African ancestry (0.71-0.8) was associated with CKD (OR: 8.8; 95% CI: 1.9-41.4). Association between *APOL1* RAs (2 vs 0/1) with CKD was significant in those who self-reported as black (OR: 3.2; 95% CI: 1.2-9.0), and in the overall study population (OR: 4.1; 95% CI: 1.5-11.2). These findings remained significant when adjusted for proportion of African ancestry (OR: 3.5, 95% CI: 1.2- 10, overall population), suggesting that the *APOL1* association is specific for this gene rather than simply tracking African ancestry.
Conclusions: Carriage of 2 *APOL1* risk alleles increases risk for CKD in youth with perinatal-HIV.

*Data presented in abstract based on data available by January 1st, 2014

BACKGROUND

- Genetic variants in the *APOL1 gene*, encoding apolipoprotein L1, and located in chromosome 22, are strongly associated with chronic kidney disease in individuals with recent sub-Saharan African ancestry[1]
- The strongest association between APOL1 variants and chronic kidney disease in adults are for HIV-associated nephropathy, specifically collapsing glomerulopathy, with odds ratio (OR) reported as 29 in the United States[2]
- Other factors interact with these risk alleles, including the environment, with HIV being the strongest risk factor identified to date, to produce kidney disease
- The increased risk is mediated, at least in part, by two *APOL1* gene variants, G1 (S342G/I384M) and G2 (NY 388/9 deletion)
  - They operate in a largely recessive manner
  - Have a combined allele frequency of 35% in the African American population[3]
  - Have a frequency as high as 62% in the Yoruba tribe in Nigeria
- A case control study design is frequently used in genetic association studies
- Race is usually identified though self reporting in most studies
- Continental ancestry, determined through genotyping, may not always match with self-reported race and ethnicity in populations with significant admixture, leading to confounding in interpretation of results[4]

OBJECTIVES

1. To estimate the frequency of the *APOL1* risk alleles in a U.S. cohort of children with perinatal HIV-1 infection
2. To examine the association of one and two *APOL1* risk alleles with chronic kidney disease in children and youth with perinatal HIV-1 infection

METHODS

Study Population

The Adolescent Master Protocol (AMP), a study within PHACS

- A prospective cohort study, IRB-approved and conducted at 15 sites within the United States and Puerto Rico
- Identifies the effects of HIV infection and antiretroviral therapy (ART) in pre-adolescents & adolescents
- Enrolled subjects 7-16 years old with perinatal HIV infection
- Enrolled the first subject on 3/26/2007 and completed subject accrual on 11/4/2009, with a total of 451 participants
- Required study visits at regular intervals in the clinic during which study-related procedures were performed
- Had informed consent and assent for participation, including genetic testing of repository specimens, obtained from parents and study participants

Definitions and Statistical Methods

Chronic Kidney Disease (CKD) was the main study outcome and cases were defined as a combination of ≥1 of the following criteria:

A. Persistent proteinuria: Two or more sequential urine protein/creatinine ratios (UP/C) ≥0.2g/g not followed by a UP/C <0.2g/g OR two or more sequential urine dipstick protein ≥1+ not followed by a urine dipstick protein <1+

B. Clinical diagnosis of chronic kidney disease: One of the following

- Nephropathy
- Nephrotic syndrome
- Chronic renal failure
- Focal segmental glomerulosclerosis

C. Persistent low GFR: Two or more sequential estimated glomerular filtration rates (eGFRs) <60 mL/min/1.73 m^2 not followed by a eGFR ≥60 mL/min/1.73 m^2. For children <18 years of age, the bedside Schwartz equation was used to calculate eGFR. For those ≥18 years of age, the CKD-EPI 2009 eGFR equation was used.

AUC HIV Viral load: The area-under-the-curve viral load, expressed as $\log_{10}$ copy-years, was calculated using the trapezoidal method[5].

***APOL1* Genotyping.**

•Salivary DNA obtained from AMP subjects was whole genome amplified by the laboratory of Stephen Spector at UCSD

•*APOL1* risk alleles were genotyped by Taqman PCR by Cheryl Winkler, at the Center for Cancer Research, National Cancer Institute, NIH

•*APOL1* genotypes were summarized as follows:

- Zero or one (0/1) risk allele: G0 (wt), G1 or G2
- Any two (2) risk alleles: G1/G1, G2/G2, G1/G2

Proportion of Ancestry that is African.

•Continental ancestry was determined by genotyping 41 ancestry-informative markers on PBMCs by real time PCR - Stephen Spector's lab (UCSD)

•Classified as African, European, American, South/Central Asian, South/West Asian, East Asian, and Oceania

Study Design and Analysis. A nested case control study within the AMP cohort. CKD cases were identified using the above criteria, as was a control comparison group with no clinical history of renal disease and all measured UP/Cs <0.2g/g, protein dipsticks <1+ and eGFRs ≥80 mL/min/1.73 m^2. Socio-demographic and clinical characteristics were compared between cases and comparison group using Fisher's Exact and Wilcoxon Rank Sum tests as appropriate. AUC viral load between groups was compared by matching intervals with sufficient viral load data and using bootstrapping to obtain a 95% CI. Logistic regression was used to obtain ORs for the effect of *APOL1* risk alleles on CKD.

RESULTS

Table 1: Characteristics of study population

Characteristics	Total (N=234)	CKD (N=27)	Comparison (N=207)	P-Value[1]
Age at CKD diagnosis/last visit (years)				0.005
Median	15.3	14.3	15.6	
Q1, Q3	12.5, 17.6	8.0, 16.3	13.0, 17.8	
Min, Max	0.4, 21.6	0.4, 19.7	7.1, 21.6	
Sex, N (%)				0.68
M	96 (41)	10 (37)	86 (42)	
F	138 (59)	17 (63)	121 (58)	
Race, N (%)				0.19
White	54 (23)	3 (11)	51 (25)	
Black	164 (70)	24 (89)	140 (67)	
Other	2 (1)	0 (0)	2 (1)	
Missing	14 (6)	0 (0)	14 (7)	
Ethnicity				0.17
Not Hispanic	169 (72)	23 (85)	146 (71)	
Hispanic	65 (28)	4 (15)	61 (29)	
Birth weight (grams), N (%)				0.93
<1500	9 (4)	1 (4)	8 (4)	
1500-2500	51 (22)	6 (22)	45 (22)	
>2500	132 (56)	18 (67)	114 (55)	
Missing	42 (18)	2 (7)	40 (19)	
Gestational age (weeks), N (%)				0.51
<32	13 (6)	0 (0)	13 (6)	
32-36	35 (15)	5 (19)	30 (15)	
≥37	105 (45)	13 (48)	92 (44)	
Missing	81 (35)	9 (33)	72 (35)	
Caregiver education level[2], N (%)				0.88
<High School	44 (19)	5 (19)	39 (19)	
High School or GED	60 (26)	7 (26)	53 (26)	
> High School	78 (33)	11 (41)	67 (32)	
Missing	52 (22)	4 (15)	48 (23)	
Household income[2], N (%)				0.45
<$10,000	41 (18)	8 (30)	33 (16)	
$10,001-$20,000	37 (16)	5 (18)	32 (15)	
$20,001-$40,000	49 (21)	4 (15)	45 (22)	
>$40,001	52 (22)	6 (22)	46 (22)	
Missing	55 (23)	4 (15)	51 (25)	
Passive cigarette smoke exposure[2,3], N (%)				0.48
Yes	55 (23)	8 (29)	47 (23)	
No	165 (71)	18 (67)	147 (71)	
Missing	14 (6)	1 (4)	13 (6)	
Active cigarette smoking[4], N (%)				0.30
Yes	21 (9)	4 (15)	17 (8)	
No	168 (72)	19 (70)	149 (72)	
Missing	45 (19)	4 (15)	41 (20)	
Any cigarette smoke exposure, N (%)				0.25
Yes	69 (29)	11 (41)	58 (28)	
No	123 (53)	12 (44)	111 (54)	
Missing	42 (18)	4 (15)	38 (18)	
Ever marijuana use, N (%)				0.82
Yes	62 (27)	8 (30)	54 (26)	
No	127 (54)	15 (55)	112 (54)	
Missing	45 (19)	4 (15)	41 (20)	
Frequent marijuana use[5], N(%)				0.30
Yes	21 (9)	4 (15)	17 (8)	
No	168 (72)	19 (70)	149 (72)	
Missing	45 (19)	4 (15)	41 (20)	
Tenofovir disoproxil fumarate				
Ever use, N (%)	107 (46)	11 (41)	96 (46)	0.68
Cumulative duration up to CKD diagnosis/last visit (years)				0.89
0	127 (54)	16 (59)	111 (54)	
>0-3	61 (26)	6 (22)	55 (26)	
>3	46 (20)	5 (19)	41 (20)	

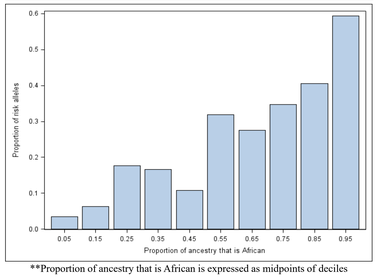
[1]Kruskal-Wallis test for continuous variables and Fisher's Exact test for categorical variables. Missing categories excluded in tests. [2]Most recent value relative to date of CKD diagnosis/last visit (i.e. could be after date of CKD diagnoses/last visit). [3]Passive smoke exposure defined as caregiver smoking an average of at least once a week or at least one cigarette per day. The highest reported level of caregiver smoking as of January 1, 2014 was used to define passive smoke exposure. [4]Active smoking defined as participant smoking at least once a week or at least one cigarette per day in the past three months. The highest level of participant smoking reported prior to or at date of CKD diagnosis/last visit was use to define active smoking. [5]Frequent marijuana use defined as use at least once a week in the past 3 months.

Table 2: Carriage of *APOL-1* risk alleles by self-reported race*

Self-reported race	N	Number of APOL1 risk variants		
		0	1	2
Black	302	132 (44%)	130 (43%)	40 (13%)
White	91	81 (89%)	10 (11%)	0 (0%)
Other	5	5 (100%)	0 (0%)	0 (0%)
Not reported	21	18 (86%)	3 (14%)	0 (0%)

*Frequency of *APOL-1* risk alleles in blacks……35%

Figure 1: Frequency of 1 and 2 *APOL-1* risk alleles by proportion of ancestry that is African**



**Proportion of ancestry that is African is expressed as midpoints of deciles

Table 3: Distribution of proportion of ancestry that is African by self-reported race and ethnicity

Self-reported Race and Ethnicity	N	Mean (SD)	Median (Q1, Q3)	Min, Max
White, Non-Hispanic	26	0.04 (0.08)	0.01 (0.01, 0.03)	0.01, 0.42
White, Hispanic	67	0.15 (0.18)	0.06 (0.02, 0.24)	0.01, 0.85
Black, Non-Hispanic	292	0.73 (0.17)	0.76 (0.69, 0.84)	0.02, 0.96
Black, Hispanic	17	0.40 (0.29)	0.36 (0.21, 0.57)	0.01, 0.92
Other, Non-Hispanic	4	0.02 (0.01)	0.02 (0.01, 0.03)	0.01, 0.04
Other, Hispanic	1	0.06		
Unknown, Non-Hispanic	1	0.12		
Unknown, Hispanic	19	0.17 (0.22)	0.08 (0.02, 0.26)	0.01, 0.80
Unknown Race and Ethnicity	1	0.02		

Figure 2: Distribution of proportion of ancestry that is African among CKD cases and the comparison group

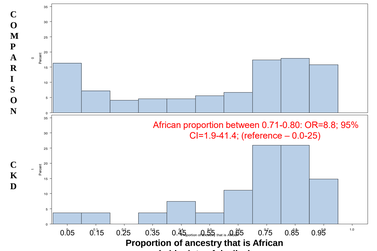


Table 4: Association between number of *APOL-1* risk variants (2 vs 0/1) and CKD in blacks, further adjusted for proportion of ancestry that is African

	N	Number of APOL1 risk variants		OR (95% CI)	P-value
		0/1	2		
Comparison	134	118 (88%)	16 (12%)	Reference	Reference
CKD (Self-reported blacks)	27	20 (74%)	7 (26%)	3.2 (1.2-9.0)	0.03
CKD (Self-reported blacks, further adjusted for proportion of ancestry that is African)	23	16 (70%)	7 (30%)	3.3 (1.1-9.6)	0.03

Characteristics of the study population (Table 1): As of data downloaded January 1st, 2014

- 27 cases of CKD
- 207 controls for comparison
- CKD cases were younger than controls (14.3 vs 15.6 years, p<0.005). This was expected, as the age of CKD participants was determined at diagnosis of CKD, while that of controls determined as of the last study visit
- No differences in other socio-demographic characteristics, including tenofovir use between the two groups

Frequency of APOL1 risk alleles (Table 2): In the whole cohort with genotyping data (N=419)

- Frequency of *APOL1* risk alleles in self-reported blacks was 35%
- Frequency of *APOL1* risk alleles in the overall population was 27%
- Two risk alleles were identified in 13% of blacks and in none of the other races
- One risk allele identified in 43% of blacks and 11% of whites
- There was a linear relationship between the frequency of one/two risk alleles and proportion of ancestry that was African (Figure 1)
  - Increasing proportions of African ancestry showed increasing frequency of risk alleles
  - Participants with 95% African Ancestry had a frequency of approximately 60%

Distribution of proportion with African ancestry and self-reported race and ethnicity (Table 3): Mean proportions of African ancestry was

- 4% in self identified whites (range 1-42%)
- 15% in self identified Hispanic whites (range 1-85%)
- 73% in self identified non-Hispanic blacks (range 2-96%)
- 40% in Hispanic blacks (range 1-92%)

There was a balanced distribution of African ancestry proportions within the comparison group, while in the CKD group this was markedly skewed toward higher proportions of African ancestry (Figure 2). Compared to African ancestry proportions of 0-0.25, those with proportions between 0.71-0.80 were 8.8 fold more likely to have CKD. When using self-reported race there was a trend for association between self-reported black race and CKD (OR: 3.0; 95% CI: 0.9-10.5).

AUC HIV viral load in cases and controls: The mean AUC viral load difference between cases and the comparison group was 4.07 $\log_{10}$ copies/ml (95% CI: -1.30-7.45).

Association between number of APOL-1 risk alleles and CKD (Table 4):

- Blacks with 2 risk alleles were 3.2 fold more likely to have CKD compared to 0 or 1 risk allele
- After adjustment for proportion of ancestry that is African, this association still persisted with an OR of 3.3 (95%CI: 1.1-9.6)

SUMMARY

- The frequency of *APOL1* risk alleles in blacks in the AMP cohort was 35%, similar to what has been observed in most African American populations
- *APOL1* allele frequency was proportional to percentage of African ancestry
- There were no significant socio-demographic and virologic differences between CKD cases and controls (comparison group) among AMP participants
- Determination of race and ethnicity by self report in the AMP cohort was not accurate: some self-reported whites had substantial admixture with African ancestry (range 1-42%) while some self-reported blacks had admixture with other races resulting in lower proportions of African ancestry (range 2-96%).
- Higher proportion of African ancestry is associated with an increased risk for CKD
- Carriage of two *APOL1* risk alleles conferred increased risk for CKD in blacks, when compared to 0/1 risk alleles
- This increased risk persisted even after adjustment for proportion of African ancestry, suggesting that it is the *APOL1* gene that is driving this risk, independent of African ancestry

CONCLUSIONS

In youth with perinatal HIV-1 infection, carriage of *APOL1* risk alleles increases risk for CKD, operating in a recessive manner.

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