APOL1 genotype-associated morphologic changes among patients with focal segmental glomerulosclerosis

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Abstract

Background The G1 and G2 alleles of apolipoprotein L1 (*APOL1*) are common in the Black population and associated with increased risk of focal segmental glomerulosclerosis (FSGS). The molecular mechanisms linking *APOL1* risk variants with FSGS are not clearly understood, and *APOL1's* natural absence in laboratory animals makes studying its pathobiology challenging.

Methods In a cohort of 90 Black patients with either FSGS or minimal change disease (MCD) enrolled in the Nephrotic Syndrome Study Network (58% pediatric onset), we used kidney biopsy traits as an intermediate outcome to help illuminate tissue-based consequences of *APOL1* risk variants and expression. We tested associations between *APOL1* risk alleles or glomerular APOL1 mRNA expression and 83 light- or electron-microscopy traits measuring structural and cellular kidney changes.

Results Under both recessive and dominant models in the FSGS patient subgroup (61%), *APOL1* risk variants were significantly correlated (defined as FDR <0.1) with decreased global mesangial hypercellularity, decreased condensation of cytoskeleton, and increased tubular microcysts. No significant correlations were detected in MCD cohort. Independent of risk alleles, glomerular *APOL1* expression in FSGS patients was not correlated with morphologic features.

Conclusions While *APOL1*-associated FSGS is associated with two risk alleles, both one and two risk alleles are associated with cellular/tissue changes in this study of FSGS patients. Our lack of discovery of a large group of tissue differences in FSGS and no significant difference in MCD may be due to the lack of power but also supports investigating whether machine learning methods may more sensitively detect *APOL1*-associated changes.

Keywords *APOL1* · Focal segmental glomerulosclerosis · Minimal change disease · Pediatric · Morphology

Introduction

Harboring two copies of the G1 and G2 alleles in apolipoprotein L1 (*APOL1*) confers significantly greater risk for nephrotic syndrome (NS) with focal segmental glomerular sclerosis (FSGS) histology [1, 2]. These *APOL1* risk variants are specific to people of recent West African ancestry, are common in

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the African-American population, and are even more common in African-Americans with FSGS [3]. From a pediatric perspective, 60–70% of African-American children with NS have a high risk (HR, two risk alleles) *APOL1* genotype. Compared to children with NS and a low risk (LR, one or two risk alleles) genotype, those with a HR genotype have a 3–10× greater likelihood of being born prematurely, present with an average 17 ml/min/1.73m^2 lower eGFR, are 1/3 less likely to achieve complete remission of proteinuria, and have a faster decline of eGFR over time [4, 5].

Altogether, this suggests that *APOL1*-associated NS is a distinct form of glomerular disease that, while most often presenting clinically as nephrotic syndrome and histologically as FSGS, behaves clinically in a more severe and intractable manner than the non-*APOL1* forms of this condition. Furthermore, the majority of Black children with NS will have *APOL1*-associated disease, meaning that a better understanding of why this disease occurs holds great potential in helping a large proportion of our patients in this population.

Massive effort has been directed toward discovering the pathobiology underlying the association between these common genetic variants and kidney disease in order to ultimately devise more rational, targeted therapies against this disease. Experiments have been done at the bench using *in vitro* systems and in laboratory organisms in whom *APOL1* has been artificially engineered into them [6–10]. In humans, *APOL1* genotype data have been combined with histological, molecular, and/or clinical data to perform association studies between genotype, mRNA and protein expression, tissue alterations, and clinical phenotype [11–14].

From a kidney morphology perspective, *APOL1* risk alleles were initially discovered through their association with the morphologic diagnosis of FSGS and hypertensive stage 5 chronic kidney disease and were linked soon thereafter to HIV-associated nephropathy (HIVAN) [15]. The continued pursuit of relationships between *APOL1* risk alleles and gene expression to kidney tissue abnormalities has revealed specific patterns of injury to the kidney, leading to potential biologic and mechanistic insights in a biologically informative way that is impossible to detect when solely associating genotype to clinical phenotype. In lupus, the *APOL1* high-risk genotype has also been linked to glomerular collapse [16]. In FSGS patients, it has been associated with more segmental and global glomerulosclerosis, interstitial fibrosis, and tubular atrophy [14]. Patients with PLA2R-associated membranous nephropathy and a high-risk *APOL1* genotype had significantly increased glomerular collapse, FSGS lesions, cystic tubular dilatation, and acute tubular injury [17]. In one study of biopsied patients with chronic kidney disease (CKD) without nephrotic syndrome, Black patients with two *APOL1* risk alleles had less obsolescent glomerulosclerosis, more solidified and disappearing glomerulosclerosis, thyroidization-type tubular atrophy, and microcystic tubular dilation [18].

Here, we hypothesized that studying kidney biopsy tissue of children and adults with NS as a function of their *APOL1* risk alleles and glomerular expression levels would lead to the discovery of a morphologic signature of *APOL1*-associated NS. To do so, we studied a cohort of 90 Black patients who underwent kidney biopsies for proteinuric kidney disease in the Nephrotic Syndrome Study Network (NEPTUNE) [19] and were found to have FSGS or minimal change disease (MCD). We integrated *APOL1* genotyping, glomerular mRNA expression levels, and research biopsy tissue measurements to test for associations between *APOL1* genotype, *APOL1* gene expression, and 83 research-based glomerular, tubulointerstitial, and vascular traits ("descriptors") noted by light and electron microscopy [20].

Methods

Study sample

NEPTUNE is an ongoing multicenter prospective observational cohort study of children and adults with proteinuria at screening of > 0.5 g/day (> 1.5 g/day in the second study phase), most of whom are undergoing a clinically-indicated kidney biopsy [19]. Blood, urine, and tissue samples were collected at the time of biopsy, along with demographic information and clinical characteristics. Only patients with MCD, FSGS, or membranous nephropathy (MN) are eligible to continue in the NEPTUNE observational study. Kidney biopsy tissue was collected at enrolling centers and sent to the NIH image coordinating center, where stained glass slides were scanned into whole slide images (WSI), and together with the digital electron microscopy (EM) images, these images were uploaded in the NEPTUNE Digital Pathology Repository (DPR) [21]. The inclusion criteria for participants in this study were (1) self-identified as Black race or had genotype-based African ancestry, (2) histologic diagnosis of FSGS or MCD, (3) *APOL1* genotyping acquired, and (4) biopsy obtained with research morphology scoring. Patients with secondary causes of FSGS (e.g., HIV infection) were not included in this study. Those individuals meeting inclusion criteria who also had glomerular mRNA transcriptomic data were included in the gene expression study described below.

Morphology descriptor outcome variables

As previously published, pathologists within the NEPTUNE consortium developed the NEPTUNE Digital Pathology Scoring System which collects quantitative data on structural changes observed in digital WSI and EM images of kidney

biopsy tissue. This system entails scoring 51 individual glomerular descriptors (which are subsequently used to calculate the percentage of glomeruli in the biopsy with each feature), 11 tubulointerstitial and vascular descriptors relating to the presence and amount of tubulointerstitial and vascular damage, and 21 EM descriptors describing the presence and quantity of abnormal ultrastructural features. These 83 descriptors were used as dependent variables in the current study.

Pathologists also identified individual glomerular descriptors that shared morphologic similarities, and these individual descriptors were combined into 21 "grouped" glomerular descriptors. We calculated the percentage of glomeruli in the biopsy with any of the features in the group and also used these grouped descriptors as dependent variables.

A list of the 83 descriptors and the 21 grouped glomerular descriptors and their definitions are published and available in tabular format [20].

Independent variables

The primary exposures of interest were *APOL1* risk genotype and glomerular *APOL1* gene expression. The number of *APOL1* risk alleles for each participant was determined by either whole genome sequencing (WGS), Sanger sequencing, or both. To measure glomerular *APOL1* gene expression, all glomeruli were manually microdissected from biopsy tissue, and RNA was extracted and sequenced (RNA-seq). RNA-seq data were normalized and transformed to log-2 counts per million (log2CPM) with the limma transformation.

Other demographic data used in the current study included age at biopsy, sex, and Hispanic ethnicity. Clinical characteristics included self/family-reported premature birth, glomerular disease diagnosis, estimated glomerular filtration rate (eGFR) at kidney biopsy, and urine protein creatinine ratio (UPCR) at biopsy. eGFR was estimated using the CKiD-Schwartz equation for children, CKD-Epi equation for adults over 26 years old, and an average of the two equations for adults between 18–26 years old [22]. If available, serum creatinine and UPCR were measured by a central laboratory using collected biosamples; otherwise, local laboratory values were used.

Statistical analysis

Demographic and clinical characteristics of participants in the study sample were described using median and interquartile range (IQR) for continuous variables and frequencies for categorical variables. We tested for differences in these characteristics across number of *APOL1* risk alleles using the Kruskal–Wallis rank sum test for continuous variables, Pearson's chi-squared test for categorical variables with at least five participants in each group, and Fisher's exact test for categorical variables with less than five participants in at least one group. To test the hypothesis that a larger number of *APOL1* risk alleles were associated with higher glomerular gene expression, we used Spearman's correlation coefficient and test.

We assessed associations between *APOL1* risk genotypes and each of 83 morphology descriptors using Spearman's correlation coefficient and test, under additive (comparing patients with 0 vs. 1 vs. 2 risk alleles) and recessive (comparing patients who are LR (0 or 1 risk alleles) vs. patients who are HR (2 risk alleles)) genetic models. Pathologists also combined related individual glomerular descriptors into grouped descriptors, and we repeated the process above using grouped descriptors as our outcomes of interest. Spearman's correlation coefficient test with FDR correction was used to assess associations between the level of glomerular *APOL1* gene expression and each morphologic descriptor. Given a smaller sample size of patients with expression and the knowledge that higher levels of expression of wildtype *APOL1* have also been shown to be associated with kidney injury, we did not adjust for or stratify by *APOL1* risk alleles in this analysis.

The primary analysis was done only among patients with a diagnosis of FSGS, given that this is the histology most strongly associated with *APOL1*, and because of this, any descriptors associated with *APOL1* in a combined cohort of MCD–FSGS may be driven more by the general FSGS descriptors rather than those specific to FSGS. However, with the rationale that *APOL1* risk alleles may lead to specific morphologic changes outside of FSGS, we also studied the association of genotype and gene expression only among the MCD patients. For any descriptors showing a significant correlation with genotype or gene expression, we additionally conducted stratified analyses by pediatric (age < 18) or adult status at the time of biopsy.

The Benjamini–Hochberg procedure was used to control false discovery rate (FDR), since it is robust even when tests are dependent. Descriptors with FDR-corrected p value less than or equal to 0.1 were considered significant.

Analyses were restricted to participants with the available data elements. Statistical analyses were conducted using R software, version 3.4.0 (R Development Core Team, Vienna, Austria).

Results

Sample characteristics

The 90 Black participants included in the current study had a median age of 16.5 years and were predominantly male (62.2%); the majority had FSGS (61.1% vs. 38.9% MCD) (Table 1). A greater proportion of participants with two *APOL1* risk alleles were born prematurely (p = 0.024). Glomerular disease diagnosis and eGFR at biopsy also

Table 1 Characteristics of cohort under investigation

		By Number of APOL1 risk alleles			
	Overall	0 risk alleles	1 risk allele	2 risk alleles	p
N	90	28	28	34	
Age at biopsy	16.5 (12.0–42.8)	13.5 (11.0–29.0)	16.5 (11.2–53.0)	17.5 (15.2–40.5)	0.276
Pediatric	54.4% (49)	60.7% (17)	53.6% (15)	50.0% (17)	0.697
Male	62.2% (56)	60.7% (17)	60.7% (17)	64.7% (22)	0.931
Hispanic	9.2% (8)	14.3% (4)	7.1% (2)	6.5% (2)	0.647
Premature	15.7% (13)	3.8% (1)	11.1% (3)	30.0% (9)	0.024
Histologic diagnosis					< 0.001
MCD	38.9% (35)	67.9% (19)	39.3% (11)	14.7% (5)	
FSGS	61.1% (55)	32.1% (9)	60.7% (17)	85.3% (29)	
Immunosuppressant use before biopsy	26.7% (24)	35.7% (10)	35.7% (10)	11.8% (4)	0.045
RAASi use before biopsy	44.4% (40)	53.6% (15)	39.3% (11)	41.2% (14)	0.498
eGFR	81.3 (45.5–106.8)	103.6 (87.1–115.0)	79.5 (41.0–98.7)	54.1 (31.8–73.6)	< 0.001
UPCR	2.5 (1.1–5.8)	2.0 (0.7–6.7)	2.0 (1.1–4.5)	3.6 (1.8–6.4)	0.289
# glomeruli in biopsy	29.0 (17.0–44.8)	31.0 (23.0–49.0)	33.0 (16.2–45.5)	21.0 (17.0–34.5)	0.125

All values are median (IQR) or % (N)

p values from Kruskal–Wallis rank sum test for continuous variables, Pearson's chi-squared test for categorical variables with at least 5 participants in each group, and Fisher's exact test for categorical variables with less than 5 participants in at least one group, compared across number of risk alleles.

Variables with missing data and the number of participants with missing data in each group (0,1,2 risk alleles): Hispanic (0,0,3), Premature (2,1,4), eGFR (0,2,6), UPCR (0,2,3), # Glomeruli (5,4,3)

differed across *APOL1* genotype ($p < 0.001$ for both): participants with no risk alleles predominantly had MCD and the highest median eGFR, whereas those with two risk alleles predominantly had FSGS and the lowest median eGFR. A greater proportion of participants with zero or one *APOL1* risk alleles had immunosuppression use prior to biopsy compared with patients with two *APOL1* risk alleles ($p = 0.045$). In this sample, number of *APOL1* risk alleles and glomerular *APOL1* gene expression were also significantly positively correlated (Fig. 1, Spearman's rank coefficient $r = 0.46$, $p = 0.02$).

APOL1 genotype and morphology

Almost all clinical associations with *APOL1* risk variants demonstrate the association of disease with two risk alleles only (a recessive model). However, a small number of clinical studies and a number of studies using molecular intermediate phenotypes have demonstrated harmful impact associated with each addition of a risk allele (additive model). Given this, we tested the risk variants' association with morphologic descriptors under each of these models in patients with FSGS. More specifically, under the recessive model, we compared correlations between 29 participants with a HR genotype and 26 with a LR genotype. Under an additive model, the comparison was between 9, 17, and 29 participants with 0, 1, and 2 risk alleles, respectively.

Among the 54 patients with FSGS, one individual glomerular descriptor one individual tubulointerstitial descriptor, and one EM descriptor were significantly associated with *APOL1* risk genotypes under both a recessive and additive model (Fig. 2, Supplemental Tables 1a and 1b). *APOL1* risk variants were significantly correlated with (1) decreased global mesangial hypercellularity—"greater than 3 mesangial cells per mesangial lobule involving > 50% of the visible mesangial regions in a glomerulus" (*additive*: $r = -0.44$, $p = 0.095$; *recessive*: $r = -0.43$, $p = 0.057$), (2) decreased condensation of the cytoskeleton—"electron-dense cytoskeleton is reorganized and condensed at the glomerular basement membrane (GBM) aspect of epithelial cell (podocyte) foot processes" (*additive*: $r = -0.39$, $p = 0.095$; *recessive:* $r = -0.39$, $p = 0.1$), and (3) increased microcysts—"presence of dilated tubules (> twice the diameter of a normal proximal tubule) containing eosinophilic amorphous material and generally accompanied by scalloping of the cast profile. The epithelium lining the microcyst is generally flattened and does not reveal brush border" (*additive*: $r = 0.38$, $p = 0.095$; *recessive*: $r = 0.43$, $p = 0.057$) (Fig. 3). There were no descriptors solely associated with *APOL1* in an additive or recessive model.

We then tested whether the significant associations of these descriptors and *APOL1* genotype discovered in FSGS patients were replicated in the 36 patients with MCD. There were no significant correlations observed. We recognized that the smaller sample size could impact significance, so we also

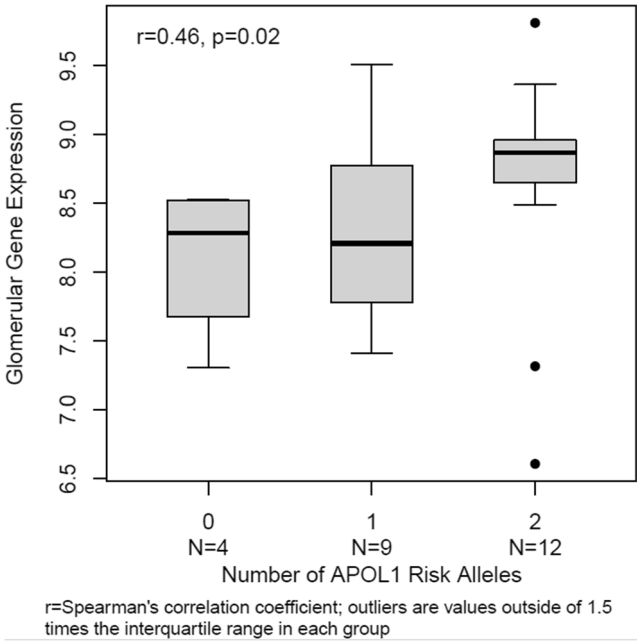

Fig 1 Glomerular *APOL1* expression as measured by log2 transformed mRNA level increases with increasing number of *APOL1* risk alleles

examined the correlation point estimates. Under the additive model, we observed, at a substantially decreased magnitude, the same direction of effect for global mesangial hypercellularity ($r = -0.17$) and condensation of the cytoskeleton ($r = -0.20$) in MCD patients. When using the MCD participants as a "discovery cohort", we did not observe any significant correlations between *APOL1* risk alleles and morphologic descriptors.

Finally, we explored the associations of these descriptors and *APOL1* genotype in pediatric and adult patients separately. Although limited sample sizes could impact both significance and the precision of the correlation point estimates, we found that the correlations for global mesangial hypercellularity were slightly stronger in pediatric patients than adult patients and the correlations for microcysts and condensation of cytoskeleton were stronger in the adult group (Supplemental Table 2).

APOL1 gene expression and morphology

Glomerular *APOL1* mRNA expression data were available for 27/55 participants with FSGS, but no morphologic descriptors were significantly correlated with glomerular *APOL1* expression (Supplemental Table 3).

Discussion

Over the past decade, epidemiologic, translational, and basic science research has led to innumerable insights about *APOL1*-associated nephrotic syndrome. We have a greater understanding of at-risk populations and the natural history of individuals affected [4, 5, 14, 15], "second hits" that potentiate *APOL1's* penetrance [7, 23], and cell structures and pathways that are involved in the disease's pathogenesis and progression [24–27]. At the same time, there are still many questions left on the table—answering them could ultimately contribute to improved patient care.

The characteristics of the participants recruited and samples collected in the NEPTUNE study provided us with a patient population and set of data with which to ask some fundamental questions about the relationship between *APOL1* genotype, glomerular expression, and kidney tissue damage in unique ways: (1) the median age of the cohort at the time of biopsy was 16 years old (54% pediatric onset overall) which gave us a chance to study the morphologic consequences of *APOL1* in a cohort younger than previously reported; (2) we restricted our cohort to only those of self-reported Black race or African genetic ancestry, which allowed us to make comparisons between risk genotypes and expression among these patients only. This prevented confounding by overall differences in genetic ancestry or by societal differences between races (e.g., structural racism against Black individuals) [28]; (3) the spectrum of patients with different number of risk alleles and available expression data gave us the opportunity to study whether single copies of risk variants and/or increased glomerular expression of *APOL1* was associated with kidney damage, as reported in other studies [9, 29–31]; (4) This is a case–case study, which allows us to ask questions about how *APOL1* may be causing a unique form of nephrotic syndrome, rather than a case–control study comparing *APOL1*-associated NS tissue to normal tissue; (5) the availability of 83 validated histologic and ultrastructural descriptors from light and electron microscopy created and measured as part of the NEPTUNE protocol allowed a level of morphologic granularity beyond that typically reported in clinical biopsy reports.

Our primary analysis focused on studying the relationship between *APOL1* genotype and morphology under a recessive model of inheritance in the subgroup of patients with FSGS. The rationale for this choice was that (1) with rare exception, published clinical studies of *APOL1's* association with nephrotic syndrome have observed harm for those carrying two, but not one, risk alleles, and (2) *APOL1* risk alleles are enriched in FSGS compared to MCD, so there is risk of spurious attribution of morphologic changes to *APOL1* genotype, rather than FSGS in general, if studying both groups of patients together.

The results of this analysis demonstrate significant correlations between an *APOL1* HR genotype with increased tubular microcysts and both a decrease in global mesangial hypercellularity and condensation of the actin cytoskeleton.

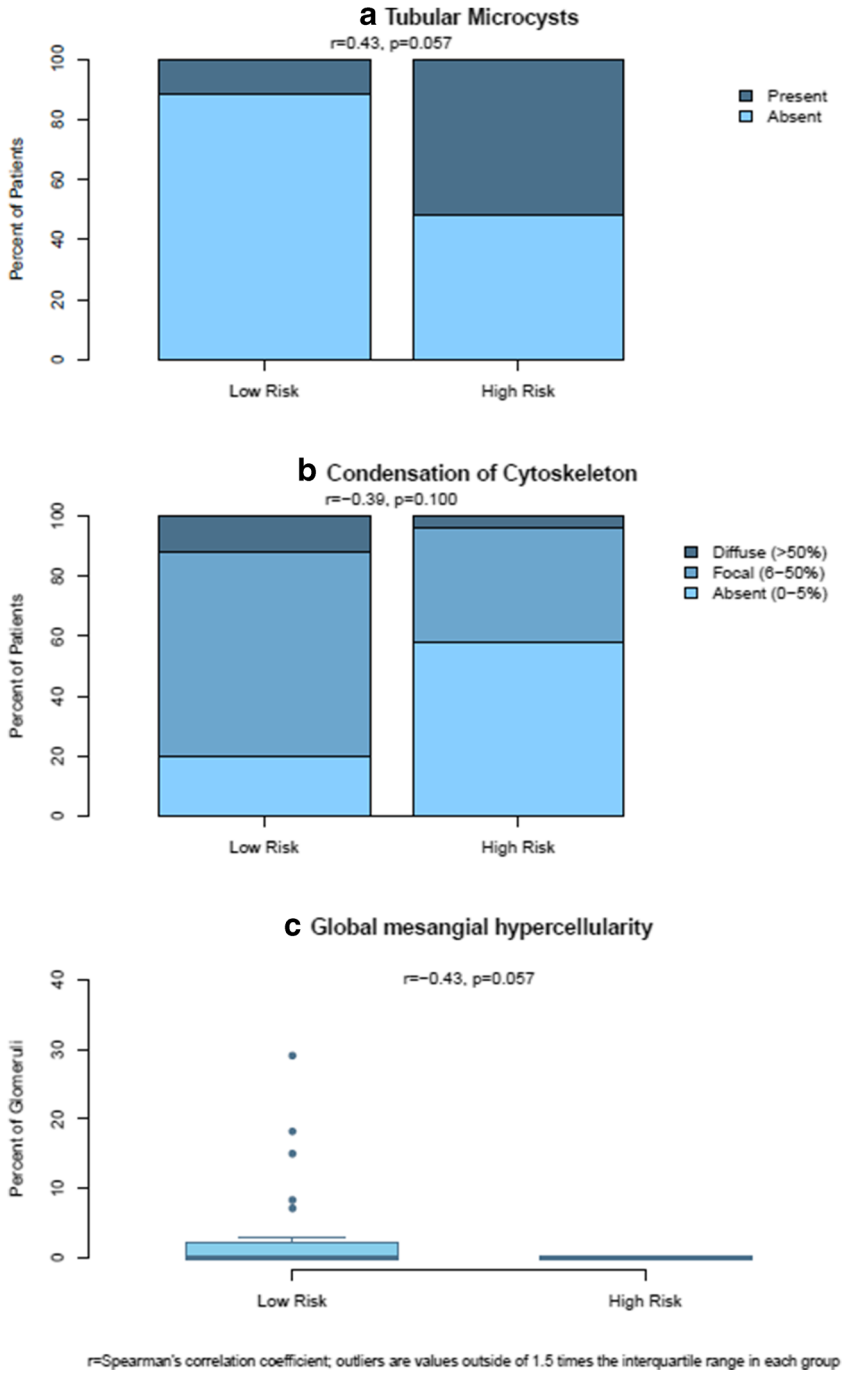

Fig 2 Morphologic descriptors associated with the *APOL1* high-risk genotype **a** presence of tubular microcysts (yes/no), **b** semiquantitative amount of condensation of the actin cytoskeleton, **c** percent of global mesangial hypercellularity

In prior studies of CKD patients with arterionephrosclerosis (and not NS), HIVAN, and collapsing glomerulonephritis with lupus, HR *APOL1* genotype was also associated with increased prevalence of microcystic tubular dilatation [18]. The authors appropriately suggested that this indicates significant tubular injury in any *APOL1*-related kidney disease, and here we extend this to FSGS as well.

Global mesangial hypercellularity, alternatively referred to as diffuse mesangial proliferation, has been alternatively considered a histologic subtype of NS ("mesangioproliferative NS") [32, 33] or as a feature observed among patients with MCD, FSGS, or other entities such as IgM or C1Q nephropathy [34, 35]. Reports of the clinical implications of global mesangial hypercellularity in patients with an NS picture vary from being associated with higher chance of remission to being associated with worse outcomes when observed in a patient with FSGS. We interpret the lower frequency of global mesangial hypercellularity observed among HR *APOL1* patients to be indicative of the different mechanisms underlying this form of FSGS. Future work incorporating our knowledge

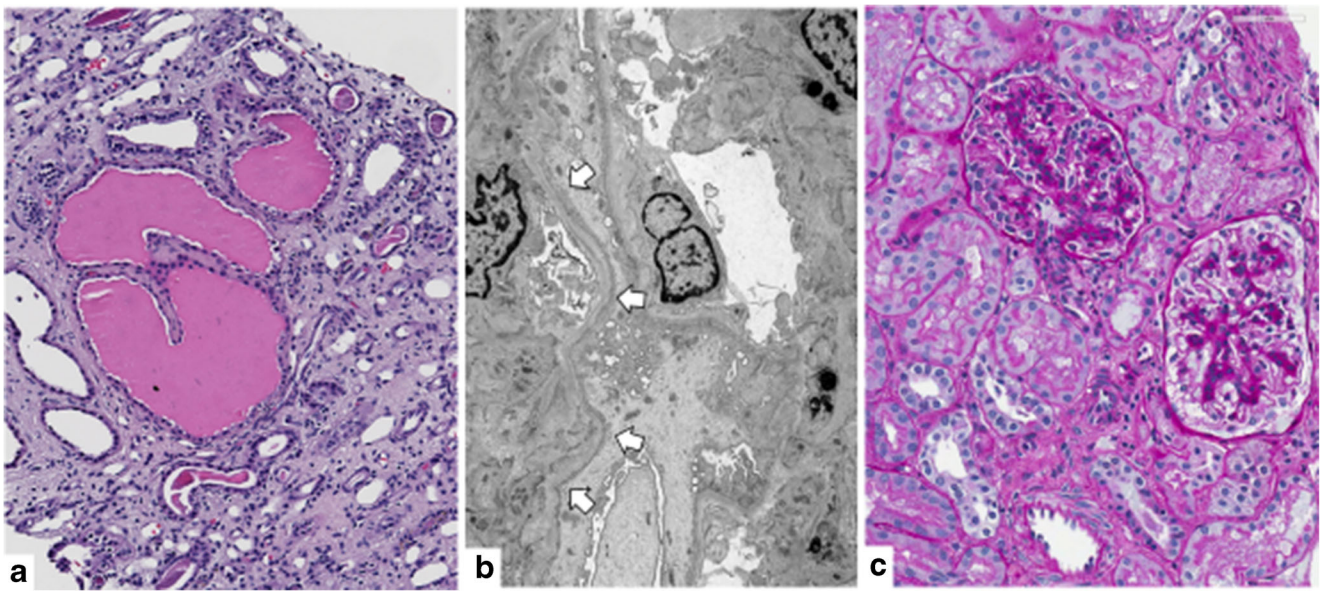

Fig 3 Representative examples of **a** tubular microcysts, **b** condensation of the actin cytoskeleton, and **c** global mesangial hypercellularity from the NEPTUNE cohort. **a** Tubular microcysts: the tubules are enlarged and have an irregular shape. They contain eosinophilic smooth proteinaceous material forming intratubular casts with scalloping of the edges of the casts. (hematoxylin and eosin stain); **b** condensation of the cytoskeleton: the cytoskeleton in podocytes with complete effacement is reorganized and condensed against the abluminal side of the podocytes facing the glomerular basement membranes (white arrow); **c** global mesangial cell hyperplasia: these 2 glomeruli reveal increased number of nuclei in the mesangium that is present in all glomerular lobules. The number of mesangial cell nuclei is 4 or more in each lobule. (Periodic acid Schiff stain)

of the pathobiology of mesangial hypercellularity may help us understand how or why these pathways are less prominent in *APOL1*-associated FSGS.

Among patients with FSGS, a HR *APOL1* genotype is also associated with less condensation of the actin cytoskeleton at the GBM aspect of the podocyte. Widespread cytoskeletal condensation seen with diffuse podocyte effacement is a classic feature of MCD [36]. But in a comparative ultrastructural study of adults with FSGS and MCD, a significantly increased level of "mat-like condensation of microfilaments" was observed among those with FSGS [37]. The reasons for less condensation in the HR state are not clear; explaining this phenomenon may enhance our understanding of its pathogenesis.

These three traits discovered using a recessive model were also significantly correlated with *APOL1* genotype under an additive model. The harm associated with one risk allele in this human study mirrors other reports that have found a deleterious impact of one risk allele, such as increased risk of HIVAN [29] and cellular damage in model systems [30, 31]. It provides further support of the relevance to humans of the potential harm coming from a single risk allele and, as reviewed elsewhere, prompts us to further understand mechanistically why this may occur [38].

While clinical studies have not reported association of *APOL1* risk alleles with MCD, we hypothesized that they would be associated with morphologic changes even in MCD and that we would detect them in this study. However, we detected no statistically significant associations at all under the recessive or additive model in the MCD subcohort. Our small sample size of MCD patients certainly resulted in less power to detect significant associations, and it may be that future studies with larger sample size will detect a signature of *APOL1* in kidneys affected by MCD.

Our availability of paired *APOL1* genotype and glomerular mRNA expression also allowed us to gain a number of insights. In contrast to our 2015 paper (which studied *APOL1* in NEPTUNE using microarray-based expression), here we found that each additional *APOL1* risk allele was associated with its increased glomerular expression. We attribute this to our current use of RNA-seq vs. previous use of microarray for measurement of expression (with its higher dynamic range of detection) and our study solely of FSGS patients rather than patients of diverse histologies prior. Second, we did not observe any significant association between increased glomerular *APOL1* expression and any morphologic changes. One explanation for this is that we were only powered to discover moderate to large changes and that the available sample size did not empower us to discover small but significant associations. Another explanation is that because our sample size did not allow us to study HR and LR *APOL1* expression's associations with morphologic outcomes separately, we were unable to detect associations that were specific to a particular genotype. Future studies of Black FSGS patients with increased sample sizes should be able to address both of these current limitations.

While our study sample size was relatively large compared with previous studies, it may have still been too small to detect some statistically significant effects, especially after controlling for false discovery rate. The sample size also limited our power and ability to adjust for all variables of interest or perform stratified analyses, particularly, the association of genotype-dependent gene expression with morphology and morphology by *APOL1* within the MCD subcohort. We were also unable to adjust or stratify by prior immunosuppressant treatment, despite the finding that a greater proportion of patients with *APOL1* LR genotype had immunosuppressant use prior to biopsy compared with patients with *APOL1* HR genotype. However, if immunosuppressant use reversed tissue damage prior to biopsy, we would expect to see fewer morphologic changes in the LR group, contradicting the greater changes we observed for condensation of cytoskeleton and global mesangial hypercellularity. Finally, we did not detect an association between these significant descriptors and any other differentially expressed genes or coexpressed gene modules. Future human-based studies, perhaps using single cell RNA-seq, larger sample sizes, and/or proteomics, may possibly be able to shed further light on these associations in a way that we could not here. Despite this limitation, the availability of *APOL1* genotype, glomerular *APOL1* gene expression, and many detailed morphologic descriptors in many glomerular disease patients is unique and a strength of our study.

By identifying associations between *APOL1* genotype, glomerular *APOL1* gene expression, and morphologic descriptors, we uncovered some known and several novel associations in an FSGS-only subcohort. Taking these observations to other human-based studies that are larger and/or have other complementary molecular datasets while in parallel pursuing them in cell lines and model systems may ultimately allow us to better understand the pathobiology underlying nephrotoxic effects of the *APOL1* HR genotype.

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Data Availability Data are available upon request through communication with corresponding authors.

Declarations

Ethics approval The work presented here followed all ethical procedures.

Consent to participate/publication All NEPTUNE participants consented to participate in the NEPTUNE studies and consented to have publications resulting from the use of their data.

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