Letter to the Editor

APOL1 Genotyping Is Incomplete without Testing for the Protective M1 Modifier p.N264K Variant

Rasheed Gbadegesin[a]   Elena Martinelli[b, c]   Yask Gupta[b, d]
David J. Friedman[e, f]   Matthew G. Sampson[f, g, h, i]   Martin R. Pollak[e, f]
Simone Sanna-Cherchi[b]

[a]Division of Nephrology, Department of Pediatrics, Duke University School of Medicine, Durham, NC, USA; [b]Department of Medicine, Columbia University Irving Medical Center, New York, NY, USA; [c]Unità Operativa Nefrologia, Azienda Ospedaliero-Universitaria di Parma, Dipartimento di Medicina e Chirurgia, Università di Parma, Parma, Italy; [d]Institute for Inflammation Medicine, University of Lübeck, Lübeck, Germany; [e]Nephrology Division, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA, USA; [f]Harvard Medical School, Boston, MA, USA; [g]Division of Pediatric Nephrology, Boston Children's Hospital, Boston, MA, USA; [h]Kidney Disease Initiative and Medical and Population Genetics Program, Broad Institute, Boston, MA, USA; [i]Division of Nephrology, Brigham and Women's Hospital, Boston, MA, USA

Focal segmental glomerulosclerosis (FSGS), a pattern of injury seen in some patients with nephrotic syndrome, is a leading cause of chronic kidney disease (CKD), especially in people of African ancestry [1]. In 2008, using the method of mapping by admixture linkage disequilibrium, two studies discovered a locus on chromosome 22 as the genetic driver of high prevalence of hypertension-associated end-stage kidney disease and FSGS in African Americans [2, 3]. Fine mapping of the region later showed that two variants in apolipoprotein L1 (*APOL1*), G1 (rs73885319: p.S342g and rs60910145: p.I384M) and G2 (rs71785313: a 6-base-pair deletion), when inherited in homozygous or compound heterozygous pattern, are driving this major risk [4, 5]. APOL1 is a component of the innate immune system targeting African trypanosomes, and G1 and G2 rose to high population frequency via positive selection due to their direct trypanolytic effects, thereby conferring resistance to deadly trypanosome infections [5]. However, the same variants can significantly increase the lifetime risk for CKD and FSGS in individuals who are homozygous or compound heterozygous for the G1 and G2 variants [5, 6]. Since this discovery, biallelic inheritance of G1 or G2 has been shown to be a large effect risk factors for kidney diseases such as FSGS, virus-mediated glomerular diseases such as human immunodeficiency virus-associated nephropathy and COVID-19 virus-associated nephropathy, and sickle cell disease nephropathy, among others [7]. However, despite these, the lifetime risk for developing FSGS and other forms of APOL1-mediated CKD in individuals with high-risk (HR) genotype is less than 20%, thus suggesting the existence of both genetic and environmental disease modifiers [8].

Correspondence to:
Rasheed Gbadegesin, rasheed.gbadegesin@duke.edu
Matthew G. Sampson, matthew.sampson@childrens.harvard.edu
Martin R. Pollak, mpollak@bidmc.harvard.edu
Simone Sanna-Cherchi, ss2517@cumc.columbia.edu

Since the original publication in 2010, attempts have been made by different investigators to identify genetic modifiers of APOL1-mediated kidney diseases. Nevertheless, most of these associations have not been validated or replicated [9–12]. On the other hand, viral infections, exposure to alpha interferon, intrarenal high expression of APOL1 protein from HR genotype (preclinical models) have all been demonstrated and robustly replicated as environmental disease modifiers [13–18]. Thus, it is imperative that, for precise translation of the role of APOL1 genetic variations in the development of FSGS and CKD, the search for genetic modifiers must continue.

Two recent studies provided data to support the existence of a modifier genetic variant in the APOL1 gene itself [19, 20]. These studies independently showed that an APOL1 coding missense variant, p.N264K (chr22: 36265628C > A; rs73885316), is able to mitigate, or even nullify, the risk for FSGS and CKD when co-inherited with APOL1-HR genotypes. Interestingly, the foundations for the human genetics work can be placed in two earlier papers. First, in 2013, physicians in Ghana treated a patient for infection with an atypical trypanosoma brucei gambiense (T.b. gambiense) that lacks the T.b. gambiense glycoprotein (TgsGP) who, surprisingly, was carrier of the APOL1-HR G2/G2 genotype that should have protected him from the infection [21]. Typical T.b. gambiense with TgsGP is resistant to lysis by human serum APOL1 protein, but the atypical strain lacking TgsGP is susceptible to lysis like other strains of trypanosoma and will, therefore, be expected to be protected by the G2/G2 genotype from clinical infection [6, 21]. In an effort to determine how the blood of this patient lost its trypanolytic effect, the authors sequenced and characterized additional APOL1 variants and pointed to the p.N264K as a loss-of-function variant that likely resulted in counteracting the protective effect of the APOL1-HR genotype by affecting its protein interaction with the endolysosomal membranes of trypanosome [21]. Then, in 2019, by studying naturally and non-naturally occurring haplotypes, we reported that the cytotoxicity of G1 and G2 alleles was significantly reduced when expressed on the haplotype defined by the p.N264K variant, thus suggesting that this variant may be protective at least in an in vitro model [22]. Altogether, these data already provided the rationale for the existence of a potentially powerful genetic modifier of APOL1-HR genotypes, but what was lacking until now was the human genetic proof.

The current studies have now provided independent and robust human genetic data supporting the protective effect of the APOL1 p.N264K missense variant against APOL1-mediated kidney disease and, especially, FSGS. In the study by Hung et al. [19], published in the Journal of the American Society of Nephrology, the group studied 121,492 individuals of African genetic ancestry participating in the Department of Veteran Affairs Million Veteran Program (MVP), to test the hypothesis that the APOL1 p.N264K variant modified the risk imparted by APOL1-HR genotypes on CKD. They reported that APOL1-HR genotypes, when co-inherited with the p.N264K reduced the risk of CKD by about 57% (OR: 0.43, 95% CI: 0.28–0.65) and ESKD by 81% (OR: 0.19, 95% CI: 0.07–0.51). They then replicated their findings in the Vanderbilt University Biobank and the National Institutes of Health All of Us program (combined $N >1$ million participants) [19]. The authors also performed functional studies to explore the mechanism underlying the protective role of the variant, using immortalized human podocytes and human embryonic kidney cell lines. Initially, they noted a substantial decrease in cellular toxicity among podocytes expressing the p.N264K variant upon introducing APOL1 G0, G1, and G2 constructs with and without the mentioned variant. In addition, their investigation revealed that cell lines expressing APOL1 G2 resulted in a robust influx of calcium. On the contrary, ion influx was efficiently inhibited in cell lines where APOL1 G2 was expressed with the p.N264K substitution, potentially suggesting defective ion conductance through the APOL1 pore in kidney cells [19]. Alternatively, the p.N264K variant may cause reduced plasma membrane permeability due to inhibition of APOL1 toxicity [21] or by affecting its interference with APOL3 control of actomyosin in podocytes [23], rather than specific inhibition of APOL1 channel activity.

In the second study, conducted by our group and published in Nature Communications, we sought to resolve the specific effect of the p.N264K on the different APOL1-HR genotypes and, especially, on the rarer and more penetrant kidney disease FSGS [20]. In fact, the p.N264K exists in two different "flavors": first, on a fraction of the non-risk and more common G0 haplotype; and, second, on a fraction of the G2-HR haplotype. It is not found on the haplotype defined by the HR G1 variant. Hence, to avoid confounding from non-risk genotypes, we selected case-control cohorts with only APOL1-HR genotypes (i.e., G1/G1, G1/G2, and G2/G2). As such, we first analyzed data from 528 FSGS and 2,606 genetically matched population controls, all with HR-APOL1 genotypes. Strikingly, in the APOL1-HR FSGS cohorts versus APOL1-HR controls with no kidney diseases, we showed 93% OR reduction from FSGS (OR = 0.07, 95% CI: 0.01–0.25), but this signal was

Gbadegesin/Martinelli/Gupta/Friedman/
Sampson/Pollak/Sanna-Cherchi

Box 1

Clinical implications of APOL1 p.N264K as genetic modifier of APOL1 G2-mediated kidney disease

- Five different haplotypes for APOL1 risk and modifier variants (G0, G0-M1, G1, G2, G2-M1)
- G2-M1 genotypes should be reclassified as non-HR genotype
- Kidneys from G2-M1 donors are at low risk of APOL1-mediated kidney disease
- M1 p.N264K provides a new target for novel therapy of APOL1-mediated kidney diseases
- Precise design of clinical trials for APOL1-mediated kidney disease based on haplotype risks

driven exclusively by the effect on G2-containing genotypes. In fact, when stratifying the cohort by APOL1 genotype (G1/G1, G2/G2, G1/G2), the p.N264K variant was only present in individuals carrying at least one G2 allele. The variant seemed to confer almost 100% risk reduction against FSGS as none of the individuals with G2/G2-N264K genotypes was observed to have FSGS (OR = 0, 95% CI: 0–0.41). Furthermore, ~86% risk reduction was observed in individuals with G1/G2 genotype (OR = 0.14, 95% CI 0.16–0.52). The specificity for the African-derived G2 haplotype was additionally supported by local ancestry analysis, showing the G2-specific protective effect of the p.N264K variant only for the African haplotype (OR = 0.12, 95% CI = 0.02–0.35, $p = 3.53 \times 10^{-6}$), while no significant effect was observed for the European haplotype (OR = 0.76, 95% CI = 0.00–12.75, $p = 0.86$). Just like the first study, we also confirmed our findings in multiple CKD cohorts [20].

Both studies reached independently similar conclusions and, together with the prior functional evidence, establish the APOL1 p.N264K missense variant as a protective allele against APOL1 G2-mediated kidney disease. Based on this almost complete abrogation of the phenotype induced by G2 allele, we here propose to call the APOL1 p.N264K "Modifier 1," or "M1," as the first robust and reproducible genetic modifier of APOL1-mediated kidney disease, particularly FSGS. In fact, the effect is so profound that the presence of M1 is associated with a reduction of risk for FSGS, CKD, and ESKD among carriers of G2-containing APOL1-HR genotypes to levels comparable to individuals with APOL1 non-risk genotypes.

These findings have multiple implications, immediate translational potential, and demonstrate the power of collaborative studies to fully harness the benefits of genomic research (Box 1). The first important conclusion is that genotyping for APOL1 is incomplete without screening for the M1 p.N264K variant (Fig. 1). In fact, based on the current

genetic knowledge, we should now consider five different APOL1 haplotypes, with respect to co-inheritance of p.N264K and the mutual exclusivity of G1 and p.N264K (Fig. 1a). As a result, the combinatorial possibilities result in many more genotypes, a total of 15, as compared to the traditional three HR and three non-HR ones, with a different associated genetic risk for FSGS and kidney disease according to the presence or absence of M1 (Fig. 1b). As such, a fraction of G2-containing genotypes that are M1-positive should be reclassified as non-HR genotypes. This, in turn, has important clinical implications: first, individuals with kidney disease and G2-containing APOL1-HR genotypes who are also M1-positive are unlikely to have APOL1-mediated FSGS, and this should prompt the caring physicians to look for additional (and potentially treatable) causes for their kidney diseases, such as immune, metabolic, toxic, structural, and other causes. Second, these findings can have an important impact for kidney transplant in terms of donor pool and correct allocation of organs, as well as for risk stratification both for living donor lifetime risk of ESKD and recipient risk for graft loss. In fact, APOL1-HR-M1 donors are likely to have similar lifetime risk for FSGS and ESKD to the one of donors with the non-HR G1/G0, G2/G0, and G0/G0 genotypes, hence expanding donors' pool. Similarly, kidney transplant recipients of an APOL1-HR-M1 kidney are likely to have low risk for developing de novo FSGS or progressive graft failure from APOL1-mediated kidney disease. Third, these findings support the efforts toward drug development for interventions that reduce APOL1 activity and toxicity [24], and incorporation of this knowledge will allow more accurate clinical trial design in which individuals with APOL1-HR-M1 genotypes should not be included in the intervention arm as cases.

These discoveries also open new hypotheses to be tested. While it is established that APOL1 G1 and G2 variants impart large risk for different form of kidney disease in a recessive mode of inheritance, it is still unclear if single heterozygous carriers are at small risk for developing some of these outcomes [25, 26] perhaps under important environmental triggers. With the incorporation of the M1 in the possible combinatorial genotypes, we now have multiple APOL1 genotypes that are functionally heterozygous HR and multiple that are being reclassified as non-HR (Fig. 1b). With adequately powered genetic studies, it is now possible to test the individual effect of each of these genotypes on the risk of kidney disease and FSGS.

The data also open more fundamental questions about the effect of M1 based on its trans or cis effect on the G1 and G2 alleles. It is parsimonious to speculate that the effect of M1 on G2 is due to a "reversal" of the gain-of-function effect of G2 by inducing a protein conformational change; therefore, the protective effect should only occur if the two variants, risk
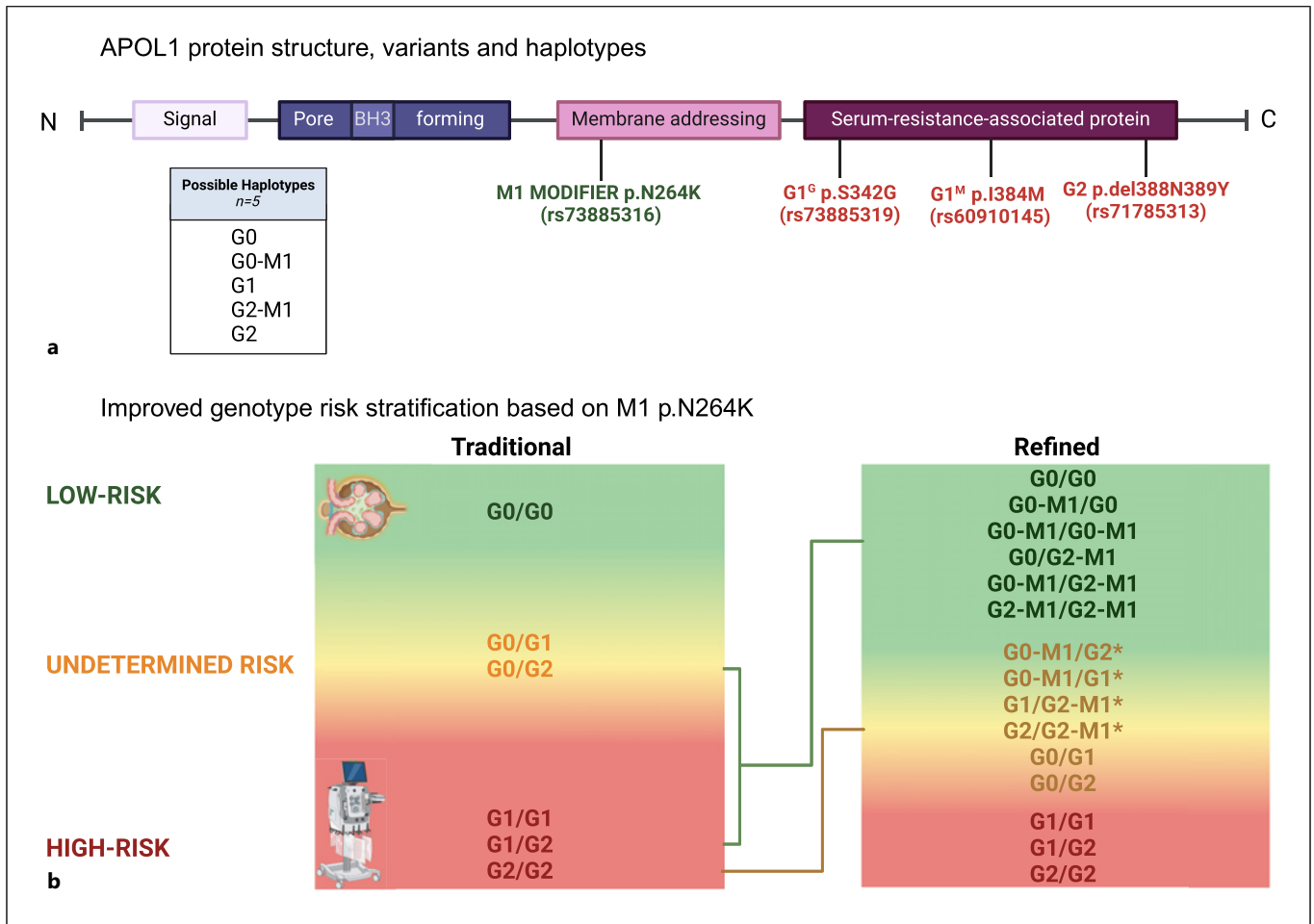
Fig. 1. Improved *APOL1* haplotype and genotype resolution based on the M1 p.N264K missense protective variant. **a** Graphical representation of APOL1 protein structure with M1, G1, and G2 variants; M1 is located in the "membrane addressing domain," while G1 and G2 are in the "serum-resistance-associated protein domain." The table shows the five different possible haplotypes resulting from the combination of these variants. It is important to note that, since M1 and G1 are mutually exclusives, a haplotype containing the two variants in cis is not possible. **b** Risk stratification into "low," "undetermined," and "high-risk" genotypes is strongly modified by the presence of M1 p.N264K protective variant. Genotypes signed with * are objects of study and could potentially be reclassified as low-risk in further studies. The cartoon has been created using BioRender at www.biorender.com.

and protective, are in cis. As a result, a G0/G2-M1 genotype is expected to be functionally the same as a G0/G0, while a G0-M1/G2 or G0-M1/G1 might actually be functionally heterozygous HR such as the more common G0/G1 and G0/G2. This hypothesis can now be tested, both functionally and with human genetics studies.

A final important consideration should be made on the impact of these findings at the population level, as this disease-modifying effect is only applicable to a fraction of individuals with *APOL1* genotypes. In our study, we utilized a dataset of 2,606 *APOL1*-HR controls of African genetic ancestry from the USA. Of those, approximately 62% (1,615 out of 2,606) were carriers of at least one G2-containing

APOL1-HR genotype, and, of these, 4.3% were found to have the M1 variant. With all the caution of this approximation, extrapolating these data to the American population, which includes approximately 40 million individuals who are self-reported African Americans (https://minorityhealth.hhs.gov/blackafrican-american-health) and hence are likely to have high fraction of African genome, we expect 5.2 million (13%) to have an *APOL1*-HR genotype and ~3.22 million of those (62%) to have a G2-containing HR genotype. If 4.3% of these individuals indeed carry the M1 protective variant, approximately 137,000 individuals of African American ancestry are estimated to be immediately reclassified from being *APOL1*-HR to non-HR.

Gbadegesin/Martinelli/Gupta/Friedman/
Sampson/Pollak/Sanna-Cherchi

In conclusion, the *APOL1* p.N264K, or M1, represents the first robust genetic modifier for APOL1-mediated kidney disease in a subset of individuals with G2-containing genotypes, and, importantly, the presence of this variant reduces the risk of *APOL1* G2-mediated FSGS and kidney disease to almost the same level as the traditional non-risk genotypes. These studies pave the way for haplotype- and genotype-aware genetic studies to fully understand the repertoire of genetic modifiers for APOL1-mediated FSGS and other CKD and their interaction with known environmental modifiers of disease.

Conflict of Interest Statement

M.R.P. and D.J.F. report research support from Vertex. The remaining authors declare no competing interests.

Author Contributions

Conceptualization: R.G., M.G.S., M.R.P., and S.S.C.; writing: R.G., E.M., and S.S.C.; graphics: E.M.; and editing: R.G., E.M., Y.G., D.J.F., M.G.S., M.R.P., and S.S.C.

References

1 Udler MS, Nadkarni GN, Belbin G, Lotay V, Wyatt C, Gottesman O, et al. Effect of genetic african ancestry on eGFR and kidney disease. J Am Soc Nephrol. 2015;26(7): 1682–92.

2 Kopp JB, Smith MW, Nelson GW, Johnson RC, Freedman BI, Bowden DW, et al. MYH9 is a major-effect risk gene for focal segmental glomerulosclerosis. Nat Genet. 2008;40(10): 1175–84.

3 Kao WH, Klag MJ, Meoni LA, Reich D, Berthier-Schaad Y, Li M, et al. MYH9 is associated with nondiabetic end-stage renal disease in African Americans. Nat Genet. 2008;40(10):1185–92.

4 Tzur S, Rosset S, Shemer R, Yudkovsky G, Selig S, Tarekegn A, et al. Missense mutations in the APOL1 gene are highly associated with end stage kidney disease risk previously attributed to the MYH9 gene. Hum Genet. 2010;128(3):345–50.

5 Genovese G, Friedman DJ, Ross MD, Lecordier L, Uzureau P, Freedman BI, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. Science. 2010;329(5993):841–5.

6 Cooper A, Ilboudo H, Alibu VP, Ravel S, Enyaru J, Weir W, et al. APOL1 renal risk variants have contrasting resistance and susceptibility associations with African trypanosomiasis. Elife. 2017;6:e25461.

7 Freedman BI, Kopp JB, Sampson MG, Susztak K. APOL1 at 10 years: progress and next steps. Kidney Int. 2021;99(6): 1296–302.

8 Friedman DJ, Pollak MR. APOL1 nephropathy: from genetics to clinical applications. Clin J Am Soc Nephrol. 2021;16(2): 294–303.

9 Cameron-Christie S, Wolock CJ, Groopman E, Petrovski S, Kamalakaran S, Povysil G, et al. Exome-based rare-variant analyses in CKD. J Am Soc Nephrol. 2019;30(6): 1109–22.

10 Zhang JY, Wang M, Tian L, Genovese G, Yan P, Wilson JG, et al. UBD modifies APOL1-induced kidney disease risk. Proc Natl Acad Sci USA. 2018;115(13):3446–51.

11 Divers J, Palmer ND, Lu L, Langefeld CD, Rocco MV, Hicks PJ, et al. Gene-gene interactions in APOL1-associated nephropathy. Nephrol Dial Transplant. 2014;29(3): 587–94.

12 Bodonyi-Kovacs G, Ma JZ, Chang J, Lipkowitz MS, Kopp JB, Winkler CA, et al. Combined effects of GSTM1 null allele and APOL1 renal risk alleles in CKD progression in the african American study of kidney disease and hypertension trial. J Am Soc Nephrol. 2016;27(10):3140–52.

13 Nichols B, Jog P, Lee JH, Blackler D, Wilmot M, D'Agati V, et al. Innate immunity pathways regulate the nephropathy gene Apolipoprotein L1. Kidney Int. 2015;87(2):332–42.

14 Kasembeli AN, Duarte R, Ramsay M, Mosiane P, Dickens C, Dix-Peek T, et al. APOL1 risk variants are strongly associated with HIV-associated nephropathy in black South Africans. J Am Soc Nephrol. 2015;26(11): 2882–90.

15 Langefeld CD, Comeau ME, Ng MCY, Guan M, Dimitrov L, Mudgal P, et al. Genome-wide association studies suggest that APOL1-environment interactions more likely trigger kidney disease in African Americans with nondiabetic nephropathy than strong APOL1-second gene interactions. Kidney Int. 2018; 94(3):599–607.

16 Pollak MR, Friedman DJ. APOL1 and APOL1-associated kidney disease: a common disease, an unusual disease gene - proceedings of the henry shavelle professorship. Glomerular Dis. 2023;3(1):75–87.

17 Chang JH, Husain SA, Santoriello D, Stokes MB, Miles CD, Foster KW, et al. Donor's APOL1 risk genotype and "second hits" associated with de novo collapsing glomerulopathy in deceased donor kidney transplant recipients: a report of 5 cases. Am J Kidney Dis. 2019;73(1):134–9.

18 Beckerman P, Bi-Karchin J, Park ASD, Qiu C, Dummer PD, Soomro I, et al. Transgenic expression of human APOL1 risk variants in podocytes induces kidney disease in mice. Nat Med. 2017;23(4):429–38.

19 Hung AM, Assimon VA, Chen HC, Yu Z, Vlasschaert C, Triozzi JL, et al. Genetic inhibition of APOL1 pore-forming function prevents APOL1-mediated kidney disease. J Am Soc Nephrol. 2023;34(11): 1889–99.

20 Gupta Y, Friedman DJ, McNulty MT, Khan A, Lane B, Wang C, et al. Strong protective effect of the APOL1 p.N264K variant against G2-associated focal segmental glomerulosclerosis and kidney disease. Nat Commun. 2023;14(1):7836.

21 Cuypers B, Lecordier L, Meehan CJ, Van den Broeck F, Imamura H, Büscher P, et al. Apolipoprotein L1 variant associated with increased susceptibility to trypanosome infection. mBio. 2016;7(2):e02198.

22 Lannon H, Shah SS, Dias L, Blackler D, Alper SL, Pollak MR, et al. Apolipoprotein L1 (APOL1) risk variant toxicity depends on the haplotype background. Kidney Int. 2019; 96(6):1303–7.

23 Uzureau S, Lecordier L, Uzureau P, Hennig D, Graversen JH, Homblé F, et al. APOL1 C-terminal variants may trigger kidney disease through interference with APOL3 control of acto-myosin. Cell Rep. 2020;30(11):3821–36 e13.

24 Egbuna O, Zimmerman B, Manos G, Fortier A, Chirieac MC, Dakin LA, et al. Inaxaplin for pro-teinuric kidney disease in persons with two APOL1 variants. N Engl J Med. 2023;388(11):969–79.

25 Freedman BI, Langefeld CD, Turner J, Núñez M, High KP, Spainhour M, et al. Association of APOL1 variants with mild kidney disease in the first-degree relatives of African American patients with non-diabetic end-stage renal disease. Kidney Int. 2012;82(7): 805–11.

26 Kopp JB, Nelson GW, Sampath K, Johnson RC, Genovese G, An P, et al. APOL1 genetic variants in focal segmental glomerulo-sclerosis and HIV-associated nephropathy. J Am Soc Nephrol. 2011;22(11):2129–37.