APOL1 risk alleles are associated with exaggerated age-related changes in glomerular number and volume in African American adults: An autopsy study.

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Running title: *APOL1* alleles and age-related changes in glomerular number.

Abstract

Objectives: *APOL1* genetic variants contribute to kidney disease in African Americans (AA). We assessed correlations between *APOL1* profiles and renal histological features in subjects without renal disease.

Methods: Glomerular number (N_{glom}) and mean glomerular volume (V_{glom}) were measured by the disector/fractionator method in kidneys of AA and other Americans (non-AA) without renal disease, undergoing autopsies in Jackson, Mississippi. *APOL1* risk alleles were genotyped and the kidney findings were evaluated in the context of those profiles.

Results: In AA age $\geq$18 years, proportions with none, one and two *APOL1* risk alleles were 38%, 43% and 19% respectively. 38% had G1 allele variants and 31% had G2 allele variants. *APOL1* positive AA appeared to have higher N_{glom} and smaller V_{glom} in early adult life, with reductions in N_{glom} and increases in V_{glom} with increasing age.

Regressions predicted an annual average loss of 8,834 (p=0.033, sex adjusted) glomeruli per single kidney over the first 38 years of adult life in AA with two risk alleles. BMIs above the group medians, (but below the "obesity" definition of $\geq$30 kg/m^2) enhanced the expression of age-related changes in N_{glom} in AA with both one and two APOL1 risk alleles.

C**onclusions:** *APOL1* risk alleles are associated with an exaggerated loss of nephrons with increasing age, probably decaying from a larger pool of smaller glomeruli in early adult life, along with enlargement of the remaining glomeruli.

These phenomena might mark mechanisms of accentuated susceptibility to kidney disease in *APOL1* positive AA.

Introduction

Recent studies have shown that the accentuated risk of African Americans (AA) for renal disease is associated strongly with *APOL1* risk alleles [1], with odds ratios of 7 for end-stage kidney disease attributed to hypertension [2], 17 for focal segmental glomerulosclerosis (FSGS) and 29 for HIV-associated nephropathy [3]. Conditions also associated with *APOL1* risk alleles include sickle nephropathy [4], collapsing HIV glomerulopathy [5,6] and with collapsing nephropathy and end-stage kidney disease associated with lupus nephritis [7,8] as well as increased allograft loss in deceased donor kidney transplants [9]. Further, the progression of chronic kidney disease (CKD) is more rapid in those with two *APOL1* risk alleles, despite remittive therapy for FSGS and control of blood pressure for hypertension-attributed CKD [2,3], with earlier institution of dialysis [10,11]. Furthermore, AA without renal disease on enrolment in the community-based ARIC study were more likely to develop CKD and progress to ESRD on follow-up if they had had two *APOL1* risk alleles, compared to those with no alleles or one allele [5]. Thus, *APOL1* risk alleles increase the risk for progressive CKD when various injurious stimuli are present, and also predispose to the de novo development of CKD.

The accentuated susceptibility of AA for kidney disease has been the focus of various hypotheses. Brenner and colleagues proposed that reduced glomerular number, present at birth, predisposes to hypertension and CKD in adult life, and suggested that the increased susceptibility of AA for diabetic nephropathy might also be related to this phenomenon [12]. However, in prior studies from the Mississippi autopsy cohort, using an unbiased disector/fractionator stereological technique to count glomeruli, we did not find significantly lower total nephron numbers (N_{glom}) in AA than in European-Americans [13]. In that study we also found that while mean glomerular volume (V_{glom}) in AA was only marginally (and

non-significantly) larger in AA than in non-AA, the value was significantly larger in AA with hypertension than in those without [13,14,15]. Moreover, our studies on individual glomerular volumes (IGV) showed more size heterogeneity and net enlargement in AA than in non-AA [16,17,18].

The intent of the autopsy series in which this study is nested was to examine microanatomy in normal kidneys. Here we describe histomorphometric phenotype in adult AA subjects in that series according to their *APOL1* risk allele profiles.

Results

DNA was successfully extracted from formalin-fixed, paraffin-embedded tissue for 98% of AA and 99% of non-AA and genotyped for the two *APOL1* renal risk missense variants comprising the G1 allele and the G2 insertion/deletion allele. Of subjects, eighteen years and over, who were successfully genotyped (159 AA and 135 non-AA), 19% (30) of AA had two risk alleles, 43% (68) had one risk allele and 38% (61) had no risk alleles. More AA females than males tended to have 2 risk alleles than zero or one (p=0.022). Among non-AA, three people (2%) had a single *APOL1* risk allele.

As shown in **Table 1**, *APOL1* genotype distributions and allele frequencies in AA were similar to those in a community-based sample of middle-aged AA, initially without renal disease, who were enrolled in the Atherosclerosis Risk in Community Study, ARIC [5]. As in the ARIC study, the genotypes in AA were in Hardy Weinberg equilibrium; although females had a slightly higher proportion of risk allele genotypes than expected, this was not significant.

Table 2 shows some characteristics of subjects by race and number of *APOL1* risk alleles. AA females with *APOL1* risk alleles tended to be younger at time of death. Females and males with two risk alleles tended towards lower weight, BMI and levels of obesity than other AAs.

However, only the younger ages at death for the dominant and additive models in females, and the lower levels of obesity for females with for the additive model were individually significant, and none of the associations were significant with Bonferroni adjustments for multiple tests.

Figure 1 shows more detail on some of these associations, with adjustment for age. The data similarly suggest an additive model for lower BMI with increasing numbers of *APOL1* risk alleles in females, while males with two alleles (ten subjects only) had the lower levels of obesity. The frequency of hypertension did not differ by number of *APOL1* alleles in females, while, among males, it was apparently highest in those with a single *APOL1* risk allele, although this was not significant (p=0.101). The data also hint at an additive model for cardiovascular deaths in males, although it was not significant (p=0.138), and conversely for deaths of misadventure.

Table 3 shows the kidney features of subjects aged 18 to 67 years. In females and males kidney weight was lowest in people with two *APOL1* risk alleles. As expected, [19, 13], females had fewer nephrons than males, by about 15% for non-AAs and 10% for AA. In AA females there was no difference in N_{glom} according to *APOL1 r*isk alleles, while N_{glom} tended to be higher in males with *APOL1* risk alleles. Aggregate V_{glom} in females tended to be smaller than in males, while the lowest V_{glom} value in both sexes was in those with two risk alleles. However, none of these associations was significant, individually, or with Bonferroni correction. Furthermore, proportions of sclerosed glomeruli, degree of cortical fibrosis and intimal thickening in remote resistance vessels (ItR) and in capacitance vessels (ItC) did not differ significantly by *APOL1* risk allele profile

Although N_{glom} and V_{glom} were not significantly correlated with *APOL1* profiles when all ages were considered together, there were apparent differences in the relationships of each to

age. **Tables 4A and 4 B** show these data for females and males. The data suggest higher baseline values of N_{glom} in young "*APOL1* positive" (one or two risk alleles as a group) AA subjects, and a ranked order of glomerular "loss" with increasing age by number of *APOL1* risk alleles in AA. The decrease is significant in females with two *APOL1* risk alleles, and but not in males with two risk alleles, due to the small sample size (n=10). A gradient is also suggested in *APOL1* positive AA females and males. V_{glom} increased with age in all female AA risk allele groups, without substantial differences between them. However, in males with two risk alleles, V_{glom} rose sharply from an apparently lower value in young adult life to higher values in middle age, compared with those with no risk alleles, while the pattern in those with one risk allele was arguably intermediate.

Figures 2A and 2B illustrate these phenomena over a more uniform age range. **Table 5** shows changes expressed in absolute values, as well as in fractional changes. These approaches give internally consistent results. The age-related reductions in N_{glom} were significant in AA subjects with two risk alleles, with one or two alleles, and in those with two G2 risk alleles (although there are only 5 subjects in this group). Age-related increases in V_{glom} were significant in those with one risk allele, two risk alleles, and with one or two risk alleles, and, marginally in those with G1/WT and G2/G2 profiles.

The data in Table 5 suggest an inverse correlation between changes in N_{glom} and V_{glom}. The relationship between the fractional change is significant by non-parametric (Kendall Tau) testing when the G2/G2 group (5 subjects only) is included (coefficient -0.73, p=0.0197), but not when that group is excluded (coefficient -0.33, p=0.215).

However, the predicted age-related changes in N_{glom} and V_{glom} were BMI dependent. **Figure 3** shows data for each AA *APOL1* risk profile group of 20 or more subjects, divided around their group-specific BMI medians. These median BMIs were all below the threshold for

obesity ($\geq$30 kg/m^2). Age-related decrements in N_{glom} were only significant in AA risk allele positive subjects with BMIs greater than their group medians. The significance of age-related V_{glom} increments associated with one or two *APOL1* risk alleles was enhanced by higher BMIs. BMI was not a significant independent predictor of N_{glom} in AA females or males, regardless of *APOL1* profiles, but addition of age*BMI interaction terms modestly improved the explained variance of N_{glom} in females with two risk alleles. BMI was an additional independent risk factor for V_{glom} in AA females with one risk allele (p=0.039), and BMI*age interaction term modestly improved the explained variance of V_{glom} in females with one and two risk alleles.

The age-related decrease in N_{glom} in *APOL1* positive people could not be readily attributed to hypertension or cardiovascular disease. **Figures 4A and 4B** show similar trends in *APOL1* positive subjects without hypertension and without a cardiovascular cause of death.

Discussion

This is the largest study of microanatomy in kidneys of AA without renal disease at autopsy, and it is the only one in which associations with *APOL1 r*isk alleles have been evaluated. The similarity of the *APOL1* risk allele frequencies to those of ARIC enrolees suggests that there is little segregation of particular *APOL1* genotypes among the AA undergoing autopsy in our series. However, the higher proportions of *APOL1* risk allele-negative AA males with deaths of misadventure, and the trend towards lower ages at deaths of *APOL1* positive females hint at some contribution of *APOL1* risk alleles to risk of natural death.

Our data suggest that certain characteristics are associated with *APOL1* risk alleles in AA. Females with two risk alleles tended towards lower weight, BMI and levels of obesity than other AA females, and both females and males with two risk alleles had lower kidney weight

than other AA. Furthermore, starting from an apparently higher baseline level of N_{glom} in early adult life, AAs with two *APOL1* risk alleles had an apparent loss of nephrons with increasing age over the next four decades. *APOL1* risk alleles were also associated with smaller average V_{glom} in early adult life as well as with more pronounced increases in V_{glom} with age. These phenomena were dependent on, or were enhanced by, higher BMIs. The trends to smaller body size, kidney size, and glomerular volume in *APOL1* homozygotes are compatible with our observations in autopsy subjects from Senegal in western Africa [20], a region with a high prevalence of *APOL1* variants [21].

Our data do not suggest that coexisting hypertension was the major driver of age-related changes in N_{glom} in subjects with two *APOL1* risk alleles. *APOL1* positive females did not have more hypertension than those without *APOL1* risk alleles, while the apparently higher levels in males were not significant. Furthermore, *APOL1* positive AAs without hypertension or cardiovascular deaths also tended towards reductions in N_{glom} with age. However, it is likely that age related reductions in N_{glom} can predispose to hypertension and cardiovascular risk, as well as renal disease, as discussed below.

Although the age-related trends in N_{glom} and V_{glom} are derived from a cross-sectional study, they probably reflect changes as an individual ages. Non-AAs and *APOL1* risk allele negative AAs appeared to have minimal loss of glomeruli with age up through their mid-50s, while some risk allele positive AAs had lower N~~glom~~ in the first 38 years of adult life. Those with two *APOL1* risk alleles "lost" a predicted average of about 350,000 nephrons per single kidney over that interval, and those with one or two alleles lost about 300,000 per single kidney. These are significant decrements in view of the mean single kidney nephron count of about 900,000 [13]. The data suggest far more serious age-related decrements of Nglom in G2/G2 subjects, but the numbers are very small. We could not detect, but cannot

exclude, an association of these age-related changes with accelerated processes of glomerulosclerosis, cortical scarring or vascular change. Although extrapolations to kidney disease are hazardous, these findings are compatible with those of Larsen et al, who found, in renal biopsies in AA with arteriolarnephrosclerosis, that a relative lack of obsolescent glomeruli, greater degrees of solidified and disappearing glomerulosclerosis, as well as less arteriolar change, distinguished those with two APOL1 risk alleles from those with no risk alleles [22].

Age-related reductions in N_{glom} and exacerbated increases in V_{glom} in *APOL1* risk allele positive subjects appear to be linked. This mimics the strong inverse correlation between N_{glom} and V_{glom} seen in all groups in this autopsy series [19, 13]. These changes could reflect compensatory hypertrophy of remaining glomeruli in the setting of progressive nephron deficiency, or glomerular loss due to intrinsic glomerular volume expansion placing glomeruli at extra risk for glomerulosclerosis [15]. In either scenario the initially smaller glomeruli in *APOL1* positive people might be at accentuated risk for damage from excessive hypertrophy. Furthermore, a recent study describes the influence of *APOL1* risk variants on enhanced podocyte necrosis through compromised lysosomal membrane permeability [23]. Much remains to be explored in this area, including, the full degree of heterogeneous individual glomerular enlargement that the estimate of average V_{glom} disguises [17, 24], and indices of podocyte number and density in the younger and older at-risk subjects [25] in the various *APOL1* subgroups.

This series is not a resource for the study of kidney disease, because such subjects were deliberately excluded. However, our findings might flag mechanisms of *APOL1*-mediated susceptibility to renal disease. Accentuated loss of N_{glom} with age in AA might contribute to earlier expression and more rapid progression of renal disease with protracted ageing

and/or with additional nephropathic insults, in a two-strike or multi-determinant disease model. This scenario is proposed for renal disease associated with sickle cell disease, with collapsing nephropathy associated with lupus or HIV and with compromised graft survival after transplantation [4-9]. *APOL1* risk variants are associated with earlier age of onset and progression to renal failure of subjects with FSGS, and prospective studies in the African American Study of Kidney Disease (AASK) and Chronic Renal Insufficiency (CRIC) cohorts show that carriage of two *APOL*1 risk variants is associated with more rapid progression to clinical endpoints in persons with renal insufficiency at study entry [2,3,5,7,8]. Moreover, AA participants without renal disease on enrolment in the ARIC study were more likely to develop CKD and to progress to renal failure if they had two *APOL1* risk alleles, compared to those with no alleles or one allele [5].

We could find no reports of exacerbation of *APOL1*-associated kidney disease by higher BMIs. If confirmed, this constitutes additional arguments for intensified surveillance of *APOL1* positive AAs, and incentives for them to remain relatively lean. It is noteworthy that the levels of BMI associated with significant age-related changes in N_{glom} and V_{glom} in this study were well below those defining "obesity" ($\geq$30 kg/m^2).

Our data hint at that hypertension and cardiovascular deaths might be higher in male AA with *APOL1* risk alleles. Ito et al. reported association of two *APOL1* risk alleles (OR~2) with atherosclerotic events in the Jackson Heart Study, with replication in the Women's Health Initiative (WHI) study [26], although this was not replicated in the large Systolic Blood Pressure Intervention Trial [27] . Numerous GWAS and admixture linkage mapping studies have not identified chromosome 22 as a locus for systolic blood pressure or hypertension, suggesting that *APOL1* variants probably do not contribute to primary hypertension.

Retrospective genetic testing of additional stored autopsy samples from AA with clear medical histories relating to hypertension, renal disease and cause of death could confirm or refute our results.

Strengths of this report are the unique study sample and approach. The duration of the sample collection (more than 10 years), the ethical considerations, and the tedious nature of the measurements, all weigh against its being repeated in this fashion.

There are several limitations in this study. The subjects constitute a convenience sample, selected for autopsy on medical and legal grounds, with a broad set of indications and underlying and coexisting conditions, ranging from deaths of misadventure to deaths with hypertension and cardiovascular disease. In addition, numbers of subjects with G1/G2, G1/G1 and G2/G2 profiles, and of males with any combination of two risk alleles are small. These factors, plus the dominance of females among those with two risk alleles, all pose analytic problems. Furthermore, the use of V_{glom}, which is an average value of glomerular volumes for a given kidney, provides no indication the variability in volumes of individual glomeruli within a given subject [13,16,17,18,24]. An additional caution is the inference that data on different people at different ages on a cross-sectional study, necessarily represent probable changes in an individual followed over a lifetime.

Despite these important limitations, this autopsy cohort has provided a unique histological resource. Findings of nephron loss in a setting of certain *APOL1* risk profiles and compensatory or causal nephron hypertrophy should provoke and direct further investigations. Development of non-invasive techniques to assess glomerular number and glomerular volume in living humans will greatly advance progress in this important field.

Concise Methods

Autopsy cohort. The right kidney was collected from 191 AA and 146 non-AA, without known renal disease, during autopsies conducted to investigate sudden or unexpected death, between 1998 and 2005, at the University of Mississippi Medical Center, Jackson, MS, USA. Subject selection and specimen collection have been previously described [15]. Race was ascertained from medical records, next-of-kin or the investigating pathologist. Subjects with known renal disease, with kidneys of significantly unequal size, and with scarred or contracted kidneys were specifically excluded, as were two subjects with unsuspected focal segmental glomerulosclerosis subsequently identified on histopathologic examination. Causes of death were documented and medical records were reviewed for a history of hypertension. Clinical and demographic characteristics were obtained from University of Mississippi Medical Centre records. Approval was obtained from the Institutional Review Board of the University of Mississippi Medical Center and the Human Research Ethics Committee of Monash University, Victoria, Australia, and consent provided by next of kin.

Stereology. Kidney tissue was sent to Monash University for stereological estimation of N_{glom} and V_{glom} and assessment of other morphological parameters. N_{glom} and V_{glom} were estimated using the physical disector/fractionator combination as previously described [28,19,29]. In brief, perfusion-fixed kidneys were sampled to provide an isotropic, uniform random sample of tissue blocks. These blocks were embedded in glycolmethacrylate and then exhaustively sectioned at 20µm. Every tenth and eleventh sections (a section pair) were then stained with PAS, and glomeruli in identical fields in each section pair were counted using the disector principle [30]. Stereological point counting at the same time on the same sampled fields allowed the calculation of V_{glom}.

Genetic analysis. DNA was isolated from formalin-fixed, paraffin-embedded tissue as previously described [31] .Genetic analysis was carried out under a protocol approved by the NIDDK Institutional Review Board (94-DK-0133). *APOL1* risk alleles were genotyped using TaqMan assays (ABI, Foster City, CA). The *APOL1* G1 allele was defined as the presence of the p.S342G variant (rs73885319A>G) and the G2 allele was defined as the deletion of p.N388Y389 (rs71785313 TTATAA/-). Genotyping of renal tissue was successfully performed on 188 AA and 144 non-AA of the autopsy cohort.

Statistical analysis. Variables were summarized using mean (standard deviation), geometric mean (95% confidence interval) or percent and presented in four groups, non-AA without risk alleles and AA with 0, 1 or 2 risk alleles. Analyses of group data were performed in Stata 13.1 (StataCorp. 2013. Stata: Release 13. Statistical Software. College Station, TX: StataCorp LP) using linear regression, logistic regression and chi-squared tests. Various transformations were applied to certain variables to optimise the normalcy of their distributions, and when applied, are specified in the legends of the data in the relevant tables. Allele group differences were tested using recessive (two vs none or one risk alleles), dominant (one or two risk alleles vs none) and additive (2>1>0 risk alleles) models, including adjustments for sex and age. Two-sided Fisher's exact tests were used to evaluate if genotype frequencies were in Hardy-Weinberg equilibria.

Renal and related physical characteristics and renal morphologic features were analysed in the context of *APOL1* risk alleles for subjects aged 18 to 67 years old at time of death. Only four AA in the entire series were older than this. The predicted annual changes of N_{glom} and V_{glom} in AA groups with various combinations of *APOL1* risk alleles were evaluated over ages

20 to 57 years, a range over which most *APOL1* profile groups had a reasonable representation.

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Statement of competing financial interests

None to declare

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Figure legends

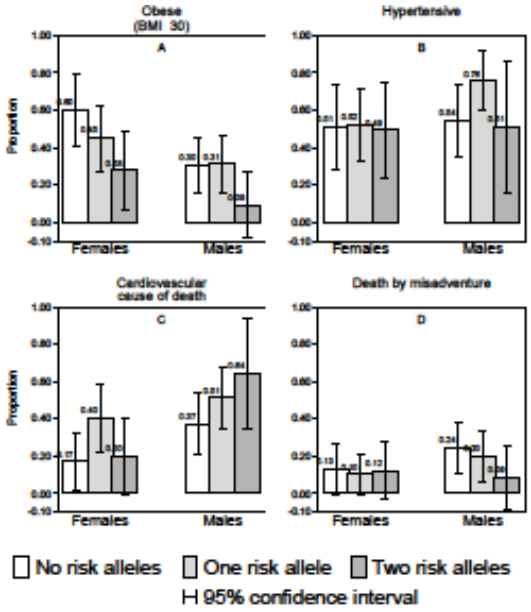

Figure 1. Proportions of obesity, hypertension, cardiovascular deaths and deaths by misadventure in African Americans age 18 to 67 years at autopsy, by sex and number of *APOL1* risk alleles, adjusted for age.

P1; P-value of F-test for linear trend (Additive model) from age-adjusted logistic regression

P2; P-value for the Dominant model from age-adjusted logistic regression

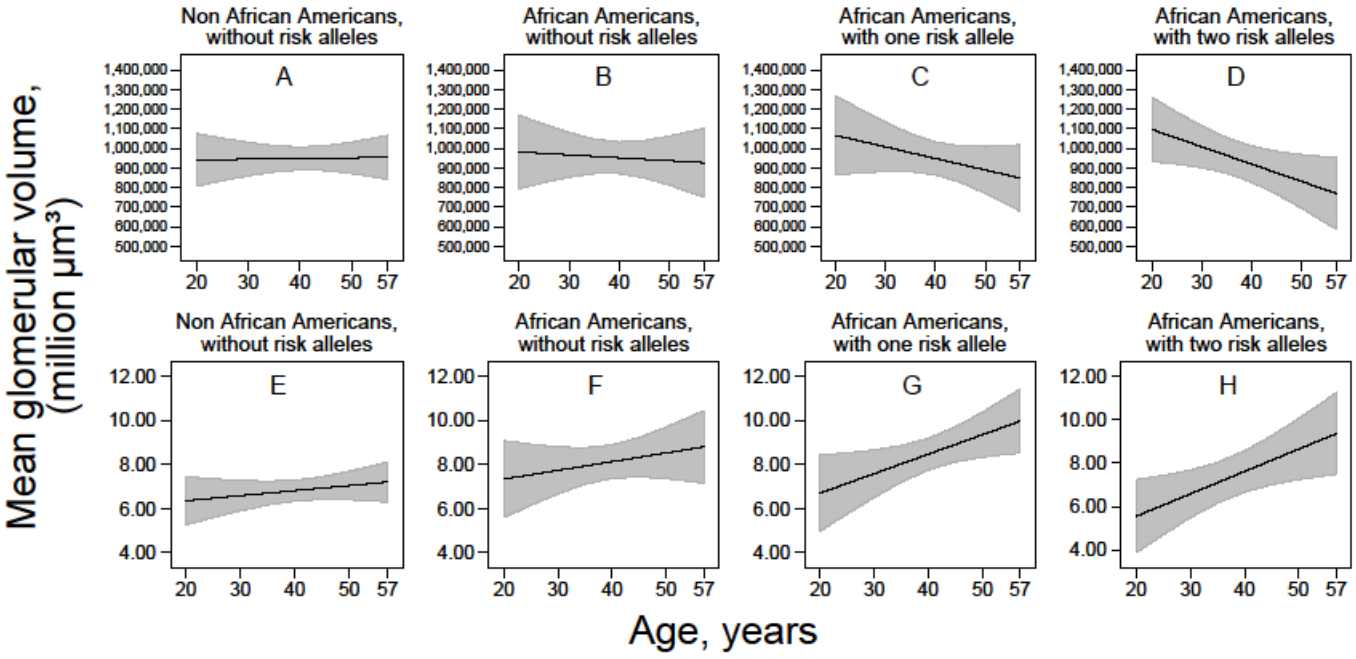

Figure 2. Age-related change in N_{glom} (A) and V_{glom} (B) at autopsy age 20 to 57 years, by race, number of *APOL1* risk allele profiles and sex, adjusted for age.

N_{glom}; estimated total number of glomeruli per kidney

V_{glom}; estimated mean glomerular volume ($\mu m^3 x 10^6$)

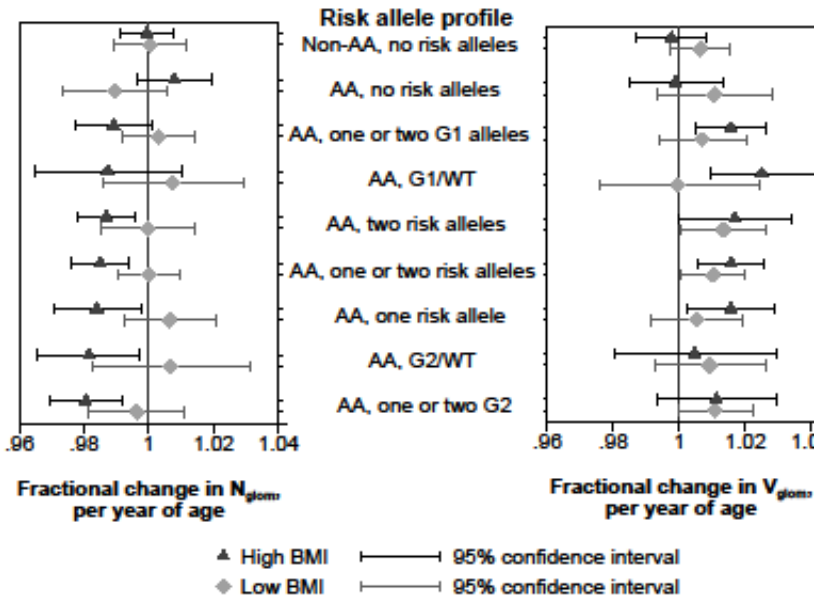

Figure 3. Comparison of fractional annual age changes at autopsy age 20 to 57 years in N_{glom} and V_{glom}, in those with BMI ≥ and < their group median, by race and *APOL1* risk allele profiles, adjusted by age and sex.

The group median BMI for AA with no risk alleles was 28.8 kg/m^2 and for AA in groups with risk alleles it ranged from 25.6 to 27.6 kg/m^2.

N_{glom}; estimated total number of glomeruli per kidney

V_{glom}; estimated mean glomerular volume ($\mu m^3 x 10^6$)

AA; African American

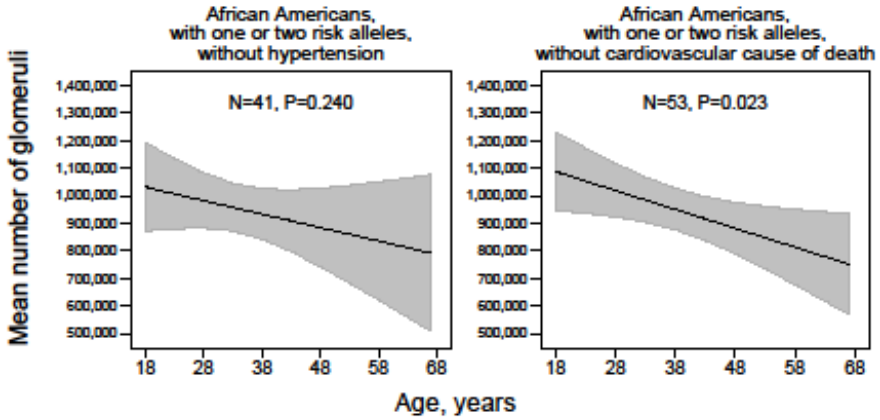

Figure 4. Age-related change in N_{glom} in African Americans with one or two *APOL1* risk alleles at autopsy age 18 to 67 years, without hypertension (A) and without a cardiovascular cause of death (B), adjusted for age and sex.

N_{glom}; estimated total number of glomeruli per kidney

N; number of subjects

P; P-value of modelled age

Table 1. African American *APOL1* genotype and risk allele frequencies, in the ARIC and current studies.

Characteristic	ARIC study [5], 45-64 yrs, n=3,067	Current study, ≥18 yrs		
		All n=159	Females n=76	Males n=83
Number of *APOL1* risk alleles, n (%)				
None	1,270 (41.4)	61(38.4)	25 (32.9)	36 (43.4)
One	1,393 (45.4)	68 (42.8)	31 (40.8)	37 (44.6)
Two	404 (13.2)	30 (18.9)	20 (26.3)	10 (12.0)
Frequencies of *APOL1* risk alleles				
G1	0.22	0.23	0.28	0.18
G2	0.13	0.17	0.18	0.16
Frequencies of *APOL1* genotypes, n (%)				
WT/WT	1,270 (41.4)	61 (38.4)	25 (32.9)	36 (43.4)
WT/G1	859 (28.0)	36 (22.6)	16 (21.1)	20 (24.1)
WT/G2	534 (17.4)	32 (20.1)	15 (19.7)	17 (20.5)
G1/G2	201 (6.6)	13 (8.2)	9 (11.8)	4 (4.8)
G1/G1	153 (5.0)	12 (7.5)	9 (11.8)	3 (3.6)
G2/G2	50 (1.6)	5 (3.1)	2 (2.6)	3 (3.6)
HWE P	0.884	0.653	0.57	1.0

WT; Wildtype (no *APOL1* risk alleles)

HWE P; P-value for Hardy-Weinberg Equilibrium, tested with two-sided Fisher's exact tests

Table 2. Characteristics of subjects at autopsy age 18 to 67 years, by race, sex and number of *APOL1* risk alleles.

Risk Allele Profile	n	Age range, yr	Age, yr, Mean (SD)	Height, cm, Mean (SD)	Weight, kg Gmean (95% CI)	BMI, kg/m2, Gmean (95% CI)	BMI ≥30, % (n)	Hyper-tension, % (n)	Cardio-vascular death, % (n)	Death by mis-adventure, % (n)
Non-African Americans, No risk alleles										
Females	46	18 to 65	42 (10.4)	163 (8.9)	75.1 (69.9-80.6)	28.4 (26.4-30.6)	35% (16)	23% (10)	24% (11)	26% (12)
Males	79	18 to 67	44 (13.0)	174 (9.2)	88.4 (82.5-94.6)	29.2 (27.4-31.0)	43% (34)	39% (30)	35% (27)	34% (27)
African American Females										
No risk alleles	25	21 to 67	45 (12.0)	166 (8.8)	82.9 (71.9-95.5)	30.4 (26.4-34.9)	60% (15)	58% (14)	24% (6)	12% (3)
One risk allele	31	19 to 57	39 (9.9)	164 (7.3)	85.1 (73.9-98.0)	31.8 (27.9-36.3)	45% (14)	48% (15)	39% (12)	13% (4)
Two risk alleles	18	20 to 57	38 (11.9)	162 (7.3)	75.0 (65.9-85.2)	28.4 (25.3-32.0)	28% (5)	44% (8)	19% (3)	17% (3)
One or two risk alleles	49	19 to 57	38 (10.6)	163 (7.3)	81.2 (73.5-89.7)	30.5 (27.8-36.1)	39% (19)	47% (23)	32% (15)	14% (7)
P, Recessive model			0.196	0.381	0.189	0.240	0.091	0.939	0.485	0.870
P, Dominant model			**0.020**	0.268	0.646	0.841	0.104	0.954	0.190	0.757
P, Additive model			**0.032**	0.249	0.269	0.388	**0.047**	0.934	0.833	0.949
African American Males										
No risk alleles	36	18 to 67	41 (13.3)	179 (9.7)	85.3 (78.4-92.8)	26.7 (24.6-29.1)	31% 11)	51% (18)	37% (13)	25% (9)
One risk allele	36	19 to 65	44 (12.4)	179 (7.7)	89.5 (81.9-97.9)	28.1 (25.9-30.6)	31% (11)	74% (26)	53% (19)	19% (7)
Two risk alleles	10	20 to 51	37 (10.9)	181 (8.2)	82.0 (67.4-99.8)	25.1 (21.5-29.5)	10% (1)	40% (4)	60% (6)	10% (1)
One or two risk alleles	46	19 to 65	42 (12.3)	179 (7.8)	87.8 (81.2-95.0)	27.4 (25.5-29.5)	26% (12)	67% (30)	54% (25)	17% (8)
P, Recessive model			0.193	0.363	0.425	0.217	0.182	0.509	0.199	0.301
P, Dominant model			0.636	0.992	0.581	0.575	0.655	0.183	0.138	0.425
P, Additive model			0.355	0.451	0.609	0.397	0.198	0.862	0.138	0.293

IQR; inter-quartile range
SD; standard deviation
Gmean; geometric mean
CI; confidence interval
Recessive model, 2 vs 0/1 risk alleles; Dominant model, 2/1 vs 0 risk alleles; Additive model, 2>1>0 risk alleles.
P; P-value; logistic regression (weight and height were log transformed) was used to test Recessive and Dominant models; logistic regression and an F-test for linear trend was used for Additive models. All tests included adjustment for age. Significance (P(α) 0.05) is shown in bold.

Table 3. Kidney features in subjects at autopsy age 18 to 67 years, by race, sex and number of *APOL1* risk alleles.

Risk Allele Profile	N	Age range, yr	Kidney weight, Mean (SD)	N_{glom}, Mean (SD)	V_{glom}, Gmean (95% CI)	GS %, Gmean (95% CI)	Cort fib%, Gmean (95% CI)	ItR, Gmean (95% CI)	ItC, Gmean (95% CI)
Non-African Americans, no risk alleles									
Females	46	18 to 65	159.6 (47.0)	865,190 (298,875)	6.4 (5.7-7.2)	1.5 (1.1-2.1)	2.8 (2.1 -3.8)	.05 (.03-.06)	.09 (.07-.12)
Males	79	18 to 67	191.2 (49.0)	993,632 (314,664)	6.5 (6.0-7.0)	1.7 (1.3-2.2)	3.4 (2.7-4.2)	.07 (.05-.08)	.10 (.09 -.13)
African American Females									
No risk alleles	25	21 to 67	164.6 (41.9)	888,707 (204,496)	7.2 (6.2-8.3)	2.5 (1.7-3.7)	4.5(3.3-6.3)	.06 (.04-.10)	.11(.08-.14)
One risk allele	31	19 to 57	165.8 (49.5)	891,476 (278,633)	7.2 (6.3-8.3)	2.7 (1.7-4.2)	4.5 (2.9-6.8)	.06 (.04-.09)	.11 (.08-.14)
Two risk alleles	18	20 to 57	149.3 (31.9)	850,073 (172,254)	6.8 (5.8-8.1)	2.0 (1.1-3.6)	3.0 (1.6-5.7)	.07 (.04-.12)	.10 (.07-.15)
One or two risk alleles	49	19 to 57	159.8 (44.3)	876,267 (243,799)	7.1 (6.4-7.9)	2.4 (1.7-3.4)	3.8 (2.7-5.4)	.07 (.05-.09)	.11 (.08-.14)
P, Recessive model			0.232	0.579	0.757	0.295	0.790	0.849	0.306
P, Dominant model			0.576	0.483	0.789	0.221	0.630	0.671	0.136
P, Additive model			0.281	0.457	0.959	0.177	0.662	0.722	0.138
African American Males									
No risk alleles	36	18 to 67	181.8 (41.4)	928,543 (328,676)	7.8 (7.0-8.7)	2,4 (1.5-3.9)	3.7 (2.4-5.7)	.11 (.08-.14)	.10 (.07-.15)
One risk allele	36	19 to 65	187.6 (47.6)	972,960 (351,069)	8.6 (7.7-9.6)	2.9 (1.9-4.3)	4.3 (3.1-5.9)	.09 (.07-.12)	.11 (.08-.14)
Two risk alleles	10	20 to 51	178.4 (50.8)	1,117,034 (347,933)	7.1 (5.5-9.2)	1.2 (0.5-3.1)	2.1 (0.9-4.5)	.04 (.02-.12)	.07(.04-.14)
One or two risk alleles	46	19 to 65	185.6 (47.9)	1,004,281 (351,684)	8.2 (7.4-9.1)	2.4 (1.7-3.5)	3.6 (2.7-4.9)	.08 (.06-.10)	.10 (.08-.12)
P, Recessive model			0.774	0.206	0.299	0.544	0.785	0.695	0.392
P, Dominant model			0.836	0.268	0.524	0.535	0.900	0.405	0.854
P, Additive model			0.865	0.156	0.513	0.477	0.789	0.544	0.441

SD; standard deviation

N_{glom}; estimated total number of glomeruli per kidney

V_{glom}; estimated mean glomerular volume ($\mu m^3 x 10^6$)

Gmean; geometric mean

CI: confidence interval

GS; glomerular sclerosis

Cort fib; cortical fibrosis (percent of cortex fibrosed)

ItR; intimal thickening ratio in remote resistance vessels

ItC; intimal thickening ratio in close compliance vessels

Recessive model, 2 vs 0/1 risk alleles; Dominant model, 2/1 vs 0 risk alleles; Additive model, 2>1>0 risk alleles.

P; P-value; linear regression of log (kidney weight, V_{glom}) or square root(N_{glom}, GS, Cort fib, ItR, ItC) transformed continuous variables were performed for Recessive and Dominant models; Additive models used an F-test for linear trend ~~test~~. All tests included adjustment for age.

Table 4A. Regression characteristics of predictions of average annual changes in N_{glom} versus age, around specified age ranges, by race, sex and number of *APOL1* risk alleles.

Risk Allele Profile	Age range, yr	N	Intercept	Coefficient (95%CI)	P
Non-African Americans, no risk alleles					
Females	18 to 65	46	1,083,710	-5,171 (-13,747 to 3,405)	0.231
Males	18 to 67	79	968,984	648 (4,851 to 6,147)	0.815
African American Females					
Zero risk alleles	21 to 67	25	825,386	1,407 (-5,942 to 8,756)	0.696
One risk allele	19 to 57	31	1,083,922	-4,971 (-15,481 to 5,538)	0.341
Two risk alleles	20 to 57	18	1,108,733	-6,898 (-13,699 to -126)	**0.046**
One or two risk alleles	19 to 57	49	1,094,762	-5,710 (-12,272 to 852)	0.087
African American Males					
Zero risk alleles	18 to 67	36	1,108,345	-4,418 (-12,915 to 4,078)	0.298
One risk allele	19 to 65	36	1,248,072	-6,316 (-15,966 to 3,333)	0.192
Two risk alleles	20 to 51	10	1,596,207	-13,128 (-36,728 to 10,471)	0.235
One or two risk alleles	19 to 65	46	1,348,860	-8,200 (-16,516 to 116)	**0.053**

N_{glom}; estimated total number of glomeruli per kidney
CI; Confidence interval
P; P-value of linear regression

Table 4B. Regression characteristics of predictions of average annual changes in V_{glom} versus age, around specified age ranges, by race, sex and number of *APOL1* risk alleles.

Risk Allele Profile	Age range, yr	N	Intercept	Coefficient (95%CI)	P
Non-African Americans, no risk alleles					
Females	18 to 65	46	5.35	0.04 -(0.04 to 0.11)	0.335
Males	18 to 67	79	5.55	0.03 (-0.01 to 0.07)	0.163
African American Females					
Zero risk alleles	21 to 67	25	8.49	-0.02 (-0.11 to 0.07)	0.654
One risk allele	19 to 57	31	4.04	0.10 (-0.01 to 0.21)	0.087
Two risk alleles	20 to 57	18	4.15	0.08 (-0.02 to .18)	0.106
One or two risk alleles	19 to 57	49	3.90	0.10 (0.02 to 0.17)	**0.017**
African American Males					
Zero risk alleles	18 to 67	36	8.27	-0.001 (-0.08 to 0.07)	0.976
One risk allele	19 to 65	36	7.36	0.04 (-0.04 to 0.11)	0.324
Two risk alleles	20 to 51	10	2.03	0.15 (-0.02 to 0.33)	0.083
One or two risk alleles	19 to 65	46	5.93	0.07 (-0.001 to 0.13)	**0.054**

V_{glom}; estimated mean glomerular volume ($\mu m^3 x 10^6$)
CI; Confidence interval
P; P-value of linear regression

Table 5. Predicted annual and fractional changes in N_{glom} and V_{glom} versus age at autopsy age 20 to 57 years, by race and *APOL1* risk allele profiles, adjusted for age and sex

		Absolute change		Fractional change	
Risk Allele Profile	**N**	**N_{glom}, Coefficient (95% CI)**	**P**	**N_{glom}, Ratio (95%CI)**	P
Non-African Americans, No risk alleles	106	340 (-5,530 to 6,210)	0.909	0.0003 (-0.0065 to 0.0072)	0.923
African Americans, No risk alleles	48	-1,480 (-10,267 to 7,306)	0.736	-0.0018 (-0.0114 to 0.0079)	0.711
African Americans, One risk allele	61	-5,885 (-14,831 to 3,060)	0.193	-0.0059 (-0.0156 to 0.0039)	0.232
African Americans, Two risk alleles	28	-8,834 (-16,676 to -791)	**0.033**	-0.0088 (-0.0164 to -0.0011)	**0.027**
African Americans, One or two risk alleles	89	-7,272 -13,498 to -1,046)	0.023*	-0.0074 (-0.0140 to -0.0008)	**0.029**
African Americans, GI/WT	32	-6,204 (-19,741 to 7,334)	0.356	-0.0049 (-0.0193 to 0.0098)	0.500
African Americans, G2/WT	29	-4,216 (-16,201 to 7,768)	0.476	-0.0058 (-0.0193 to 0.0079)	0.388
African Americans, G1/G2	12	2,114 (-8,527 to 12,755)	0.664	0.0008 (-0.0113 to 0.0130)	0.885
African Americans, G1/G1	11	-5,618 (-14,473 to 3,237)	0.182	-0.0064 (-0.0164 to 0.0038)	0.186
African Americans, G2/G2	5	-37,636 (-64,544 to -10,729)	**0.027**	-0.0291 (-0.0541 to -0.0034)	**0.040**
		V_{glom}, Coefficient (95%CI)		**V_{glom}, Ratio (95%CI)**	
Non-African Americans, No risk alleles	106	0.02 (-0.02 to 0.07)	0.341	0.0042 (-0.0025 to 0.0110)	0.220
African Americans, No risk alleles	48	0.04 (-0.04 to 0.12)	0.338	0.0053 (-0.0051 to 0.0158)	0.308
African Americans, One risk allele	61	0.09 (0.01 to 0.16)	0.024*	0.0108 (0.0014 to 0.0202)	**0.025**
African Americans, Two risk alleles	28	0.10 (0.02 to 0.19)	0.017*	0.0142 (0.0035 to 0.0251)	0.011*
African Americans, One or two risk alleles	89	0.10 (0.04 to 0.16)	0.001*	0.0129 (0.0060 to 0.0198)	<0.001*
African Americans, GI/WT	32	0.11 (0 to 0.22)	**0.050**	0.0143 (0.0003 to 0.0284)	**0.045**
African Americans, G2/WT	29	0.06 (-0.06 to 0.18)	0.317	0.0061 (-0.0073 to 0.0196)	0.359
African Americans, G1/G2	12	0.02 (-0.13 to 0.17)	0.792	1.00056 (-0.0164 to 0.0178)	0.943
African Americans, G1/G1	11	0.11 (-0.03 to 0.24)	0.112	1.014575 (-0.0028 to 0.0322)	0.089
African Americans, G2/G2	5	0.13 (0 to 0.26)	**0.051**	0.0264 (0.0047 to 0.0484)	**0.034**

N_{glom}; estimated total number of glomeruli per kidney (log transformed for fractional estimation)
V_{glom}; estimated mean glomerular volume ($\mu m^3 x 10^6$) (log transformed for fractional estimation)
CI; Confidence interval
P; P-value of linear regression
*Remain significant after Bonferroni corrections for multiple (two) comparisons)