



Ph.D. Thesis Defense

Student Presentation

Thursday, 03 December 2020 at 10:00

This seminar is open to the public via Microsoft Teams at the following link:

<https://ucy.zoom.us/j/99027018463?pwd=MTZKb0ZjbytVSFd2N1k1UzBWYUhhDZz09>

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“Alport Syndrome and Thin Basement Membrane Nephropathy: Phenotypic analysis of two novel mouse models and clinical investigation of Cypriot patients”

Alport syndrome (AS) is a hereditary nephritis leading inevitably to end stage renal disease. Until today there is no effective treatment for it. It is attributed to mutations in the *COL4A3*, *COL4A4* and *COL4A5* genes, coding for $\alpha 3$, $\alpha 4$ and $\alpha 5$ chains of collagen type IV respectively. Collagen type IV is one of the main components of the glomerular basement membrane (GBM). 85% of AS cases show an X-linked type of inheritance and 15% an autosomal recessive one. Heterozygous carriers of the autosomal recessive AS have Thin Basement Membrane Nephropathy (TBMN). Collagen IV diseases are characterized by significant genetic and phenotypic heterogeneity.

Despite the fact that most of the mutations causing AS are missense mutations, a detailed phenotypic analysis of a knockin mouse model for AS is absent from the literature. In this study we present a thorough analysis of two AS mouse models. The first one is a knockin model carrying the *Col4a3-p.G1332E* missense mutation and the second one is a compound heterozygous model carrying the *Col4a3-p.G1332E* and a *Col4a3* knocked out allele. This mutation is the homologous of the *COL4A3-p.G1334E* in humans, which is very frequent among the Cypriot heterozygous carriers of *COL4A* mutations, as a result of a founder effect. Furthermore, many AS patients are compound heterozygotes for mutations in the *COL4A3*, *COL4A4* and *COL4A5* genes.

The findings from the two mouse models are compatible with AS kidney characteristics (reduced survival, impaired biochemical markers in blood and urine indicative of kidney disease, histological findings of increased fibrosis of the glomeruli and tubulointerstitial tissue and the pathognomonic ultrastructural findings of the GBM). Of special interest are the findings from the immunofluorescence studies on kidney sections and western blot results, where it is shown that the podocytes effectively secrete the mutant $\alpha3\alpha4\alpha5$ protomer to the GBM, which is then probably cleaved. The effective secretion of the mutant type IV collagen molecules to the GBM is probably associated with the milder phenotype exhibited by the two mouse models compared to the *Col4a3* knockout model described in the past. It seems that the presence of the defective $\alpha3\alpha4\alpha5$ partly “rescues” the phenotype compared to the total absence of it from the GBM.

Additionally, recruitment of Cypriot patients with heterozygous *COL4A3/COL4A4* mutations and TBMN was performed. These patients were fully characterized based on clinical and laboratory findings at two time points, 2,5 years apart. Furthermore, a classification of their kidney disease severity was done based on the presence of impaired kidney function and/or clinically significant proteinuria. Once more it was confirmed that TBMN in Cypriots is not a benign condition and its’ progression is slow, giving the chance for therapeutic interventions.

In parallel, blood for DNA, serum and plasma isolation was collected as well as urine samples. The biological material was stored at the Biobank of the University of Cyprus. Combined with the meticulous phenotypic analysis of the TBMN patients, this material proves to be valuable for future use in finding modifier genes and diagnostic/prognostic biomarkers.