From man to fish: What can Zebrafish tell us about ApoL1 nephropathy?

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Abstract. Background: Risk variant Apolipoprotein L1 (G1/G2) are strongly associated with a spectrum of kidney disease in people of recent African descent. The mechanism of ApoL1 nephropathy is unknown. Podocytes and/or endothelial cells are the presumed target kidney cells. Given the close homology in structure and function of zebrafish (ZF) pronephros and human nephron, we studied the effect of podocyte-specific or endothelium-specific expression of ApoL1 (G0, G1, or G2) on the structure and function of ZF pronephros.

Methods: Wild type (G0) or risk variant ApoL1 (G1/G2) were expressed in podocyte-specific or endothelium-specific under podocin/Flk promoters, respectively, using Gal4-UAS system. Structural pronephric changes were studied with light and electron microscopy (EM). Proteinuria was assayed by measuring renal excretion of GFP-vitamin D binding protein. Puromycin aminonucleoside (PAN) was used as inducer of podocyte injury. Results: Endothelial-specific transgenic expression of G1/G2 is associated with endothelial injury indicated by endothelial cell swelling, segmental early double contours, and loss of endothelium fenestrae. Podocyte specific expression of G1 is associated with segmental podocyte foot process effacement and irregularities relative to G0. Despite the histological changes, the expression of G1/G2 alone in podocyte or endothelium compartment is not associated with edema, proteinuria, or gross whole fish phenotype. Moreover, PAN produced equal pericardial edema in all transgenic fish as well as nontransgenic controls. Conclusions: Transgenic expression human ApoL1 (G1/G2) is associated with histologic abnormalities in ZF glomeruli but is insufficient to cause quantifiable renal dysfunction. This finding supports the necessity of a “second hit” in the pathogenesis/progression of ApoL1-associated nephropathy.

Introduction

The incidence of end-stage renal disease (ESRD) among African Americans is three to five fold that of European Americans, despite similar prevalence of CKD. This excess risk of ESRD is attributable largely to two mutations in the Apolipoprotein L1 gene that encodes ApoL1 protein, which is a component of human HDL [1, 2]. The mutations consist of a pair of amino acid alterations: a serine to glycine substitution at position 342 and an isoleucine to methionine substitution at position 384 (referred to as G1), and a deletion of two amino acids, asparagine at position 388 and tyrosine at position 389 (called G2) [1]. ApoL1 G1 and G2 mutations arose in sub-Saharan West Africa 5 – 10,000 years ago, where, in heterozygous state, they confer the evolutionary benefit of protection against Trypanosoma brucei rhodesiense infection [1, 3]. Not surprisingly, the frequency of G1 and G2 among Africans and people of recent African ancestry is high. Nearly 1/3 of Yoruba and a quarter of Ibo in Nigeria have two copies of these two risk alleles [4]. Individuals of recent African ancestry who have two copies of G1 and/or G2 ApoL1 have significantly higher risk of developing HIV-associated nephropathy (HIVAN), focal segmental glomerulosclerosis (FSGS), and lupus nephritis [1, 5, 6]. When they develop CKD, persons with two copies of G1 and/or G2 also progress rapidly, nearly 10 years sooner, to ESRD [7, 8, 9]. Additionally, transplanted kidneys from deceased African American donors with two copies of APOL1 risk variants are also more likely to fail sooner in the transplant recipients [10].
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The mechanism by which these ApoL1 mutations result in kidney disease remains unknown. The fact that ApoL1 gene is present only in human and some higher primates but absent in physiologically-relevant experimental animal models has slowed progress in ApoL1 research. The zebrafish has a protein that is only 28% identical with human ApoL1. It is unclear if this protein is functionally homologous with human ApoL1. Given the known structural and functional similarity between zebrafish pronephric glomeruli and human glomeruli [11], we hypothesized that transgenic expression of human ApoL1 in zebrafish could provide an avenue for understanding the nephropathy associated with G1/G2 ApoL1, and could also provide a tool for screening small molecules that could inhibit the toxicity of ApoL1 variants. In this report, we describe the generation and initial characterization of transgenic zebrafish that express human ApoL1.

Material and methods

Generation of ApoL1 transgenic zebrafish

We generated ApoL1 transgenic zebrafish using the well-characterized Gal4/UAS system. Briefly, the system has two parts: the Gal4 gene, which encodes the yeast transcription activator protein, Gal4, and the upstream activation sequence (UAS), the enhancer to which Gal4 binds to activate whatever gene is cloned in front on UAS. Transgenic zebrafish that constitutively express Gal4 under promoters of ubiquitin, or podocin, or Flk were crossed with zebrafish carrying a DNA construct in which ApoL1 variants were cloned behind UAS element. Gal4 zebrafish have green hearts while UAS-ApoL1 fish have green eye. Progeny of a successful cross yields zebrafish with green eyes and green hearts.

Quantitative polymerase chain reaction (PCR)

Isolated mRNA from 10 – 20 zebrafish was reverse transcribed to cDNA. Quantitative polymerase chain reaction was performed. Human ApoL1 primer (fwd): 5’-GGTGGGCTCAGGAGCTGGAGGA-3’, (Rvs): 5’-AGTTCTTGTCGCCCTGCAAGA-3’. Zebrafish 18S primer (fwd) 5’-CACTTGTCCCTAAGAAGTTGCA-3’, (Rvs): 5’-GGGTGATTCCGATAACGAACG-3’.

Western blotting

Whole zebrafish lysate was prepared by homogenizing 10 – 20 zebrafish in RIPA buffer. 20 microgram protein was resolved by SDS PAGE. Resolved protein was transferred to PVDF and probed with ApoL1 antibody (Sigma, St. Louis, MO, USA) or β-actin (Santa Cruz Biotechnology, Dallas, TX, USA).

Results

To determine if the transgenic zebrafish generated with the Gal4/UAS system ex-
pressed human ApoL1, we measured ApoL1 mRNA (cDNA) with quantitative PCR and ApoL1 protein by western blotting analysis. As shown in Figure 2A, B, the Gal4/UAS system produced comparable expression of ApoL1 mRNA and proteins in zebrafish either ubiquitously (under ubiquitin promoter), or specifically in the podocyte under the podocin promoter. Similar results were seen when ApoL1 was in endothelia cell under Flk promoter (data not shown). As expected, control zebrafish lacking ApoL1 transgene did not express ApoL1 mRNA or protein.

Analysis of zebrafish pronephros with electron microscopy shows that expression of risk variants ApoL1 (G1, or G2) in endothelia compartment results in endothelial injury as indicated by segmental early double contours, endothelial cell swelling, and loss of endothelium fenestrae (Figure 2). This endothelial injury was absent in zebrafish expressing wild type (G0) ApoL1 nor seen in control zebrafish. Similarly, expression of risk variants ApoL1 G1 in the podocyte is associated with podocyte injury as indicated by podocyte foot process effacement (Figure 3).

To determine the effect of the histological changes associated with expression of risk variants ApoL1 (G1 or G2) on the function of zebrafish pronephros, we asked if expression of ApoL1 G1 or G2 results in increased proteinuria in the zebrafish. Similar to human response to podocyte injury, zebrafish also develop proteinuria as a result of kidney injury [12]. Despite the histologic evidence of podocyte injury in ApoL1 G1 or G2 expressing zebrafish, there was no detectable proteinuria (data not shown).

We next asked if expression of G1 or G2 ApoL1 in zebrafish pronephros potentiates glomerular injury caused by Puromycin aminonucleoside (PAN). First we modified

### Summary of EM Findings by a blinded pathologist

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<th>isolated loss of SD</th>
<th>segmental EC swelling</th>
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Figure 2. Transgenic expression of risk variants ApoL1 in the endothelium is associated with endothelial injury. FP = foot process; SD = standard deviation; EC = endocapillary.
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Discussion

In this study, by successfully expressing human ApoL1 (G0/G1/G2) in zebrafish, we discovered that expression of kidney-disease-associated ApoL1 variants (G1 or G2) result in podocyte and endothelial injury as indicated by podocyte foot process effacement and segmental double contours, respectively. In contrast, expression of wild type ApoL1 (G0) is not associated with podocyte or endothelial injury. These findings suggest that solely expressing G1 or G2 ApoL1 in distinct glomerular compartments (i.e., podocyte or endothelial cell) is sufficient to produce injury in those cellular compartments. This raises the possibility that onset of ApoL1 nephropathy in humans may also be preceded by upregulation of risk variants ApoL1. Further investigation is required to test this hypothesis.

Despite the histologic changes associated with G1 or G2 ApoL1, there is no gross phenotypic evidence of proteinuria or edema. The reason for this is not apparent. Unlike human, zebrafish regenerate their pronephros following injury. This regenerative potential could limit the effect of injury associated with risk variants ApoL1. It is also possible that zebrafish lack cellular components that make the risk variants ApoL1 fully toxic to human kidneys. Moreover, manifestation of nephrotoxicity of the risk variants ApoL1 may require the presence of a specific second hit other than Puromycin aminonucleoside.

Acknowledgments

This work was supported by NIH Grants MD007898, TK32-DK007199 and T32-DK07540.

References


Figure 3. Transgenic expression of risk variants ApoL1 in the podocyte is associated with focal effacement of podocyte foot process.

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