ESRF	**Experiment title:** Effects on renal cysts growth of the combined calcimimetics and tolvaptan treatment in animal models of human Polycystic Kidney Disease using X-ray microtomography.	**Experiment number**: MD-1093
Beamline: ID17	**Date of experiment**: from: 23 Feb 2018 to: 28 Feb 2018	**Date of report**: 12/3/2018
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Report:

During the beamtime allocated for the experiment MD-1093 (proposal n. 85565) we have been able to analyze 12 kidneys deriving from control rats or rat model of ADPKD (PCK rat). Autosomal dominant polycystic kidney disease (ADPKD) is a genetic disease characterized by the progressive growth of renal cysts causing disruption of renal architectures. Tolvaptan, a selective antagonist of the vasopressin V2 receptor (V2R), is the only approved drug able to slow cyst progression in patients. Our aim was to analyze the tolvaptan treatment effects on renal cysts growth in animal models of human ADPKD, using high-resolution X-ray microtomography for early evaluation of the pathological renal remodeling and therapeutic effects.

The experiment was performed at a pixel size of 3.1 μm, with a photon energy of 38 keV. We used 10.5 shifts to analyze 4 kidneys derived from 4 healthy rats (CTR), 4 kidneys from 4 PCK rats, and 4 kidneys from 4 PCK rats treated with 0.03% tolvaptan for 6 weeks.

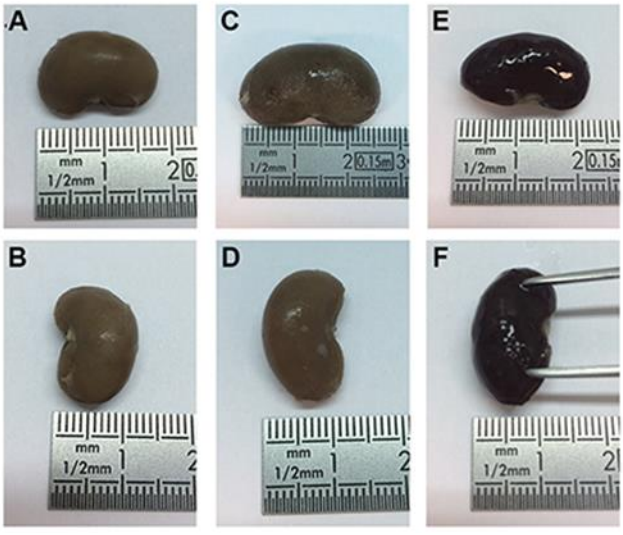

Fig. 1 *Representative images of a kidney from a healthy rat (A, B), a PCK rat (C, D) and a PCK rat treated with tolvaptan (E, F).*

	HEIGHT	WIDTH
CTR kidneys	1.9 ± 0.04	1.2 ± 0.03
PCK kidneys	2.5 ± 0.14	1.6 ± 0.09
Tolvaptan treated PCK kidneys	2.2 ± 0.06	1.3 ± 0.08

Tab. 1 *Height and width of the analyzed kidneys, expressed in cm (means ± SEM).*

At macroscopic analysis, PCK kidneys were significantly heavier and larger compared to control (Tab. 1). Of note, kidneys from PCK rats treated with tolvaptan showed intermediate size and weight with respect to CTR and non-treated PCK rats, probably due to the reduction of cyst growth after tolvaptan treatment. Representative pictures of the three experimental conditions are showed in Fig. 1.

X-ray microtomography revealed that the increase in PCK kidneys volume was mainly due to the expansion of the medulla for the presence of large cysts. Figure 2 shows representative X-ray microtomography images of kidneys from the 3 experimental conditions. The cysts affecting PCK kidneys are mainly restricted to the medullary region (Fig. 2C), but equally large and numerous also in a 7.2 mm more distal scan (Fig. 2D). Interestingly, PCK rats treated with tolvaptan display an intermediate phenotype, with smaller cysts mostly located in the medulla (Fig. 2E), which appear fewer in the distal section (Fig. 2F), where the percentage of apparently normal parenchima is much more evident, resulting in a significant reduction of the total kidney volume compared to PCK non-treated rats. Fig. 3 shows a mid-sagittal reconstruction of a PCK kidney affected by cysts in the entire length. Image analysis suggest that large cysts originate from fusion of neighboring smaller cysts.

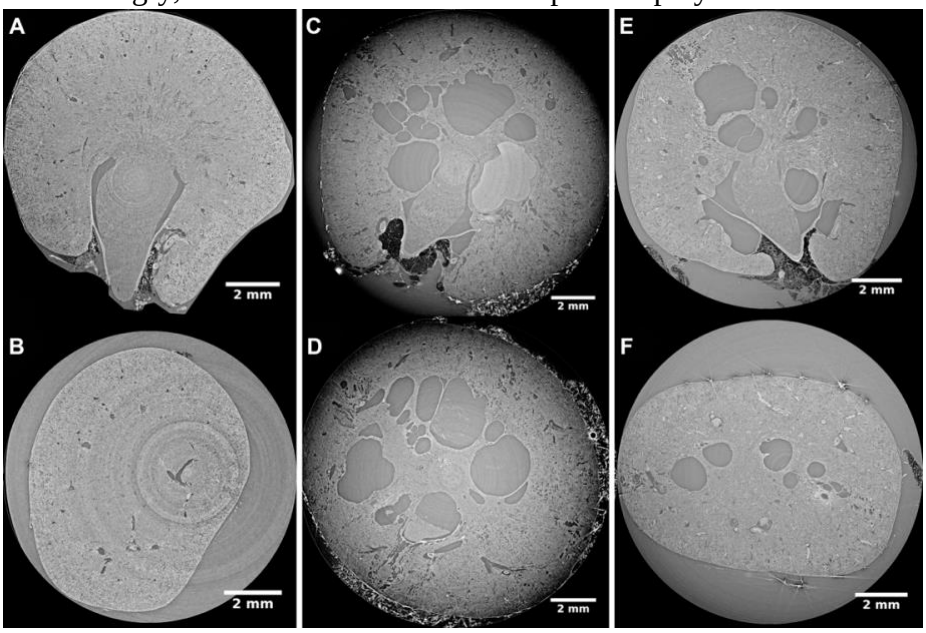

Fig. 2. *Representative scanning micro-CT images from control (A, B), PCK (C, D) and PCK rats treated with tolvaptan (E, F). A, C and E show images taken at the renal hilum. B, D and F show images 7.2 mm more distal.*

Together these results indicate that X-ray microtomography could provide novel information regarding the cysts structure and distribution in PKD and the effects of a therapeutic intervention.

The initial aim of this proposal was to evaluate by X-ray microtomography the therapeutic effect on renal cyst growth of tolvaptan combined to calcimimetics, allosteric CaSR activators, which have been proposed as possible therapeutic target, but due to technical reasons, we could not test PCK rats treated with calcimimetics and with the dual therapy tolvaptan+calcimimetics. For these reasons, we have reduced the allocated beamtime (from 15 to 9 shifts). Moreover, we were assigned beamtime only in the monochromatic hutch, so we were not able to use the 0.7-micron optics requested in this proposal.

New submitted proposal MD-1198.

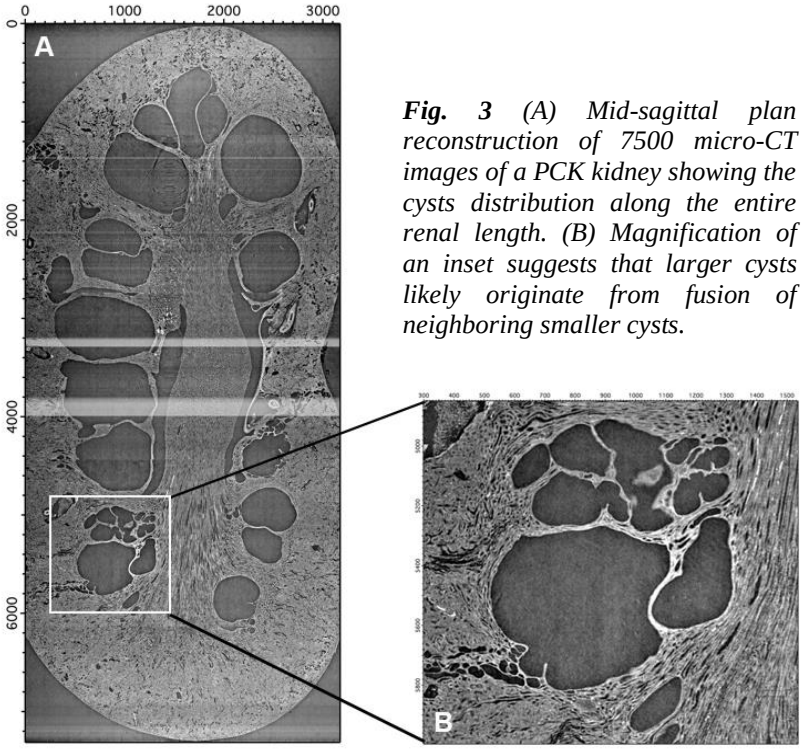

Fig. 3 *(A) Mid-sagittal plan reconstruction of 7500 micro-CT images of a PCK kidney showing the cysts distribution along the entire renal length. (B) Magnification of an inset suggests that larger cysts likely originate from fusion of neighboring smaller cysts.*