COVID-19 infection causes collapse of glomerular capillaries and loss of podocytes, terminating in a severe kidney disease called COVID-19 associated nephropathy (COVAN). The underlying mechanism of COVAN is unknown. We hypothesized that cytokines induced by COVID-19 trigger expression of pathogenic APOL1 via JAK-STAT signaling, resulting in podocyte loss and COVAN phenotype. Here, based on nine biopsy-proven COVAN cases, we demonstrated for the first time that \textit{APOL1} protein is abundantly expressed in podocytes and glomerular endothelial cells (GECs) of COVAN kidneys but not in controls. Moreover, a majority (77.8\%) of COVAN patients carried two \textit{APOL1} risk alleles. We showed that recombinant cytokines induced by SARS-CoV-2 act synergistically to drive \textit{APOL1} expression through the JAK-STAT pathway in primary human podocytes, GECs, and kidney micro-organoids derived from a carrier of two \textit{APOL1} risk alleles but was blocked by JAK1/2-inhibitor, baricitinib. We demonstrated for the first time that cytokine-induced JAK-STAT-APOL1 signaling reduced the viability of kidney organoid podocytes but was rescued by baricitinib. Together, our results support the conclusion that COVID-19-induced cytokines are sufficient to drive COVAN-associated podocytopathy via JAK-STAT-APOL1 signaling and that JAK-inhibitor could block this pathogenic process. These findings suggest that JAK-inhibitors may have therapeutic benefits for managing cytokine-induced APOL1-mediated podocytopathy.
JAK inhibitor blocks COVID-19-cytokine-induced JAK-STAT-APOL1 signaling in glomerular cells and podocytopathy in human kidney organoids

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Abstract

COVID-19 infection causes collapse of glomerular capillaries and loss of podocytes, terminating in a severe kidney disease called COVID-19 associated nephropathy (COVAN). The underlying mechanism of COVAN is unknown. We hypothesized that cytokines induced by COVID-19 trigger expression of pathogenic APOL1 via JAK-STAT signaling, resulting in podocyte loss and COVAN phenotype. Here, based on nine biopsy-proven COVAN cases, we demonstrated for the first time that APOL1 protein is abundantly expressed in podocytes and glomerular endothelial cells (GECs) of COVAN kidneys but not in controls. Moreover, a majority (77.8%) of COVAN patients carried two APOL1 risk alleles. We showed that recombinant cytokines induced by SARS-CoV-2 act synergistically to drive APOL1 expression through the JAK-STAT pathway in primary human podocytes, GECs, and kidney micro-organoids derived from a carrier of two APOL1 risk alleles but was blocked by JAK1/2-inhibitor, baricitinib. We demonstrated for the first time that cytokine-induced JAK-STAT-APOL1 signaling reduced the viability of kidney organoid podocytes but was rescued by baricitinib. Together, our results support the conclusion that COVID-19-induced cytokines are sufficient to drive COVAN-associated podocytopathy via JAK-STAT-APOL1 signaling and that JAK-inhibitor could block this pathogenic process. These findings suggest that JAK-inhibitors may have therapeutic benefits for managing cytokine-induced APOL1-mediated podocytopathy.
Introduction

Kidney failure is a devastating complication of COVID-19 infection. Up to 50% of inpatient and 70% of intensive care unit COVID-19 admissions are complicated by acute kidney injury (AKI), which in turn increases mortality by 30-50% (1, 2). A kidney biopsy case series revealed that collapsing glomerulopathy is the most common histopathologic diagnosis in COVID-19-associated AKI (3). A distinctive feature of COVID-19 associated collapsing glomerulopathy (abbreviated COVAN) is its near-exclusive predilection for African Americans or Blacks who carry two risk alleles of Apolipoprotein L1 (APOL1) (3, 4). The two risk alleles (named G1 and G2) emerged as coding variants in the APOL1 gene and confer protection against African trypanosomiasis. However, carriage of G1G1, G2G2, or G1G2 (termed high-risk genotypes) increases the risk of a spectrum of kidney disease and explains much of the excess risk of non-diabetic kidney disease among African Americans (5-8). An estimated 13% of African Americans carry high-risk APOL1 genotypes (7). During the COVID-19 pandemic, studies found that a remarkable 92% of biopsy-proven COVAN cases were in carriers of high-risk APOL1 genotypes, 61% of whom required dialysis at presentation (3, 9). These findings establish APOL1 variants as a major contributor to the racial disparity in COVID-19 health outcomes. Despite this impressive association, the cellular mechanism that connects high-risk APOL1 genotypes to SARS-CoV-2 infection and pathogenesis of collapsing glomerulopathy of COVAN remains unknown.

The strong epidemiologic association between high risk APOL1 genotype and COVAN have led to the hypothesis that COVID-19-induced expression of APOL1 G1 or G2 in podocytes and glomerular endothelial cells—the kidney cells impacted in collapsing glomerulopathy—drives pathogenesis of COVAN. This hypothesis was supported by recent reports that transgenic overexpression of APOL1 risk alleles in mouse podocytes or glomerular endothelial cells caused podocytopathy, endotheliopathy, glomerulopathy, and clinical manifestations of kidney failure (10-14). These murine disease models suggest that the mechanism that underlies COVID-19-induced APOL1 expression would be a potential therapeutic target for COVAN. However, there are two important unknowns. One, it is unknown whether
*APOL1* protein expression is upregulated in glomeruli of patients with COVAN. Two, it is unknown whether SARS-CoV-2 induces *APOL1* expression directly by viral infection of kidney cells or indirectly via the effects of SARS-CoV-2-induced cytokine storm.

The failure to detect SARS-CoV-2 in kidney biopsies of COVAN patients provides indirect support for the hypothesis that COVAN likely results from the effects of cytokine storm rather than from direct viral infection of kidney parenchyma. The occasional detection of SARS-CoV-2 viral particles has been in autopsy kidney specimen in which the confounding effect of tissue autolysis could not be excluded (3, 4, 15, 16). Several inflammatory cytokines and chemokines have been noted to be upregulated in the sera of patients with COVID-19 and/or COVAN (4, 17, 18). This list includes cytokines such as interferons alpha, beta, gamma and TNF which are previously known to upregulate *APOL1* expression. However, the list also includes several inflammatory cytokines that are robustly upregulated by COVID-19 infection. It is unknown whether these cytokines have additive, synergistic, or antagonistic effects on *APOL1* expression and associated podocytopathy.

In the current study, we addressed these knowledge gaps by leveraging kidney biopsies obtained from nine patients with COVAN and two human control kidneys to investigate whether and where *APOL1* protein is expressed in COVAN and control kidneys. We profiled eighteen COVID-19-induced cytokines to identify eight cytokines that were sufficient, in the absence of SARS-CoV-2, to synergistically induce *APOL1* expression in primary human glomerular cells and cause podocytopathy in human kidney micro-organoids. This study not only offers the first proof in human-derived experimental model that COVID-19 cytokine storm induces *APOL1* expression and podocytopathy, it also identifies the common signaling pathway that mediates the pathogenic effects. This study has implications that could impact strategies for screening and treating COVAN in Black and Hispanic patients. It raises question about safety of supplemental interferons as COVID-19 therapy in Black and Hispanic individuals who carry high-risk *APOL1* genotypes (19, 20).
Results

APOL1 expression is upregulated in podocytes and glomerular endothelial cells of COVAN patients.

To investigate whether patients with biopsy-proven diagnosis of COVAN have elevated expression of APOL1 protein in their podocytes and glomerular endothelial cells (GECs), we performed immunohistochemical co-staining of APOL1, synaptopodin (an actin-associated protein of differentiated podocytes), and CD31 (an endothelial cell marker) on kidney biopsies of two patients with COVAN diagnosis (Figure 1). APOL1 expression was abundant in glomeruli of both case 1, who was biopsied ten months after COVID-19 diagnosis (Figure 1A-F), and case 6, who was biopsied nine days after COVID-19 diagnosis (Figure 1G-L). In both patients, there was strong APOL1 staining in synaptopodin-positive podocytes (arrow; Figure 1B, E, H, and K) and along CD31-positive glomerular endothelium (arrowhead; Figure 1C, F, I, and L). The presence of APOL1 protein in podocytes and GECs at time 9 days and 10 months after diagnosis of COVID-19 infection suggests that APOL1 expression is induced early and may persist in the glomeruli for several months, long after the triggering COVID-19 infection has resolved.

APOL1 expression is upregulated in biopsy tissue of COVAN cases but not in controls. To further evaluate the generalizability of these immunohistochemical findings, we identified a total of nine COVAN cases with available biopsy tissue for genotyping and IHC (Figure 2) as well as two control patients, including one autopsy control of a patient who had COVID-19 infection but did not develop AKI (Figure 2B and 2C). The classic histopathologic features of COVAN included glomerular capillary tuft collapse with adjacent podocyte hypertrophy and proliferation, often with the podocyte protein reabsorption droplets associated with glomerular proteinuria (Supple Fig1 and Supple Fig2). APOL1 IHC staining was absent in all glomeruli of controls (Figure A-C) but present in glomeruli of all nine COVAN patients (Figure 2D-X). APOL1 staining was abundant in the cytoplasm of podocytes, GECs, and in some parietal epithelial cells (Figure 2N, hashed circle) (Figure1, Figure 2 and Suppl Fig3). APOL1 protein could be seen in glomeruli with open capillaries as well as in areas of glomerular
collapse. The apparent presence of APOL1 protein within some capillary lumen likely represented circulating APOL1, which is produced primarily by the liver (21). Moreover, APOL1 was also seen in peritubular capillaries and in injured tubular epithelial cells (asterisks). The specificity and significance of this latter finding is unclear. Notably, seven of the nine COVAN cases carried high risk APOL1 genotype (Figure 2D-V). The other two cases, case 9 and case 4, carried low-risk G0G0 genotype (Figure 2W and 2X). Despite being of low-risk genotype, case 9 APOL1 expression was comparable to those of the seven high-risk cases (Figure 2W). There were only two glomeruli in case 4 kidney biopsy slide, and APOL1 expression was lower in these glomeruli (Figure 2X). Together, these findings demonstrate that the basal kidney APOL1 expression is low in glomeruli of individuals without glomerular injury, even when the individual has COVID-19 infection; whereas APOL1 expression becomes upregulated in podocytes and GECs in the setting of COVAN in 89% of our cases.

As shown in Table 1, seven of the nine patients (77.8%) with biopsy-proven COVAN self-identified as African American. Six of these seven patients (85.7%) carried high-risk APOL1 genotype (four G1G1, one G1G2, one G2G2). By comparison, 13% of African Americans carry high-risk APOL1 genotype. The remaining two patients self-identified as White Hispanic but notably, one of them also carried high-risk APOL1 genotype. In total, seven of the nine COVAN patients (77.8%) carried high-risk APOL1 genotype. The median age of the cases was 51 years (range 37-60). All nine cases developed acute kidney injury and had varying degrees of proteinuria, ranging from subnephrotic to nephrotic range (1.4-14 g/24hrs). Most biopsies were performed at least 1 month after COVID-19 infection, with the exception of case 6 who was biopsied 9 days after positive PCR test. One patient’s biopsy was not pursued until 10 months after infection secondary to incomplete recovery. All nine patient biopsies exhibited collapsing glomerulopathy, tubular injury, and interstitial inflammation. Endothelial tubular reticular inclusions were not observed in any of the cases. Notably, in the two COVAN biopsies that were tested for direct SARS-CoV-2 viral infection by IHC and in situ hybridization, no virus was detected (data not shown).
Recombinant COVID-19-Induced cytokines synergistically upregulate APOL1 expression in primary human glomerular endothelial cells and podocytes. To investigate whether COVID-19-induced cytokine storm is sufficient to trigger APOL1 expression in human glomerular cells, we cultured primary human podocytes isolated from deceased donor kidney and primary human glomerular endothelial cells (GECs) in 1 of 18 cytokines and chemokines previously reported to be elevated in the serum of patients with SARS-CoV-2 (Figure 3A) (4, 17). Podocyte identity was confirmed with multiple podocyte markers including Wilms tumor1, synaptopodin, nephrin, and podocalyxin (Figure 3B and Suppl Fig 4A-B). GEC identity was confirmed by expression of PECAM1 relative to human embryonic kidney 293 cells (HEK) (Figure 3C). Induced APOL1 expression was quantitated by qPCR and immunoblot after 48hr treatment in GECs (Figure 3D and 3F) and podocytes (Figure 3E and 3G). Consistent with prior report (22), we found that interferons (gamma > beta > alpha) robustly induced expression of APOL1 in both GECs and in podocytes. Similarly, we found that TNF also induced a modest APOL1 expression in GECs and podocytes. Unexpectedly, we found that three cytokines—IL-6, IL-1β, and IL-18, which were previously unrecognized as inducers of APOL1 expression, also individually induced modest APOL1 expression in GECs or podocytes. Notably, the combination of all 18 recombinant cytokines produced a synergistic upregulation of APOL1 that was an order of magnitude higher than that produced by any of the interferons alone (Figure 3D). These effects were not only observed with cytokine concentration of 50ng/mL (Figure 3D and 3E) but also at 20ng/mL and 10ng/mL (Supplemental Figure 5). Cytokine conditions inducing >1.5 fold APOL1 transcript compared to media-treated control were further analyzed for significance. Significance was assessed using unpaired t-test with Holm-Sidak correction for multiple comparisons. P-values reported are the adjusted p-values. These results expand the list of physiologic cytokines that are capable of inducing APOL1 expression beyond the well-recognized interferons and TNF. Importantly, the findings also suggest that the synergy of COVID-19-induced cytokines may be more relevant for APOL1 regulation than the impact of an isolated cytokine alone.
**JAK-STAT signaling mediates COVID-19-cytokine-induced APOL1 expression.** We next investigated whether COVID-19 induced cytokines upregulate APOL1 expression via a common intracellular signaling pathway that could be exploited as a therapeutic target. It was previously reported that interferon induction of APOL1 is mediated by JAK-STAT1/2 (22, 23). Signaling through the IL-6 receptor has been shown to be mediated by STAT3, and both IL-1β and TNF are reported to indirectly activate STAT3 (24, 25). Of interest, Meliambro et al recently reported upregulation of phospho-STAT3 in the biopsy tissue of a case of COVAN and HIV-associated nephropathy (HIVAN) compared to control (26). Based on this background information, we hypothesized that the JAK1/2-STAT1/2/3 pathways are the primary mediators of the effects of COVID-19-induced cytokines in driving APOL1 expression. To test this hypothesis, we determined the state of these signaling pathways by measuring the phosphorylated STAT1, 2, and 3 in lysates of GECs after culturing them in individual or combined cytokines for 48 hours (Figure 3F). Type I interferons (IFNα and IFNβ) increased phosphorylation of STAT1-3 while IFNγ upregulated phosphorylation of STAT 1 and 3. IL-1β, TNF, and IL-6 increased phosphorylation only of STAT3. Combined-cytokines increased phosphorylation of STAT1-3. Knowing that JAK1 and JAK2 are the primary upstream protein kinases that phosphorylate STAT1-3, we hypothesized that inhibition of JAK1/2 would block APOL1 expression induced by “all cytokines”. Consistent with this prediction, we found that JAK1/2-specific inhibitor, baricitinib, significantly reduced APOL1 mRNA and APOL1 expression by all-cytokine treated GECs and primary podocytes (Figure 3D-G). Together, these results demonstrate that JAK-STAT signaling is the primary pathway that mediates COVID-19-cytokine-induced APOL1 expression.

**COVID-19-induced cytokines are sufficient to drive APOL1 expression in human iPSC-derived kidney micro-organoids via JAK-STAT pathway.** Human kidney micro-organoid is a proven platform for modeling human kidney disease and facilitating clinical translation. We asked whether the results we obtained from primary human podocytes and GECs were generalizable and validated by human-derived kidney micro-organoid model. Therefore, we generated kidney micro-organoids from induced
pluripotent stem cells (iPSCs) of an African American carrier of G1G2 APOL1 genotype to investigate APOL1 regulation, expression, and outcomes in this model (Figure 4A). We cultured kidney micro-organoidss in IFNγ 10ng/mL or a combination of eight cytokines (IFNγ, IFNα, IFNβ, IL-18, IL-8, IL-6, TNF, IL-1β) each at 10ng/mL either in the absence or presence of baricitinib, 10µM for 24 hours. These eight cytokines were chosen due to their observed regulation of APOL1 expression in the preceding experiments. Podocytes and tubular epithelial cells in the kidney micro-organoids were marker-confirmed (Figure 4B). Consistent with literature (27), endothelial cells were underrepresented in the kidney micro-organoids (data not shown). We discovered that basal APOL1 protein expression was low in micro-organoids. IFNγ treatment induced substantial APOL1 expression, with highest intensity co-localized to areas of podocyte marker, podocalyxin. The cocktail of cytokines induced an outsized and robust APOL1 expression throughout the micro-organoid structure when compared to other treatments. Groups treated with IFNγ plus baricitinib and all cytokines plus baricitinib showed no APOL1 expression, consistent with complete inhibition of cytokine effect. The APOL1 expression in kidney micro-organoid podocytes was reminiscent of that seen in podocytes of COVAN patients. However, unlike COVAN kidneys in which no significant APOL1 expression was seen in healthy tubular epithelial cells, kidney micro-organoid E-Cadherin-positive tubular epithelial cells expressed APOL1. This difference could be due to differences in membrane cytokine receptors or epigenetic factors that impact protein expression in the immature tubules of the kidney micro-organoids. In summary, human iPSC-derived kidney micro-organoids cultured with COVID-19-induced cytokines show robust upregulation of pathogenic G1G2 APOL1 protein and the expression was blocked by inhibition of the JAK-STAT signaling.

Cytokine-induced JAK-STAT-APOL1 signaling reduced the viability of kidney micro-organoid podocytes which was rescued by JAK-inhibitor. Finally, we asked if the G1G2 APOL1 expressed in kidney micro-organoid impairs podocyte viability. We hypothesized that cytokine-induced variant APOL1 protein would cause podocyte loss—a hallmark phenotype of COVAN. To test this hypothesis, we isolated
podocytes from kidney micro-organoids generated from iPSCs of a carrier of G1G2. The podocytes were cultured in IFNγ (10ng/mL), or combination of eight cytokines (10ng/mL each), both in the presence and absence of baricitinib (10µM) for 96 hours (Figure 5A and B). Cytokine treatment robustly induced \textit{APOL1} expression and this expression was blocked by baricitinib, consistent with our earlier experiments (Figure 5C). Concordantly, cytokine treatment caused significant podocyte loss as indicated by viability assay and total cellular ATP (Figure 5D and 5E). Remarkably, baricitinib completely rescued the cytokine-induced podocyte loss. Together, these results support the conclusion that COVID-19-induced cytokines trigger JAK-STAT-APOL1 signaling which in turn causes podocyte injury and loss. The protective effect of JAK-inhibition on podocyte viability strongly supports this hypothesis.
Discussion

The major conclusions of the current study are that several COVID-19-induced cytokines beyond interferons act synergistically via JAK-STAT signaling to drive pathogenic APOL1 expression, resulting in podocyte injury and loss which is blocked by JAK inhibition. Based on a case series, we demonstrate for the first time that APOL1 protein is abundantly expressed in podocytes and GECs of patients diagnosed with COVAN but not in the glomeruli of healthy controls nor of COVID-19-positive but COVAN-negative control. In three experimental models, we demonstrate that recombinant cytokines upregulated in COVID-19 infection are sufficient to drive robust APOL1 expression, and unexpectedly, that the strong synergism produced by combination of cytokines was mediated predominantly through a common intracellular signaling pathway. Collectively, our experimental evidence strongly supports a causal relationship between cytokine-induced JAK-STAT-APOL1 signaling and in vivo COVAN glomerular phenotype, and supports further investigation into this therapeutic target.

The increased frequency of high-risk APOL1 genotype (77.8%) among patients with COVAN that we report here correlates with a recent international multi-center pathology review that reported high-risk APOL1 genotype in 91.7% of COVAN patients (3). Given that the frequency of high-risk APOL1 genotype in the general African American population is 13% (28), discovering a frequency of 77-90% in COVAN is profound and comparable to the 60-70% frequency reported in HIV-associated nephropathy (HIVAN) (28-31). The existence of the remaining 20-30% of COVAN (and HIVAN) cases who do not carry high risk APOL1 genotypes suggests the possibility of an APOL1-independent pathomechanism or the possibility that in some cases COVID-19-induced supraphysiologic expression of G0 APOL1 may also cause podocytopathy. Parsing these possibilities will require further studies. Nevertheless, we previously demonstrated in human embryonic kidney (HEK) cells with tetracycline-inducible APOL1 expression system that cytotoxicity of APOL1 is both variant-and dose-dependent (32, 33). Dose-dependent APOL1 cytotoxicity was also reported by other investigators in similar cell-based systems (34). Moreover, APOL1 transgenic mouse models have not only validated the causal link between
APOL1 risk alleles and podocyte injury, but have demonstrated that the degree of podocytopathy correlated with APOL1 expression levels (10, 12-14, 35). Our discovery that eight out of nine COVAN cases demonstrated robust glomerular APOL1 expression relative to controls and the evidence that expression of endogenous APOL1 risk alleles causes podocytopathy in human kidney micro-organoids supports the causal link between APOL1 and podocytopathy. Conversely, the lack of APOL1 in the glomeruli of COVID-19 positive but AKI-negative G0G0 autopsy control suggests that COVID-19 infection without APOL1 induction is not a sufficient driver of COVAN disease.

Our results shift the current paradigm of interferon-induced APOL1-nephropathy. Several examples in which high interferon states caused collapsing glomerulopathy in carriers of high risk APOL1 genotype led to a paradigm that privileged interferons as the chief second-hit triggers of APOL1-mediated glomerulopathy (31, 36, 37). This paradigm was further reinforced by the fact that interferon alpha, beta, and gamma induced APOL1 expression in cultured podocyte and endothelial cell lines (22). However, this architype is challenged by the observation that interferons are not always elevated in serum of patients with COVID-19 infection and COVAN, whereas other cytokines including IL-6, IL-1β, and IL-18 are increased (4, 17, 18). In the current study, we demonstrated that even in the absence of interferons, these non-interferon cytokines individually and collectively induced robust APOL1 expression in human podocytes and GECs while also highlighting a previously unappreciated synergism. By demonstrating that JAK-STAT signaling is the central mediator of these combined cytokine effects, our results provide a plausible explanation for how the COVID-19 cytokine storm drives APOL1 expression and the high incidence of collapsing glomerulopathy seen in patients with risk variant APOL1 and COVID-19 infection. These findings may have implications for other APOL1-mediated nephropathies.

While our results show that COVID-19-induced cytokines are sufficient to induce APOL1 expression and cause the loss of kidney micro-organoid podocytes, they do not exclude the possibility that SARS-CoV-2 may also directly infect kidney cells as it was recently reported (38, 39). However, SARS-CoV-2 in human kidney tissue has only been demonstrated in autopsy specimen in which the confounding
contribution of tissue autolysis could not be excluded (3, 4, 15, 16). Most reports from kidney biopsies of patients with COVAN have failed to detect SARs-CoV-2 virus despite using sensitive methods (3, 4), including two of the nine cases in the current study which were tested by immunohistochemistry and in situ hybridization.

By demonstrating cytokine-induced podocytopathy, our kidney micro-organoid model diverged from recently published kidney organoid model of APOL1-mediated kidney disease by Liu et al who generated kidney organoids from CRISPR-edited iPSCs of a non-African donor in which G0 APOL1 alleles were edited to G1 alleles but on a G0 genetic background. While interferon gamma-induced APOL1 expression in their organoids, it did not cause cytotoxicity (40). The lack of cytotoxicity in their kidney organoid model could be explained by the less toxic genetic background on which G1 mutations were superimposed. It is known that cytotoxicity of APOL1 haplotype is affected by its genetic background (41). The kidney micro-organoid in the present study was generated from unedited iPSCs of an African American carrier of G1G2 genotype. Preservation of the native genetic haplotype may have contributed to the APOL1-associated cytotoxicity seen in our kidney micro-organoids.

Furthermore, in contrast to the upregulated APOL1 protein expression we report here, a recent study found no difference in APOL1 mRNA level in the kidney biopsy of one patient with COVAN compared to healthy controls (26). This disagreement in our results may be explained by the transient nature of APOL1 mRNA relative to protein, especially when biopsies were obtained several weeks after the initial diagnosis of COVID-19 infection when the acute effects of COVID-19-induced cytokine storm and corresponding mRNA expression profile may have dissipated. It is conceivable that the farther one is from the COVID-19 induced cytokine storm, the weaker the acute phase reactants downstream of the cytokine receptor become, including phosphorylated STAT proteins and APOL1 mRNA. Our results suggest that the induced APOL1 protein persists beyond APOL1 mRNA and phosphorylated STATs.
This study has three major clinical implications. One, they underscore the need to genotype Black or Hispanic individuals found to have collapsing glomerulopathy in the context of active or recent COVID-19 infection. However, if kidney biopsy is not feasible or possible, *APOL1* genotyping of Black or Hispanic COVID-19 infected individuals with new or worsening proteinuria and AKI is also likely to be high yield. Secondly, because multiple COVID-19-induced cytokines redundantly activate the JAK-STAT pathway to induce *APOL1* expression, a therapeutic strategy based on selective removal or inhibition of any one cytokine is unlikely to be effective in preventing or treating COVAN. Currently, baricitinib is only authorized for use under an emergency use authorization for treatment of COVID-19 requiring supplemental oxygen. Therefore, its potential as a treatment for COVAN requires serious consideration, especially for Black and Hispanic carriers of high risk *APOL1* genotypes who have COVID-19 infection. Lastly, based on the evidence that interferon deficiency is associated with severe COVID-19 infection, it was proposed that interferon be administered as therapy for COVID-19. At the time of this writing, according to ClinicalTrials.gov, thirty-six clinical trials of interferon as therapy in COVID-19 are either ongoing or completed. In contrast to the rationale behind these clinical trials, our results caution against administration of interferons as treatment for COVID-19 infection in carriers of high risk *APOL1* genotype because interferons could upregulate expression of pathogenic *APOL1* in the kidney and precipitate COVAN.

Our study has some limitations. Our COVAN case series of nine biopsy-proven collapsing glomerulopathy cases is a relatively small sample size and may have underestimated the association between high risk genotype and COVAN or been subject to sampling bias. The logistical challenge of obtaining kidney biopsies from patients with active COVID-19 infection in acute care setting limits the frequency of kidney biopsies in this population. The same factor is likely responsible for the fact that most of the biopsies in this study were performed several weeks to months after the initial diagnosis of COVID-19 infection. Analysis of kidney biopsies obtained closer to the infectious trigger may provide additional insights into early cellular phenotypes. Additionally, the current study does not identify who
and when to treat COVAN with JAK inhibitors. Answers to these questions and the determination of the efficacy of JAK inhibition as treatment for COVAN will be the focus of future investigations.

This work highlights the association of high-risk APOL1 genotype and the role of JAK-STAT-APOL1 signaling in the development of COVAN. As our understanding of the COVID-19 pandemic evolves, there is an urgent need to increase clinician and public health awareness about the renal complications of COVID-19. There is also an urgent need for COVAN therapy. Our study offers new data on JAK inhibitors as strong therapeutic candidates for APOL1-associated COVAN.
Methods

Antibodies and reagents. Primary antibodies against the following proteins were used: APOL1 rabbit anti-human [Genentech, 3.1C1&3.7D6; Western blot [WB] 1:5000 (final concentration 0.05 ug/mL); Genentech, 5.17D12 [IHC] 1:4000 (final concentration 0.95 ug/mL) according to recent report (42)]; APOL1 mouse anti-human [Genentech, 4.17A5; Immunofluorescence [IF] 1:2000 (final concentration 2.13 ug/mL)]; GAPDH mouse anti-human (Santa Cruz Biotechnology, sc47724; WB 1:200); Vinculin mouse anti-human (Sigma-Aldrich, V9131; WB 1:200); NEPH1 mouse anti-human (Santa Cruz Biotechnology, sc373787; WB 1:300); WT1 rabbit anti-human (Abcam, ab89901; WB 1:1000); STAT1 mouse anti-human (Cell Signaling Technology [CST], 9176s; WB 1:1000); STAT2 rabbit anti-human (CST, 72604: WB 1:1000); STAT3 mouse anti-human (CST, 9139; WB 1:1000); Phosphorylated-STAT1 (Y701) rabbit anti-human (CST, 9167; WB 1:1000); Phosphorylated-STAT2 (Y690) rabbit anti-human (CST, 88410; WB 1:1000); Phosphorylated-STAT3 (Y705) rabbit anti-human (CST, 9145; WB 1:2000); PODXL goat anti-human (R&D, AF1658; IF 1:500), E-Cadherin rabbit anti-human (Cell Signaling, 3195S; IF 1:200), NEPH1 mouse anti-human (Santa Cruz, sc-373787; IF 1:100). Secondary antibodies included goat anti-rabbit IgG, HRP-linked antibody (CST, 7074s; WB 1:1000); horse anti-mouse IgG, HRP-linked antibody (CST, 7076s; WB 1:1000); Alexa Fluor 488 conjugated donkey anti-mouse (Jackson ImmunoResearch, 715-546-150; IF 1:1000), Alexa Fluor 594 conjugated donkey anti-goat (Jackson ImmunoResearch, 705-585-147; IF 1:1000), and Alexa Fluor 405 conjugated donkey anti-rabbit (Thermo Scientific, A48258; 1:000). For qRT-PCR, TaqMan Gene Expression Assays included APOL1 (Hs01066280_m1), GAPDH (Hs03929097_g1), and PECAM-1 (Hs00169777_m1).

Kidney Immunohistochemistry staining for APOL1, synaptopodin and CD31. Starting with formalin fixed paraffin embedded kidney biopsy slides, antigen retrieval was performed with (EDTA solution at pH 8.0) for 56 minutes at 100 °C. Primary antibodies to APOL1 (5.17D12, Genentech), synaptopodin (Progen Biotechnik, 61094) and CD31 (Cell Signaling, 3528s) were applied at 1:4000 (final concentration 0.95 ug/mL), 1:100 and 1:1600, respectively for 60 minutes at 36 °C. Ready-to-use HQ-conjugated
secondary anti-rabbit multimers (760-4815) were incubated for 12 minutes at 36 °C. This was followed by addition of anti-HQ HRP for 12 min. DAB (760-159) was incubated for 5 minutes at room temperature. Tissue section was counterstained hematoxylin (760-2021) for 4 min at room temperature. Bluing reagent (760-2037) was added for 4 minutes at room temperature.

Cytokine treatment of cultured cells. Primary human glomerular endothelial cells, primary podocytes subcultured from human donor kidney, and organoid-derived podocytes were treated with 50ng/mL, 20ng/mL, or 10ng/mL concentration of the following cytokines: Recombinant Human CXCL9 (Biolegend, 578102); Recombinant Human CXCL10 (Biolegend, 573502); Recombinant Human CXCL13 (Biolegend, 573502); IL-1β (PeproTech, 200-01B); IL-15 (PeproTech, 200-15); IL-18 (Biolegend, 592102); IL-8 (Biolegend, 574202); Interferon (IFN) alpha 1 (Sigma-Aldrich, SRP4596); IFN beta (Peprotech, 300-02BC); IFN gamma (Sigma-Aldrich, I17001); IL-6 (Peprotech, 200-06); TNF-alpha (Peprotech, 300-01A); MCP-1 (CCL2) (Peprotech, 300-04); MIP-1alpha (CCL3) (Peprotech, 300-08); IL-10 (Peprotech, 200-10); IL-7 (Peprotech, 200-07); IL-2 (Peprotech, 200-02); G-CSF (Peprotech, 300-23). Standardized cytokine concentration of 50ng/mL was pre-determined based on precedent from human cell treatments evaluating cytokine shock syndromes in COVID-19 infection (17). Follow-up experiments used concentrations of 20ng/mL and 10ng/mL. Subsequent treatments, including organoid and organoid-derived podocyte experiments, were performed using 10ng/mL. JAK 1/2 inhibitor, baricitinib (INCB028050) (Selleckchem, S2851), was used at 10uM final concentration in all experiments.

Primary human glomerular endothelial cell culture. Frozen stock of low-risk (G0G0) primary human glomerular endothelial cells were purchased from Celprogen (36066-05), thawed and cultured in human glomerular endothelial primary cell culture complete media with serum, antibiotic free (Celprogen, M36066-05SA) on plates coated with proprietary extracellular matrix (Celprogen, E36066-05-PD10 and E36066-05-12Well) in humidified environment at 37°C and 5% CO₂. Cells were passaged using 1X
Trypsin EDTA (Celprogen, T1509-014). Cells were used for experiments between passages 2 to 4. Cells were validated by qRT-PCR showing enrichment in PECAM1 gene expression (endothelial cell marker, also known as CD31).

**Glomerular isolation from donor human kidney and podocyte subculture.** Human donor kidney was procured through the National Disease Research Interchange (NDRI). The donor APOL1 genotype was G0G1. Glomerular isolation was performed using the sieve method adapted from prior publication (43, 44). Briefly, working on ice in a sterile hood, the kidney was first decapsulated and cut in half mid-sagittally. The medulla was dissected away, leaving the cortex remaining. The cortex was then minced and passed sequentially through stainless steel mesh sieves (sizes 425µm, 250µm) and collected on top of a third sieve (150µm) while washing frequently with pre-cooled Phosphate Buffered Saline (PBS) with 1% BSA (without calcium and magnesium) (Endecotts, Sieves 100SIW.150, 100SIW.250, 100SIW.425). Glomeruli were collected, centrifuged, and resuspended in 5mL PBS. 10µL of sample were stained with NucBlue Live ReadyProbes Reagent (Hoechst 33342) (Thermo Fisher Scientific, R37605) and visualized by light microscopy. Glomeruli were then incubated in digestion buffer [DMEM/F12, 1mg/mL each of (collagenase I, IV, and V), DNAse I (50 U/mL or 50 µg/mL)] at 37°C for 1hr to obtain a single cell suspension (Thermo Fisher Scientific, 10565018; StemCell Technologies, 07415m 07426, 07430; SigmaAldrich, 11284932001). DMEM/F12 with 10%FBS (R&D Systems, S10350H) was added to stop digestion and the sample was centrifuged at 450g x5minutes at 4°C. Isolated podocytes were subcultured in Advanced RPMI (Fischer Scientific, MT10040CV) with 10%FBS and 1%penicillin-streptomycin (Thermo Fisher Scientific, 15070063) on vitronectin coated plates (StemCell Technologies, 07004) at 37°C and 5% CO₂. Subcultured cells were visible at ~5 days and were treated after 2 weeks in culture.

**Protein extraction and Western blotting.** All culture plates and samples were maintained at 4°C. Monolayer of cells were lysed and harvested with Cell Lysis Buffer (Cell Signaling, 9803) with complete mini protease inhibitor and phosphatase inhibitor (Sigma-Aldrich, 04693159001, 04906837001).
Samples were sonicated at level 4 for 10 seconds each, centrifuged at 12000rpm x5 min, supernatant was collected in new Eppendorf tube, and protein concentration was determined by BCA protein assay (Pierce, 23225). Protein lysates were diluted with 4x LaemmLi sample buffer+ 2-mercaptoethanol (BioRad, 1610747; Thermo Fisher, 21985023) and heated at 95-100°C for 5 minutes. Protein lysates were then separated using Criterion TGX stain-free gels (4-20%) and transferred using Bio-Rad Trans-Blot Turbo transfer system. Transferred membranes were blocked for 1hr in 3% nonfat milk in Tris-buffered saline and incubated with specific primary antibodies overnight at 4°C. Subsequent day after standard washing, membranes were incubated with horseradish peroxidase-conjugated secondary antibody for minimum 1hr at room temp prior to imaging with Bio-Rad ChemiDoc MP Imaging System per manufacturer’s instructions.

**RNA extraction and qRT-PCR.** RNA was isolated from cell monolayer using RLT lysis buffer and Qiagen RNEasy Mini Kit (74106) per manufacturer’s instructions. RNA was transcribed into cDNA using Invitrogen SuperScript IV Reverse Transcriptase reagents and protocol (Thermo Fisher, 18091050). qRT-PCR reactions were performed with Applied Biosystems TaqMan Fast Advanced Master Mix (Fischer Scientific, 44-445-57) and Gene Expression Assays using QuantStudio 6 Flex System (Thermo Fisher Scientific) using ∆∆Ct method with GAPDH as reference gene.

**Genotyping.** DNA was extracted from FFPE tissue blocks using the QIAamp DNA FFPE Tissue Kit (Qiagen, 56404) per manufacturer’s protocol. Genotyping was performed using Applied Biosystems Taqman allelic discrimination assays for G1 SNP (p.Ser342Gly) and G2 polymorphism (p.Asn388_Tyr389del) using QuantStudio 6 Flex System. This assay has 100% analytic specificity and an analytic sensitivity (limit of detection) of 1.0ng DNA for the detection of APOL1 risk variants in DNA extracted from peripheral blood monocytes. Assays were previously validated by comparing results to direct sequencing (Sanger sequencing). Genotyping quality control measures included the use of technical replicates, 100% matching of positive controls of each genotype, and negative controls.
**Derivation of G1G2 patient iPSC kidney micro-organoid.** Kidney micro-organoids were derived per published protocol (27), with some modifications. Briefly, induced human pluripotent stem cells (iPSC) were dissociated into single cells using TrypLE Select and seeded onto vitronectin-coated plates in StemFlex media (Thermo Fisher Scientific, A3349401) with 10uM Rho kinase inhibitor (Tocris Bioscience, 1254) at a density of 11,000-14,000 cells/cm² and cultured in humidified environment at 37°C and 5% CO₂. Cells were then transitioned to TeSR-E6 media (Stemcell Technologies, 05946) with 8 µM CHIR99021 (Tocris Bioscience, 4423) for 4 days. From day 5 to day 7, cells were treated with 200 ng/mL FGF9, 1 µg/mL heparin and 1 µM CHIR99021. On day 7, cells were washed with PBS and dissociated with TrypLE. Dissociated cells were then washed with plain DMEM and centrifuged at 300xg for 5 minutes. Cell pellet was resuspended in Stage1 media [TeSR-E6 containing 200 ng/mL FGF9, 1 µg/mL heparin, 1 µM CHIR99021, 0.1% PVA, 10 µM Rho kinase inhibitor (Tocris Bioscience)] and transferred to a 24-well AggreWell 400 plate (Stemcell, 34411) at approximately 1.2 million cells/well. The plate was then centrifuged at 100xg for 3 minutes and incubated for 48hrs at 37°C and 5% CO₂ in standard cell culture incubator. After 48 hours (day7+2), organoids from the AggreWell were transferred to 6-well low attachment plate with Stage2 media [TeSR-E6 containing 200 ng/mL FGF9, 1 µg/mL heparin, 1 µM CHIR99021, 0.1% PVA] on orbital shaker inside cell incubator for another 72 hours. From day 7+5 onwards, all organoids were refreshed with Stage3 media [TeSR-E6 containing 0.1% PVA] on alternative days until used for experiments.

**Podocyte isolation from micro-organoid.** Isolation of glomeruli from kidney organoids was adapted from previously described protocol (45). Briefly, groups of iPSC-derived kidney organoids with an initial starting cell number of 1.2x10⁶ iPSCs were dissociated by incubation with TrypLE select (Thermo Fisher) for 5 min at 37 °C. Gentle mixing using a 1 mL pipette was applied every 2-3 min to aid dissociation. Per group, a single 70 µm cell strainer (PluriSelect) was placed onto a 50 mL tube (Falcon) and the mesh hydrated with PBS. The cell solution was added to the using a 1 mL pipette, allowing flow through of the solution by gravity. Using the plunger from a 1 mL sterile syringe, the remaining cell
solution captured on the strainer was gently pushed through the mesh. The strainer was washed with PBS and discarded, keeping the cell flow-through. The cell flow-through was then pipetted onto a pre-hydrated 40 µm cell strainer (Pluriselect) placed onto a fresh 50 mL tube (Falcon) allowing single cells to flow through by gravity and washing the sieve extensively with PBS to remove any remaining single cells. The largest glomeruli were then collected from the 40 µm cell strainer by inverting the sieve onto a fresh 50 mL tube, and washed using PBS to flush out the captured glomeruli. This process was repeated using flow-through from 40 µm process using the final 30 µm cell strainer (PluriSelect) to collect the smaller glomeruli. Isolated glomeruli from IPSC-derived kidney organoids were cultured on vitronectin coated plates with advanced RPMI 1640 containing 10% FBS in standard cell culture incubator at 37 °C plus 5% CO2. The media were refreshed every other day until cells were used for experiments.

Immunofluorescence staining, micro-organoid. Kidney micro-organoids were washed with PBS and fixed in 4% PFA in PBS for 30-45 min on ice. Fixed organoids were then washed three times with PBS, and incubated in 30% sucrose in PBS overnight at 4°C. Organoids were embedded in OCT and subjected to 10 µm cryosectioning with a Laica Cryostat. Organoids cryosections were washed three times with PBS and then blocked with blocking buffer 1 (1% fish gelatin, 2% donkey serum, 0.3% Triton X-100 in PBS) for one hour at room temperature. Next, cryosections were incubated with primary antibodies in blocking buffer 1 overnight at 4°C. Cryosections were washed three times with PBS. After wash, cryosections were incubated with secondary antibodies in blocking buffer 1 for 2 hours at room temperature. After five washes with PBS, the cryosections were mounted with Prolong Glass antifade mounting solution (Thermo Scientific, P36980). Fluorescent images were generated using an ECHO microscope.

Immunofluorescence staining, podocyte. Podocyte cultures were washed with PBS and fixed in 4% PFA in PBS for 10-15 min at room temperature. After fixation, cells were washed three times with PBS and blocked in blocking buffer 2 (1% fisher gelatin, 2% donkey serum, 0.1% saponin in PBS) for 30-60
Cells were incubated with primary antibodies in blocking buffer 2 for 2 hours at room temperature (or overnight at 4°C). Cells were washed three times with blocking buffer 2, and incubated with secondary antibodies in blocking buffer 2 for 1 hour at room temperature. After three washes with blocking buffer 2 and one final wash with PBS, the cells were mounted with Drop-n-Stain EverBrite mounting medium (Biotium, 23008). Fluorescent images were captured using an ECHO microscope.

**Viability Testing.** For cell viability and ATP measurements, organoid-derived podocytes were plated onto vitronectin-coated 96-well plate in 100uL media (StemCell Technologies, 07004; Corning, CLS3603) and cultured in Advanced RPMI+10%FBS+1%PS (Fischer Scientific, MT10040CV; Thermo Fisher Scientific, 15070063) in humidified environment at 37°C and 5% CO₂. Organoid-derived podocytes were simultaneously plated onto 6-well plate to be used for RNA extraction for qPCR and onto 24-well plate to be used for immunohistochemistry (IHC). Cells were treated at ~80% confluency with six conditions [control, IFNG (10ng/mL), IFNG (10ng/mL) + baricitinib (final concentration 10uM), All Cytokines (10ng/mL), and All Cytokines (10ng/mL) + baricitinib (final concentration 10uM)]. Media was changed Q48hrs and cells were evaluated at 96hrs. 96-well plate was processed using Promega cell viability and ATP assays as further mentioned below; 6-well plate was processed for RNA extraction, cDNA synthesis, and qRT-PCR; and 24-well plate was fixed in 4% PFA for IHC. CellTiter-Fluor™ Cell Viability Assay (Promega, G6080) and CellTiter-Glo® 2.0 Assay (Promega, G9241) were performed using standard protocol instructions provided by manufacturer. The assays were multiplexed per protocol. Fluorescence (non-lytic protease assay) and luminescence (lytic ATP assay) were measured using SpectramaxM3 fluorometer. Difference in measures was determined by Student’s unpaired t-test.

**Statistics.** All data are presented as mean ± SD. GraphPad Prism 8.3.1 software was used for data analysis. Cytokine conditions inducing >1.5 fold APOL1 transcript compared to media-treated control were analyzed for significance using unpaired t-test with Holm-Sidak correction for multiple comparisons. P-values reported are the adjusted p-values. Significance was set at p<0.05.
Study approval. This study was approved by the Institutional Review Board (IRB) of Duke University, North Carolina. Patient informed consent was not required by the IRB because the case portion of this study was a retrospective review of clinical and archived pathologic material only. Human kidney for glomerular isolation and podocyte subculture was procured through the National Disease Research Interchange (NDRI); this human tissue research was also pre-approved through Duke’s IRB. NDRI requires all tissue source sites obtain informed consent from tissue donor or surrogate.

Author Contributions
SEN, GL, SD, KS, and DS performed experiments and edited manuscript. AW and DT performed and interpreted histopathology, IHC, and edited manuscript. GH contributed essential reagents and edited manuscript. SEN and OAO designed the study, analyzed and interpreted data, designed figures, and wrote and edited manuscript.

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References


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Figure 1. APOL1 expression is upregulated in podocytes and glomerular endothelial cells (GECs) of patients with COVAN. Immunohistochemistry for APOL1 in biopsy tissue from (A-F) case 1 and (G-L) case 6. (B, E, H, K) Co-staining of APOL1 with podocyte marker, synaptopodin. (C,F,I,L) Co-staining of APOL1 with endothelial marker, CD31. Arrow represents podocyte. Arrowhead represents GEC.
Figure 2

Figure 2. APOL1 expression is upregulated in biopsy tissue of COVAN cases but not in controls. Immunohistochemistry (IHC) of (A) wedge biopsy from control 1 and (B-C) autopsy specimen from control 2 shows no APOL1 staining. IHC of APOL1 in (D-F) case 1, (G-I) case 2, (J-L) case 3, (M-O) case 5, (P-R) case 6, (S-U) case 7, (V) case 8, (W) case 9, (X) case 4.
Figure 3. Cytokine storm synergistically induces \textit{APOL1} expression in primary human glomerular endothelial cells and podocytes. JAK-STAT signaling mediates COVID-19-cytokine-induced \textit{APOL1} expression.

A. Experimental design: Primary human podocytes isolated from donor kidney and primary glomerular endothelial cells (GECs) were cultured with or without component cytokines to determine the effect on cellular \textit{APOL1} expression.

B. Positive immunofluorescent staining of podocytes for Wilms tumor1 (podocyte marker). Additional marker staining found in Supplemental Figure4.

C. Quantitative PCR analysis of GECs compared to human embryonic kidney 293 cells (HEK) showing enrichment in PECAM1 gene expression (endothelial marker, also known as CD31). Data are expressed as mean +/- SD, n=3. Significance difference assessed by t-test, with significance set at p<0.05.

D. Quantitative PCR analysis of \textit{APOL1} mRNA transcript level in GECs and (E) podocytes treated x48hrs with specified individual cytokines, combination of cytokines, and combination of cytokines plus JAK-inhibitor (baricitinib). \textit{GAPDH} was used for normalization. All cytokine concentrations were 50ng/mL. Cytokine conditions inducing >1.5 fold \textit{APOL1} transcript compared to media-treated control were further analyzed for significance using unpaired t-test with Holm-Sidak correction for multiple comparisons. \textit{P}-values reported are the adjusted \textit{p}-values. Significance was set at \textit{p}<0.05. Data are expressed as mean +/- SD, with n=6 for podocyte control and n=3 for all others. Cytokine conditions inducing significantly different \textit{APOL1} expression compared to control are indicated in red.

F. Western blot analysis of \textit{APOL1}, phosphorylated-STAT1-3, and total-STAT1-3 in GECs after 48hr treatment with indicated cytokines, combination of cytokines, and combination of cytokines plus JAK-inhibitor (baricitinib). GAPDH used as housekeeping protein for comparator.

G. Western blot analysis of \textit{APOL1} in podocytes after 48hr treatment with individual cytokines, combination of cytokines, and combination of cytokines plus baricitinib. Vinculin used as housekeeping protein for comparator. NEPH1 and WT1 are podocyte markers.
Figure 4. COVID-19-induced cytokines are sufficient to drive APOL1 expression in human iPSC kidney micro-organoids which is blocked by inhibition of the JAK-STAT-APOL1 axis.

A. Experimental design: We created kidney micro-organoids from induced pluripotent stem cells (iPSCs) from individual with G1G2 genotype. Organoids were cultured with or without component cytokines and baricitinib.

B. Immunohistochemistry (IHC) for APOL1, podocyte marker podocalyxin (PODXL), and kidney tubule marker E-Cadherin in micro-organoids treated with media alone; IFNγ alone; IFNγ plus baricitinib; all cytokines; or all cytokines plus baricitinib. Cytokines were used at concentration 10ng/mL. Baricitinib final concentration was 10μM. Scale bar: 140 μm.
Figure 5

A. Kidney Micro-organoid Isolate Glomerulus Culture Podocyte from Glom 96hrs +/- Cytokines +/- Baricitinib Measure Viability

B. Bright field DAPI NEPH1 DAPI NEPH1

Control

IFNγ

IFNγ + baricitinib

All Cytokines

All Cytokines + baricitinib

C. APOL1 Expression (96hrs)

D. Viability (96hrs)

E. ATP (96hrs)
Figure 5. Cytokine-induced APOL1 expression correlates with significantly decreased viability and cellular metabolism in organoid-derived podocytes (genotype G1G2). Cells are rescued by co-administration with JAK-inhibitor.

A. Schemata summarizing the isolation of organoid-derived glomeruli and subculture of podocytes followed by treatment with or without cytokines and baricitinib with subsequent measures in APOL1, viability, and immunofluorescence.

B. Representative immunofluorescent staining of DAPI and NEPH1 in organoid-derived podocytes treated x96hrs with specified treatment conditions; n=4 biological replicates. All cytokine concentrations were 10ng/mL. Baricitinib 10uM final concentration was used. Scale bar: 140µm.

C. Relative APOL1 mRNA levels in organoid-derived podocytes treated x96hrs IFNγ (10ng/mL), combination of cytokines (10ng/mL), IFNγ plus JAK-inhibitor (baricitinib 10uM), and combination of cytokines plus baricitinib (10uM) vs media-treated control. GAPDH was used for normalization. Analysis was performed using t-test with significance set at p<0.05. Data are expressed as mean +/-SD, n=3.

D. Relative Fluorescence Units (RFU) were measured after performing viability assay in organoid-derived podocytes treated x96hrs with IFNγ (10ng/mL), combination of cytokines (10ng/mL each), IFNγ plus JAK-inhibitor (baricitinib 10µM), and combination of cytokines plus baricitinib (10µM) vs media-treated control. Data are expressed as mean +/-SD. Significance was determined by student’s t-test with alpha 0.05; n=3 represent independent biological replicates.

E. Relative Luminescence Units (RLU) were measured to quantitate ATP as indicator of metabolically active cells in organoid-derived podocytes treated x96hrs with IFNγ (10ng/mL), combination of cytokines (10ng/mL each), IFNγ plus JAK-inhibitor (baricitinib 10µM), and combination of cytokines plus baricitinib (10µM) vs media-treated control. Data are expressed as mean +/-SD. Significance was determined by student’s t-tests with alpha 0.05; n=3 represent independent biological replicates.
Graphical Abstract
Supplemental Figure 1. COVID-19 associated collapsing glomerulopathy (COVAN) with capillary tuft wrinkling, retraction with collapse, and adjacent epithelial cell hypertrophy and proliferation.

A. Case 1, Jones methenamine silver stain
B. Case 2, Periodic acid-Schiff
C. Case 4, Periodic acid-Schiff
D. Case 5, Jones methenamine silver stain
E. Case 8, Periodic acid-Schiff
F. Case 9, Periodic acid-Schiff
Supplemental Figure 2

Supplemental Figure 2. Podocyte protein reabsorption droplets shown by albumin immunofluorescence in Case 2.
Supplemental Figure 3. IHC of APOL1 (shown in brown color) for Case 1. APOL1 staining can be seen in the podocyte cytoplasm (blue circles) and in parietal epithelial cell (green dashed circle). Purple staining in right panel is synaptopodin.
Supplemental Figure 4A

Supplemental Figure 4. Primary human podocyte identity confirmed by immunofluorescent staining.

A. Positive immunofluorescent staining of podocyte markers (synaptopodin and nephrin) in primary human podocytes.
Supplemental Figure 4. Primary human podocyte identity confirmed by immunofluorescent staining.

B. Positive immunofluorescent staining of podocyte marker (podocalyxin) and negative staining for endothelial cell marker (CD31 or PECAM-1).
Supplemental Figure 5. Quantitative real-time PCR analysis of APOL1 mRNA transcript level in GECs treated x48hrs with specified individual cytokines and combination of cytokines at 20ng/mL and 10ng/mL concentrations compared to media-treated controls. GAPDH was used for normalization. Cytokine conditions inducing >1.5 fold APOL1 transcript compared to media-treated control were further analyzed for significance using unpaired t-test with Holm-Sidak correction for multiple comparisons. P-values reported are the adjusted p-values. Significance was set at p<0.05. Data are expressed as mean +/- SD, n=3. Cytokine conditions inducing significantly different APOL1 expression compared to control are indicated in red.