Prevalence of Mendelian Kidney Disease Among Patients With High-Risk *APOL1* Genotypes Undergoing Commercial Genetic Testing in the United States

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Introduction: Among individuals with high-risk *APOL1* genotypes, the lifetime risk of developing kidney failure is ∼15%, indicating that other genetic variants or nongenetic modifiers likely contribute substantially to an individual patient's risk of progressive kidney disease. Here, we estimate the prevalence and distribution of Mendelian kidney diseases among patients with high-risk *APOL1* genotypes undergoing commercial genetic testing in the United States.

Methods: We analyzed clinical exome sequencing data from 15,181 individuals undergoing commercial genetic testing for Mendelian kidney disease in the United States from 2020 to 2021. We identified patients with high-risk *APOL1* genotypes by the presence of G1/G1, G1/G2, or G2/G2 alleles. Patients carrying single risk APOL1 alleles were identified as G1/G0, G2/G0; the remainder of patients were G0/G0. We estimated the prevalence and distribution of Mendelian kidney disease stratified by *APOL1* genotype and genetically predicted ancestry.

Results: Of 15,181 patients, 3119 had genetic testing results consistent with a molecular diagnosis of Mendelian kidney disease (20.5%). Of 15,181 patients, 1035 (6.8%) had high-risk *APOL1* genotypes. Among patients with recent genomic African ancestry, the prevalence of Mendelian kidney diseases was lower in those with high-risk *APOL1* genotypes (9.6%; *n* = 91/944) compared with single risk *APOL1* allele carriers (13.6%; *n* = 198/1453) and those with G0/G0 *APOL1* genotypes (16.6%; *n* = 213/1281). Among patients with Mendelian kidney disease and recent genomic African ancestry, we observed differences in the prevalence of pathogenic/likely pathogenic variants in *PKD1* (19.8% in high-risk vs. 30.2% in low-risk genotypes), and *COL4A4* (24.2% in high-risk vs. 10.5% in low-risk genotypes).

Conclusion: In this selected population of patients undergoing clinical genetic testing, we found evidence of Mendelian kidney disease in ∼10% patients with high-risk *APOL1* genotypes.

Americans with recent African ancestry are at increased risk of kidney failure compared with Americans of European descent, and much of this excess risk has been attributed to *APOL1*-mediated kidney disease.[1] Two risk alleles in *APOL1*, the gene encoding apolipoprotein-L1, have been identified as risk factors for the development and progression of chronic kidney disease.[2] These coding variants, termed G1 and G2, are present relatively at high frequencies among people of recent African descent in part because of the positive natural selection of these alleles as they confer a protective advantage against the trypanosomes that cause African sleeping sickness.[3,4] Approximately 13% of the Americans with recent African ancestry carry high-risk APOL1 genotypes, defined as having 2 high-risk alleles in APOL1 (G1/G1, G1/G2, or G2/G2).[4] The risk of developing kidney disease is 3- to 30-fold higher among persons with *APOL1* high-risk genotypes than those not carrying any *APOL1* risk alleles.[5] However, among persons with high-risk *APOL1* genotypes, the lifetime risk of kidney failure is ∼15%,

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indicating that other genetic variants, or nongenetic modifiers, including environmental factors, likely contribute substantially to an individual patient's risk of kidney disease.[1,2]

Recent studies have demonstrated that ∼10% of adult patients with chronic kidney disease have Mendelian forms of kidney disease,[6] and as such, clinical genetic testing to identify monogenic kidney diseases has become increasingly common.[7] The interaction between APOL1 risk alleles and other causes of kidney disease is an area of active research. For example, several studies have demonstrated that the presence of APOL1 high-risk genotypes is associated with worse disease trajectories than for patients with otherwise unrelated forms of kidney disease, and especially glomerular diseases (e.g., membranous nephropathy and systemic lupus erythematosus).[8-10] The relationship between APOL1 risk alleles and Mendelian kidney diseases, in contrast, has been understudied.

Here, we analyze real-world data from a large, ethnically diverse cohort of over 15,000 patients undergoing commercial clinical genetic testing for Mendelian kidney disease in the United States. We characterize the estimated prevalence and distribution of Mendelian kidney diseases in this cohort, stratified by APOL1 genotype, with the hypothesis that Mendelian kidney diseases may contribute to kidney disease among persons with high-risk APOL1 genotypes.

METHODS

This is a cross-sectional study of consented patients undergoing commercial genetic testing from April 1, 2020, to December 30, 2021. Patients were referred for genetic testing by their respective clinical providers. Demographic and clinical information collected at the time of testing includes age, race/ethnicity (either self-reported or designated by the clinical provider), sex (female or male), transplant status (yes/no), and International Classification of Diseases diagnostic codes. We received an exemption from institutional review board review (study ID 20099-03) from Ethical & Independent Review Services, Corte Madera, California. All data were deidentified to protect patient privacy. We excluded data from patients with missing information on age and/or sex. We also excluded persons undergoing clinical genetic testing in the setting of kidney donation.

Designated or Self-reported Race/Ethnicity

The following race and ethnicity categories were present on the intake form: African American, Ashkenazi Jewish, Caucasian, East Asian, French Canadian/Cajun, Hispanic, Mediterranean, Other, Sephardic Jewish, South Asian, and South-East Asian. To achieve a

sufficiently large sample size for further analysis, certain race/ethnic categories, namely French Canadian/Cajun, Sephardic Jewish, and Ashkenazi Jewish, were combined with the category labeled as "Other" because of the limited number of individuals. We have opted to use "European American" in place of "Caucasian' throughout this study to align with more accurate ethnic descriptors.[11,12] The term "Multiple" refers to samples with >1 self-reported race and ethnicity category.

Sequencing

Genomic DNA was extracted from either the individual's whole blood or saliva and processed for hybrid capture-based next-generation sequencing. Massively parallel sequencing was performed at 150 base-pairs, paired-end reads on a clinical exome backbone. The percentage of coverage was determined at a minimum of 20× per gene on the panel and Sanger sequencing was used to fill in regions of low coverage. The sequencing data were aligned to the GRCh37/hg19 genome assembly, and the variants were called and annotated using a bioinformatics pipeline that applied the Genome Analysis Toolkit framework.[13] Single nucleotide variants, insertions and deletions, and copy number variants were detected using this assay. Orthogonal methods for confirmation of variants included Sanger sequencing for single nucleotide variants and insertions and deletions and quantitative polymerase chain reaction or multiplex-ligation dependent probe amplification for copy number variants.

Genetic Ancestry Determination

The population structure and admixture analyses were conducted using the continental ancestry groups from the combined, harmonized databases of the Human Genome Diversity Project and the 1000 Genomes Project[14] We included single nucleotide variants with a minor allele frequency >0.05 and missing in <5% of individuals within our dataset to determine ancestry. We retained samples for further analysis with an overall genotype rate >90% (i.e., missing <10% of selected single nucleotide variants). We performed principal component analysis using SNPW 8 version 2[10] and PLINK v1.9,[15] and estimated global genomic ancestry proportions using Admixture v1.3[16] focusing on the following populations: African, admixed American, Central South Asia, East Asian, and European.

In additional analyses, we assigned a single ancestry group to each individual patient via a clustering method derived from the principal component analysis dimensions. We used the first 10 principal components

and applied the Uniform Manifold Approximation and Projection algorithm, via the Umap package in R.[17] The Uniform Manifold Approximation and Projection outputs were clustered using the Hierarchical Density-Based Spatial Clustering of Applications with Noise algorithm, via the dB scan R package.[18] Five distinct clusters were identified, corresponding to inferred continental ancestries. These clusters aligned well with the predominant admixture proportions for their respective ancestry groups. We were also able to categorize samples with unknown self-designated or self-reported race/ethnicity into 1 of the 5 distinct ancestry clusters. Therefore, individuals with recent African ancestry were defined as those clustered within the African genetic cluster according to the first 10 principal components in the principal component analysis. This approach was similarly used to define the remaining ancestry groups.

Interpretation of Genetic Variants

Variants were analyzed in 343 genes associated with Mendelian forms of kidney disease (Supplementary Table S1), including the risk alleles associated with APOL1. Detected germline variants were classified using a 5-tier classification system (B, benign; LB, likely benign; VUS, variant of uncertain significance; LP, likely pathogenic; and P, pathogenic) in accordance with the American College of Medical Genetics and Genomics and the Association for Molecular Pathology guidelines at the time of testing.[19] As part of routine clinical care, reports of identified variants and their relevant American College of Medical Genetics and Genomics and the Association for Molecular Pathology classifications were returned to the referring clinicians and patients at the time of testing.

Identifying Patients with Mendelian Kidney Diseases

Accurate diagnosis of Mendelian forms of kidney disease requires genotype-phenotype correlation based on variant classification information combined with the mode of inheritance for the disease. We classified individuals as having Mendelian kidney disease based on the presence of P/LP variants, meeting criteria informed by empirical data.[19] We considered a test "positive" or consistent with a molecular diagnosis of Mendelian kidney disease if (i) the individual has a heterozygous or hemizygous P/LP variant in a gene associated with a dominant or X-linked inheritance pattern; or (ii) the patient has 2 P/LP variants (compound heterozygous or homozygous) in a gene with a recessive inheritance pattern. For genes with both dominant and recessive patterns of inheritance, we considered each variant's specific phenotype, and the

associated pattern of inheritance was considered. For HBB, only results consistent with autosomal recessive sickle cell anemia were included as "positive." We considered the individuals harboring heterozygous P/LP variants in genes associated with an autosomal recessive inheritance as carriers. The remaining samples were considered negative.[19]

APOL1 Genotype Status Definition

We assessed 2 APOL1 high-risk alleles: (i) G1 (NM_003661.4:c.[1024A>G;1152T>G]) defined by the presence of 2 missense variants p.S342G (rs73885319) and p.I384M (rs60910145) that are nearly always in linkage disequilibrium; (ii) G2 (NM_003661.4:c.1164_1169del) defined by the presence of an in-frame deletion of 2 amino acid residues at codons 388 and 389, respectively (rs71785313). We defined APOL1 high-risk genotypes by the presence of 2 high-risk alleles (G1/G1, G1/G2, or G2/G2). We defined single risk APOL1 allele carriers by the presence of only 1 risk allele (G1/G0 or G2/G0). The lack of APOL1 risk alleles was denoted as (G0/G0). We deliberately considered single risk APOL1 allele carriers because, by definition, these patients have recent African ancestry.

Statistical Analysis

Continuous variables were summarized by means, medians, and interquartile ranges, where appropriate. Comparisons of continuous variables across groups were made via Wilcoxon rank sum test. We described categorical variables using proportions and compared groups using Fisher's exact test. A P-value <0.05 was considered statistically significant. Analyses were conducted in R version 4.1.2.

RESULTS

Prevalence of Mendelian Disease in the Overall Cohort

A total of 15,532 individuals underwent clinical genetic testing from April 1, 2020, to December 30, 2021, of whom 15,181 (97.7%) had sufficient data for further analysis (Figure 1). The median age at the time of testing was 47 years (25%, 75% range 30–62 years), with ~50% of the patients identified as female (Table 1). Fewer than 3% ($n = 492$) had received a kidney transplant before the time of testing. Of 15,181 patients, 3119 had a molecular diagnosis of Mendelian kidney disease (20.5%; Table 1), defined by the presence of pathogenic or likely pathogenic variants (P/LP) consistent with the inheritance pattern of the specific disease. Of the total, 5049 (33.3%) patients were classified as carriers of Mendelian kidney disease, and 7013 (46.2%) were negative for P/LP variants in any of the
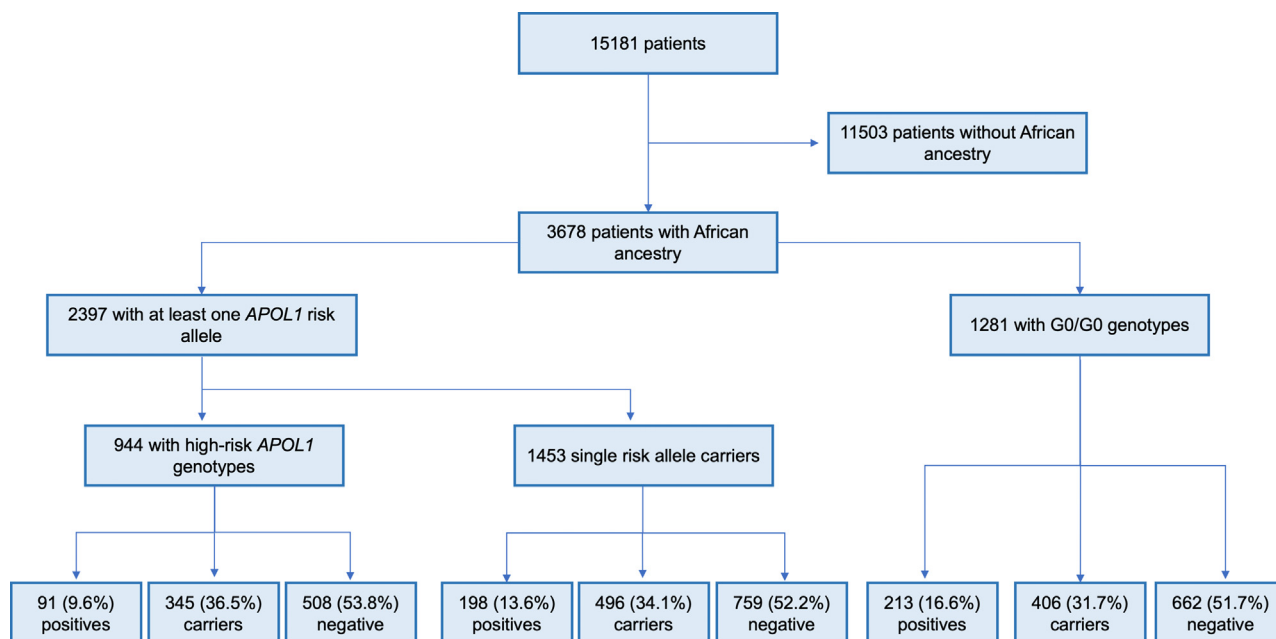
Figure 1. Flowchart of patients included in this study. Positive samples refer to the number of patients with Mendelian kidney diseases. Carriers were defined as patients harboring heterozygous P/LP (pathogenic/likely pathogenic) variants in genes associated with a recessive inheritance pattern. The remaining samples were considered negative.

343 genes evaluated (Supplementary Table S1). Patients with Mendelian kidney disease were younger on average, at the time of testing, compared with carriers or patients with negative test results (40 vs. 48 years, $P <0.0005$; Table 1).

Self-reported or Designated Race/Ethnicity and Genomic Ancestry in the Overall Cohort

Based on the self-reported or designated race/ethnicity of each patient, the majority of samples were collected from European Americans (37.1%, $n = 5718$), followed by African Americans (19.2%, $n = 2960$), and Hispanic persons (12.9%, $n = 1989$), whereas the remaining samples were collected from persons from other race/ethnicities (29.7%, $n = 4514$; Table 1). Two hundred forty-one (1.6%) individuals had a self-reported multiracial/multiethnic designation and 23% of patients in the overall cohort had unknown race/ethnicity information. We used a panel of ancestry informative markers from the clinical exome sequencing data to predict each patient's genetic ancestry. We found that 49.1% ($n = 7458$) of patients were assigned to the European genetic cluster, 24.2% ($n = 3678$) to the African genetic cluster, 15.2% ($n = 2313$) to the Admixed American cluster, and 7.5% ($n = 1137$) to the Asian cluster. A total of 595 samples (3.9%) were excluded from this analysis because of quality control steps (Table 1). Admixture analysis revealed that the patients in the African cluster had a median inferred continental African ancestry of 70% (interquartile range: 63%–76%) (Supplementary Table S2).

Approximately 74% ($n = 2718$) of individuals in the African ancestry cluster had a self-reported or designated race/ethnicity of (Supplementary Table S3). Overall, self-reported or designated race/ethnicity was highly concordant with genetic ancestry (Supplementary Figure S1 and Supplementary Tables S2–S4).

Prevalence of APOL1 Risk Alleles in the Overall Cohort

Of 15,181 individuals, 1035 (6.8%) had high-risk *APOL1* genotypes (Figure 1). Homozygous haplotypes G1/G1 and G2/G2 accounted for 43.7% ($n = 452$) and 12.9% ($n = 134$) of patients, respectively; 449 patients with high-risk *APOL1* genotypes had 1 G1 allele and 1 G2 allele (43.4%; G1/G2). Of the total, 1687 (11.1%) patients were single-risk *APOL1* allele carriers, of which 1064 were with G1 (63.1%), and 623 with G2 (36.9%); 12459 patients were G0/G0 (82.1%).

The majority of patients with high-risk *APOL1* genotypes had self-reported or designated African American race (815/1035; 78.9%; Supplementary Table S5). Single-risk allele carriers had a high proportion of self-reported African American race as well (1158/1687; 68.6%; Supplementary Table S5). As expected, individuals carrying *APOL1* variants predominantly overlapped with the African ancestry cluster from reference continental population samples (Supplementary Figure S2). However, individuals with G0/G0 genotypes had a much lower proportion of self-reported African American race (987/12459; 7.9%),

Table 1. Clinical and demographic characteristics of patients, stratified by Mendelian kidney disease status in the overall cohort

Category	Total[a]	Mendelian Disease[b]	Carriers[b]	Negative[b]
N	15181 (100)	3119 (20.5)	5049 (33.3)	7013 (46.2)
Gender & Age				
Female	7606 (50.1)	1723 (22.7)	2454 (32.3)	3429 (45.1)
Male	7575 (49.9)	1396 (18.4)	2595 (34.3)	3584 (47.3)
Median age yr (IQR)	47 (30–62)	40 (23–56)	49 (32–64)	48 (31–63)
Self-reported race & ethnicity				
African American	2960 (19.2)	377 (12.7)	1017 (34.4)	1566 (52.9)
East Asian	230 (1.5)	62 (27)	64 (27.8)	104 (45.2)
European American	5718 (37.1)	1303 (22.8)	2076 (36.3)	2339 (40.9)
Hispanic	1989 (12.9)	437 (22)	502 (25.2)	1050 (52.8)
Mediterranean	93 (0.6)	16 (17.2)	33 (35.5)	44 (47.3)
Other	570 (3.7)	129 (22.6)	185 (32.5)	256 (44.9)
South-East Asian	330 (2.1)	76 (23)	90 (27.3)	164 (49.7)
Unknown	3542 (23)	775 (21.9)	1171 (33.1)	1596 (45.1)
Genetic cluster				
Admixed American	2313 (15.2)	501 (21.7)	598 (25.9)	1214 (52.5)
African	3678 (24.2)	502 (13.6)	1247 (33.9)	1929 (52.4)
Central-South Asian	368 (2.4)	79 (21.5)	107 (29.1)	182 (49.5)
East Asian	769 (5.1)	175 (22.8)	208 (27)	386 (50.2)
European	7458 (49.1)	1777 (23.8)	2726 (36.6)	2955 (39.6)
Unclustered samples[c]	595 (3.9)	85 (14.3)	163 (27.4)	347 (58.3)
Clinical diagnosis[d]				
Chronic kidney disease	8267 (49.1)	1620 (19.6)	2824 (34.2)	3823 (46.2)
Cystic kidney disease	1466 (8.7)	658 (44.9)	398 (27.1)	410 (28)
End-stage kidney disease	1616 (9.6)	222 (13.7)	524 (32.4)	870 (53.8)
Hematuria	584 (3.5)	169 (28.9)	165 (28.3)	250 (42.8)
Hypertension	467 (2.8)	49 (10.5)	147 (31.5)	271 (58)
Kidney transplant	432 (2.6)	73 (16.9)	138 (31.9)	221 (51.2)
Proteinuria/nephrotic syndrome	1024 (6.1)	167 (16.3)	354 (34.6)	503 (49.1)
Others	1554 (9.2)	305 (19.6)	518 (33.3)	731 (47)
None available	1431 (8.5)	269 (18.8)	506 (35.4)	656 (45.8)

IQR, interquartile range.
[a]column-based proportions.
[b]row-based proportions.
[c]No. of Samples excluded during PCA filtering steps due to missing the minimum number of single-nucleotide variants after quality control.
[d]Clinical diagnoses are based on ICD codes, summarized into the categories presented. The total count exceeds the number of unique patients due to the presence of multiple ICD codes in some patients.

because this group also included samples from non-African clusters. When considering genetically predicted ancestry, 91.2% of patients with high-risk *APOL1* genotypes ($n = 944/1035$), 86.1% of patients with single-risk alleles ($n = 1453/1687$), and 10.3% of patient with G0/G0 were assigned to the African genetic cluster ($n = 1281/12459$; Supplementary Figure S3 and Supplementary Table S5). Given that high-risk *APOL1* genotypes only occur in a background of recent African ancestry, and non-African individuals are unlikely to carry *APOL1* risk variants, we focused our analysis exclusively on the samples within the African ancestry cluster.

Prevalence of Mendelian Kidney Disease Stratified by APOL1 Genotype Among Patients with Recent African Ancestry

We subset patients from the overall cohort who belonged to the African genetic cluster for further analysis ($n = 3678/15181$; 24.2%, Table 1). Of 3678 patients with recent African ancestry, 502 had a diagnosis of Mendelian kidney disease (Table 2), a prevalence lower than in those with recent European ancestry (13.6% vs. 23.8%, $P <0.00001$; Supplementary Table S5). Of the total, 1247 (33.9%) of the patients with recent African ancestry were classified as carriers of Mendelian kidney disease, and 1929 (52.4%) were negative (Table 2).

Considering *APOL1* genotypes, 25.6% of patients in the genetic African ancestry cluster had high-risk *APOL1* genotypes ($n = 944$), 39.5% were single risk allele carriers ($n = 1453$), and 34.8% had G0/G0 ($n = 1281$; Table 2 and Supplementary Figure S3). The prevalence of Mendelian kidney disease varied across *APOL1* genotypes among patients in the African genetic cluster: 9.6% of patients with high-risk *APOL1* genotypes had a concomitant diagnosis of Mendelian kidney disease compared with 13.6% of single risk

Table 2. Clinical and demographic characteristics among patients with recent African ancestry, stratified by Mendelian kidney disease status

Categories	All	High risk genotype	Single risk allele carriers	G0/G0
	3678	944	1453	1281
Gender & age				
Female	1776 (48.3)	435 (46.1)	691 (47.6)	650 (50.7)
Male	1902 (51.7)	509 (53.9)	762 (52.4)	631 (49.3)
Age yr (IQR)	46 (31–60)	45 (32–57)	47 (31–61)	47 (27–61)
Mendelian status				
Affected	502 (13.6)	91 (9.6)	198 (13.6)	213 (16.6)
Carrier	1247 (33.9)	345 (36.5)	496 (34.1)	406 (31.7)
Negative	1929 (52.4)	508 (53.8)	759 (52.2)	662 (51.7)
Self-reported race & ethnicity				
African American	2718 (73.9)	746 (79)	1093 (75.2)	879 (68.6)
East Asian	1 (0)	0 (0)	0 (0)	1 (0.1)
European American	51 (1.4)	6 (0.6)	13 (0.9)	32 (2.5)
Hispanic	115 (3.1)	14 (1.5)	27 (1.9)	74 (5.8)
Mediterranean	1 (0)	0 (0)	0 (0)	1 (0.1)
Other	107 (2.9)	27 (2.9)	34 (2.3)	46 (3.6)
Unknown	685 (18.6)	151 (16)	286 (19.7)	248 (19.4)
Genetic admixture proportion				
AFR	0.7 (0.63–0.76)	0.72 (0.66–0.77)	0.7 (0.63–0.76)	0.69 (0.59–0.76)
AMR	0.01 (0–0.06)	0.02 (0–0.06)	0.01 (0–0.05)	0.02 (0–0.06)
CSA	0 (0–0.04)	0 (0–0.04)	0 (0–0.04)	0 (0–0.05)
EAS	0.01 (0–0.05)	0 (0–0.05)	0.01 (0–0.05)	0.01 (0–0.05)
EUR	0.22 (0.15–0.29)	0.2 (0.15–0.27)	0.22 (0.15–0.29)	0.23 (0.16–0.31)
Clinical diagnosis[a]				
Chronic kidney disease	2136	528 (24.7)	866 (40.5)	742 (34.7)
End-stage kidney disease	572	221 (38.6)	203 (35.5)	148 (25.9)
Proteinuria/nephrotic syndrome	279	73 (26.2)	111 (39.8)	95 (34.1)
Cystic kidney disease	233	33 (14.2)	97 (41.6)	103 (44.2)
Hypertension	145	27 (18.6)	57 (39.3)	61 (42.1)
Hematuria	85	16 (18.8)	36 (42.4)	33 (38.8)
Kidney transplant	61	22 (36.1)	20 (32.8)	19 (31.1)
Others	272	54 (19.9)	95 (34.9)	123 (45.2)
None available	286	66 (23.1)	119 (41.6)	101 (35.3)

AFR, African; AMR, Admixed American; CSA, Central/South Asian; EAS, East Asian; EUR, European.
[a]Clinical diagnoses are based on ICD codes.

allele carriers and 16.6% of patients with G0/G0 (Table 2; P <0.01 for single risk allele vs. high-risk, P <0.00001 for G0/G0 vs. high-risk). We observed a higher prevalence of high-risk *APOL1* genotypes (n = 853/3176, 26.9%) among patients without Mendelian kidney disease when compared with patients with Mendelian kidney disease (n = 91/502; 18.1%, P <0.0012).

Patients with Mendelian kidney disease had similar ages at the time of testing across *APOL1* genotypes (36–41 years) and tended to be younger than patients without Mendelian kidney disease (Table 3). We did not find evidence that patients with high-risk APOL1 genotypes and Mendelian kidney disease present for clinical genetic testing at younger ages than patients without high-risk *APOL1* genotypes and Mendelian kidney disease (Table 3, P = 0.357 for single risk allele vs. high-risk; P = 0.409 for G0/G0 vs. high risk).

Distribution of Common Mendelian Kidney Diseases by APOL1 Genotype Among Patients with Recent African Ancestry

We grouped Mendelian forms of kidney disease into 5 nonexclusive categories: (i) cystic and tubulointerstitial diseases, (ii) glomerular diseases, (iii) tubulopathies and other tubular diseases, (iv) congenital anomalies of the kidney and urinary tract and other structural diseases, and (v) complement-related kidney diseases (Supplementary Table S1). Among patients with recent African ancestry and Mendelian kidney disease, 40.7% had cystic and tubulointerstitial diseases, 33.4% had glomerular diseases, 15.7% tubulopathies and other tubular diseases, 8.8% had congenital anomalies of the kidney and urinary tract and other structural diseases and 1.4% had complement-related diseases.

The prevalence of cystic and tubulointerstitial diseases was significantly different between patients with high-risk *APOL1* genotypes (n = 29/944, 3.07%) and

Table 3. Demographic data of patients included, stratified by presence of APOL1 risk alleles among patients with recent genomic African ancestry

| Category	All patients	High-risk APOL1 genotype				Single risk allele carriers				G0/G0			
		All	Positive	Carrier	Negative	All	Positive	Carrier	Negative	All	Positive	Carrier	Negative
N	3678	944 (25.7)	91 (9.6)	345 (36.5)	508 (53.8)	1453 (39.5)	198 (13.6)	496 (34.1)	759 (52.2)	1281 (34.8)	213 (16.6)	406 (31.7)	662 (51.7)
Gender & Age													
Female	1776	435 (24.5)	53 (12.2)	145 (33.3)	237 (54.5)	691 (38.9)	112 (16.2)	223 (32.3)	356 (51.5)	650 (36.6)	122 (18.8)	194 (29.8)	334 (51.4)
Male	1902	509 (26.8)	38 (7.5)	200 (39.3)	271 (53.2)	762 (40.1)	86 (11.3)	273 (35.8)	403 (52.9)	631 (33.2)	91 (14.4)	212 (33.6)	328 (52)
Median Age yr (IQR)	46 (31–60)	45 (32–57)	37 (20–52)	46 (35–57)	46 (33–59)	47 (31–61)	41 (23–54)	47 (31–63)	48 (34–61)	47 (27–61)	36 (14–50)	49 (34–64)	49 (29–63)
Self-reported race & ethnicity													
African American	2768	758 (27.4)	71 (9.4)	281 (37.1)	406 (53.6)	1112 (40.2)	148 (13.3)	386 (34.7)	578 (52)	898 (32.4)	139 (15.5)	291 (32.4)	468 (52.1)
East Asian	1	0	0	0	0	0	0	0	0	1 (100)	0	0	1 (100)
European American	69	9 (13)	1 (11.1)	3 (33.3)	5 (55.6)	21 (30.4)	4 (19)	6 (28.6)	11 (52.4)	39 (56.5)	5 (12.8)	8 (20.5)	26 (66.7)
Hispanic	137	18 (13.1)	0	10 (55.6)	8 (44.4)	34 (24.8)	9 (26.5)	12 (35.3)	13 (38.2)	85 (62)	19 (22.4)	23 (27.1)	43 (50.6)
Mediterranean	2	0	0	0	0	1 (50)	0	0	1 (100)	1 (50)	0	1 (100)	0
Other	70	21 (30)	2 (9.5)	10 (47.6)	9 (42.9)	17 (24.3)	2 (11.8)	8 (47.1)	7 (41.2)	32 (45.7)	4 (12.5)	10 (31.2)	18 (56.2)
South-East Asian	2	0	0	0	0	1 (50)	0	0	1 (100)	1 (50)	1 (100)	0	0
Unknown	685	151 (22)	18 (11.9)	49 (32.5)	84 (55.6)	286 (41.8)	38 (13.3)	94 (32.9)	154 (53.8)	248 (36.2)	47 (19)	82 (33.1)	119 (48)

low-risk genotypes ($n = 206/2734$, 7.53%, including both single risk allele carriers and G0/G0, $P <0.00001$; Supplementary Table S6). In contrast, the prevalence of Mendelian glomerular diseases was higher in those with high-risk APOL1 genotypes (53.85%, $n = 49/91$) than those with low-risk genotypes (35.04%, $n = 144/411$, $P <0.004$; Supplementary Table S6).

The differences in the prevalence of glomerular and cystic/tubulointerstitial diseases are predominantly driven by variants identified in COL4A4 and PKD1, respectively. We found that the prevalence of Alport syndrome/thin basement membrane disease because of P/LP variants in COL4A4 varied across APOL1 genotypes, comprising 24.2% of patients with Mendelian kidney and high-risk APOL1 genotypes and 10.5% of patients with low risk APOL1 genotypes (including both G0/G0 and single-risk allele carriers, $P = 0.0009$). Similarly, the prevalence of autosomal dominant polycystic kidney disease because of P/LP variants in PKD1 was 30.2% among patients with Mendelian kidney disease and low risk APOL1 genotypes, and 19.8% among patients with Mendelian kidney disease and high-risk APOL1 genotypes ($P < 0.05$). The most common forms of Mendelian kidney disease across the APOL1 genotypes are presented in Figure 2 and Supplementary Figure S4. The full list of Mendelian kidney diseases is presented in Supplementary Table S7.

DISCUSSION

Because the lifetime risk of developing kidney failure among persons with high-risk APOL1 genotypes is ~15%, there is a need to understand how other genetic and nongenetic modifiers contribute to the risk of kidney disease and kidney disease progression.[20] Here, we studied Mendelian kidney disease in individuals with high-risk APOL1 genotypes using genomic data derived from a multiethnic cohort of >15,000 patients who underwent commercial clinical genetic testing for Mendelian kidney disease in the United States.

We found that 9.6% of patients with high-risk APOL1 genotypes who underwent genetic testing in this cohort had concurrent Mendelian kidney diseases. To our knowledge, only 1 single-center study ($n = 239$ patients with high-risk APOL1 genotypes) has explored the prevalence of Mendelian kidney disease among patients with chronic kidney disease and APOL1 high-risk genotypes (excluding patients with congenital anomalies of the kidney and urinary tract and polycystic kidney disease),[20] demonstrating that 2.5% of these patients had concurrent Mendelian kidney diseases. In contrast, our cohort of patients undergoing commercial clinical genetic testing had a much higher prevalence, likely explained by different
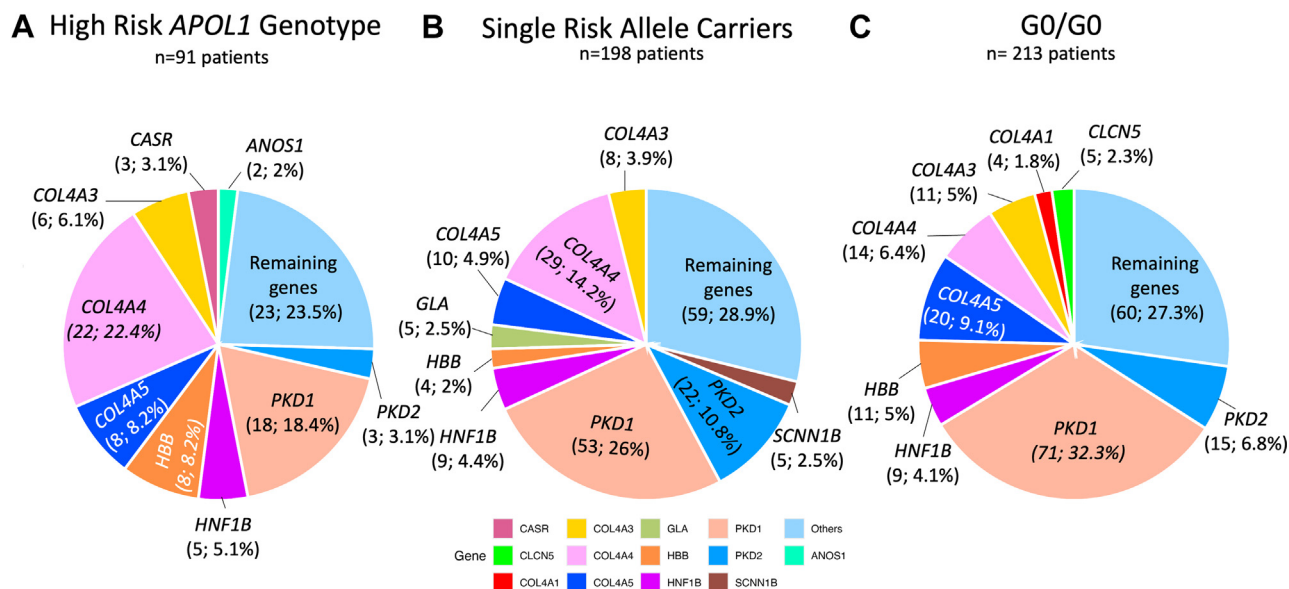
Figure 2. Most common genes affected among patients with recent African ancestry and Mendelian kidney diseases stratified by APOL1 genotype. Proportional representation (*n*, %) of most common genes in patients with recent African ancestry and (a) high-risk APOL1 genotypes, (b) single-risk allele carriers and (c) G0/G0 patients.

patient selection in our highly selected cohort of patients undergoing clinical genetic testing. Nonetheless, our data highlights the potential contribution of Mendelian forms of kidney disease in patients with high-risk *APOL1* genotypes. The majority of patients with high-risk *APOL1* genotypes and concurrent Mendelian kidney diseases in our cohort had glomerular diseases (53.85%), dominated by variants in Alport syndrome genes: *COL4A3*, *COL4A4* and *COL4A5*. Because patients with *APOL1*-mediated kidney disease and Alport syndrome can present with overlapping clinical (e.g., hypertension and proteinuria) and histologic findings on kidney biopsy (e.g., focal segmental glomerulosclerosis), this finding may support consideration of broader diagnostic genetic testing in a subset of patients with high-risk *APOL1* genotypes.

Our analysis also demonstrated that patients with recent African ancestry had lower prevalence of Mendelian kidney disease than patients with more remote African Ancestry or patients without African ancestry. This finding aligns with existing research indicating a generally lower diagnostic yield for genetic diseases in African American patients with kidney disease.[21-23] Such discrepancies might be attributed to the underrepresentation of African Americans in genomic sequencing studies.[23-25] This work highlights the potential need for more equitable genetic disease identification across racial and ethnic groups, including African Americans, a critical area for future research.

We found differences in the distribution of Mendelian kidney diseases among patients with recent

African ancestry and different *APOL1* genotypes. Specifically, we found that P/LP variants in *PKD1* were less frequent, and P/LP variants in *COL4A4* were more frequent, among patients with high-risk *APOL1* genotypes compared to single risk allele carriers or those with G0/G0. These findings are interpreted with caution due to the relatively small number of patients evaluated (e.g., 91 individuals with Mendelian kidney disease and high-risk *APOL1* genotypes), and the highly selected nature of the cohort. Future studies are warranted to evaluate how the type of Mendelian kidney disease may vary with *APOL1* genotype status.

Several limitations in this study should be acknowledged. First, our cohort is composed of a highly selected population of patients undergoing clinical genetic testing for Mendelian kidney disease; we had limited clinical information, and we can only infer (but not document) that these patients had kidney disease and that a genetic cause was contemplated. As such, we should be cautious in extrapolating these findings to unrestricted populations of patients with chronic kidney disease. The lack of clinical information prevents genotype-phenotype correlation because we have no information on any patient's phenotype. Despite this limitation, we note that variants classified as P/LP meet stringent criteria, and in a patient population selected for clinical genetic testing, the likelihood that such variants contribute causally to a patient's kidney disease would be high. Second, our analysis focused on a set of 343 genes known to be associated with kidney disease, which, although extensive, may not encompass all possible genetic

variants contributing to kidney disease. We also cannot rule out Mendelian kidney disease in negative cases because there may be clinically significant variants within the 343 genes that have either not been captured by the assay or not classified as P/LP based on the variant classification guidelines, which are subject to updates over time.

Overall, our data suggest that Mendelian kidney disease may be an important contributor in a fraction of patients with high-risk *APOL1* genotypes. Future research to confirm these findings in diverse, unselected cohorts, and to further elucidate the interaction between Mendelian kidney disease and *APOL1* risk alleles is needed.

DISCLOSURE

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AUTHORS CONTRIBUTIONS

RSFJ developed the research idea and study design, did the data analysis and interpretation, as well as statistical analysis. SP developed theresearch idea and study design, data analysis and interpretation. LV developed the research idea and study design, data analysis and interpretation. NS developed research idea and study design, data analysis and interpretation. VB worked on data interpretation. GMC worked on data interpretation. DKK developed the research idea and study design, data analysis and interpretation. VC developed the research idea and study design, data analysis and interpretation, statistical analysis. Each author contributed important intellectual content during manuscript drafting or revision and agrees to be personally accountable for the individual's own contributions and to ensure that questions pertaining to the accuracy or integrity of any portion of the work, even one in which the author was not directly involved, are appropriately investigated and resolved, including with documentation in the literature if appropriate.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Comparison of self-reported or designated race/ethnicity with genomic ancestry derived from HGDP and 1KG.

Figure S2. Population Stratification of individuals harboring APOL1 risk alleles.

Figure S3. Flowchart of patients with recent African ancestry included in this study.

Figure S4. Most common genes among all patients with Mendelian kidney diseases stratified by APOL1 genotype included in this study.

Table S1. Comprehensive list of 343 genes implicated in Mendelian kidney diseases.

Table S2. Distribution of global ancestry proportions across five continental populations in genetic clusters from principal component analysis.

Table S3. Comparative analysis of self-reported or designated race/ethnicity and genetic clusters derived from principal component analysis.

Table S4. Global ancestry proportions in five continental populations based on self-reported or designated race/ethnicity.

Table S5. Demographic data of patients stratified by presence of APOL1 risk alleles in the overall cohort.

Table S6. Prevalence and association between APOL1 high-risk genotype and APOL1 low-risk genotypes among the Mendelian kidney disease categories in patients with recent African ancestry.

Table S7. Frequency of Mendelian kidney disease stratified by APOL1 genotype in the overall cohort.

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