

APOL1 Genetic Variants in Focal Segmental Glomerulosclerosis and HIV-Associated Nephropathy

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ABSTRACT

Trypanolytic variants in *APOL1*, which encodes apolipoprotein L1, associate with kidney disease in African Americans, but whether *APOL1*-associated glomerular disease has a distinct clinical phenotype is unknown. Here we determined *APOL1* genotypes for 271 African American cases, 168 European American cases, and 939 control subjects. In a recessive model, *APOL1* variants conferred seventeen-fold higher odds (95% CI 11 to 26) for focal segmental glomerulosclerosis (FSGS) and twenty-nine-fold higher odds (95% CI 13 to 68) for HIV-associated nephropathy (HIVAN). FSGS associated with two *APOL1* risk alleles associated with earlier age of onset ($P = 0.01$) and faster progression to ESRD ($P < 0.01$) but similar sensitivity to steroids compared with other subjects. Individuals with two *APOL1* risk alleles have an estimated 4% lifetime risk for developing FSGS, and untreated HIV-infected individuals have a 50% risk for developing HIVAN. The effect of carrying two *APOL1* risk alleles explains 18% of FSGS and 35% of HIVAN; alternatively, eliminating this effect would reduce FSGS and HIVAN by 67%. A survey of world populations indicated that the *APOL1* kidney risk alleles are present only on African chromosomes. In summary, African Americans carrying two *APOL1* risk alleles have a greatly increased risk for glomerular disease, and *APOL1*-associated FSGS occurs earlier and progresses to ESRD more rapidly. These data add to the evidence base required to determine whether genetic testing for *APOL1* has a use in clinical practice.

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African Americans have a fourfold increased risk for end-stage kidney disease (ESKD) arising from the three leading causes of chronic kidney disease (CKD): diabetic nephropathy, hypertension-attributed CKD, and glomerulonephritis.¹⁻³ Two admixture mapping studies identified a locus on chromosome 22 with association with focal segmental glomerulosclerosis (FSGS), HIV-associated nephropathy (HIVAN, which, histologically, is manifested as collapsing glomerulopathy), and pooled nondiabetic ESKD cases, and this was extended to hypertension-attributed ESKD.^{4,5} The admixture peak was centered on *MYH9*, encoding nonmuscle myosin IIA heavy chain, and intronic single nucleotide polymorphisms (SNPs) in dbSNP were found to be highly associated with these kidney diseases. Fine mapping and resequencing efforts did not reveal obvious causal variation.^{6,7} Recently, the major source of genetic risk for African American nondiabetic ESRD and FSGS was localized to *APOL1*, encoding apolipoprotein L1 (ApoL1), 14kb from *MYH9*.⁸ FSGS and hypertension-attributed ESKD were associated with two *APOL1* risk alleles: G1, comprising two missense variants (S342G and I384M), and G2, which has a 6 base pair (bp) in-frame deletion (N388del:Y389del); G1 and G2 are mutually exclusive, never occurring on the same chromosome.⁸ These variants were strongly associated with sporadic FSGS in either the homozygous or compound heterozygous state.⁸ The effect of *APOL1* variants has not been determined for HIVAN, affecting approximately 10% of HIV-infected African Americans before the initiation of combined antiretroviral therapy.⁹

APOL1 and *MYH9* are adjacent genes on Chromosome 22, in a region that shows signatures of both historical selection at the time of human and nonhuman divergence and more recent selection within certain African populations.^{8,10-14} *APOL1* is unique to certain higher primates but is not found in nonprimate species. Apolipoprotein L1 (ApoL1) lyses *Trypanosoma brucei brucei*, a cause of disease in a broad range of mammals; because of *APOL1*, humans are resistant to *T. b. brucei* infection.¹⁵ Both *T. b. rhodensiense* and *T. b. gambiense*, the causes of human African sleeping sickness, have evolved independent mechanisms to avoid lysis by ApoL1. There is suggestive evidence of a recent incomplete, selective sweep of the region, which manifests as extended linkage disequilibrium in the *MYH9-APOL1* region in certain African populations and high allele frequency divergence, likely due to selection acting on the *APOL1* gene.^{12,14} *T. b. rhodensiense* carries a serum resistance-associated (SRA) protein that binds and inactivates the wild type ApoL1 protein; the G1 and G2 allelic variants, occurring near and in the SRA binding site, respectively, restore the ability of human serum to lyse *T. b. rhodensiense*. This appears to represent heterozygous advantage, where protection against trypanosomal infection for heterozygotes comes at the cost of increased risk of kidney disease in homozygotes or compound heterozygotes for *APOL1* G1 and G2 alleles.⁸ These kidney risk alleles are very common (the frequency is approximately 35%) in African Americans and in Yoruba from Nigeria, but the distribution of G1 and G2

alleles has been reported only for a small number African and world populations.¹⁶

In the present report, we present a comprehensive analysis of *APOL1* genetic variation in both African Americans and European Americans with FSGS and HIVAN, and correlate genotype with clinical phenotype. This is the first report to directly test the statistical association of *APOL1* genetic variation with biopsy-proven, HIV-associated kidney disease (collapsing glomerulopathy) in a case control study. We also show the frequency distribution of *APOL1* variants in well-defined human populations using DNA samples from the Human Genome Diversity Panel (HGDP) and HapMap. As approximately two-thirds of people with HIV disease live in sub-Saharan Africa, and the prevalence of clinically evident kidney diseases rises as patients survive longer,¹⁷ these findings have considerable relevance to understanding population differences for kidney disease and for future clinical studies to assess the efficacy of genetic screening in selecting therapy or determining prognosis.

RESULTS

To more precisely identify the effect size of the *APOL1* variants with FSGS and to determine the effect of these variants on HIVAN, *APOL1* genotypes and haplotypes were obtained for 1378 African American and European American study participants (Figure 1 and Table 1). Allele and haplotype frequencies for African American cases and controls are shown in Figure 1. Allele frequencies in the African American normal donor controls were 23% for G1 and 13% for G2. Because a subset of our

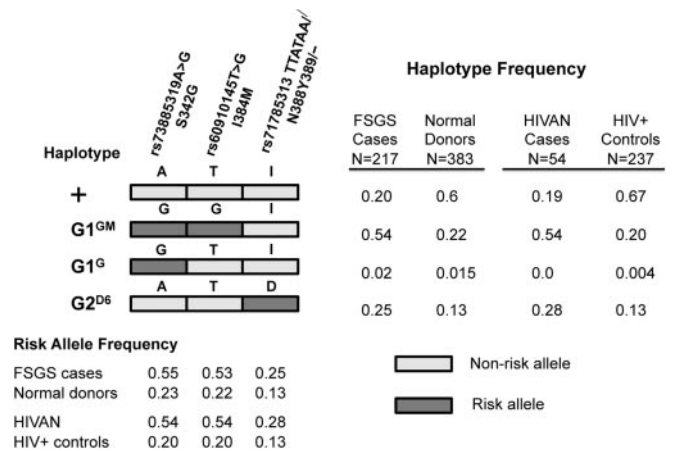


Figure 1. Distribution of haplotypes and risk alleles. Four *APOL1* haplotypes were observed in the African American study group: the major haplotype, denoted as (+) and comprised of three nonrisk alleles; the G1^{GM} haplotype with two missense risk alleles; the rare G1^{G+} haplotype with one missense risk allele; and the G2^{D6} haplotype with the 6 bp deletion risk allele. The risk allele and haplotype frequencies are shown for FSGS and HIVAN case and control groups in African Americans, including healthy blood donors and HIV+ individuals with no kidney disease.

Table 1. Demographic characteristics of FSGS and HIVAN cases and controls

Population Group	Cases	n	Age of Onset (yr)	Controls	n	Age at Blood Donation (yr)	Total
African Americans	Primary FSGS	217	34 ± 14	Normal donors	383	44 ± 8	600
African Americans	HIVAN	54	36 ± 7	HIV + hypernormals	237	44 ± 6	291
European Americans	Primary FSGS	168	33 ± 17	Normal donors	319	50 ± 9	487
Total		439			939		1378

This table summarizes numbers and age distribution of the six groups of subjects studied, as mean ± SD. Hypernormal controls are those that have HIV disease for >8 yr and are lacking evidence of CKD (elevated creatinine or proteinuria). FSGS, focal segmental glomerulosclerosis; HIVAN, HIV-associated collapsing glomerulopathy.

FSGS cases and controls was used in the Genovese *et al.* study,⁸ we first replicated the reported associations using an independent and nonoverlapping group of primary (idiopathic) FSGS cases ($n = 77$) and controls ($n = 310$) under the recessive model for *APOL1* G1 and/or G2 (OR = 42.2 (95% CI 13.1, 68.5)). Next, we used the entire group of primary FSGS cases ($n = 217$) and controls ($n = 383$), as well as 54 biopsy-proven HIVAN cases and 237 hypernormal controls, to more precisely estimate the effect sizes for the alleles and their haplotypes, and to extend the associations to biopsy proven HIVAN. The G1 and G2 risk alleles are in complete negative linkage disequilibrium (LD) and never appear together on the same chromosome (Figure 1); a recombination event between the physically proximal G1 and G2 variants that would bring them together on the same chromosome has not been observed in this dataset or previously reported.⁸ Therefore, to determine the effect size of each of the alleles, it is necessary to stratify the analysis by the other allele (Table 2). These analyses show strong and essentially equivalent associations for individuals carrying two copies of G1, two copies of G2, or one copy of each; because of the complete negative LD, an individual can carry no more than two copies of G1 and/or G2. The odds ratios for carrying two copies of G1 and/or G2 were 17 (95% CI 11, 26) for primary FSGS and 29 (95% CI 13, 68) for HIVAN. There was a marginally significant association, with a much smaller effect size, for carriers of a single copy of the G1 allele without the G2 allele (OR 1.9, 95% CI 1.01, 3.5) for the combined FSGS and HIVAN, but no significant association for carriers of a single copy of G2. *APOL1* was resequenced in all our controls and cases, but no rare or private nonsynonymous variants were observed to contribute to this modest single copy gene effect (data not shown). These associations and levels of statistical support were nearly identical in a logistic regression analysis adjusting for age of onset (data not shown).

When we did the same analyses, but without accounting for the presence or absence of a risk allele on the alternative chromosome, we noted strong artifactual associations for heterozygotes of *APOL1* G1 or G2 (Supplementary Table 1), due to the unaccounted presence of the alternative risk allele on the second haplotype. As can be seen by comparing the results in Supplementary Table 1 to those in Table 2, this apparent dominant or additive association is misleading.

The *APOL1* G1 and G2 variants were rare in European Americans: Among 320 controls, one G2 (frequency of ap-

proximately 0.3%) and four G1 (frequency of approximately 1.3%) heterozygotes were observed. Among the 165 European American FSGS cases, two G2 heterozygotes, one G1 heterozygote, and one G1 homozygote were observed. A principal components analysis of ancestry in a previous study showed that three of these four cases had substantial African admixture (>80%).⁵

Onset ages for primary FSGS were highly variable. We considered that FSGS associated with a specific genetic lesion represented by homozygosity or compound heterozygosity for *APOL1* risk alleles would plausibly have a different age distribution than other forms of primary FSGS, which could represent a broader spectrum of underlying causes. Among 182 African Americans for whom age of onset data were available, the mean ± SD age of onset for those with 0 or 1 *APOL1* risk alleles was 37.6 ± 16.0 yr ($n = 64$), compared with 31.7 ± 11.9 yr for those with 2 *APOL1* risk alleles ($n = 118$; $P = 0.01$, t test with unequal variances) (Figure 2). The variance of age on onset was 45% less in the *APOL1* risk genotype ($P = 0.003$, F test for equality of variance). Onset ages were 39.2 ± 14.4 yr ($n = 22$) for those with 0 *APOL1* risk alleles, and 36.7 ± 17.0 ($n = 42$) for those with 1 *APOL1* risk allele ($P > 0.2$ for tests of equality of means and variances). As shown in Table 3, 70% of individuals with two *APOL1* risk alleles experience onset of FSGS between the ages of 15 to 39 compared with 42% of carriers of 0 or 1 risk alleles ($P = 0.0003$, FET). Together these data support the idea that FSGS among *APOL1* high-risk individuals is a distinct disease with earlier onset and tendency to present during the teenage years and early adulthood.

FSGS associated with nearly all Mendelian genetic mutations is resistant to glucocorticoid therapy, including those associated with the most common recessive gene, *NPHS2* (podocin).¹⁸ Among African American FSGS cases, 56 had received at least 8 wk of glucocorticoid therapy, the minimum required to determine responsiveness. The frequency of steroid sensitivity was 29% (12/42) in African American subjects with two *APOL1* risk alleles who were given an adequate trial of glucocorticoid therapy, and 33% (5/15) in subjects with zero or one *APOL1* risk allele ($P > 0.5$). Due to limited number of informative subjects, we cannot rule out a modest influence on steroid sensitivity in carriers of two *APOL1* variants.

We analyzed the duration from disease onset to ESKD, defined as estimated GFR <15 ml/min/1.73m², chronic dialysis, or kidney transplantation among 92 African American FSGS

Table 2. Independent effects of the APOL1 G1 and G2 risk alleles

Risk Allele	Stratum	Number Case/Control	1 vs. 0 Risk Allele		2 vs. 0 Risk Alleles		2 vs. 1 Risk Alleles		Recessive Model 2 vs. 1 or 0 Risk Alleles	
			OR (CI)	P	OR (CI)	P	OR (CI)	P	OR (CI)	P
HIV-associated collapsing glomerulopathy (n = 54) and hyper-normal controls (n = 237)										
G1	No G2	27/180	1.9 (0.5, 8)	0.37	47.4 (11.9, 231.5)	4.9 × 10 ⁻¹¹	24.3 (6.6, 105.4)	2.2 × 10 ⁻⁸	35.2 (10.7, 132.6)	2.3 × 10 ⁻¹¹
G2	No G1	11/150	1.4 (0.2, 7.5)	0.70	14.4 (1.7, 116.3)	0.007	10.1 (1.02, 105.9)	0.02	13.1 (1.7, 93.7)	0.007
G1/G2	G1/G2 ^b	26/113	—	—	44.9 (12.9, 192.1)	2.2 × 10 ⁻¹³	—	—	—	—
G1 or G2	All	54/237	1.8 (0.5, 6.8)	0.42	40.4 (13.7, 148.4)	4.10 × 10 ⁻¹⁸	23.2 (9.5, 61.6)	1.4 × 10 ⁻¹⁶	29.2 (13.1, 68.5)	6 × 10 ⁻²²
African American idiopathic FSGS cases (n = 217) and controls (n = 383)										
G1	No G2	125/286	1.9 (1, 3.5)	0.05	23.2 (12, 46.7)	7.3 × 10 ⁻²⁹	11.6 (6.0, 23.2)	4.7 × 10 ⁻¹⁷	17.4 (9.8, 31.7)	4.3 × 10 ⁻³⁰
G2	No G1	56/234	1.0 (0.4, 2.3)	1.0	25.1 (8.8, 83.3)	7.8 × 10 ⁻¹³	17.0 (5.1, 65.4)	8.5 × 10 ⁻⁸	25.3 (9.2, 81.7)	1.4 × 10 ⁻¹³
G1/G2	G1/G2	82/186	—	—	16.7 (8.5, 34)	7.3 × 10 ⁻²¹	—	—	—	—
G1 or G2	All	217/383	1.4 (0.8–2.6)	0.26	20.6 (11.8–37)	6.7 × 10 ⁻³⁸	14.6 (8.7, 25.1)	6.4 × 10 ⁻²³	16.9 (11–26.5)	1.3 × 10 ⁻⁴⁸

The APOL1 risk alleles are referred to as follows: G1, S342G mutation; G2, 6 bp deletion (N388del;Y389del). Stratum refers to analysis that included a subset of genotypes (e.g., all cases and controls that lacked the G2 allele or G1 allele). The strata do not add to the total due to overlap between strata. G1/G2 compound heterozygotes were compared to subjects lacking both G1 and G2 alleles. P values were determined by Fisher exact test. As shown, the data best fit a recessive mode of inheritance, with marginal evidence for a dominant effect of the G1 allele in idiopathic FSGS.

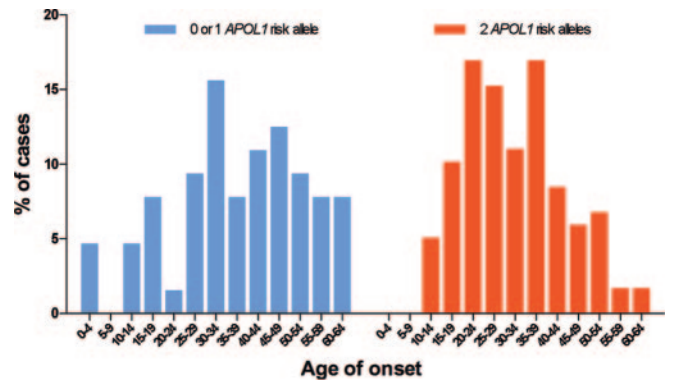


Figure 2. Distribution of age of onset of primary FSGS for APOL1 risk allele groups. Age of onset of primary FSGS is presented for African Americans with 0 or 1 APOL1 risk alleles (n = 63) and with 2 APOL1 risk alleles (n = 117). Height of bars represents the percentage of subjects of each group falling in each age group. As shown, APOL1-associated FSGS tends to present at a younger age and at a narrower onset age range, with 70% of cases presenting between 15 and 39 versus 43% of carriers of 0 or 1 risk alleles (P = 0.0009) (SD of presentation age in years, 11.8 versus 16.2, P = 0.004).

Table 3. Onset age for primary FSGS in African Americans stratified by G1 or G2 APOL1 risk alleles

Onset Age (years)	0 or 1 APOL1 Risk Alleles (n = 64)	2 APOL1 Risk Alleles (n = 118)
< 15	9%	5%
15–39	42%	70%
≥ 40	48%	25%

The distribution of onset ages for primary FSGS onset is shown. P = 0.0003 for the 3x2 contingency table by Fisher’s exact test.

patients who were evaluated at the National Institutes of Health (NIH) Clinical Center (Figure 3). Progression to ESKD was significantly faster among subjects with two APOL1 risk alleles, with a hazard ratio = 2.3 (95% CI 1.43, 4).

Among African American subjects, there were exceptions to the reported observation that the two SNPs defining the G1 risk allele are in absolute positive linkage disequilibrium.⁸ An infrequent haplotype carrying the risk allele for G1^{S342G} and the common allele at G1^{I384M} was observed. This haplotype, G1^{G+}, carried by seven controls and seven cases, accounts for less than 1% of the APOL1 haplotypes in the combined control groups. The common haplotype (+) containing the major frequency alleles for the two SNPs, and for the 6-bp insertion deletion, was most frequent in the controls (approximately 65%). In the HIVAN and FSGS case groups, the most frequent haplotype was G1^{GM} (54%) and the second most frequent was G2^{Δ6} (25% and 28%, respectively). The G1^{GM} and G2^{Δ6} haplotypes were less frequent (20 to 22%, and 13%, respectively) in both African American control groups (Figure 1).

To determine whether both G1 mutant alleles (342G and 384M) or only G1^{S342G} contribute kidney disease risk, we compared the effect sizes of the four G1 haplotype combinations (+/G^{GM}, +/G1^{G+}, G1^{GM}/G1^{GM}, G1^{GM}/G^{G+}) to the reference

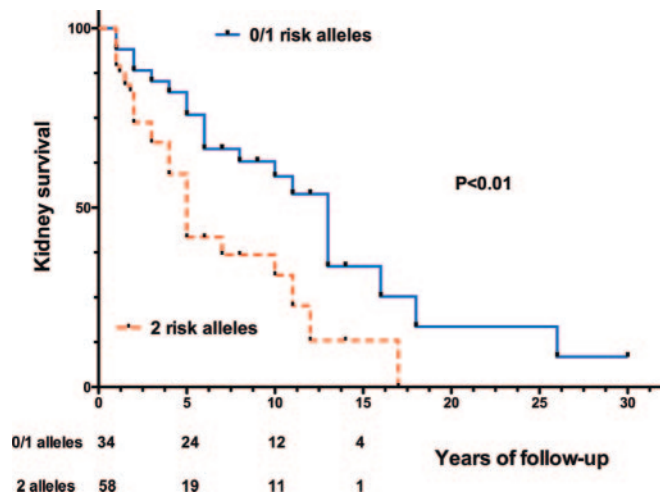


Figure 3. *APOL1* variants and kidney survival. The Kaplan-Meier plot depicts the kidney survival among 92 African American patients with primary FSGS, with the number of subjects at risk at each time point shown. The curves differed by the log rank test ($P < 0.01$). The hazard ratio for progression was 2.3 (95% CI 1.43, 4), and median survival was 5 yr for subjects with 2 *APOL1* risk alleles, compared with 13 yr for subjects with 0 or 1 *APOL1* risk allele. Survival curves were similar for 0 and 1 risk allele subjects ($P > 0.05$) and are therefore combined.

group (+/+). Due to the large effect of $G1^{342G}$, the relatively few individuals with the $G1^{G+}$ haplotype were sufficient to show that this haplotype had an effect size similar to the $G1^{GM}$ risk haplotype but different from the common (+) haplotype ($P = 0.02$) (Figure 4). We were unable to test the risk associated with $G1^{G+}/G2$ or the $G1^{G+}/G1^{G+}$ genotypes (carried by one individual and by no individuals, respectively), but the $G1^{GM}/G^{G+}$ association strongly suggests that the single $G1^{S342G}$ allele determines the G1 pathogenic phenotype, and it is sufficient to type just $G1^{S342G}$ and the G2 insertion/deletion to assess kidney disease risk.

Previous studies had shown that the G1 and G2 *APOL1* risk alleles were most common in western and southern Africa.^{8,16} We determined the distribution of *APOL1* risk alleles in DNA samples from 1024 individuals from 51 discrete human populations in HDGP, and in an additional 60 Yoruba from Nigeria and 90 Luhya from Kenya in the HapMap Project. We also include 702 African and European Americans controls from the state of Maryland. These results, displayed in Figure 5 and Supplementary Table 2, indicate that the G1 and G2 variants are differentially distributed throughout Africa. The G1 allele is most frequent in West Africa, whereas the G2 allele is less frequent but more widely distributed.

The attributable risk for homozygotes or compound heterozygotes for *APOL1* G1 and G2 risk alleles, in the recessive genetic model, is 68% for both FSGS and HIVAN. The explained fractions for G1 and G2 are 18% for FSGS and 35% for HIVAN. These are strikingly high values; for comparison, the explained fraction of smoking for lung cancer is 10 to 12%.^{19,20} Similarly, *APOL1* genotype is estimated to have a large effect

on lifetime risk of FSGS or HIVAN. For HIVAN, assuming a population lifetime risk for the development of HIVAN of 10.0% for HIV-infected African Americans who have not received antiretroviral therapy, the lifetime risks are 2.5%, 4.0%, and 50.0% in carriers of 0, 1, or 2 *APOL1* risk alleles, respectively. For FSGS, assuming an African American population lifetime risk of 0.8%,²¹ the lifetime risks are 0.2%, 0.3%, and 4.25% for these genotypes.

DISCUSSION

We investigated the association of *APOL1* genetic variation in FSGS and HIVAN and have several findings. First, we show, for the first time, that *APOL1* G1 and G2 alleles are strongly associated with HIVAN and confirm their association with primary FSGS; the associations are best described by a recessive model. We have more precisely determined the true effect sizes for these alleles for primary FSGS and HIVAN, with OR of 17 and 29, respectively, in a recessive model. These are the strongest effect sizes discovered, to date, for common variants with a complex disease.²² Second, we show that the $G1^{384M}$ allele is probably not required for pathogenesis, as the $G1^{G+}$ haplotype containing just the $G1^{342G}$ allele can complement the $G1^{GM}$ haplotype containing both variant alleles; we lack data for the other possible combinations ($G1^{G+}/G1^{G+}$ or $G1^{G+}/G2$). Third, we found no significant differences in glucocorticoid sensitivity in subjects who had received an adequate therapeutic trial. While the numbers of subjects are relatively small, they provide no evidence to support withholding glucocorticoid therapy in individuals carrying two *APOL1* risk alleles. Fourth, we show that the age of onset is significantly earlier for G1 and/or G2 homozygotes or compound heterozygotes. Fifth, we have shown that kidney survival is significantly shorter for individuals with primary FSGS who are carrying two *APOL1* risk alleles. Sixth, explained fractions for *APOL1* variants are notably high, at 18% for FSGS and 35% for HIVAN; the variants increase the risk of developing primary FSGS and HIVAN by approximately twentyfold compared with individuals lacking two risk alleles. We note that estimates for HIVAN were based on data from the era before the widespread use of effective antiviral therapy, and that the epidemiology of HIV-associated kidney disease has and continues to change. Finally, we show that both the G1 and G2 risk alleles are widely distributed across sub-Saharan Africa, but both kidney disease risk alleles are absent from populations outside of sub-Saharan Africa and in populations without African ancestry.

The mechanism by which *APOL1* variants cause kidney disease remains unknown. Apolipoprotein L1 is a circulating protein, a component of high-density lipoprotein (HDL) class 3, particularly the dense subset (class 3a), and is largely absent from other classes. HDL3 has the particular function of protecting low-density lipoprotein (LDL) particles from oxidation.²³ Whether apolipoprotein L1 has a role in this antioxidant function is unknown. Likewise, it remains to be determined

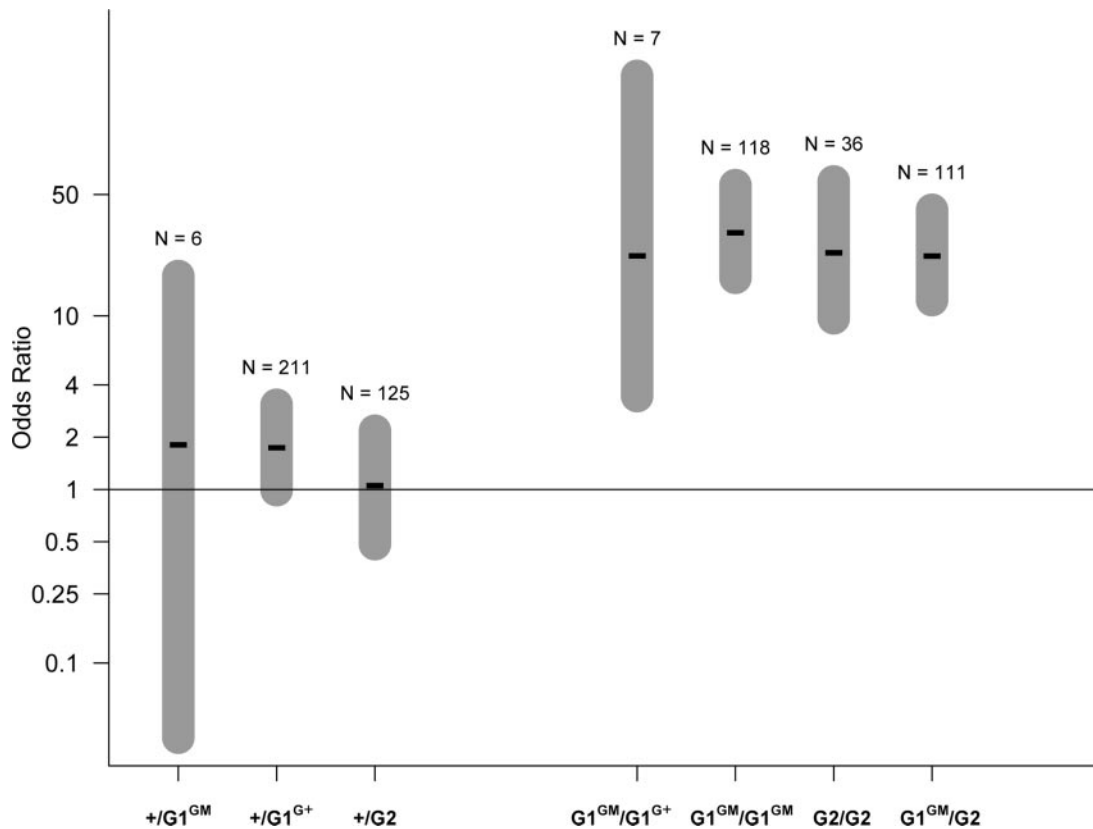


Figure 4. Odds ratios for the effects of the *APOL1* G1 and G2 alleles. Odds ratios with confidence intervals for association of different G1–G2 genotypes with combined FSGS/HIVAN cases compared with subjects carrying no G1 or G2 risk alleles (+/+). Heterozygous carriers of the risk alleles do not show significant association with FSGS/HIVAN cases, with the exception that combined $+/G1^{GM}$ and $+/G1^{G+}$ was marginally significant (OR 1.8, $P = 0.02$). By contrast, dual heterozygous and homozygous individuals showed a consistent association. In this analysis, the rare $G1^{G+}$ haplotype is distinguished from the common $G1^{GM}$ haplotype and $G1^{G+}$ is able to complement $G1^{GM}$, resulting in disease association for this genotype. A meaningful odds ratio for $G1^{G+}/G2$, carried by one subject, could not be determined.

whether circulating apoL1, kidney-expressed apoL1, or both contribute to kidney injury. As noted above, the G1 and G2 variants are located in the C-terminal domain (residues 342 to 398) of apolipoproteinL1, the binding region for the trypanosomal SRA.¹⁵ The function of this domain in human physiology is unknown, but the fact that both pathogenic variants occur in this domain may be a clue to kidney disease pathogenesis. Structural studies will be required to determine how these variants may alter domain structure. The data presented here indicate that I384M mutation does not contribute to pathogenesis. Until *APOL1* variants are directly implicated in kidney disease by experimental techniques such as expression in transgenic animals or injection of recombinant protein into animals, it remains possible that kidney injury is due not to *APOL1* variants but to another gene in linkage disequilibrium. However, only a few SNPs in the region are in moderate to strong linkage disequilibrium ($r^2 > 0.6$) with G1 and G2, and these are six or more orders of magnitude less statistically significant than the *APOL1* variants. The 1000 Genomes Project has not yet revealed plausible alternative candidates to *APOL1* G1 and G2 in the region.²⁴

APOL1 genetic variation is an attractive candidate for personalized medicine. Our data suggest a striking increase in disease prevalence among *APOL1* risk variant homozygotes. We estimate the lifetime incidence for HIVAN of 50% among HIV-infected African Americans with two *APOL1* risk alleles who are not receiving antiviral therapy. Future studies will be required to demonstrate whether genetic testing could play a role in the initiation of antiretroviral therapy in an individual who does not otherwise meet the criteria. Before such a recommendation can be made, an appropriate controlled trial is needed. We estimate the lifetime incidence for primary FSGS among African Americans with two *APOL1* risk alleles to be 4%. Some important questions remain, including whether the initial phase of *APOL1*-associated FSGS may involve a sustained period of microalbuminuria or how best to treat these individuals when they are glucocorticoid-resistant.

In conclusion, *APOL1* genetic variation is a powerful contributor to risk for FSGS and HIVAN. The attributable risk and explained fraction for *APOL1* variants are quantitatively comparable to the role of smoking in non-small-cell lung cancer risk. While only 12 to 13% of African Americans carry two

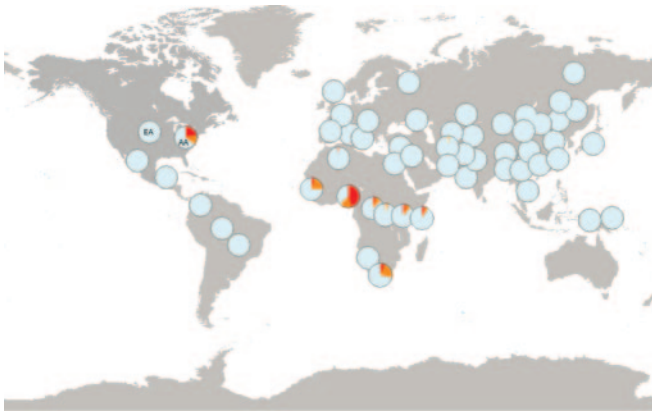


Figure 5. Worldwide frequency distribution of *APOL1* variants G1 and G2. Genotypes of G1 and G2 are determined for 51 populations in the Centre d'Etude du Polymorphisme Humain (CEPH) HGDP, for the HapMap Luhya population from Kenya (International HapMap Project [Phase II]), and for African Americans (AA) and European American (EA) controls in the NIH FSGS study cohort. The allele frequencies of G1 (red), G2 (orange), and wild-type alleles (light blue) in each population are depicted in pie charts overlaid upon a world map. Allele frequencies for G1 and G2 for each population are shown in Supplementary Table 2.

APOL1 risk alleles, these individuals have an estimated 4% lifetime risk for FSGS, and when they develop FSGS, they tend to progress to ESKD rapidly. Further studies will be required to determine whether early detection could alter the clinical course, and thus provide a rationale for genetic screening in the context of personalized medicine.

CONCISE METHODS

Study Participants

The study includes three case-control groups, two of which were self-described as African American and one of which was self-described as European American, as shown in Table 1. Cases included African Americans with biopsy-proven primary FSGS ($n = 217$) or biopsy-proven HIVAN, defined histologically as HIV-associated collapsing glomerulopathy ($n = 54$), and European Americans with biopsy-proven primary FSGS ($n = 168$). The cases and controls have been previously described.^{5,25} Primary FSGS and HIVAN cases were enrolled in the NIH FSGS Genetic Study from 22 academic medical centers in the United States. Institutional review boards at each collaborating medical center approved study protocols and each subject provided written informed consent. Renal diagnoses at each institution were made according to standard pathologic criteria. For simplicity, primary collapsing glomerulopathy was classified under primary FSGS. We excluded subjects judged to have postadaptive FSGS as a result of reflux nephropathy, reduced renal mass, sickle cell nephropathy, or morbid obesity and FSGS due to medications.

Onset age was defined as the age of proteinuria onset. Glucocorticoid sensitivity was defined as complete or partial remission in subjects who had received at least 8 wk of daily or alternate-day glucocor-

ticoid therapy.²⁶ Glucocorticoid sensitivity was evaluable for 56 African Americans with FSGS (26% of the total number of cases); the other subjects did not receive the required course of glucocorticoids.

DNA Samples for Global Diversity

DNA samples from the HGDP, the Foundation Jean Dausset-CEPH, Paris, France,²⁷ the Yoruba trios from Nigeria, and the Luhya samples from Kenya, both sets taken from the International HapMap Project, were used to determine G1 and G2 allele frequencies; the DNA and cell lines were obtained from the Coriell Institute (Camden, New Jersey). Genotypes were determined for 1024 individuals from 51 distinct ethnic groups from HGDP, and 60 Yoruba parents and 90 Luhya for G1 (rs73885319 (S342G) and rs60910145 (I384M), and the G2 6 bp insertion/deletion (indel) (rs71785313).

Terminology

For *APOL1* haplotypes, we use the terms $G1^{G+}$ (harboring the minor frequency risk allele at S342G only) and $G1^{GM}$ (harboring the minor frequency risk alleles S342G plus I384M). The G2 haplotype harboring the 6 bp in-frame deletion is termed $G2^{\Delta 6}$. For *APOL1* alleles, we use the terms G1, the risk allele for S342G that occurs on two G1 haplotypes ($G1^{G+}$ and $G1^{GM}$), and G2 for the deletion allele (Figure 1). We use “+” to designate the most frequent (nonrisk) state for haplotypes and alleles.

Genotyping

APOL1 SNPs (rs73885319 and rs60910145) and the G2 indel (rs717185313) were genotyped by TaqMan assays (ABI, Foster City, California). Each SNP was tested for deviations from Hardy-Weinberg expectations (HWE) among control subjects using a χ^2 goodness-of-fit test. All of the tested SNPs confirmed to HWE in the normal donor control groups.

Statistical Methods

Fisher's exact test (FET) was used to compare individuals with one or two risk alleles (G1 or G2) versus zero or one risk alleles (+/+), and also for the recessive model comparing individuals with G1/G1, G2/G2, or G1/G2 genotypes to individuals with +/G1, for primary FSGS and HIVAN. A similar analysis was performed comparing the $G1^{GM}$ and $G1^{G+}$ haplotypes and their diplotypes.

For the analysis of age of onset of FSGS, an *F* test (*R* function var.test) was used to test for inequality of the variances of the two groups with and without *APOL1* risk genotypes; a Welch two-sample *t* test was used to compare the means (*R* function t.test). Difference in kidney survival was analyzed with the log-rank test (Mantel-Cox), using Prism (Graph Pad, San Diego, California). For attributable risk, explained fractions, and lifetime estimate risks, population contingency tables were extrapolated from case-control data by scaling the number of controls to yield risks of 0.8% for FSGS and 10% (among untreated HIV infected subjects) for HIVAN, based on estimates of African American population lifetime risk.^{20,28,29}

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DISCLOSURES

None.

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- See related editorial, "Apolipoprotein L1 and the Genetic Basis for Racial Disparity in Chronic Kidney Disease," on pages 1955–1958.
- Supplemental information for this article is available online at <http://www.jasn.org/>.