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4D MRI of polycystic kidneys from rapamycin-treated Glis3-deficient mice

Luke Xie^{1,2}, Yi Qi¹, Ergys Subashi^{1,3}, Grace Liao⁴, Laura Miller DeGraff⁴, Anton M. Jetten⁴, and G. Allan Johnson^{1,2}

¹Center for In Vivo Microscopy, Department of Radiology, Duke University Medical Center, Durham, North Carolina 27710

²Department of Biomedical Engineering, Duke University, Durham, North Carolina, 27708

³Medical Physics Graduate Program, Duke University Medical Center, Durham, North Carolina, 27710

⁴Cell Biology Section, Immunity, Inflammation and Disease Laboratory, Division of Intramural Research, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina, 27709

Abstract

Polycystic kidney disease (PKD) is a life-threatening disease that leads to a grotesque enlargement of the kidney and significant loss of function. Several imaging studies with MRI have demonstrated that cyst size in polycystic kidneys can determine disease severity and progression. In the present study, we found that while kidney volume and cyst volume decreased with drug treatment, renal function did not improve with treatment. Here, we applied dynamic contrast-enhanced MRI to study PKD in a Glis3-deficient mouse model. Cysts from this model have a wide range of sizes and develop at an early age. To capture this crucial stage and assess cysts in detail, we imaged during early development (3 to 17 weeks) and applied high spatiotemporal resolution MRI (125×125×125 cubic microns every 7.7 seconds). A drug treatment with rapamycin (also known as sirolimus) was applied to determine whether disease progression could be halted. The effect and synergy (interaction) of aging and treatment were evaluated using an analysis of variance (ANOVA). Structural measurements including kidney volume, cyst volume, and cyst-kidney volume ratio changed significantly with age. Drug treatment significantly decreased these metrics. Functional measurements of time-to-peak (TTP) mean and TTP variance were determined. TTP mean did not change with age, while TTP variance increased with age. The treatment of rapamycin generally did not affect these functional metrics. Synergistic effects of treatment and age were not found for any measurements. Together, the size and volume ratio of cysts decreased with drug treatment, while renal function remained the same. Quantifying renal structure and function with MRI can comprehensively assess the pathophysiology of PKD and response to treatment.

Keywords

Small animal preclinical imaging; magnetic resonance imaging; magnetic resonance microscopy; 3D dynamic contrast-enhanced; polycystic kidney disease; Glis3 protein

Introduction

Polycystic kidney disease (PKD) is a life-threatening genetic disorder that leads to a grotesque enlargement of the kidney. One particular type of PKD, autosomal dominant PKD, has affected tens of millions of people worldwide (1). If undetected and untreated, this disease can cause end-stage renal disease, which requires dialysis or kidney transplantation (2,3). Measures of renal function have been used to study and diagnose the disease. Indicators of renal function include serum biomarkers, urinary albumin, and biomarkers for glomerular filtration rate (GFR) (4–7). However, traditional biomarkers have no predictive value in early stages of the polycystic disease (8).

Several studies have demonstrated the utility of imaging, especially MRI, to study the structure of cysts and the size of kidneys in polycystic disease (8–11). Some of these studies have suggested that the size and distribution of cysts can help indicate glomerular and tubular function. Other studies have indicated that the volume progression in certain types of PKD can be a major factor in determining clinical outcomes (12–15). However, in the present study, we found that the cyst size and volume progression describe only one part of the disease. The motivation for of this work was to determine whether both structure and function measured with MRI could comprehensively assess PKD.

We applied dynamic contrast-enhanced (DCE) MRI to study PKD in a Glis3-deficient mouse model. GLI-similar (Glis)1–3 proteins play critical roles in several physiological and disease processes (16). Glis2 and Glis3 have been implicated in pathologies such as diabetes, nephropathy, hypothyroidism, glaucoma, and liver fibrosis (17,18). When Glis3 is knocked out, the kidney exhibits renal cysts and dilation of tubules and collecting ducts (19,20). Cysts from this model have a wide distribution of sizes and develop at an early age through adulthood. For this reason, we imaged mice during early development (3 to 17 weeks of age) and applied high spatiotemporal resolution MRI ($125 \times 125 \times 125 \mu\text{m}^3$ every 7.7 seconds) to evaluate cystic kidneys in detail. We used an MRI methodology described in our previous work (21). Both renal structure and function were measured with age. Rapamycin, a mammalian target of rapamycin (mTOR) kinase inhibitor (22), has been used to reduce cyst growth (10,23). We used this therapy to determine whether our imaging tools could detect changes in disease progression. The MR imaging method biomarker developed here is the first to examine structure and function of cystic kidneys in relation to aging, disease progression, and treatment. These imaging biomarkers can be used for drug discovery studies of PKD and can be applied to other models of renal pathophysiology such as diabetic nephropathy, chronic kidney disease, and acute kidney injury. The methods have been developed with a specific focus on the potential of translational use.

Methods

Biological support

The C57BL/6 Glis3-null mice were generated using methods described previously (19,24). Heterozygous mice were imported from the Cell Biology Section at the National Institute of Environmental Health Sciences (NIEHS) to the Center for In Vivo Microscopy, Duke University Medical Center for breeding and imaging. The heterozygous mice were crossed to obtain homozygous Glis3-null mice and littermate wild type mice. Genotyping from tail biopsy specimens was performed to confirm the animal allele. All animal experiments were conducted in accordance with a protocol approved by the Duke Institutional Animal Use and Care Committee and the NIEHS Animal Care and Use Committee, and adhered to the NIH Guide for the Care and Use of Laboratory Animals.

Wild type mice and mutant Glis3-null mice were each separated into a rapamycin-treated and untreated cohort. Rapamycin, an mTOR inhibitor (Sirolimus R-5000, LC Laboratories, Woburn, MA), was used to limit the progression of cyst development (22). Rapamycin was stored in dimethyl sulfoxide (DMSO) and injected in a solution of DMSO, ethanol, and water. A dose of 5 mg/kg/day was injected in the treated cohort. This concentration has been used previously (11,25). A vehicle solution (21.5% DMSO, 21.5% ethanol, and 57% water) was injected in the untreated cohort. Both the treatment and vehicle groups were injected and monitored daily. A total of 24 animals were divided into 4 cohorts: 1) wild type treated with vehicle (n=5); 2) wild type treated with rapamycin (n=5); 3) Glis3-null mutants treated with vehicle (n=7); and 4) Glis3-null mutants treated with rapamycin (n=7).

All animals were imaged longitudinally over 17 weeks of age (3, 5, 7, 9, 13, 17 weeks). Animals were provided free access to food and water before imaging studies. While imaging, the animals were anesthetized under isoflurane and were breathing freely. Three-dimensional printing (Stratasys Dimension, Eden Prairie, MN) was used to make custom parts for the nose cone and for proper positioning of the animal inside the cryogenic surface coil, which was originally designed for brain imaging.

Gadolinium-based contrast agent (gadofosveset trisodium, Lantheus Medical Imaging, Billerica, MA) was injected as a bolus via tail vein catheter with a dose of 0.03 mmol/kg (similar to clinical dose) at a rate of approximately 0.35mL/min. Contrast was injected using an automatic syringe pump (KD Scientific, Holliston, MA).

Magnetic resonance imaging (MRI)

We used the MRI methodology described in our previous work (21). Briefly, MRI was performed on a 7 T, 20-cm bore magnet (Bruker BioSpec 70/20 USR, Billerica, MA). A high-sensitivity cryogenic RF coil was used for transmission and reception (Bruker CryoProbe). The active region of the cryogenic coil was focused on one of the kidneys (left) allowing us to acquire both high temporal and spatial resolution.

A custom interleaved radial sequence (center out 3D ultrashort echo time [UTE]) was implemented on Bruker ParaVision 5.1 to allow for keyhole imaging. Thirteen uniform-interleaved subvolumes of Fourier space were sampled (total views=40222, polar

undersampling=2, TR=2.5 ms, TE=20 μ s, FA=10°, BW=100kHz). Radial keyhole images were reconstructed by sharing the projections from the unique subvolumes via a sliding window approach (26). This yielded a 3D image (160^3 voxels, $125^3 \mu\text{m}^3$ resolution, i.e., voxels of 1.95 nL) every 7.7 seconds over a 50-minute time course (390 time points) of contrast enhancement and clearance. Contrast agent was injected on the 10th 3D image (77 seconds). This point is considered time 0 for subsequent calculations.

This study yielded $160 \times 160 \times 160 \times 390$ pixels per scan (6.4 GB) for 24 mice over 17 weeks of development (922 GB). A pre-contrast and a post-contrast image were acquired ($160 \times 160 \times 160 \times 104$, 1.7 GB each), which were averaged in the 4th dimension to create enhanced anatomical images. These images had high SNR and were used for subsequent image processing. The total dataset (4D with age) was 1.4 TB.

Image processing

A bias field correction was applied to reduce inhomogeneities of the B_1 field from the cryogenic surface coil. This bias field was calculated from the additional post-contrast 3D image. The nonparametric non-uniform intensity normalization algorithm (N4ITK) (27) was used for the correction. The convergence threshold was set at 0.00004 for both the 1st-level (original resolution, up to 500 iterations) and 2nd-level (half the resolution, up to 500 iterations). The entire 4D DCE dataset was corrected by dividing the image at each time point by the bias field (a scalar 3D image). The bias field correction process is shown in Fig. S1 (see Supporting Information).

The individual 3D images from the 4D array were registered to a common post-contrast 3D image to reduce motion artifacts (see Fig. S2 in Supporting Information). Images were registered using FMRIB Software Library (FSL, <http://www.fmrib.ox.ac.uk/fsl>) with correlation ratio, affine transform, hanning sinc interpolation, and a search range of -60 to 60 in all three axes. A maximum change due to registration in this study was: 1.7° for rotation, 3.1 mm for translation, 1.0% for scale, and 0.016% for skew. The improvement due to registration is shown in Fig. S3 (see Supporting Information).

Analysis

The kidney and cysts were segmented to analyze changes as a function of age. Normal kidneys (wild type mice) were segmented following procedures described previously (21). Polycystic kidneys (Glis3-null mice) were segmented by subtracting the pre-contrast image from the post-contrast image. This difference image had higher intensities in the kidney compared to the background tissue. Thresholding was used to isolate (create a mask) of the kidney in the difference image. Since the cyst regions remained dark in the difference image, additional morphological operations (filling and closing) were used to add cysts to the kidney mask.

The kidney region from a time-averaged DCE dataset was then used to separate cysts from normal kidney tissue. Cysts were segmented using Atropos in Advanced Normalization Tools (ANTs) (28) by employing a finite mixture modeling approach with 4 k-means clusters for initialization. The convergence threshold was set at 0.001 (up to 7 iterations) and the Markov random field smoothing factor was set at 0.05 (neighborhood of $1 \times 1 \times 1$). The

resulting 4 clusters were split into a cystic image mask (cluster 1) and a non-cystic tissue mask (clusters 2–4). Segmented regions can be seen in Fig. S4 (see Supporting Information).

Time-to-peak (TTP) functional maps were determined from the time-intensity curves of the DCE datasets (Fig. S5 in Supporting Information). TTP was calculated from the time of injection (at 77 seconds or the 10th 3D image) to peak enhancement. The TTP distribution values (mean and variance) in the non-cystic tissue area were used to analyze changes with age. An example distribution plot of TTP values is shown in Fig. S6 (see Supporting Information).

Segmented kidneys, isolated cysts, and TTP values were determined as a function of age. Kidney volume was determined for all animal cohorts. Four Five measurements determined from the mutant cohorts (Glis3-null with vehicle and Glis3-null with rapamycin) include: 1) cyst volume; 2) cyst volume percent of the entire kidney; 3) TTP mean in tissue area; and 4) TTP variance in tissue area. Structural changes (kidney volume, cyst volume, and cyst percent) and a functional changes (TTP mean) were fitted to a polynomial function of 2nd order (29). TTP variance was fitted to a linear function. Error estimates (standard deviation of the error) of the functions were determined at the 95% confidence interval for predicting a future observation. The goodness of fit was evaluated using an adjusted R² for the linear and 2nd order polynomial functions. The *f*-statistic was determined for each fitted function.

One-way ANOVA (analysis of variance) was performed to determine whether measurements (kidney volume, cyst volume, cyst percent, TTP mean, TTP variance) changed with age. Two-way ANOVA was performed to determine whether the same measurements changed with drug treatment or age. Synergistic (interaction X1*X2) effects were determined between treatment (X1) and age (X2). A three-way ANOVA was performed to determine whether kidney volume was affected by polycystic disease, treatment, age, synergistically by disease and treatment, synergistically by disease and age, or synergistically by treatment and age. A significance level of 0.05 or lower (p-value) was used to reject the null hypothesis that the samples from the measurements are drawn from populations with the same mean.

Histology

Conventional histology was obtained from additional animals to help confirm cysts found in MRI. Animals (n=3) were perfusion-fixed and preserved at 5, 13, and 17 weeks of age. Kidneys were sectioned in the coronal plane, stored in 10% formalin, embedded in paraffin, and sectioned at 5- μ m thickness. Sections were stained with Hematoxylin and Eosin (H&E). Slides were digitally scanned using brightfield contrast on an Axioskop 2 FS microscope (Carl Zeiss Microscopy, Thornwood, NY). Images were acquired at 1.02 μ m using a 10x objective and tiling was required to cover the entire kidney section. Correction was applied to remove the shading effects and non-uniformities in the tiled images. MR images were manually registered to histology images to enable comparison of renal cysts from age-matched animals.

Results

The contrast enhancement and clearance behavior was significantly different in the polycystic Glis3-null mice compared to wild type. This can be seen in the DCE time course shown in Fig. 1. The first row shows a representative time series (7.7 seconds to 50 minutes) in the wild type kidney. The second row shows a representative time series in the polycystic kidney. In the wild type mice, the contrast enhancement occurs in less than 2 minutes and the clearance is mostly complete by 50 minutes. On the other hand, the polycystic kidney continues to accumulate contrast agent up to 50 minutes.

The anatomical images before and after contrast injection can be seen in Fig. 2, which shows 7 slices from the 3D image (160×160×160 voxels). Fig. 2A shows the volume before contrast and Fig. 2B shows the volume after contrast. Contrast agent accumulates in the tissue parenchyma, with very little accumulation in the cysts (3). The content of the cysts is mostly water (long T_1) and cysts appear as dark spheres in both the pre-injection and post-injection images. The kidney tissue containing contrast agent (short T_1) appears bright in the post-injection image. Cysts are indicated by arrows in Fig. 2 and compared with age-matched mice in histology (Fig. S7 in Supporting Information).

An overview of representative kidney images from animal cohorts as function of age is shown in Fig. 3. The first row shows kidneys from Glis3-null vehicle-treated mice; second row shows kidneys from Glis3-null rapamycin-treated mice; third row shows kidneys from wild type vehicle-treated mice; and fourth row shows kidneys from wild type rapamycin-treated mice. Each image is time averaged from the DCE dataset. Images from each cohort are registered to the first age point (3 weeks). One particular cyst is indicated by arrows in the Glis3-null treated mice. This cyst expands up to 7 weeks and regresses around 9 weeks.

Structure and function

Renal cysts (Glis3-null mice) were segmented from the time-averaged DCE datasets via 4 k-means clusters. The results of the cyst segmentation are shown in Fig. 4. Cysts are shown as a yellow-orange volume rendering overlaid on a slice of the kidney volume. The top row shows kidneys from Glis3-null vehicle-treated mice through development (3 to 17 weeks). The bottom row shows kidneys from Glis3-null rapamycin-treated mice. Cysts are distributed throughout the volume of the kidneys. Cyst size and kidney volume in the vehicle cohort are bigger and increase at a faster rate.

Functional time-to-peak (TTP) maps were determined from the time-intensity curves (Glis3-null mice). Time-intensity curves from wild type mice was reported previously (21). TTP maps as a function of age (3 to 17 weeks) are shown in Fig. 5. Top row shows kidneys from vehicle-treated mice and bottom row shows kidneys from rapamycin-treated mice. Areas of high intensity (delayed peak enhancement times) are spread out throughout the kidney, including the cortical and medullary regions.

Changes with age and treatment

Structural and functional changes of the kidney with age (3 to 17 weeks) are shown as log plots in Fig. 6. Fig. 6A shows kidney volumes in all cohorts (Glis3-null vehicle, Glis3-null

rapamycin, wild type vehicle, and wild type rapamycin). Remaining plots show measurements in the mutant cohorts only (Glis3-null vehicle and Glis3-null rapamycin). Fig. 6B shows cyst volumes. Fig. 6C shows cyst volume percent of the whole kidney. Fig. 6D shows TTP mean and Fig. 6EF shows TTP variance.

Structural changes (kidney volume, cyst volume, and cyst volume percent) were modeled by a 2nd-order polynomial function. The functional change of TTP mean was also modeled by a 2nd-order polynomial function. TTP variance was modeled by a linear function. Bands surrounding the fitted functions show the 95% confidence interval in Fig. 6. Fitted functions, adjusted residuals, and *f*-statistics for the Glis3-null cohorts are included in Table 1. In general, the *f*-statistics were largest when a 2nd-order polynomial function was used to fit structural measurements and one functional measurements (TTP mean). The *f*-statistic was largest when a linear function was used to fit TTP variance.

One-way ANOVA tests were performed to determine whether measurements changed significantly with age. Two-way ANOVA tests were performed to determine the effect of drug treatment. Detailed ANOVA tables (one-way, two-way, and three-way analysis) are included in the Supporting Information. A summary of the one-way ANOVA is included in Table 2 and two-way ANOVA is included in Table 3 for the Glis3-null cohorts. Generally, kidney volume, cyst volume, cyst volume percent, and TTP variance changed significantly with age. However, TTP mean did not change significantly with age. Kidney volume, cyst volume, and cyst volume percent, and TTP sum changed significantly with drug treatment. However, the drug did not affect TTP mean or TTP variance. There was no synergistic (interaction) effect of treatment and age on all six measurements.

Discussion

In this study, we developed an imaging method to study polycystic disease in a Glis3 knockout model. Drug treatment was applied to determine whether the disease progression could be altered and detected. Our methods employed high spatiotemporal resolution ($125 \times 125 \times 125 \mu\text{m}^3$ every 7.7 seconds) to evaluate renal cysts (structure) and contrast enhancement (function) at microscopic detail. Kidney volume, cyst volume, cyst volume percent, TTP mean, and TTP variance were measured to thoroughly assess renal structure and function. The effect of therapy on these metrics was quantified using ANOVA tests.

Comparison of structural and functional changes

Structural measurements (kidney volume, cyst volume, and cyst volume percent) changed depending on the animal cohort, drug treatment, and age. Kidney volume changed significantly with age in all four cohorts including wild type vehicle, wild type rapamycin, Glis3-null vehicle, and Glis3-null rapamycin. The two-way ANOVA revealed that while the drug treatment did not affect kidney volume in wild type mice, drug treatment significantly affected kidney volume in Glis3-null mice. The three-way ANOVA (Table S4 in Supporting Information) confirmed that the drug treatment only affected the Glis3-null cohort. In addition to kidney volume, cyst volume changed significantly with age in both Glis3-null vehicle and Glis3-null rapamycin cohorts. Drug treatment significantly reduced cyst volume in Glis3-null mice. Interestingly, cyst volume percent (ratio of cyst to kidney) did not

increase with age when animals were treated with rapamycin. These findings suggest that the therapy had a positive effect in slowing down the progression of cysts and kidney volume.

Functional measurements (TTP mean, and variance) in the Glis3-null mice changed depending on treatment and age. TTP mean did not change significantly with age in Glis3-null vehicle and Glis3-null rapamycin cohorts. The two-way ANOVA revealed that neither age nor treatment affected TTP mean. Lastly, TTP variance changed significantly with age in treated and vehicle cohorts. Yet, the treatment of rapamycin did not affect TTP variance.

Functional measurements revealed a different story from that of structural measurements. Generally, functional metrics determined with TTP values were unaffected by therapy. Considering the time it took to maximally enhance the contrast agent, the kidney's ability to filter and concentrate was significantly compromised in polycystic compared to normal kidneys. Time to maximally enhance contrast agent or TTP values remained at about 30 minutes throughout development in cystic kidneys, while intensities peaked before 2 minutes in normal kidneys. The variance of TTP values increased with age. The increase in the variation of TTP suggests that there are nephron tubules working harder to concentrate the agent (shorter TTP) and nephron tubules with reduced function (longer TTP). On average, the nephrons are working about the same (TTP mean). The increasing distribution and spread of functioning nephrons can be due to glomerular hyperfiltration, which occurs to compensate for cyst expansion and probable nephron loss in polycystic kidneys (30,31). Together, these findings suggest that the therapy did not improve kidney function, and hyperfiltration and nephron loss continued with age.

Study comparison

A number of MRI studies have demonstrated the value of kidney and cyst size as indicators of disease progression in polycystic kidneys (8–10). Reichardt et al. (11) showed that the ratio of cyst-to-kidney volume remained stable with rapamycin treatment. Similarly, we found that the kidney volume, cyst volume, and cyst volume percent changed significantly with rapamycin. Furthermore, cyst volume and cyst volume percent also showed regression with treatment after 9 weeks of age. While volume changes with treatment demonstrated an important outcome, these structural measurements describe only one aspect of the entire disease. In this study, we measured peak intensity times with TTP to examine function. We found that the mean TTP and the TTP variance did not change with drug therapy. The increase of TTP variance suggests probable hyperfiltration to compensate for nephron loss. However, the filtering and concentrating function of the cystic kidney is extremely poor (TTP of 30 minutes or more) compared to control (TTP less than 2 minutes). This functional decline may explain the reduced lifespan in the Glis3-null mouse model (19). The expansion of renal cysts was reduced; however, the function of filtering contrast did not improve with therapy.

Renal function can be measured using traditional biomarkers such glomerular filtration rate (GFR) with ethylenediaminetetraacetic acid (EDTA), serum creatinine, or inulin (32,33). However, traditional GFR can be inaccurate and does not decrease substantially with the onset and progression of renal cysts (4,8). MRI can be a valuable tool to assess renal

function (34–39). One study showed the decline of renal function with MRI-based GFR in polycystic kidneys (31). Here, MRI-GFR decreased from control animals to two models of polycystic kidney disease (PKD-Mhn at 10.5 months and PCK rats at 7.5 months). Similarly, we found that renal function was compromised in polycystic Glis3-deficient mice (slow TTP) compared to wild type mice (fast TTP) throughout development. To our knowledge, this is the first MRI study to assess both structure and function of cystic kidneys as a function of age and treatment. In our results, TTP was significantly slower as early as 3 weeks. The TTP was approximately 30 minutes in non-cystic areas of polycystic kidneys, while TTP was less than 2 minutes even in the slowest inner medullary region of normal kidneys (21). Measures of renal function with TTP can be a sensitive imaging metric of polycystic disease.

Technical considerations

One of the limitations of the present study arises from the use of the cryogenic surface coil. The non-uniform excitation (B_1) of the coil makes it difficult to quantify T_1 changes for determining gadolinium concentrations. Accurate concentrations are necessary for kinetic modeling, rate constants, and MRI-based GFR (40–42). The major benefit of the cryogenic coil is a much higher SNR, which allows very high spatiotemporal resolutions. The high spatiotemporal resolution was important for two reasons. One, the high spatial resolution allowed for accurate segmentation of smaller cysts. Two, the high temporal resolution captured animal movement through the time series of the DCE dataset. This movement or motion artifact can then be corrected using image registration. Together, the high spatiotemporal resolution was critical for accurate segmentation of the kidney and cysts. In turn, accurate functional measurements in the non-cystic regions were achieved.

Radial keyhole imaging must be carefully designed to reduce potential artifacts. Effects can be magnified in T_2^* -weighted GRE sequences with long echo times (43). The cutoff window or cutoff frequency for keyhole imaging can affect the fidelity of the reference data. To mitigate some of these problems, we used an interleaved radial sequence with ultrashort echo times (20 μ s) and a cutoff frequency satisfying the Nyquist criterion (26).

Conclusion

The DCE-MRI data acquired here provided a comprehensive assessment of polycystic kidneys from Glis3-deficient mice. The effect and synergy (interaction) of aging and rapamycin treatment were evaluated with ANOVA. The size and volume ratio of cysts decreased with drug treatment, while renal function remained unaffected. Both structure and function were essential in describing the pathophysiology and progression of polycystic kidney disease. The MR imaging features of structure and function can reveal the efficacy of diverse intervention and therapy in future studies, and provide a tool for evaluating drugs that could have clinical impact.

Representative 3D datasets and Supporting Information are available through CIVMSpace, our method for sharing information with the scientific community (<http://www.civm.duhs.duke.edu/lx201407>).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of abbreviations

ANOVA	Analysis of variance
DCE	Dynamic contrast-enhanced
DMSO	Dimethyl sulfoxide
EDTA	Ethylenediaminetetraacetic acid
GFR	Glomerular filtration rate
mTOR	Mammalian target of rapamycin
PKD	Polycystic kidney disease
RF	Radiofrequency
TTP	Time-to-peak
UTE	Ultrashort echo time

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Fig. 1. Comparison of DCE datasets from a wild type and a Glis3-null mouse (both vehicle at 17 weeks). Six time points from 390 (50 min) show representative contrast enhancement and clearance in the kidneys. Each time point is a 3D image ($125 \times 125 \times 125 \mu\text{m}^3$). Scale bars, 1 mm.

Fig. 2.
Anatomical images of a Glis3-null mouse (vehicle, 17 weeks) before and after contrast agent injection. A: images before contrast shown every 10 slices or 1.25 mm. B: images after contrast. Arrows point to corresponding cysts in A and B.

Fig. 3. Overview of the data. Animals were imaged over 17 weeks of development (at 3, 5, 7, 9, 13, and 17 weeks). First row: Glis3-null mice treated with vehicle injection (n=7). Second row: Glis3-null mice treated with rapamycin (n=7). Arrows point to one cyst through development. Third row: wild type mice treated with vehicle (n=5). Fourth row: wild type mice treated with rapamycin (n=5). Scale bar, 1 mm.

Fig. 4.
Volume rendering of segmented cysts from Glis3-null mice (vehicle and treated). Volume rendering shown in yellow-orange is overlaid on a slice of the kidney volume.

Fig. 5. TTP heat map of kidneys from Glis3-null mice (vehicle and treated). Dark areas correspond to short TTPs and bright areas correspond to long TTPs.

Fig. 6.

Log plots showing volume and TTP changes with age. A: kidney volume in Glis3-null with vehicle (G3-V), Glis3-null with rapamycin (G3-R), wild type with vehicle (WT-V), and wild type with rapamycin (WT-R). B: cyst volume in Glis3-null mice. C: cyst volume percent (cyst to kidney volume). TTP mean measured in non-cystic tissue of Glis3-null mice. EF: TTP variance. All measurements are fitted with a second-order polynomial function (black line in A–D), except TTP variance is fitted with a linear function (black line in E). Gray band shows the 95% confidence interval.

Table 1

Linear and polynomial fit of age (weeks) to measurements. G3-V=Glis3-null vehicle, G3-R=Glis3-null rapamycin, and x =age (weeks).

		Function	Adjusted residuals	f -statistic
Kidney volume (mm ³)	G3-V	$-3.2x^2 + 110x + 150$	0.97	82
	G3-R	$-4.0x^2 + 97x + 38$	0.93	36
Cyst volume (mm ³)	G3-V	$-1.8x^2 + 57x - 47$	0.93	36
	G3-R	$-1.2x^2 + 30x - 24$	0.90	24
Cyst volume percent (%)	G3-V	$-0.10x^2 + 2.8x + 16$	0.84	14
	G3-R	$-0.059x^2 + 1.5x + 15$	0.68	6.2
TTP mean (min)	G3-V	$0.075x^2 - 1.2x + 32$	0.90	23
	G3-R	$0.041x^2 - 0.58x + 31$	0.23	1.8
TTP variance (mm ²)	G3-V	$7.7x + 150$	0.77	18
	G3-R	$6.7x + 150$	0.77	18

Table 2

Summary of one-way ANOVA in Glis3-null mice. X=age.

	Kidney volume	Cyst volume	Cyst volume percent	TTP mean	TTP variance
X					
	Glis3-null vehicle	*	*	*	*
	Glis3-null rapamycin	*	*		*

* Statistically significant (p<0.05)

Table 3

Summary of two-way ANOVA in Glis3-null mice. X1=treatment. X2=age.

	Kidney volume	Cyst volume	Cyst volume percent	TTP mean	TTP variance
X1	*	*	*		
X2	*	*	*		*
X1 * X2					

* Statistically significant (p<0.05)