Kidney360

Apolipoprotein L1 Gene Testing Comes of Age

Barry I. Freedman[1] *and Chris P. Larsen*[2]

KIDNEY360 1: 58–61, 2020. doi: https://doi.org/10.34067/KID.0000162019

The landscape in nephrology changed dramatically in 2010 with the discovery of genetic association between two coding renal-risk variants (RRVs) in the apo L1 gene (*APOL1*) and CKD (1). The *APOL1* G1 and G2 nephropathy-associated variants arose <10,000 years ago and are present almost exclusively in individuals with recent African ancestry, those with similar genetic makeup to populations currently residing in Africa. The frequencies of these variants rapidly increased, likely due to positive selection for protection afforded from *Trypanosoma brucei rhodesiense* infection, a parasite causing African sleeping sickness. Due to the trans-Atlantic slave trade, approximately 39% of African Americans possess one *APOL1* RRV and 13% possess two. In contrast, hemoglobin S (*HbS*) gene variants are also common in African Americans; however, <8% inherit one or two *HbS* variants.

Ancestry-based variation in *APOL1* contributes to the excess risk of ESKD in African Americans, more rapid failure of transplanted kidneys from African American deceased donors, and higher rates of nephropathy in African American living-kidney donors (2,3). The kidney disease historically attributed to hypertension in nondiabetic African Americans with low-level proteinuria also proved to be *APOL1* associated, with biopsy findings including solidified glomerulosclerosis and thyroidization-type tubular atrophy (4). In addition, the *APOL1* spectrum of kidney disease includes FSGS, HIV-associated nephropathy (HIVAN), severe lupus nephritis, and sickle cell nephropathy (5). *APOL1* RRVs are associated with nephropathy in individuals with recent African ancestry residing in the United States, Africa, Europe, South America, and the Caribbean. Beyond genetic association, *APOL1* RRVs were shown to directly cause kidney disease in a transgenic mouse model (6). Recent studies show that *APOL1* kidney risk variants do not associate with cardiovascular disease (7,8). Despite the evidence that *APOL1* risk variants lead to kidney disease, the precise pathogenic mechanisms are not known. Data suggest a toxic gain of function conferred by RRVs and there is evidence to support that early mitochondrial injury may initiate cell death and scarring (9,10).

These findings are changing clinical practice and informing basic research. This *Kidney360* Perspective reviews potential indications for *APOL1* genotyping (Table 1). The field is rapidly evolving and developments will likely necessitate more frequent testing in the future. As opposed to diseases resulting from multiple mutations in a single gene that require testing several affected family members (*e.g.*, autosomal dominant polycystic kidney disease), *APOL1* genotyping is simple, relatively inexpensive, and can be performed in hours. Although *APOL1*-associated nephropathy is inherited in an autosomal recessive fashion, only a minority with high-risk genotypes (two *APOL1* RRVs: G1G1, G2G2, or G1G2) develops CKD in their lifetime. Disease modifiers exist. HIV infection and IFN administration are examples of *APOL1*-environment interactions leading to kidney disease.

Before ordering *APOL1* testing, physicians must counsel patients about privacy concerns and interpretation of results. The Genetic Information and Nondiscrimination Act of 2008 protects privacy and prevents discrimination based on the results of genetic testing in employment and health insurance. To reduce anxiety, individuals should be informed that the majority with *APOL1* high-risk genotypes, normal kidney function, and no albuminuria will not develop CKD. Importantly, patients should be advised to reduce exposure to modifiable kidney disease risk factors regardless of their genotype. This includes a heart healthy diet, active lifestyle, and smoking avoidance. Armed with this information, interpretation of *APOL1* genotype results should be straightforward for patients and clinicians.

APOL1 genotyping is often performed in the setting of kidney transplantation (2). After living-kidney donation, African Americans develop advanced nephropathy at higher rates than European Americans. Data suggest an important role for *APOL1* in this disparity (3). Many transplant programs perform *APOL1* genotyping in the evaluation of living-kidney donor candidates with recent African ancestry, particularly younger donors. This is an attempt to minimize future nephropathy by avoiding nephrectomies in currently healthy individuals with high-risk genotypes.

Kidneys transplanted from African American deceased donors fail more rapidly than those from European American deceased donors (2). As a result, African American deceased-donor kidneys are generally perceived to be at higher risk for shortened allograft survival. Decisions on the allocation of deceased-donor kidneys are largely based on the assessment of organ quality using the Kidney Donor

[1]Department of Internal Medicine, Section on Nephrology, Wake Forest School of Medicine, Winston-Salem, North Carolina; and [2]Arkana Laboratories, Little Rock, Arkansas

Correspondence: Dr. Barry I. Freedman, Section on Nephrology, Wake Forest School of Medicine, 1 Medical Center Boulevard, Winston-Salem, NC 27157-1053. Email: bfreedma@wakehealth.edu

Table 1. Clinical scenarios in which *APOL1* genotyping may be useful

Clinic Location	Potential Indication	Comments
Transplant clinic	Evaluation of living-kidney donor candidates	Await NIH APOLLO Study results
Nephrology clinic	Improve compliance in HIV^+ patients at risk for HIV-associated nephropathy	Highly active antiretroviral therapy curative
Nephrology clinic	Improve compliance in patients with SLE at risk for severe lupus nephritis or ESKD	
Nephrology clinic	Detect possible nondiabetic nephropathy in individuals with T2D and CKD	In those who have not undergone a diagnostic kidney biopsy
Nephrology clinic	Patients with bland chronic injury or FSGS on kidney biopsy	Provides more definitive diagnosis of underlying etiology
Medicine clinic	Prior to IFN administration	
Family planning	Screen parents from families with multiple members having ESKD	
Clinical research	Test novel therapies for *APOL1* nephropathy	APOL1 small molecule inhibitors and antisense oligonucleotides may soon be tested
Clinical research	Rapid evaluation of deceased donors before allocation of kidneys for transplantation	Await NIH APOLLO Study results

Scenarios are limited to individuals with recent African ancestry. NIH, National Institutes of Health; APOLLO, *APOL1* Long-term Kidney Transplantation Outcomes; T2D, type 2 diabetes.

Risk Index (KDRI). The KDRI analyzes ten factors in deceased donors, including "race/ethnicity." Retrospective data supports replacement of African American race/ethnicity in the KDRI with *APOL1* genotype. Approximately 15% of African American deceased donors in a recent report possessed *APOL1* high-risk genotypes and their kidneys were at risk for shorter allograft survival (2). In contrast, 85% of African American deceased donors had *APOL1* low-risk genotypes and their kidneys function for similar durations as those from European American donors. *APOL1* genotypes, as opposed to nonbiologic classifications of race imposed by society, should be used in clinical decision making. This is important when disease-causing genetic variants are known and distribute unequally across populations. Julian and colleagues (reviewed in reference 2) showed that KDRI scores would markedly improve in approximately 85% of African American deceased-donor kidneys when *APOL1* genotypes are considered. This could reduce numbers of discarded kidneys, yield more kidney transplants, improve outcomes, and reduce costs in ESKD. *APOL1* genotyping could also improve safety in living-kidney donation from African Americans. These concepts remain controversial in the transplant community. Therefore, they are under study in the National Institutes of Health–sponsored prospective *APOL1* Long-term Kidney Transplantation Outcomes (APOLLO) Study (11).

Although *APOL1* kidney risk haplotypes are associated with more rapid kidney function decline, variability in the trajectory of decline limits the use of genotyping for individual prediction in the general population (12). Intensive efforts are underway to identify patients with CKD who will have more rapid progression to ESKD or face higher risk for associated complications. This would permit maximization of therapy in high-risk individuals, including prescribing renin-angiotensin-aldosterone system blockade and statins. Many algorithms use artificial intelligence based on variables in the electronic medical record to determine risk; some include *APOL1* genotypes and plasma biomarker concentrations (13). Initial results appear promising and validation studies are underway.

There are no evidence-based management protocols for patients diagnosed with *APOL1*-associated nephropathy at this time. In general, patients should be managed according to guidelines for the pattern of disease found on kidney biopsy. This would include consideration of immunosuppression for biopsies showing FSGS and lupus nephritis, and typical preventative management for patients with CKD. However, the knowledge of *APOL1* status informs prognosis and will facilitate engagement in clinical trials when they become available. A recent report (14) described an *APOL1* antisense oligonucleotide with potential to be developed as a therapeutic and Vertex Pharmaceuticals issued a press release indicating plans to start clinical development of a small molecule inhibitor of APOL1 (https://investors.vrtx.com). If these novel treatments prove safe and effective, many more patients with and at risk for *APOL1*-associated nephropathy will likely undergo genotyping.

APOL1 genotypes are important determinants of risk for HIVAN in patients of African ancestry with HIV infection and severe lupus nephritis in patients with SLE (15). Genetic testing provides the opportunity for physicians to discuss whether patients with HIV or SLE who display suboptimal compliance have *APOL1* high-risk genotypes. These data could prove useful in attempts to improve compliance. Antiretroviral medication in patients with *APOL1* high-risk genotypes and HIV infection prevents HIVAN. Genetic testing could also be useful in patients with recent African ancestry who require IFN therapy. These scenarios demonstrate that *APOL1*-associated kidney diseases can be prevented by treating modifiable environmental factors. *APOL1* genotyping may also prove useful for family planning purposes.

Although CKD progresses more rapidly in individuals who are diabetic and have *APOL1* high-risk genotypes, *APOL1* RRVs do not associate with diabetic kidney disease (DKD). Without a kidney biopsy, it may be challenging to differentiate *APOL1*-associated FSGS from DKD in African Americans with proteinuria; both disorders occur more frequently in the African American population. Excluding African Americans with clinically diagnosed diabetic ESKD

(lacking a kidney biopsy) with *APOL1* high-risk genotypes appears to enrich for DKD by yielding homogeneous cases (16). Thus, genotyping with a follow-up kidney biopsy could be useful to ensure diabetic African Americans enrolled in DKD treatment trials do not have coincident *APOL1*-associated CKD.

Finally, more widespread *APOL1* genotyping would enable patients to have knowledge of the underlying pathogenesis of their disease. The increased risk of ESKD in African Americans is often attributed to diabetes and hypertension; however, we now know that much of this risk relates to *APOL1* (5). Although clinician scientists appreciate this at the population level, a diagnosis of *APOL1* nephropathy is less often made in patients. A recent study gathering informed input from African Americans through community deliberations found strong support for *APOL1* testing in kidney transplantation and patient care. The authors characterized the sentiment of the group as "you are just now telling us about this?"; 64% endorsed offering testing to all African American patients, whereas only 5% saw no personal or clinical benefit (17). The increased awareness that comes with knowledge of risk for *APOL1* kidney disease would enable activism, just as many patients with genetic disease have organized to support research and awareness. It also enables patients to know if they could meet eligibility criteria for clinical trials.

Only recently has a role for *APOL1* genotyping in common complex kidney disease been appreciated. Screening for *APOL1* high-risk genotypes may assist with identifying living-kidney donor candidates at heightened risk for ESKD, counseling patients with HIV infection and SLE to improve compliance, better gauging risk for progressive CKD, and family planning. Ongoing studies will determine whether allocation of deceased-donor kidneys from individuals with recent African ancestry would benefit by including *APOL1* genotype and the effectiveness of novel therapies for *APOL1* nephropathy. Once effective therapies to prevent progression of *APOL1* nephropathy are available, more widespread *APOL1* genotyping will likely be indicated. It may then become important to consider testing individuals with CKD who do not report having recent African ancestry. A small percentage may possess unrecognized African ancestry and have *APOL1* high-risk genotypes. In contrast, *APOL1* RRVs are rare in individuals without kidney disease who report European ancestry. Testing for *APOL1* genotypes in these individuals, including deceased kidney donors, is unlikely to be cost-effective or alter decision making.

Author Contributions

B. Freedman and C. Larsen wrote the original draft.

Disclosures

Wake Forest University Health Sciences and B. Freedman have rights to an issued United States patent related to *APOL1* genetic testing (www.apol1genetest.com). B. Freedman is a consultant for AstraZeneca and Renalytix AI. C. Larsen has nothing to disclose.

Funding

This work is supported by the National Institutes of Health grants R01 MD009055 and U01 DK116041 (to Dr. Freedman).

References

1. Genovese G, Friedman DJ, Ross MD, Lecordier L, Uzureau P, Freedman BI, Bowden DW, Langefeld CD, Oleksyk TK, Uscinski Knob AL, Bernhardy AJ, Hicks PJ, Nelson GW, Vanhollebeke B, Winkler CA, Kopp JB, Pays E, Pollak MR: Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science* 329: 841–845, 2010
2. Freedman BI, Locke JE, Reeves-Daniel AM, Julian BA: Apolipoprotein L1 gene effects on kidney transplantation. *Semin Nephrol* 37: 530–537, 2017
3. Doshi MD, Ortigosa-Goggins M, Garg AX, Li L, Poggio ED, Winkler CA, Kopp JB: *APOL1* genotype and renal function of Black living donors. *J Am Soc Nephrol* 29: 1309–1316, 2018
4. Larsen CP, Beggs ML, Saeed M, Ambruzs JM, Cossey LN, Messias NC, Walker PD, Freedman BI: Histopathologic findings associated with APOL1 risk variants in chronic kidney disease. *Mod Pathol* 28: 95–102, 2015
5. Freedman BI, Limou S, Ma L, Kopp JB: APOL1-associated nephropathy: A key contributor to racial disparities in CKD. *Am J Kidney Dis* 72[Suppl 1]: S8–S16, 2018
6. Beckerman P, Bi-Karchin J, Park AS, Qiu C, Dummer PD, Soomro I, Boustany-Kari CM, Pullen SS, Miner JH, Hu CA, Rohacs T, Inoue K, Ishibe S, Saleem MA, Palmer MB, Cuervo AM, Kopp JB, Susztak K: Transgenic expression of human APOL1 risk variants in podocytes induces kidney disease in mice. *Nat Med* 23: 429–438, 2017
7. Grams ME, Surapaneni A, Ballew SH, Appel LJ, Boerwinkle E, Boulware LE, Chen TK, Coresh J, Cushman M, Divers J, Gutiérrez OM, Irvin MR, Ix JH, Kopp JB, Kuller LH, Langefeld CD, Lipkowitz MS, Mukamal KJ, Musani SK, Naik RP, Pajewski NM, Peralta CA, Tin A, Wassel CL, Wilson JG, Winkler CA, Young BA, Zakai NA, Freedman BI: *APOL1* kidney risk variants and cardiovascular disease: An individual participant data meta-analysis. *J Am Soc Nephrol* 30: 2027–2036, 2019
8. Bick AG, Akwo E, Robinson-Cohen C, Lee K, Lynch J, Assimes TL, DuVall S, Edwards T, Fang H, Freiberg SM, Giri A, Huffman JE, Huang J, Hull L, Kember RL, Klarin D, Lee JS, Levin M, Miller DR, Natarajan P, Saleheen D, Shao Q, Sun YV, Tang H, Wilson O, Chang KM, Cho K, Concato J, Gaziano JM, Kathiresan S, O'Donnell CJ, Rader DJ, Tsao PS, Wilson PW, Hung AM, Damrauer SM; VA Million Veteran Program: Association of *APOL1* risk alleles With cardiovascular disease in blacks in the million veteran program. *Circulation* 140: 1031–1040, 2019
9. Ma L, Chou JW, Snipes JA, Bharadwaj MS, Craddock AL, Cheng D, Weckerle A, Petrovic S, Hicks PJ, Hemal AK, Hawkins GA, Miller LD, Molina AJ, Langefeld CD, Murea M, Parks JS, Freedman BI: *APOL1* renal-risk variants induce mitochondrial dysfunction. *J Am Soc Nephrol* 28: 1093–1105, 2017
10. Shah SS, Lannon H, Dias L, Zhang JY, Alper SL, Pollak MR, Friedman DJ: APOL1 kidney risk variants induce cell death *via* mitochondrial translocation and opening of the mitochondrial permeability transition pore [published online ahead of print September 26, 2019]. *J Am Soc Nephrol* doi:10.1681/ASN.2019020114
11. Freedman BI, Moxey-Mims M: The *APOL1* long-term kidney transplantation outcomes network-APOLLO. *Clin J Am Soc Nephrol* 13: 940–942, 2018
12. Grams ME, Rebholz CM, Chen Y, Rawlings AM, Estrella MM, Selvin E, Appel LJ, Tin A, Coresh J: Race, APOL1 risk, and eGFR decline in the general population. *J Am Soc Nephrol* 27: 2842–2850, 2016
13. Nadkarni GN, Chauhan K, Verghese DA, Parikh CR, Do R, Horowitz CR, Bottinger EP, Coca SG: Plasma biomarkers are associated with renal outcomes in individuals with APOL1 risk variants. *Kidney Int* 93: 1409–1416, 2018
14. Aghajan M, Booten SL, Althage M, Hart CE, Ericsson A, Maxvall I, Ochaba J, Menschik-Lundin A, Hartleib J, Kuntz S, Gattis D, Ahlström C, Watt AT, Engelhardt JA, Monia BP, Magnone MC, Guo S: Antisense oligonucleotide treatment ameliorates IFN-γ-induced proteinuria in APOL1-transgenic mice. *JCI Insight* 4: 126124, 2019
15. Nicholas Cossey L, Larsen CP, Liapis H: Collapsing glomerulopathy: A 30-year perspective and single, large center experience. *Clin Kidney J* 10: 443–449, 2017
16. Freedman BI, Langefeld CD, Lu L, Divers J, Comeau ME, Kopp JB, Winkler CA, Nelson GW, Johnson RC, Palmer ND, Hicks PJ,

Bostrom MA, Cooke JN, McDonough CW, Bowden DW: Differential effects of MYH9 and APOL1 risk variants on FRMD3 Association with Diabetic ESRD in African Americans. *PLoS Genet* 7: e1002150, 2011

17. Umeukeje EM, Young BA, Fullerton SM, Cavanaugh K, Owens D, Wilson JG, Burke W, Blacksher E: You are just now telling us about this? African American perspectives of testing for genetic susceptibility to kidney disease. *J Am Soc Nephrol* 30: 526–530, 2019