APOL1 allelic variants are associated with lower age of dialysis initiation and thereby increased dialysis vintage in African and Hispanic Americans with non-diabetic end-stage kidney disease

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Abstract

Background. The APOL1 G1 and G2 genetic variants make a major contribution to the African ancestry risk for a number of common forms of non-diabetic end-stage kidney disease (ESKD). We sought to clarify the relationship of APOL1 variants with age of dialysis initiation and dialysis vintage (defined by the time between dialysis initiation and sample collection) in African and Hispanic Americans, diabetic and non-diabetic ESKD.

Methods. We examined APOL1 genotypes in 995 African and Hispanic American dialysis patients with diabetic and non-diabetic ESKD.

Results. The mean age of dialysis initiation for non-diabetic African-American patients with two APOL1 risk alleles was 48.1 years, >9 years earlier than those without APOL1 risk alleles (t-test, P = 0.0003). Similar results were found in the non-diabetic Hispanic American cohort, but not in the diabetic cohorts. G1 heterozygotes showed a 5.3-year lower mean age of dialysis initiation (t-test, P = 0.0452), but G2 heterozygotes did not show such an effect. At the age of 70, 92% of individuals with two APOL1 risk alleles had already initiated dialysis, compared with 76% of the patients without APOL1 risk alleles. Although two APOL1 risk alleles are also associated with ~2 years increased in dialysis vintage, further analysis showed that this increase is fully explained by earlier age of dialysis initiation.

Conclusions. Two APOL1 risk alleles significantly predict lower age of dialysis initiation and thereby increased dialysis vintage in non-diabetic ESKD African and Hispanic Americans, but not in diabetic ESKD. A single APOL1 G1, but not G2, risk allele also lowers the age of dialysis initiation, apparently consistent with gain of injury or loss of function mechanisms. Hence, APOL1 mutations produce a distinct category of kidney disease that manifests at younger ages in African ancestry populations.

Keywords: African-Americans; APOL1; dialysis; Hispanic-Americans; non-diabetic kidney disease

Introduction

The incidence rate for end-stage kidney disease (ESKD) due to hypertension in African-Americans above the age of 65 is four times greater than for European Americans. However, this disparity is even more prominent under the age of 60, in which the incidence rate of African-Americans is >10 times greater compared with European Americans [1]; (Supplemental Figures S1 and S2). Initial studies, which identified the chromosome 22 risk region, pointed to clusters of non-coding polymorphic variants in the MYH9 gene as being most strongly associated with
African ancestry non-diabetic kidney disease risk [2, 3]. One such cluster consisted of a group of Single Nucleotide Polymorphisms (SNPs), which were in strong linkage disequilibrium (LD) with each other and were termed the E-1 haplotype SNPs [2–5]. Subsequent studies identified functional variants in the neighboring APOL1 gene, as being even more strongly associated with disease risk and which explained most or all of the risk that have been assigned to the E-haplotype SNPs [6, 7]. APOL1 mutations have recently been associated with non-diabetic ESKD [7, 8], hypertensive nephropathy [8], HIV-associated nephropathy (HIVAN) [9], non-monogenic idiopathic focal-segmental glomerulosclerosis (FSGS) [6, 9] and transplant nephropathy [10], but not associated with diabetic ESKD [2, 3, 11] and IgA nephropathy [12].

The risk allelic variants in APOL1 comprise a haplotype of two missense mutations in almost perfect LD (rs73885319 [Ser342Gly] and rs60910145 [Ile384Met]) designated as G1 and another 6bp deletion (rs71785313 [388–389 Asn–Tyr del]) designated as G2 [6, 7]. The G1 and G2 mutations are not observed together on the same chromosome as a combined haplotype, indicating that they have arisen independently on separate phylogenetic branches [4]. These allelic variants rose to high frequency in populations of West and Central Africa by virtue of the adaptive advantage that they provide (In Yoruba from Nigeria, the allele frequency of G1 is 46% and of G2 is 7% [13]) [4]. Even in the heterozygote state, these allelic variants protect from lethal forms of sleeping sickness mediated by the Trypanosoma brucei rhodesiense species [6]. The alleles now appear in high frequencies also among Americans with African heritage, whose ancestry includes nearly half a millennium of tragic slavery and forced migration to the Americas from these very regions in Africa, with accompanying admixture with European ancestry populations in African-Americans [4, 7] and in Hispanic Americans [14]. However, these allelic variants are nearly absent in Eastern Africa [4, 7, 15] and outside of Africa [9]. The most striking risk of APOL1 variants for ESKD has been reported under a recessive inheritance mode in individuals carrying two APOL1 risk alleles: G1:G1, G2:G2 or a compound heterozygote state of G1:G2, with impressive odds ratio that reaches as high as 29 in HIVAN [9]. While the African-American population frequency of these two risk allele diplotype is ~12% [6, 7], it is dramatically higher in different ESKD etiologies: 47% in hypertension-attributed ESKD [6], 66% in FSGS [6, 9] and 67% in HIVAN [9]. In addition, some, but not all, case-control association studies show a possible weaker additive effect for the G1 heterozygous state [6, 7, 9, 12] but not for the G2 heterozygous state [9].

The 5-year survival of incident dialysis patients is 32%, less than the survival in many forms of cancer [16, 17]. Survival remains low despite advances in dialysis technology. Approximately half of the ESKD deaths are caused by cardiovascular or infectious events [18]. Various attempts to increase survival of patients on dialysis, by increasing dialysis dosages [19] or the aggressive treatment of risk factors such as hyperlipidemia with statins, have not met with the degree of success seen in other clinical settings [20]. Recent studies show that earlier dialysis initiation does not itself improve survival [21, 22]. However, it is well-documented that patients who initiate renal replacement therapy at ages <40 have a survival advantage compared to older age groups [23]. Of great interest, it has been reported that risk of death following initiation of dialysis is nearly 45% lower in African-Americans compared to European Americans [24, 25]. At the same time, in the corresponding non-dialysis general populations, the mortality from cardiovascular disease is considerably higher among African-Americans compared to European Americans [26–28]. The decreased mortality of African-Americans on dialysis therapy, in contrast to their increased mortality in the general population, has been called the ‘dialysis survival paradox’. This finding, consistently seen in numerous studies, has never been adequately explained.

Recent studies have shown that APOL1 risk variants are associated with lower age of dialysis initiation in African-American dialysis patients [9, 29]. In a study focusing on FSOS, Kopp et al. [9] showed a significantly younger age of dialysis initiation associated with the two APOL1 risk alleles (G1:G1 or G1:G2 or G2:G2, mean age 31.7), compared with the 0 or 1 APOL1 risk allele state (Wt:Wt or Wt:G1 or Wt:G2, mean age 37.6). A study of non-diabetic ESKD patients by Kanji et al. [29] demonstrated a significant difference of approximately 10 years in the age of dialysis initiation between those who carried two APOL1 risk alleles (G1:G1 mean age 49.0 and G1:G2 mean age 49.3) and those without risk alleles (Wt:Wt, mean age 61.8). In addition, lower mean age of dialysis initiation was also observed for G2 homozygotes and for G1 heterozygotes, but these were not reported to reach statistical significance in this study [29].

Since APOL1 risk variants are now known to make a substantial contribution to the burden of chronic kidney disease (CKD) among African-Americans [4], we sought to confirm the effect of African APOL1 risk variants on the age of dialysis initiation and to clarify their possible relationship with dialysis vintage in African ancestry diabetic and non-diabetic dialysis patients. We postulated that APOL1 risk variant, CKD etiology and younger age of dialysis initiation might be a factor which importantly leads to the ‘dialysis survival paradox’. We demonstrated that non-diabetic ESKD individuals with two APOL1 risk alleles initiate dialysis 9–12 years earlier in comparison with those patients without risk alleles, in both African and Hispanic American patient groups. In addition, we showed a single-allele effect for the G1 variants, which is associated with a significant reduction in the age of dialysis onset for G1 heterozygotes, but we did not detect a similar heterozygote effect for the G2 variant in non-diabetic African-Americans. For the diabetic ESKD sample set, we did not observe a significant effect of two APOL1 risk alleles on the age of dialysis initiation in either African Americans or Hispanic Americans. Examination of the effect of the APOL1 allelic state on dialysis survival revealed a significantly higher frequency of two APOL1 risk alleles in non-diabetic African-Americans with increased dialysis vintage. Further analysis shows this not to be a direct effect of APOL1 on survival but rather is consistent with initiation of dialysis at a younger age.
Materials and methods

We examined a previously described ESKD sample set of 955 individuals that includes African and Hispanic Americans, non-diabetic and diabetic ESKD patients on hemodialysis [5, 7, 30]. Clinical characteristics of the sample sets are shown in Table 1. Samples were collected at dialysis clinics affiliated with the Cabrini Medical Center in New York City, NY. The DNA sampling was approved by the Cabrini Institutional Review Board, and all study participants provided written informed consent. We collected different clinical characteristics from the patients by direct questioning or by reviewing medical records, including: height, weight, age, gender, place of birth, parental place of birth, self-reported ethnicity, kidney function status, kidney biopsy diagnosis when available, the date of the first day on dialysis, hypertension, diabetes and HIV status. The self-identified Hispanic American subjects in the current study were mostly of Puerto Rican, Dominican and other Caribbean parental ancestry, with few subjects of Mexican–American ancestry. It should be noted that there are well-described differences in genome-wide ancestry structure in different Hispanic communities [14].

The definition of non-diabetic ESKD in this study refers to patients who usually have hypertension, but do not have diabetes (Type 1 and 2), HIV infection or other known specific etiologies of kidney disease. Participants with diabetes were defined based on: self-reporting of the patient, known treatment with insulin or oral hypoglycemic agents and/or medical history. ESKD subjects with congenital, obstructive, cancer-related kidney diseases or with known monogenic forms of kidney disease (e.g. autosomal dominant polycystic kidney disease, Alport syndrome) were excluded from the sample set.

For estimating global African ancestry, we used former genotypic data that were obtained by KASPar genotyping of 40 genome-wide ancestry informative markers (Supplementary Table S1) [30]. The frequency of each marker in different populations was available from HapMap [32] and from a novel dataset [30]. Global ancestry was calculated by maximum likelihood approach assuming a multinomial distribution for the

Table 1. Clinical and APOL1 genotype data for sample groups

<table>
<thead>
<tr>
<th></th>
<th>African-Americans</th>
<th>Hispanic Americans</th>
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<tbody>
<tr>
<td></td>
<td>Non-diabetic ESKD</td>
<td>Diabetic ESKD</td>
</tr>
<tr>
<td>Sample size (n)</td>
<td>358</td>
<td>408</td>
</tr>
<tr>
<td>Frequency of females</td>
<td>42</td>
<td>52</td>
</tr>
<tr>
<td>Mean African ancestry (%)</td>
<td>81 ± 16</td>
<td>83 ± 17</td>
</tr>
<tr>
<td>Mean BMI (±SD)</td>
<td>26.0 ± 6.5</td>
<td>28.1 ± 7.5</td>
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<tr>
<td>Overall risk alleles (%)</td>
<td>39</td>
<td>22</td>
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Mean age on dialysis initiation (years ± SD, n, raw P-value and q-value in comparison to Wt:Wt)

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<tr>
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<th>Diabetic ESKD</th>
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<tbody>
<tr>
<td>Overall</td>
<td>52.6 ± 15.5, 357</td>
<td>60.2 ± 12.4, 407</td>
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<tr>
<td>Wt:Wt</td>
<td>57.4 ± 16.1, 85</td>
<td>60.4 ± 12.5, 149</td>
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<tr>
<td>Wt:G1</td>
<td>52.1 ± 15.6, 82, 0.0151, 0.0452 [0.0410]</td>
<td>63.0 ± 10.7, 103, 0.9607, &gt;0.1</td>
</tr>
<tr>
<td>Wt:G2</td>
<td>58.0 ± 13.7, 50, 0.5789, &gt;0.1 [&gt;0.1]</td>
<td>59.1 ± 12.2, 64, 0.2458, &gt;0.1</td>
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<tr>
<td>G1:G1</td>
<td>48.6 ± 14.8, 63, 0.0004, 0.0017 [1.0 × 10⁻⁵]</td>
<td>59.1 ± 14.2, 44, 0.3042, &gt;0.1</td>
</tr>
<tr>
<td>G1:G2</td>
<td>47.8 ± 14.5, 59, 0.0001, 0.0011 [3.3 × 10⁻⁶]</td>
<td>55.5 ± 12.6, 38, 0.0197, 0.0474</td>
</tr>
<tr>
<td>G2:G2</td>
<td>47.7 ± 12.2, 18, 0.0034, 0.0016 [0.0082]</td>
<td>59.2 ± 15.6, 9, 0.4180, &gt;0.1</td>
</tr>
<tr>
<td>Two APOL1 risk alleles</td>
<td>48.1 ± 14.3, 140</td>
<td>57.7 ± 13.7, 91, 0.0625, &gt;0.1</td>
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Mean dialysis vintage (years ± SD, n, raw P-value and q-value in comparison to Wt:Wt)

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<th>Diabetic ESKD</th>
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<tr>
<td>Overall</td>
<td>4.8 ± 4.3, 356</td>
<td>3.6 ± 3.3, 408</td>
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<tr>
<td>Wt:Wt</td>
<td>3.8 ± 3.6, 84</td>
<td>3.6 ± 3.2, 150</td>
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<tr>
<td>Wt:G1</td>
<td>4.8 ± 3.8, 81</td>
<td>3.7 ± 3.4, 103, 0.0494, &gt;0.1</td>
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<tr>
<td>Wt:G2</td>
<td>4.2 ± 4.5, 50</td>
<td>3.3 ± 2.9, 64, 0.4427, &gt;0.1</td>
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<tr>
<td>G1:G1</td>
<td>5.7 ± 5.3, 64</td>
<td>3.4 ± 3.1, 44, 0.7605, &gt;0.1</td>
</tr>
<tr>
<td>G1:G2</td>
<td>5.3 ± 3.7, 59</td>
<td>0.0070, 0.0536</td>
</tr>
<tr>
<td>G2:G2</td>
<td>5.8 ± 4.6, 16</td>
<td>4.1 ± 2.7, 9, 0.3684, &gt;0.1</td>
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<tr>
<td>Two APOL1 risk alleles</td>
<td>5.6 ± 5.0, 141</td>
<td>3.7 ± 3.5, 91, 0.4493, &gt;0.1</td>
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Mean dialysis vintage (years ± SD, n, raw P-value and q-value in comparison to Wt:Wt)

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<td>Overall</td>
<td>4.6 ± 4.6, 47</td>
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NA, not available.

bFor comparison, in African-American (n = 144) and Hispanic American (n = 378) healthy controls, the frequencies for the two APOL1 risk alleles are 13 and 2%, for the non-risk genotype (Wt:Wt) are 38 and 81% and for the Wt:G1 genotype are 28 and 10%, respectively (data not shown).

'q-values are the FDR adjusted P-values following multiple correction [31]. These results are also presented in Supplementary Figures S5–S6. Significant results are marked with bold fonts. P-values were calculated in comparison to the APOL1 non-risk state genotype group (Wt:Wt), with one sided t-test without equal variance assumption. In squared brackets, P-values followed by meta-analysis with previously published results by Kanji et al. [29].
APOL1 allelic variants and age of dialysis initiation

Results

Population characteristics of the sample sets that were examined in this study are shown in Table 1. We studied 995 ESKD individuals on dialysis: non-diabetic African-Americans (n = 358), diabetic African-Americans (n = 408), non-diabetic Hispanic Americans (n = 98) and diabetic Hispanic Americans (n = 131). We and others have previously reported that Hispanic Americans have a much lower average West African genome-wide ancestry (~29% in our sample set) compared with African-Americans (~82%) [30, 35]. In the African-American (AA) and Hispanic Americans (HIS) sample sets, the frequency of individuals who carry two APOL1 risk alleles (G1:G1 or G1:G2 or G2:G2) is significantly higher in the non-diabetic ESKD patients (39%; 20%) compared with the diabetic ESKD patients (3.5%; 20%). In some cases, we used the enzymes Psil or Tsp509I for RFLP genotyping of the G2 mutation. To genotype APOL1 variants, we also occasionally used Sanger sequencing of the DNA fragment that contains G1 and G2 mutations [7].

The initiation age of dialysis was calculated by subtracting the time between the birth dates from the starting dialysis dates. Dialysis vintage was defined as the duration of time between the first day of starting dialysis and the day of sample collection. We use dialysis vintage as a surrogate measure of dialysis survival, recognizing and fully acknowledging that many additional factors (e.g. transplantation rate, longevity characteristics and others) may also have important interacting and additional influences [34]. It also should be noted that the measurement of dialysis vintage reflects an underestimation of dialysis survival since individuals who are designated as having 'short dialysis vintage' might actually survive much longer if followed longitudinally. Statistical analyses were performed with the software SPSS version 16. In order to test the variables that affect dialysis vintage, we performed a linear regression that included the following covariates: age of dialysis initiation, APOL1 genotype, body mass index (BMI) and percentage of African ancestry. We were not able to include other variables in this analysis, not available to us for this sample set, that could also affect dialysis vintage, such as: socioeconomic factors, previous hospitalizations and transplantation rate, among others [34]. In order to obtain P-values that are adjusted for multiple comparisons, we applied a false discovery rate (FDR) method to the raw P-values obtained by one-sided t-test (without assuming equal variance), such FDR corrected P-values are also named q-values [31]. This procedure was applied to our main analysis of the association between APOL1 genotypes and mean age of dialysis onset or mean dialysis vintage, where we corrected for performing 24 tests overall in both sample sets (Table 1). In addition, Fisher’s combined probability test was used to combine our results with those of a previously published study [29].

The non-diabetic African-American ESKD sample set was stratified first by age of dialysis initiation. The frequency of patients who carry two APOL1 risk alleles was 45% in the group who initiated dialysis before 60 years of age (n = 249) compared to 25% in the group of patients who initiated dialysis after the age of 60 years (n = 108) (χ² = 13.12, P-value = 0.0003). We also compared the mean age of dialysis initiation for the different APOL1 genotypes in our sample sets (Table 1, Figure 1). We found a significant difference between the age of dialysis initiation in the non-diabetic African-American individuals who did not carry any APOL1 risk alleles (Wt:Wt, mean age 57.4 ± 16.1) compared to those who carry two G1 risk alleles (G1:G1, mean age 48.6 ± 14.8, t-test FDR corrected P = 0.0017), compound heterozygotes (G1:G2, mean age 47.8 ± 14.5, t-test FDR corrected P = 0.0011) and G2 homozygotes (G2:G2, mean age 47.7 ± 12.2, t-test FDR corrected P = 0.0116). The mean age of dialysis initiation for all non-diabetic African-Americans who carry two APOL1 risk alleles (G1:G1 or G1:G2 or G2:G2) was 48.1 ± 14.3 years (n = 140), >9 years younger in comparison to those without these risk alleles (t-test FDR corrected P = 0.0003). Meta-analysis with Fisher’s method for combining P-values that was performed with the results of Kanji et al. [29] strengthens these observations (Table 1). As evident in Supplemental Figure S3, the age distribution of dialysis initiation for this subset of patients is shifted by ~10 years in those with 2 APOL1 risk alleles compared to those with zero APOL1 risk alleles. As evident in Supplemental Figure S4, the cumulative proportion of patients who initiated dialysis before the age of 70 is 92% for the two APOL1 risk allele state, 85% for G1 heterozygotes and 76% with no APOL1 risk alleles. The cumulative proportion of patients who initiated dialysis before the age of 75 is 96% for the two APOL1 risk allele state, 94% for the G1 heterozygotes and 84% for those without APOL1 risk alleles. We also examined the same question in a sample set of non-diabetic Hispanic Americans (n = 98). Even with this relatively small sample number, we observed a significant difference between the age of dialysis initiation in individuals who did not carry any APOL1 risk alleles (Wt:Wt, mean age 53.1 ± 15.5) and those who carry two G1 risk alleles or compound heterozygotes (Table 1). The
mean age of dialysis initiation for patients who carry two \textit{APOL1} risk alleles (G1:G1 or G1:G2 or G2:G2) was 41.0 ± 8.3 years, 12 years earlier in comparison to those without risk alleles (\textit{t}-test FDR corrected \( P = 0.0003 \)). Notably, we found that the mean age of individuals who did not carry any \textit{APOL1} risk alleles (Wt:Wt) was not significantly different between Hispanic and African-Americans (\textit{t}-test \( P > 0.1 \) n.s.), however, the mean age of those who carry two-risk alleles was significantly lower in the Hispanic Americans (41.0 ± 8.3) compared with African-Americans (48.1 ± 14.3, \textit{t}-test \( P = 0.0028 \)), consistent with our previously reported larger effect of \textit{APOL1} risk alleles in Hispanic Americans [30].

Following the non-significant trend reported in Kanji et al. [29], we also sought to clarify a possible effect of the heterozygous G1 state on age of dialysis initiation. Notably, we found that dialysis initiation occurred at an age of ~5 years younger for G1 heterozygous non-diabetic African-Americans (Wt:G1, mean age 52.1 ± 15.6) compared with those that do not carry any risk allele (Wt:Wt, mean age 57.4 ± 16.1; FDR corrected \( P = 0.0452 \)). Combining the data as shown, using Fisher’s approach, we provided further evidence for a G1 heterozygote effect on age of dialysis initiation, reinforcing the results from our sample set alone (Table 1). This meta-analysis lowered the \( P \)-value reported by Kanji et al. [29] (\( P = 0.152 \)) to the combined \( P \)-value of \( P = 0.0411 \) for the G1 heterozygous single-risk allele state. No such difference in age at initiation of dialysis was observed for G2 heterozygotes (Wt:G2, mean age 58.0 ± 13.7, \( P > 0.1 \)). In the diabetic African-American samples and in the non-diabetic Hispanic American samples, we did not find a significant difference between the heterozygous states and individuals who did not carry any \textit{APOL1} risk alleles (\textit{t}-tests \( P > 0.1 \)). In the diabetic Hispanic American samples, we found a borderline significantly lower mean age for the heterozygous G1 (mean age, 53.7 ± 12.2, \textit{t}-test FDR corrected \( P = 0.0474 \)) but not for the two \textit{APOL1} risk allele individuals, due to the low frequency of \textit{APOL1} in that population. An additional or larger cohort of Hispanic Americans would be needed to clarify this association and determine its reliability and general applicability across the varied Hispanic heritage communities in different regions of the USA. We did not observe a significant effect of the G2 heterozygous state (Wt:G2) on dialysis initiation age in any of the sample sets (\textit{t}-test \( P > 0.1 \)).

We also investigated the effect of \textit{APOL1} mutations on dialysis vintage (Table 1). We found that in African-Americans with non-diabetic ESKD on dialysis for >5 years (\( n = 126 \)), almost half (48%) carry two \textit{APOL1} risk alleles in comparison to patients on dialysis for <2 years, where only 30% possess two \textit{APOL1} risk alleles (\( n = 108 \), Pearson \( \chi^2 \) \( P = 0.0034 \), Supplementary Figure S7). We also found a significant difference of almost 2 years in the average dialysis vintage between those who do not carry \textit{APOL1} risk alleles (Wt:Wt, mean 3.8 ± 3.6 years) compared to those who carry two risk alleles (G1:G1 or G1:G2 or G2:G2, mean 5.6 ± 5.0, \textit{t}-test FDR corrected \( P = 0.0230 \)). We did not observe a significant difference in dialysis vintage between the \textit{APOL1} G1 or G2 risk allele heterozygotes and no risk allele individuals in all sample sets. To determine whether the apparent association of the two \textit{APOL1} risk allele state with increased dialysis vintage is a primary \textit{APOL1} effect or rather related to the younger age of dialysis initiation, our study employed a linear regression analysis to the African-American samples. The regression included the following covariates: age of dialysis initiation, \textit{APOL1} genotype, BMI and percentage of African ancestry. It should be noted that other factors not available to us and that might also be associated with dialysis vintage, as noted in the Materials and methods, were not included in this analysis. For the non-diabetic African-American samples, the factors that showed a significant effect on dialysis vintage were age of dialysis initiation (\( \beta = -0.08, P = 1.81 \times 10^{-7} \)) and BMI (\( \beta = -0.09, P = 0.01 \)). In the linear regression analysis for the diabetic African-American samples, the only factor that showed an effect on dialysis vintage was the age of dialysis initiation (\( \beta = -0.06, P = 4.98 \times 10^{-6} \)). Similarly, in the Hispanic American samples, the age of dialysis initiation was also the only factor required to explain dialysis vintage, as indicated by linear regression performed on both the non-diabetic (\( \beta = -0.136, P = 1.48 \times 10^{-4} \)) and diabetic (\( \beta = -0.35, P = 0.046 \)) Hispanic Americans. There was no residual significant effect of \textit{APOL1} risk alleles on dialysis vintage independent of age of dialysis initiation. Accordingly, the mean age of dialysis initiation in diabetic and non-diabetic African-Americans is significantly lower among dialysis patients who have been on dialysis >5 years (57.1 ± 11, 47.5 ± 14.2) compared with dialysis patients who have been on dialysis <5 years (61.4 ± 12.7; 55.3 ± 15.5, Supplementary Figure S8).

\section*{Discussion}

The current study shows that African-American patients with non-diabetic ESKD with two \textit{APOL1} risk alleles (G1:G1 or G2:G1 or G2:G2) initiate dialysis at a mean age that is some 9 years earlier compared to those without \textit{APOL1} risk alleles (Wt:Wt). Concomitantly, ~45% of dialysis patients who initiated dialysis under the age of 60 have two \textit{APOL1} risk alleles compared with only ~25% among those who initiated dialysis after the age of 60 years of age. This difference was also evident in another cohort of non-diabetic ESKD Hispanic American patients, in whom a 12-year earlier age of dialysis onset was found in the group with two \textit{APOL1} risk alleles compared to those without \textit{APOL1} risk alleles. Our results are consistent with the findings of Kopp et al. [9] in a study of African-Americans with FSGS, who recently reported that African-American patients who carry two \textit{APOL1} risk alleles started dialysis at a mean age of 31.7 years, whereas patients who carry 0 or 1 \textit{APOL1} risk alleles started dialysis at a later mean age of 37.6 years. Since the focus of that one was on FSGS, which affects younger individuals compared to the other etiologies of ESKD associated with \textit{APOL1} risk alleles, it is not surprising that the overall age of dialysis initiation was younger in that study compared to the current one. Our results are also consistent with the findings of Kanji et al. [29] who also studied non-diabetic African-American ESKD patients and showed an almost 10 year difference in dialysis initiation age between the non-risk group with mean age of 61.8 versus the group with two \textit{APOL1} risk alleles with mean age of 49.0.
years. The finding that below the age of 75, 96% of the dialysis patients with two APOL1 risk alleles already initiated dialysis compared with 84% of the patients without APOL1 risk alleles, further indicates that APOL1 associated nephropathies affect mostly young non-diabetic African-Americans, while older patients are more likely to initiate dialysis due to end-stage kidney disease of other causes.

We also demonstrated a significant heterozygous effect for the G1 risk allele. Here too, the mean age of dialysis initiation is significantly younger by some 5 years in the group with genotype Wt:G1 compared to those without APOL1 risk alleles (Wt:Wt). A similar, however, statistically non-significant difference of 6 years was also seen by Kanji et al. [29] who used a conservative multiple comparisons correction. We did not detect a similar G1 heterozygous effect in the non-diabetic Hispanic cohort, possibly because of the smaller sample size in a population with a lower APOL1 risk allele frequency and hence lower statistical power. The effect of G1 heterozygosity was also suggested by the weaker but still significant additive effect that has been reported for this haplotype in several previous case-control association studies of kidney disease phenotypes [6, 7, 9, 12]. This is of importance to the design of case-control association studies of kidney disease etiology.

The finding that below the age of 75, 96% of the patients with two APOL1 risk alleles, further indicates that APOL1 associated nephropathies affect mostly young non-diabetic African-Americans, while older patients are more likely to initiate dialysis due to end-stage kidney disease of other causes.

In most studies, APOL1 mutations have not been associated with diabetic ESKD [2, 3, 11], and indeed, in the current study, diabetic ESKD individuals with two APOL1 risk alleles did not show significantly lower mean age of dialysis initiation, in both sample sets of African-Americans and Hispanic Americans. Interestingly, the frequency of two APOL1 risk mutations in the African-American diabetic ESKD patients under 45 was significantly higher (37%) compared with patients over 45 (20%, Supplementary Figure S9). This high frequency may reflect a misclassification of kidney disease etiology as diabetic ESKD, when in reality the disease is related to an APOL1 nephropathy, as has been previously surmised [39]. Genotyping of APOL1 mutations, in diabetic individuals with ESKD who are under the age of 45, may thus provide a more precise diagnosis of kidney disease etiology.

We also found a higher frequency of APOL1 risk alleles in individuals who have been on dialysis for a longer time (dialysis vintage). Almost half of the African-American non-diabetic ESKD patients who had been on dialysis for >5 years had two APOL1 risk alleles, compared with 30% in patients who had been on dialysis <2 years. Accordingly, in this population, patients with two APOL1 risk alleles have almost 2 years longer dialysis vintage (5.6 years) compared with those who do not carry any risk allele (3.8 years). This apparent interaction between APOL1 risk alleles and dialysis vintage (an indication of dialysis survival) was further evaluated using a multivariate analysis, which showed that the main covariate that affects dialysis vintage, based on the information available to us in our sample set, is age of dialysis initiation and not the APOL1 state per se (Figure 2). We find that by being associated with onset of dialysis at an earlier age, APOL1 risk alleles appear to confer an indirectly increased survival, as older dialysis patients have an expected decreased dialysis survival [23, 40]. Nevertheless, a recent study by Kucirka et al. [41] showed that young non-diabetic African-Americans with ESKD have higher mortality compared with European Americans. It now becomes both possible and important to determine whether there is a relationship of APOL1 risk nephropathies to this mortality discrepancy.

In conclusion, we confirm the observation that APOL1 risk alleles are associated with lower age of dialysis initiation in non-diabetic ESKD patients with two APOL1 risk alleles, 9 years earlier than those without APOL1 risk alleles in African-Americans and 12 years earlier in Hispanic Americans. This confirmation, in a second population group with an overall reduced percentage of African ancestry, may broaden the potential implications of APOL1 genotyping to other populations at potential risk. We also showed a clearly evident G1 heterozygous effect (Wt:G1) on a clinical renal disease
phenotype, namely—mean age of dialysis initiation in African-Americans, with the mechanistic implications that such a finding brings with it. It is also notable and consistent that we did not find an effect of APOL1 risk alleles on the age of dialysis initiation in diabetic ESKD patients. In the non-diabetic African-American patients above the age of 70, 92% of those who had two APOL1 risk alleles had already initiated dialysis compared with 76% of the patients without APOL1 risk alleles, indicating that older patients are more likely to reach dialysis due to non-APOL1 associated causes of ESKD. We can suggest that for African ancestry patients who have reached the age of 70 years with two APOL1 risk alleles without kidney disease, there is less concern regarding kidney failure due to APOL1 nephropathy. We additionally showed a high frequency of APOL1 mutations in individuals that have longer dialysis vintage and demonstrated that this survival advantage can be fully attributed to a lower age of dialysis initiation. While not likely, any possible direct effect of APOL1 on dialysis survival needs to be examined in longitudinal studies and in larger cohorts. Our findings demonstrate that APOL1 mutations produce a unique and distinct type of kidney disease that manifests itself at significantly younger ages in non-diabetic individuals of African ancestry.

Supplementary data

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

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Conflict of interest statement. None declared.

(See related article by Freedman and Langefeld. The new era of APOL1-associated glomerulosclerosis. Nephrol Dial Transplant 2012; 27: 1288–1291.)

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