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Advances in our understanding of the pathogenesis of HIV-1 associated nephropathy in children

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Abstract

Childhood HIV-1 associated nephropathy (HIVAN) is a clinical and renal histological disease characterized by heavy proteinuria associated with focal and segmental glomerular sclerosis and/or mesangial hyperplasia in combination with microcystic tubular dilatation. These lesions lead to renal enlargement and rapid progression to kidney failure. Children of African ancestry have a unique susceptibility to developing HIVAN. It is estimated that approximately 300,000 HIV-infected children living in the sub-Saharan Africa could develop HIVAN if they do not receive appropriate antiretroviral therapy. This article discusses recent developments and controversies related to the pathogenesis of childhood HIVAN. The role of host genetic factors, including the newly identified variants in the *APOL1* gene, is discussed in the context of previous studies that established the pathological paradigm for HIVAN, and our current understanding of the functional genomics analysis. Hopefully, these advances will provide new research opportunities to generate better treatments for children with HIVAN.

Keywords

African–American; *APOL1*; autophagic cell death; autophagy; Duffy antigen; genetic susceptibility; HIVAN; kidney failure; nonsynonymous SNP

African–Americans represent approximately 65% of all children with HIV-1 infection or AIDS in the USA [1,2]. HIV-1 associated nephropathy (HIVAN) affects the clinical outcome, quality of life, and survival of HIV-infected children [1,2]. Unfortunately, despite the effective antiretroviral therapies (ART) available, we continue to see newly diagnosed children with HIVAN in the USA. In addition, an estimated 2.3 million children living with HIV in sub-Saharan Africa, are at high risk of developing HIVAN if they do not receive appropriate ART [2]. Moreover, young children represent a unique group in which to study

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the pathogenesis of HIVAN because they are usually infected through vertical transmission and do not develop many of the confounding comorbidities and renal diseases seen in adult patients [1,2]. For all these reasons, it is necessary to understand the basic mechanisms involved in the pathogenesis of childhood HIVAN, in order to develop better strategies to prevent or ameliorate the progression of this renal disease. This review will highlight the progress made during the last few years, and discuss how these findings may affect our understanding of HIVAN in children. Other pediatric renal diseases associated with HIV infection, including IgA nephropathy, immune complex glomerulonephritis and proliferative glomerulonephritis, are not part of HIVAN, and will not be discussed in this review.

Clinical & renal histological lesions

HIV-1 associated nephropathy is a clinical and renal histological disease characterized by the presence of heavy proteinuria and nephrotic syndrome leading to a rapid progression to chronic renal failure [1,3,4]. The classic renal pathological findings in HIVAN are focal and segmental glomerulosclerosis (FSGS) with hypertrophy and hyperplasia of glomerular epithelial cells, tubuloreticular inclusions in renal endothelial cells, and microcystic tubules filled with proteinaceous material and infiltrating mononuclear cells [1,3,4]. Immunofluorescence microscopic findings are negative or non-specific [1,3,4]. The microcystic tubular changes lead to renal enlargement, a finding that contrasts with the small fibrotic kidneys typically seen in patients with chronic renal disease of other etiologies. Another feature characteristic of HIVAN in adults is the presence of collapsing glomerulopathy. However, this lesion can be seen in HIV-negative patients, and children usually develop the classic type of FSGS [1,2]. In addition, at the onset of nephrotic proteinuria, children may only exhibit mesangial hyperplasia in combination with microcystic tubular dilatation (Figure 1) [1,2]. It is worth mentioning the possibility that in some cases, what is being seen as mesangial proliferation could in fact be collapsing FSGS disguised. However, children with mesangial hyperplasia progress at a slower rate when compared to those with classic or collapsing FSGS [1]. As previously discussed [5], it is unclear whether mesangial cells can be infected by HIV-1 *in vivo*, and more studies are needed to understand the pathogenesis of mesangial hyperplasia in HIV-infected children. In addition, it remains to be determined whether these changes could be the first step toward the development of FSGS, or whether they can evolve as an independent pathogenic process associated with the viral infection. In summary, HIVAN is a similar condition in adults and children, but children may respond to HIV-1 in a different manner because their tissues and immune system are undergoing growth and developmental changes. In addition, HIV-infected children are usually not exposed to many of the several comorbidities, drugs, or risk factors that affect adult patients.

Role of HIV-genes in HIVAN

The HIV-1 genome contains at least nine genes that encode viral proteins [6]. At least four of these genes, *env*, *tat*, *nef* and *vpr*, have been linked to the pathogenesis of the renal disease [5,7,8]. The *env* gene encodes for HIV-1 proteins gp120 and gp41, which constitute the viral envelope and form the surface unit of HIV-1 as it ‘buds’ out from cells [6,9]. gp120 binds to chemokine receptors and induces signaling pathways in many cell types [6]. Tat is a transcription factor that is essential for viral replication, but can also be released and taken up by uninfected cells, where it can bind to heparan sulfate proteoglycans (HSPGs) and activate several host genes [5,10]. Both gp120 and Tat are present in the circulation of HIV-infected patients and can activate the immune system and induce endothelial dysfunction [9,11]. The *nef* gene product helps HIV-1 escape host immunity by downregulating CD4 and other cell surface receptors [6]. Nef also activates several signaling pathways involved in cell growth and differentiation [12], and can induce the proliferation and de-differentiation

of immortalized murine podocytes [13]. In addition, Nef can also affect the expression of the tumor suppression gene p53 [14], which normally inhibits the proliferation of podocytes [15]. However, to date, the mitogenic effect of Nef has not been demonstrated in cultured human podocytes. Vpr has multiple effects on host cells, including regulation of cell arrest, apoptosis, cytokine production, and can act as a transcriptional activator or repressor [6]. Vpr can facilitate the entry of viral DNA and proteins into the nucleus [16,17]. In summary, the exact mechanism by which HIV genes affect the outcome of HIVAN in children is not fully understood.

Host genetic variations in people with HIVAN

Host genetic factors appear to play an essential role in the pathogenesis of HIVAN [18–20]. People of African descent are at markedly, >18-fold-increased risk for developing HIVAN compared with people of European descent [18]. One of the first host genetic factors explored to explain the susceptibility of people from African ancestry to develop HIVAN, was the 'Duffy negative phenotype' [21,22]. This phenotype is determined by a mutation in the Duffy antigen receptor for chemokines (DARC), which disrupts the binding site for a transcription factor (GATA-1) that is required to express DARC on the surface of red blood cells [23]. Previous studies showed that DARC may promote the clearance of and/or neutralize the activity of inflammatory chemokines [24], and can bind HIV-1 particles [25–27]. Thus, people carrying the Duffy/DARC negative genotype might not be able to neutralize circulating chemokines and/or HIV-1 in an effective manner. Children with HIVAN show increased expression of DARC in glomerular endothelial and tubular epithelial cells [21], and these changes may facilitate the recruitment of HIV-infected cells, and increase its renal toxicity. However, one study done in HIV-infected adults found no association between the Duffy negative phenotype and HIVAN [22]. Alternatively, recent studies have explored the role of Duffy negative phenotype in the progression of HIV infection [27–31]. To date, the causative link between the Duffy/DARC negative phenotype and the potential outcome of HIVAN or HIV infection is unclear, and more studies are needed to determine how DARC may affect the clinical outcome of childhood HIVAN.

Host genetic factors in a region of chromosome 22q12 in HIVAN

Familial clustering of various kidney diseases including hypertension and diabetic-attributed end-stage kidney disease (ESKD), idiopathic FSGS, and HIVAN are often observed among populations of African descent. In the past few years, linkage analysis and mappingm by admixture linkage disequilibrium have localized a region in chromosome 22q12 that includes the *MYH9* gene, and harbors risk variants associated with FSGS, ESKD, and HIVAN in people of African ancestry [18,20,32]. *MYH9* encodes nonmuscle myosin IIA heavy chain, a cytoskeletal motor protein expressed in many cell types, including glomerular podocytes. Dense mapping of *MYH9* has identified individual single nucleotide polymorphisms (SNPs) and groups of such SNPs as haplotypes that were found to be highly associated with ESKD risk phenotypes [33]. Previously, a number of mutations in the coding exons of *MYH9* have been identified in patients with dominantly inherited giant platelet syndrome [34,35]. Two specific *MYH9* variants (rs5750250 or S-haplotype and rs11912763 or F-haplotype), both in noncoding introns, were designated as most strongly predictive for ESKD on the basis of Receiver Operating Characteristic analysis. Despite intensive efforts including resequencing of the *MYH9* gene, no functional/pathological causative mutations have been identified in people with HIVAN or idiopathic FSGS. Interestingly, a recent study showed that although C57BL/6 mice carrying a podocyte-specific deletion of the *MYH9* gene did not develop proteinuria or renal insufficiency, they were predisposed to develop glomerulosclerosis when stressed with adriamycin [36]. These findings suggest that lack of expression of MYH9 in podocytes, together with a second (environmental or genetic) provocation/stress, could induce renal disease.

In 2010, using mappingm by admixture linkage disequilibrium and database mining in 1000 Genomes Project, three independent studies demonstrated that human *APOL1* variants are associated with FSGS and ESKD [19,37,38]. In sum, two haplotypes, harboring three coding sequence mutations of *APOL1*, have been identified as risk variants. The first one, termed G1, is a two-nonsynonymous-SNP haplotype (rs73885319 (A→G; S342G) and rs60910145 (G→T; I384M). The second one, termed G2, is a two-codon-deletion haplotype (rs71785313 (6-bp in frame deletion; DN388Y389). All three of these mutations are located in the last/7th exon. In addition, it has been demonstrated that the distribution of the *APOL1* G1 haplotype in African populations is consistent with the pattern of African ancestry risk of developing ESKD previously attributed to *MYH9* [38]. Importantly, both G1 and G2 variants, located approximately 14 kb 3′ downstream from *MYH9*, are in linkage disequilibrium with common *MYH9* SNPs known to confer a greater risk of FSGS and nondiabetic ESRD. Based on odds ratios and p values, *APOL1* risk variants are more strongly associated with ESKD than the leading *MYH9* risk variants (S-haplotype and F-haplotype). Furthermore, we have recently reported that apolipoprotein L1 (ApoL1) is a novel BH3-only, phospholipid-binding, pro-death protein that, when overexpressed intracellularly, induces autophagy and autophagic cell death in all cell types examined thus far, including those originating from normal kidney and cancerous tissues [39–41]. However, the normal function of *APOL1*, and/or the possible pathological consequences of *APOL1* risk alleles in kidney cells have not been investigated. To explore this issue, we conducted a functional genomics analysis using NCBI RefSeqs Build 37.2, and showed that the human *APOL1* gene encodes at least four putative human mRNA variants, from which three protein isoforms, ApoL1.a, ApoL1.b and ApoL1.c., can be made. ApoL1.a, a 398 amino-acid (aa) polypeptide, is the most abundant and relatively well-studied protein (Figure 2). We expect that the functional expression system we have established previously [39] will be valuable to further characterize the roles of the wild type, G1 and G2 alleles in kidney cells.

The first report describing the cloning and characterization of human ApoL1 was published in 1997 [42]. This study showed that extracellular ApoL1 interacts with apolipoprotein A1 (ApoA1), as a component of circulating high density lipoprotein complexes in human plasma [42]. Subsequently, using immunoblot and immunohistochemical analyses, we and others have shown the intracellular expression and localization of ApoL1 in a number of cells and tissues [39,40,201]. In general, kidney tubular epithelial cells, pancreatic exocrine granular cells and smooth muscle cells have strong ApoL1 expression, whereas other cell types have weak to very low expression. As an illustration (Figure 3), a kidney section showed a remarkable expression of ApoL1 in tubular epithelial cells. In addition, expression of *APOL1* can be induced by TNF-α, IFN-γ and p53 in various cell types [40]. Taken together, these findings suggest that cytokines released by HIV-infected cells may upregulate the expression of ApoL1 in renal tubular epithelial cells. Furthermore, as discussed above, it has been proposed that HIVAN arises due to HIV-1-induced dysregulation of glomerular parietal epithelial cells and podocytes. Interestingly, a recent report showed that podocytes have high levels of autophagic homeostasis for cellular integrity and survival [43]. Autophagy-deficient podocytes exhibited glomerulopathy in aging mice, suggesting that autophagy is a major protective means to maintain functional podocytes and therefore kidneys [43]. It is conceivable that if ApoL1 plays an important role in regulating autophagy in kidney cells, both gain-of-function and loss-of-function mutations of *APOL1* may disrupt ApoL1-dependent cellular homeostasis, leading to apoptosis, and other forms of cell death. In this manner, in patients homozygous for *APOL1* G1 or G2 alleles, HIV-1 may act as a second provocation stress to trigger apoptosis in renal epithelial cells and induce HIVAN. Moreover, our advanced genomic and SNP analysis identified additional nine nonsynonymous-SNPs and two frameshift deletion variants in the coding exons of the human *APOL1* structural gene, *N95K*, *G96R*, *K142fs*, *E150K*, *N176S*, *M218I*,

R255K, *N264K*, *L266fs*, *G270D* and *D337N* (Figure 2). It is logical to speculate that the protein products of some of these alleles of APOL1 may have altered activity, stability or subcellular localization, resulting in cytotoxicity.

With regard to other extracellular functions of ApoL1, intensive studies conducted by a number of parasite-focused groups demonstrated that ApoL1, functioning as the trypanolytic factor, kills the African trypanosome *Trypanosoma brucei brucei*, except two subspecies adapted to humans, *T. b. rhodesiense*, *T. b. gambiense* [44]. Resistance to ApoL1 is conferred by a trypanosomal protein known as serum resistance-associated protein (SRA), which is a lysosomal protein that interacts strongly with a carboxy-terminal α-helix of ApoL1 [45]. ApoL1 is taken up through the endocytic pathway into the lysosome of trypanosomes, and causes osmotic swelling of the lysosome until the trypanosome dies [46]. Interestingly, the two risk alleles of *APOL1* in ESKD in African–Americans, *S342G*/*I384M* (G1) and Δ*N388Y389* (G2), are in the C-terminal domain (Figure 2), which interacts with trypanosomal SRA. Consequently, these two mutant alleles lose binding affinity with SRA and are trypanolytic. Importantly, the I384M mutation is also present in the leucine zipper domain and therefore may disrupt the interaction of ApoL1 with other human proteins that lead to pathological consequences. However, whether extracellular or intracellular ApoL1 proteins produced by these two haplotypes are ‘toxic’ to kidney cells is currently unknown. It is worth noting that there are two other protein isoforms of ApoL1 that can be generated from alternative splicing, ApoL1.b (414 aa), 16 aa longer than ApoL1.a at the N-terminus, and ApoL1.c (380 aa) missing a 18 aa motif close to the N-terminus of ApoL1.a (Figure 2). The function of both isoforms is not clearly understood. Thus, future studies need to elucidate the normal function of ApoL1 isoforms in kidney cells, the crosstalk between ApoL1, HIV-1, and several host inflammatory proteins, as well as the clinicopathological consequences of G1, G2 and other nonsynoynmous mutations of *APOL1*.

Finally, *APOL1* may affect other functions that are involved in the pathogenesis of kidney disease and hypertension, including lipid metabolism, chronic inflammation and endothelial dysfunction [40]. *APOL1* is strongly induced by INF-γ and TNF-α in cultured endothelial cells [40]. To date, very little is known about the role of *APOL1* in the pathogenesis of hypertension or chronic kidney failure in children. We speculate that the long-term metabolic consequences of ART in children, may cause changes in lipid metabolism, endothelial dysfunction, and renal injury *per se*, and therefore, patients carrying the *APOL1* risk genotype may be a higher risk of developing hypertension and chronic renal disease in adulthood.

Host genetic factors in HIV-Tg_{26} mice

A few years after HIVAN was recognized as a new renal disease, three HIV-transgenic (Tg) founding lines were generated and used to support the notion that the expression of viral genes in renal epithelial cells can induce the full HIVAN phenotype in the absence of viral replication and immune dysregulation [47]. One of these mouse lines, named Tg_{26}, was used to show that HIV-1 genes, in combination with endogenous host growth factors, can induce ‘uncontrolled’ hyperplasia of renal tubular epithelial cells, leading to renal enlargement [48]. Since the role of genetic factors in the pathogenesis of HIVAN was studied in HIV-Tg_{26} mice [49,50], we will review relevant features of this animal model.

The Tg_{26} mouse line carries a replication-deficient, Δ gag/pol, pNL4–3 HIV proviral genomic DNA driven by the endogenous HIV-1 promoter [47]. The mRNA expression of HIV is detected in a wide range of cells, including renal glomerular and tubular epithelial cells [47]. Viral proteins cannot however, be detected in the circulation or peripheral blood mononuclear cells. HIV-Tg_{26} mice are born with normal kidneys, and develop a progressive

HIVAN-like renal disease. Homozygous mice are usually runted and die during the first month of life, sometimes without developing renal disease [51]. Many heterozygous Tg_{26} mice develop renal disease by approximately 40 days of life, however, not all mice develop renal disease [48]. During the initial stages of the renal disease, Tg_{26} mice show moderate proteinuria in association with glomerular and tubular epithelial injury [48]. Subsequently, these mice develop global or focal segmental glomerulosclerosis in combination with microcystic tubular dilatation, leading to renal enlargement. This stage is characterized by the presence of proliferating glomerular and tubular epithelial cells that are not well differentiated and express very low levels of HIV-1 transcripts [48]. Usually, mice died of uremia and/or ascites by approximately 60–80 days of life. Elegant studies done in different mouse strains carrying the Tg_{26} transgene, confirmed the notion that host genetic factors play a critical role in the pathogenesis of HIVAN [16,49,50,52]. For example, while Tg_{26} mice generated on the FVB/N mouse strain are susceptible to develop renal disease, Tg_{26} mice bred onto the mixed FBV/CAST background mouse strain do not developing renal disease [16]. Furthermore, these mice were used to identify several genetic loci responsible for the penetrance of the renal disease [16,50,52]. Taken together, these studies support the notion that in some mouse strains, the expression of HIV-1 transcripts in renal epithelial cells is not sufficient to induce the full HIVAN phenotype.

HIV-Tg rats

This model was generated in Sprague–Dawley rat carrying the Tg_{26} Δ gag/pol pNL4–3 HIV-transgene [53]. HIV-Tg rats develop a progressive AIDS-like disease, including immunological dysfunction, nephropathy, muscle wasting and skin lesions [53,54]. One advantage of the rat model, relative to HIV-Tg_{26} mice, is that the Tg_{26} transgene is more efficiently expressed in rat peripheral blood mononuclear cells, and that gp120 and Tat can be detected in the circulation. In the kidney, only glomerular and tubular epithelial cells express viral transcripts, and these rats develop microcystic tubular dilatation and FSGS. In addition, HIV-Tg rats develop two renal features frequently seen in HIV-infected children; prominent mesangial hyperplasia and renal vascular lesions [54]. Based on these findings, it appears that the pathogenesis of the renal disease in HIV-Tg rats is probably multifactorial, involving the expression of viral transcripts in renal epithelial cells, and the recruitment of circulating viral proteins, heparin binding growth factors, and mononuclear cells expressing HIV genes in the kidney [54]. More recently, HIV-Tg rats were used to show that HIV-1 proteins can induce oxidative stress in several tissues [55–57]. Free iron was also found accumulated in diseased kidneys [58]. Interestingly, HIV-infected children with renal disease also show elevated urinary levels of iron and iron-related proteins, including neutrophil gelatinase-associated lipocalin (NGAL) [58]. Thus, it is tempting to speculate that the accumulation of iron may accelerate the progression of the renal disease by causing oxidative stress. If this hypothesis is correct, both the urinary levels of iron and NGAL might become promising candidate biomarkers to identify HIV-infected children with renal disease and iron accumulation. In support of this notion, one study in HIV-Tg_{26} mice reveals renal iron accumulation in HIVAN, and upregulation of NGAL in renal tubular epithelial cells undergoing microcystic changes [59]. This study concluded that the urinary NGAL levels may mark the presence of renal microcysts in people with HIVAN, and could aid in the noninvasive diagnosis of HIVAN [59]. In summary, iron could become a therapeutic target against HIVAN, and more studies are needed to determine how much iron supplement should be given to HIV-infected children with renal disease. Alternatively, another study performed in HIV-infected children, showed that the urinary levels of EGF, FGF-2, and matrix metalloproteinase-2, could become a reliable biomarker profile to identify and follow the renal outcome of children with HIVAN [60].

Role of renal & circulating factors in childhood HIVAN

Several Tg mouse models demonstrate that the selective expression of HIV-1 genes in podocytes, including *Nef* and *Vpr*, play an important role in the pathogenesis of HIVAN [7,61]. These findings, however, do not exclude the possibility that circulating factors might play an additional role in the pathogenesis of childhood HIVAN, since the processes of viral infection, replication, and immunological response to the virus are all bypassed in HIV-Tg mice. For example, HIV-Tg mice do not develop tubuloreticular inclusion in renal endothelial cells, which is a typical renal feature caused by the host immune response to the virus in people with HIVAN. In addition, HIV-infected children develop progressive endothelial injury in response to a high viral load, circulating viral proteins, and cytokines release by the host immune response [5], and all these factors may affect the outcome of HIVAN. Moreover, the forced expression of high levels of HIV-genes in podocytes may cause renal injury by a different mechanism to that seen in HIV-infected children. Alternatively, Tg mice expressing the full HIV genome, or HIV-*nef*, under the control of the human CD4 promoter, which restricts the expression of HIV-1 genes mainly to circulating immune cells, can also develop renal microcysts and FSGS [44,62–67]. These findings challenge the notion that the expression of HIV-genes in renal epithelial cells is a necessary condition for developing HIVAN, and suggest that renal infiltrating mononuclear cells carrying HIV-1 genes, and/or cytokines released by these cells, can induce HIVAN without establishing a productive infection of renal epithelial cells. In summary, it is possible that both renal and systemic factors are needed to induce the full HIVAN phenotype in HIV-infected children.

Infection of renal epithelial cells

One critical question that remains to be answered to understand the pathogenesis of childhood HIVAN, is how HIV-1 infects renal epithelial cells *in vivo*. One study showed that cultured human renal tubular epithelial cells harvested from HIV-infected children can be infected via a CD4-independent process [68]. This event, however, results in the generation of very low levels of viral proteins [68]. Moreover, a high viral load was needed to infect these cells, and the mechanism mediating the process of viral entry remains a mystery [68]. Renal epithelial cells harvested from HIV-infected children do not express detectable protein levels of CD4, the primary HIV-1 receptor, or the coreceptors CCR-5 or CXCR-4. Nevertheless, cell–cell contact interactions with mononuclear cells, facilitate the transfer and entry of HIV-1 to renal epithelial cells [68]. Considering that children with HIVAN show a remarkable upregulation of renal HSPG [69], it is tempting to speculate that HSPGs facilitate the entry of HIV-1 into renal epithelial cells through endocytic pathways, as demonstrated in cultured endothelial cells [70]. Thus, HIV-1 may use HSPGs as low-affinity attachment receptors to concentrate viral particles close to specific entry receptors [70,71]. In support of this notion, a recent study showed that sulfated polysaccharides can block the transfer of viruses from T cells to cultured renal tubular epithelial cells by a mechanism that is not yet clearly understood [72]. Alternatively, macrophages harvested from HIV-infected children express CD4 and HIV coreceptors, and produce high levels of viral proteins [68]. These cells can transfer mature viral particles to renal epithelial cells by a Trojan-horse like mechanism [68], and may establish a clinically relevant renal reservoir in HIV-infected children [5].

In recent years, a variety of cellular binding proteins have been found to be associated with the entry of HIV-1 into human renal glomerular and tubular epithelial cell lines. Among these, the C-type lectin receptor DEC-205 can facilitate the entry of HIV-1 into the HK2 tubular epithelial cell line [73,74]. However, these cells were able to sustain a nonproductive silent infection for a short period of time. In a similar manner, the DC-specific ICAM-3-

grabbing nonintegrin can mediate the internalization of HIV-1 into human podocytes [75], but these cells were also not productively infected. To date, it is unclear whether renal epithelial cells from HIV-infected children express significant protein levels of DEC-205 or DC-specific ICAM-3-grabbing nonintegrin. Alternatively, cholesterol and glycosphingolipids within lipid rafts can facilitate the entry of HIV-1 into several cell types, including cultured human podocytes [76]. Interestingly, recent work found that the HIV-1 envelope glycoprotein gp120 can bind with high affinity to the glycosphingolipid globotriaosylceramide (Gb_3), which is expressed within 'nonraft' fractions in human renal tubular epithelial cells [77]. Renal sections harvested from HIV-Tg_{26} mice and HIV-infected children with renal disease show a high content of Gb_3 in renal tubules [78,79]. Thus, it is tempting to speculate that Gb_3 and other glycosphingolipids may modulate the binding and entry of HIV-1 into renal tubular epithelial cells [79]. In other cell types, viral infectivity can be affected by the reciprocal interactions between X4 and R5 viruses and Gb_3 expression [80]. In addition, recent findings in mice and humans suggest that glycosphingolipids may play a role in the formation of renal cysts [81]. Thus, further experiments are needed to define whether pharmacological manipulation of the expression of Gb_3 and other renal glycosphingolipids may provide a new approach to treat HIVAN. We also hope that future studies will elucidate the conditions and receptors needed to establish a productive infection of human podocytes and tubular epithelial cells *in vivo*.

Proliferating renal epithelial cells & childhood HIVAN

As discussed above, one distinctive feature of HIVAN is the presence of renal microcysts and proliferating glomerular epithelial cells [1,2]. The basic mechanisms responsible for these changes are not completely understood, and have been the subject of extensive studies and controversies. One current leading hypothesis proposes that HIV-1 can induce a productive infection of podocytes, leading to the expression of Nef and subsequent activation of several proliferative signaling pathways [12]. This hypothesis, however, is based on studies done in conditionally immortalized murine podocytes [13] and/or Tg mice [16]. Thus, they need to be validated in human epithelial cells and renal sections harvested from HIV-infected children. At the present time, we do not know whether mature human podocytes have the capacity to de-differentiate and proliferate when exposed to HIV-1 *in vivo*, and these events have not been reproduced in cultured human podocytes. Moreover, cultured human podocytes cannot be productively infected with HIV-1 [75,82–84], and it is unclear how these cells may produce Nef or other HIV proteins. Alternatively, podocytes exposed to HIV-1 may die, be shed in the urine, and be replaced by glomerular parietal epithelial cells [85,86] or renal stems cells [87–89]. This scenario could have implications for future therapies and is a promising field of research.

Previous studies in HIV-Tg_{26} mice and rats support the hypothesis that the renal epithelial proliferative changes characteristic of HIVAN, are a late event in the pathogenesis of the disease [48,54]. As discussed before, the expression of HIV-genes during the early stages of the renal disease in HIV-Tg_{26} mice induces renal epithelial injury [48]. Subsequently, these changes appear to trigger an exaggerated proliferative response involving undifferentiated renal epithelial cells that express almost undetectable levels of HIV-transcripts [48,54]. This response seems to be driven, at least partially, by the renal accumulation of heparin-binding growth factors, including FGF-2 [48,54] and VEGF-A [90]. HIV-Tat, acting from the extracellular space, can facilitate the release of FGF-2 from cultured human podocytes [91] and increase their proliferation and permeability, acting in synergy with VEGF-A [92]. FGF-2 increases the attachment of HIV-infected cells to renal tubular epithelial cells harvested from HIV-infected children [93], and can induce renal microcystic changes in young rodents [94]. Moreover, the serum levels of basic FGF-2 increase in correlation with the progression of AIDS [95], and the urinary levels of FGF-2 are elevated in children with

HIVAN [60]. Renal sections from people with HIVAN and HIV-Tg_{26} mice show an up-regulated expression of VEGF-A in podocytes [90]. Experimental data suggest that too much or too little VEGF-A release by podocytes can cause proteinuria and renal disease [96,97]. For example, young mice overexpressing $VEGF_{164}$ selectively in podocytes can develop collapsing glomerulopathy [96]. In addition, these heparin-binding growth factors stimulate signaling pathways that are known to be activated in patients with HIVAN [98,99]. Finally, cytokines released by HIV-infected mononuclear cells can also modulate the outcome of HIVAN [65,100,101]. Insight into the molecular and signaling mechanisms that are activated by these factors is rapidly expanding, and this information will facilitate our understanding of childhood HIVAN.

Conclusion

After more than 25 years of research, the scientific community has made significant progress to understand the pathogenesis of HIVAN. However, we need a better understanding of the mechanisms by which HIV-1 induces renal injury in order to prevent the development of cardiovascular complications and chronic renal failure in HIV-infected children. Recent studies show that host genetic factors play an essential role in HIVAN. Thus, we need to define how these factors modulate the infection and survival of renal cells *in vivo*, and determine the role of APOL1 in this process. Moreover, it is necessary to elucidate what intrinsic renal factors modulate the proliferation of renal epithelial cells, and why these cells continue to proliferate out of control. The role of circulating viral proteins, immune response, heparin-binding growth factors, and iron accumulation, should be also explored in more depth. Hopefully, all these studies will lead to better strategies to improve the quality of life of HIV-infected children.

Future perspective

During the early years of the AIDS epidemic, children with HIVAN progressed very rapidly to end stage renal disease and/or died before they developed chronic renal failure [5]. Subsequently, the early treatment with ART lead to the reduction of viral burden and has improved the outcome of all AIDS-defining conditions [5]. To date, ART is the best treatment available to prevent the development and/or block the progression of proteinuria and HIVAN [2,102]. Recent excellent articles have discussed the efficacy of ART to prevent and/or treat HIVAN [102–105], and therefore this topic will not be further discussed. We anticipate that the virological success of ART promise a longer lifespan for HIV-infected children, however, the long-term cardiovascular and renal consequences of ART are unknown. Angiotensin converting enzyme inhibitors or receptor blockers, may provide additional therapeutic benefits, but should be used with caution in young children with hypoalbuminemia, salt-wasting disorders, diarrhea and other gastrointestinal problems [2,102]. Measurements of renal function should be made in the patients on a serial basis to follow their progression. Overall, we anticipate that the medical community will continue to face several obstacles to improve the clinical outcome of HIV-infected children. In the USA, the prevalence of HIVAN in children may increase, reflecting the improved survival and increased number of HIV-infected teenagers that become noncompliant with ART. A very large number of children living in the sub-Saharan Africa may develop HIVAN if they do not receive appropriate ART. Among other challenges, the emergence of recombinant viruses that are resistant to ART must be considered. In addition, ART may induce renal injury *per se* [105], and HIV-infected children develop more severe cardiovascular complications during dialysis [106]. We expect that future studies will elucidate the role of host genetic factors, including the *APOL1* polymorphisms, and improve our understanding of childhood HIVAN.

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Executive summary

Definition & clinical outcome of childhood HIV-1 associated nephropathy

- Childhood HIV-1 associated nephropathy (HIVAN) is a renal disease characterized by the presence of heavy proteinuria and rapid progression to chronic renal failure.
- Renal histological findings show mesangial hyperplasia and/or focal segmental glomerulosclerosis in combination with microcystic tubular dilatation.
- Children of African ancestry show a unique susceptibility to developing HIVAN.

Role of HIV genes in HIVAN

- The expression of viral genes in the kidney of HIV-transgenic (Tg) rodents can induce HIVAN in the absence of viral replication.
- At least for HIV-1 genes, *env*, *tat*, *nef* and *vpr*, have been found to play a role in the pathogenesis of HIVAN.
- *nef* and *vpr* appear to play a critical role in the pathogenesis of HIVAN in HIV-Tg mice, however, their role in childhood HIVAN is unclear at the present time.

Host genetic variations & HIVAN

- Studies in HIV-Tg_{26} mice show that host genetic factors play a critical role in the pathogenesis of HIVAN.
- The causative link between the Duffy/Duffy antigen receptor for chemokines negative phenotype and outcome of HIVAN or HIV infection is unclear at the present time.
- There is a remarkable association between noncoding genetic variants in the *MHY9* gene and the presence of focal and segmental glomerulosclerosis in African–Americans with HIVAN.

Role of APOL1 in HIVAN

- Three coding-sequence mutations of the human *APOL1* gene have been linked to HIVAN, idiopathic focal and segmental glomerulosclerosis, and end-stage kidney disease.
- Recent linkage analysis using data generated by the 1000 Genome Project suggested that the risk alleles of *APOL1* are more strongly associated with HIVAN than those of *MYH9*.
- The functional consequences of those *APOL1* risk alleles in kidney cells have not been elucidated.
- The *APOL1* gene encodes three protein isoforms and is highly expressed in kidney tubular epithelial cells.

Circulating factors & childhood HIVAN

- HIV-Tg mouse models cannot exclude the possibility that circulating factors might play a role in childhood HIVAN, since the processes of viral infection, replication, and immunological response to HIV-1 are all bypassed in transgenic mice.

- HIV-Tg mice carrying HIV genes under the control of the CD4 promoter, which restricts the expression of the transgene mainly to immune cells, can develop a HIVAN-like disease.
- The HIV-Tg rat model suggests that circulating viral proteins and the accumulation of iron in diseased kidneys may play a pathological role by generating oxidative stress.

Infection of renal epithelial cells

- Renal tubular epithelial cells cultured from children with HIVAN can be productively infected with primary isolates harvested from children with HIVAN by a CD4-independent mechanism.
- Macrophages can transfer mature viral particles to cultured renal tubular epithelial cells by a Trojan horse-like mechanism, and may establish a clinically relevant renal HIV-1 reservoir.
- Heparan sulfated proteoglycans, DEC-205, DC-specific ICAM-3-grabbing nonintegrin, lipid rafts and glycosphingolipids can modulate the process of HIV-1 entry into cultured renal epithelial cells.

Proliferation of renal epithelial cells

- *nef* induces the proliferation of conditionally immortalized murine podocytes. These findings have not been confirmed in primary human renal epithelial cells harvested from HIV-infected children.
- The heparin-binding growth factors, FGF-2 and VEGF-A, alone or in combination with HIV-Tat, can induce the proliferation of cultured human podocytes and tubular epithelial cells, and may play a role in the pathogenesis of childhood HIVAN.
- The urinary levels of FGF-2 may become a promising biomarker to follow the outcome of childhood HIVAN.

Challenges facing the medical community to improve our understanding of childhood HIVAN

- The role of genetic variations in *APOL-1* and other candidate genes needs to be studied in depth.
- The conditions and receptors that are required to establish a productive infection of human podocytes and tubular epithelial cells *in vivo* need to be defined.
- A better understanding of the mechanism by which HIV-1 induces mesangial hyperplasia, renal epithelial injury and proliferation of human renal epithelial cells is needed.
- The roles of circulating viral proteins, heparin-binding growth factors and immune response to the virus need further investigation in children.
- New biomarkers are needed to follow the outcome of childhood HIVAN, and to define how much iron supplementation should be given to children with HIVAN.

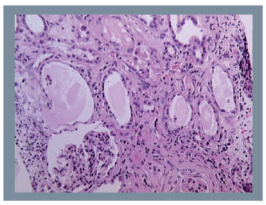

Figure 1.
Representative hematoxylin and eosin staining of a renal biopsy from a child with HIV associated nephropathy.

Figure 2. Sequences of three human ApoL1 isoforms and their naturally occurring nonsynonymous alleles

ApoL1.a, a 398 amino-acid (aa) polypeptide, is shown in its complete sequence. Resulting from alternatively splicing, ApoL1.b, 414 aa, is 16 aa longer than ApoL1.a at the N-terminal (first line), while ApoL1.c, 380 aa, is missing a 18 aa motif (underlined) close to the N-terminal of ApoL1.a. Thus far, 14 naturally occurring, nonsynonymous alleles of *APOL1* have been identified. According to residue numbers of ApoL1.a, they are N95K, G96R, K142fs, E150K, N176S, M218I, R255K, N264K, L266fs, G270D, D337N, S342G, I384M and ΔN388Y389 (aa changes are underwritten). The BH3 (aa 158–166) and leucine zipper (aa 365–392) domains are in bold and underlined. The two risk alleles of APOL1 in ESKD in African–Americans, S342G/I384M and ΔN388Y389, are in the C-terminal domain (aa 338–398), which interacts with trypanomosal serum resistance-associated protein. I384M mutation is also present in the leucine zipper domain.

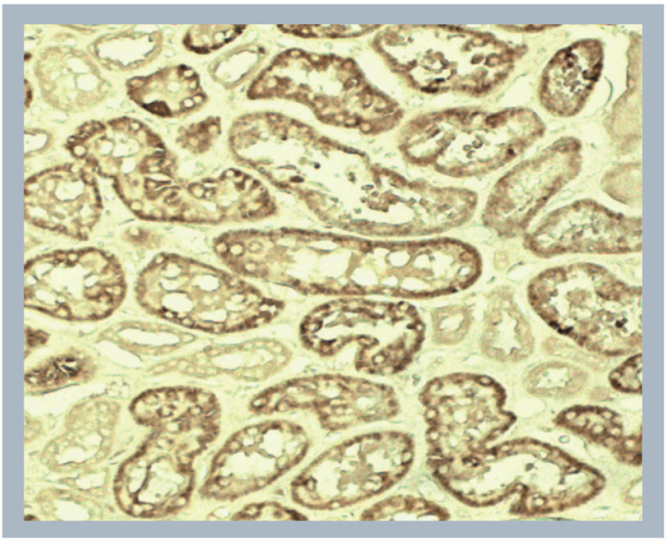

Figure 3. Representative immunohistochemistry staining showing ApoL1 expression in human renal tubular epithelial cells

The ApoL1 antibody was generated and used as previously described [34].