Original Articles

Two non-invasive GFR-estimation methods in rat models of polycystic kidney disease: 3.0 Tesla dynamic contrast-enhanced MRI and optical imaging

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Abstract

Background. The aim of this study was the assessment of kidney morphology and glomerular filtration rate (GFR) in rat models of polycystic kidney disease and a healthy control group of Sprague–Dawley rats (SD rats). The performance of two non-invasive GFR estimation methods—3.0 Tesla magnetic resonance imaging (MRI) and optical imaging were investigated. Data of GFR assessment was compared to surrogate markers of kidney function and renal histology.

Methods. Optical imaging of GFR was performed transcutaneously in a small animal imaging system with the fluorescent renal marker fluorescein-isothiocyanate-labelled-sinistrin. Morphologic and dynamic renal imaging was done on a clinical 3.0T MR scanner. Renal perfusion analysis was performed with a two-compartment filtration model.

Results. The healthy SD rats showed physiological levels of creatinine and urea, indicating normal kidney function. These parameters were elevated in the small animal groups of polycystic kidney disease. For the calculation of perfusion and filtration parameters of kidney function in MRI, a 2D turbo FLASH sequence was performed and allowed to distinguish between normal GFR of healthy rats and reduced GFR of rats with polycystic kidney disease. Also, MRI GFR varied among two different rat strains of polycystic kidney disease, according to their status of renal function impairment. Optical imaging GFR confirmed higher GFR values in healthy rats compared to ill rats but did not show different results among the two rat strains of polycystic kidney disease. For this reason, MRI and optical imaging GFR estimation presented an intra-method bias.

Conclusions. Both non-invasive estimation methods of GFR, MRI and optical imaging, can differentiate between healthy rats and animals with limited kidney function. Furthermore, optical imaging, unlike MRI, seems to consider that disease progression with increase of renal polycystic deterioration does not correlate with decrease of GFR in the initial stage of compensatory hyperfiltration.

Keywords: contrast-enhanced MR imaging; glomerular filtration rate; optical imaging; PKD-rat; PCK-rat

Introduction

In autosomal dominant polycystic kidney disease (PCK and PKD disease), a hereditary renal disorder characterized by the development of renal and extrarenal cysts combined with progressive interstitial fibrosis and tubular atrophy, rapid deterioration of kidney function can occur [1–4]. Polycystic kidney disease can induce nephroangiosclerosis which is an important cause of end-stage renal disease, morbidity and mortality in children as well as in adults [5]. Especially in patients with inherited systemic disease, gradual cyst expansion causes renal enlargement which progresses to kidney failure. According to the current understanding of the epidemiology of polycystic kidney disease, compensatory glomerular hyperfiltration is a common finding in the initial phase of the disease during cyst formation and probable nephron loss [6]. The disease is also characterized by an initial long asymptomatic phase despite gradual increase of kidney weight and cyst size with late onset of renal dysfunction [6–8]. Several dynamic contrast-enhanced magnetic resonance imaging (MRI) protocols have been established in order to measure and image kidney perfusion; moreover, there have been efforts to assess glomerular filtration rate (GFR) from this data in recent years [5, 9–13]. Dynamic contrast-enhanced MRI represents a non-invasive imaging method offering high spatial resolution and avoiding radiation exposure for the patient. At the same time, MRI can provide detailed morphological and anatomical information on the kidney. In patients with polycystic kidney disease, disease progression is age dependent. Extensive renal cyst formations combined with increased renal volume finally lead to kidney failure [3, 14–16]. Understanding the cystogenesis of polycystic kidney disease and measurement of disease progression is...
necessary for correct diagnosis and the establishment of appropriate treatment options in order to avoid end-stage renal disease.

The intention of our study was to assess kidney morphology and function according to disease severity in small animal models of polycystic kidney disease (PKC [17] and PKD/Mhm rats [18]). We compared renal insufficient PCK and PKD/Mhm rats in comparison to healthy Sprague–Dawley rats (SD rats). Image-based MR measurements on a clinical 3.0 Tesla scanner, previously published on a paca mouse model, were applied for investigation of kidney function in rats [19]. Moreover, functional analysis of the GFR of the animals was assessed by transcatheter GFR determination using optical imaging, previously introduced in the preclinical setup [20]. Data obtained was compared with haemodynamic parameters of kidney function and histopathological findings of the kidneys.

Materials and methods

Animals

The studies were conducted in accordance with federal and local laws. The experimental study population was comprised of 23 male rats which were housed in groups of 3–4 in macrolon cages at the animal laboratory facilities of our institution. All animals had free access to standard food and water (Sniff GmbH experimental animal diet R/M-H). The animals were divided into three groups: (i) PCK rats (n = 8) with a mean age of 7.5 months (7–8 months) and a mean weight of 457 g (430–487 g); (ii) PKD/Mhm rats (n = 8) with a mean age of 10.5 months (9–12 months) and a mean weight of 516 g (495–538 g); (iii) SD rats (n = 7), all 6 months old, with a mean weight of 459 g (413–485 g). The SD rats were purchased from Janvier, Le Genest-Saint-Ise, France. PCK and PKD/Mhm rats were obtained from our own breeding facility. PCK and PKD/Mhm animal models resemble human polycystic kidney disease. The PCK rats develop renal cysts with focal interstitial fibrosis and inflammation affecting the liver as well [17]. The PKD/Mhm rats are characterized by polycystic kidneys and progressive uraemia and proteinuria [18].

Blood sampling

One week before transcatheter optical imaging and MR measurements of GFR, 600 μl of retrobulbar venous blood was collected under isoflurane anaesthesia. Using an autoanalyser (Hitachi 917E; Roche Diagnostics), creatinine (mg/dL) and urea levels (mg/dL) were determined in the separated plasma.

Optical imaging protocol

Optical imaging of GFR was performed transcatheterly on the rat ear using the fluorescent renal marker fluorescein-isothiocyanate-labelled sinistrin (FITC-S) and a small animal imager (Maestro Small Animal Imaging System; CRI Corporation, Woburn, MA), as described previously [20]. FITC-S was dissolved in phosphate-buffered saline (400 mg/mL) for tail vein injection [doseage 673 mg/kg body weight (b.w.)]. The animals were positioned in the small animal imager and covered with a non-fluorescent sheet, only exposing the depilated rat ear to the focus of the CCD camera. For image acquisition, a long pass filter starting at 515 nm (CRI Corporation) at an exposure time of 10 ms was applied. After intravenous (i.v.) injection of the fluorescent dye, pictures were taken in 2 min intervals up to 120 min. For quantification of the fluorescence signals, a region of interest (ROI) was drawn over the whole ear of the rat using the Maestro 2p20 software (CRI Corporation) [20]. Determination of half-life and GFR was performed using a one compartment model and applying a conversion formula [20].

MRI protocol

Renal MR examinations were carried out on a clinical 3.0 T scanner (Siemens MAGNETOM Trio; Siemens Healthcare Sector, Erlangen, Germany) equipped with a 32 receiver channel using a dedicated 8-element whole-body small animal coil for rats (Rapid Biomedical, Rimpar, Germany). After a combined intraperitoneal Rompun® 2% (xylazine-hydrochloride 5 mg/kg b.w.) and Ketamin® 10% (ketaminhydrochloride 100 mg/kg b.w.) anaesthesia, the rats were placed tail first supine on the animal holder platform in order to minimize motion artefacts. A 26-gauge catheter was inserted into the external jugular vein for contrast material application. A dedicated delivery system with a total length of 1.2 m and 0.28 mm inner diameter was connected to the external jugular vein catheter and fixed with a ligature. The delivery system was filled with 1 ml gadodate meglumine 0.5 mmol/ml (Dotarem®; Guerbert GmbH, Sulzbach, Germany) diluted in 30 ml sodium chloride. For contrast-enhanced imaging, the rats received 2 ml/kg b.w. diluted contrast agent. After completion of the pre-contrast sequences (see imaging protocol), gadodate meglumine was applied i.v. and the T1-w 3D volume-interpolated breathhold exam (VIBE) sequence was repeated in two orientations.

For assessment of renal morphology in the animals, following MR sequence protocol was applied:

(1) T1-w 3D VIBE sequence using parallel imaging (PI) with an acceleration factor of 2 (slices/slab 26, slice thickness 2.0 mm, TR 5.19 ms, TE 2.1 ms, flip angle 12.5°, spatial resolution 0.7 × 0.5 × 2.0 mm³, acquisition time 1:33 min axial, 0.39 min coronal) in axial and coronal orientation pre- and post-contrast.

(2) T2-w 2D half-Fourier acquired turbo spin echo (HASTE) sequence (distraction factor 20%, slices 22, slice thickness 2.0 mm, TR 1000 ms, TE 130 ms, flip angle 120°, spatial resolution 0.8 × 0.6 × 2.0 mm³, PI factor 2, acquisition time 0.22 min) in coronal orientation.

(3) T2-w 2D BLADE-TSE sequence with fat saturation (distaction factor 20%, slices 30, slice thickness 1.5 mm, TR 3640 ms, TE 139 ms, flip angle 120°, spatial resolution 0.5 × 0.5 × 1.5 mm³, PI factor 2, acquisition time 3:21 min axial, 4:56 min coronal) in axial and coronal orientation.

(4) T2-w 3D SPACE sequence (slabs 1, slices/slab 72, slice thickness 0.62 mm, TR 2000 ms, TE 130 ms, spatial resolution 0.6 × 0.6 × 0.6 mm³, PI factor 2, acquisition time 4:56 min) in coronal orientation.

Due to previous experience with small animals [19, 21], dynamic imaging for GFR estimation was performed with a time resolved 2D SR-Turbo FLASH sequence (temporal resolution four slices/800 μs, acquisition time 7.00 min, voxel size 1.0 × 1.0 × 2.5 mm³, distance factor 21%, three coronal and one axial slice, slice thickness 2.5 mm, TR 273 ms, TE 2 ms, TI 99 ms, flip angle 15°, matrix 128 × 127). The total scan time for each animal was 40 min.

Analysis of kidney function parameters

Renal anatomy was evaluated by visual inspection of the MR images. Postprocessing of the SR turbo FLASH data was performed offline using PMI 0.4 written in IDL (Version 6.3). For kidney function analysis, MR renal perfusion and filtration measurements with a published two-compartment model were applied [13, 22–24]. After data import, the whole cortex ROIs were individually defined on the middle coronal slice of the plasma flow (PF) maps with manual exclusion of extracortical and cystic regions. If motion-related problems occurred with the renal ROI, ROI position was corrected manually. For determination of the arterial input function, an ROI was centrally placed inside the abdominal aorta of the rats on an axial slice. Care was taken to define the ROI as small as possible within the aortic lumen to reduce partial volume effects [25]. Signal time courses were averaged over the cortical regions and contrast concentrations were approximated by signal enhancement after subtraction of the baseline from the dynamic signal [13]. Analysis of MR–GFR was performed by fitting the data to a two-compartment model.

Calculation of GFR yielded the following parameters: PF (mL/min/100 mL), plasma volume (mL/100 mL), plasma mean transit time (seconds), tubular flow (TF mL/min/100 mL), tubular mean transit time (seconds), extraction fraction (%), ROI volume (mL) and ROI GFR (mL/min). Calculation of cortex GFR was performed by multiplication of the tubular flow with ROI volume (ROI GFR mL/min = TF mL/min/100 mL × ROI volume mL).

Histology

Renal weight and histopathology were evaluated after organ fixation in 4% phosphate-buffered formalin solution. The poles and middle parts of the kidneys were embedded in paraffin. Cross-sections of 3 μm were cut and stained with haematoxylin–eosin. Renal medulla and cortex were analysed microscopically (software: Leica QWIN). Cyst-fibrosis ratio was assessed.
MRT and optical imaging GFR estimation in rats

in the PCK and the PKD/Mhm rats. Cyst formation was scored with a five-grade scale (Grade 0, no cysts; Grade 1, very few small/middle-sized cysts; Grade 2, few middle-sized/large cysts; Grade 3, multiple middle-sized/large cysts; Grade 4, vast amount of middle-sized/large cysts; Grade 5, formation of cystic mesh). Fibrosis was scored with a four-grade scale (Grade 0, no fibrosis; Grade 1, mild fibrosis, Grade 2, moderate fibrosis, Grade 3, massive fibrosis; Grade 4, massive fibrosis with interstitial inflammation) [26].

Results

The kidney function surrogate parameters plasma creatinine and urea demonstrated physiological state in SD rats (urea 41.6 ± 3.1 mg/dL; creatinine 0.28 ± 0.07 mg/dL) and comparably elevated levels in the PKD/Mhm rats (urea 97.7 ± 6.7 mg/dL; creatinine 0.58 ± 0.07 mg/dL) and the PCK rats (urea 87.1 ± 7.7 mg/dL; creatinine 0.58 ± 0.1 mg/dL). GFR assessment by optical imaging revealed a mean GFR in the healthy SD rats of 1.08 ± 0.13 mL/min/100 g b.w., 0.67 ± 0.12 mL/min/100 g b.w. in the PKD/Mhm rats and 0.66 ± 0.14 mL/min/100 g b.w. in the PCK rats (Figure 1A and C).

The time resolved 2D SR-turbo FLASH sequence was suited for semiquantitative calculation of perfusion and filtration parameters applying a two-compartment model. Different kidney function perfusion and filtration parameters for each study group were recorded (Table 1). The SD rats showed signal time courses resembling an initial first pass of the contrast material bolus, followed by a recirculation and a cortical elimination phase. Semiquantitative parameters of kidney function of the SD rats were not deteriorated compared to that of the PKD/Mhm and the PCK rats with worst renal function parameters. Mean calculated values for cortex GFR, normalized on 100 g b.w., were 0.084 ± 0.019 mL/min/100 g b.w. for the healthy SD rats, 0.033 ± 0.012 mL/min/100 g b.w. for the PKD/Mhm rats and only 0.010 ± 0.003 mL/min/100 g b.w. for the PCK rats with the worst kidney function (Figure 1).

MRT and optical imaging GFR values equally confirmed the highest GFR in the SD rats and reduced renal function in the PKD/Mhm rats but with a tremendous intra- method bias 0.76 ± 0.2 mL/min/100 g b.w. concerning overall GFR values (Figure 2). For the PCK rats, optical imaging revealed comparable GFR values as calculated for the PKD/Mhm rats. In contrast, MRT–GFR estimation resulted in remarkably lower GFR values for the PCK rats compared to the PKD/Mhm rats (Figure 2).

For assessment of renal anatomy, the T2-w 2D BLADE-TSE sequence with fat saturation proved the most sensitive sequence, as published previously [19]. Delineation and demarcation of small cystic changes in the kidneys were achieved in detail due to improved spatial resolution (Figure 3B). The T2-w 2D HASTE sequence suffered from blurring artifacts, decreasing the detection of very small cystic lesions in all animal groups (Figure 3A). The T1-w 3D VIBE sequences prior and post-contrast were very valuable for assessment of kidney morphology (Figure 3C). Homogenous renal parenchymal status was displayed in all the sequences in the SD rats (Figure 3A–C, SD rats). Renal cortex and medulla of the PKD/Mhm rats was characterized by small cystic lesions additionally presenting with right-sided hydronephrosis in one animal, best visible on the VIBE sequence post-contrast (Figure 3A–C, PKD/Mhm rats). The PCK rats presented with massively enlarged kidneys, major hepatic and renal cyst formations and limited renal contrast uptake in the VIBE sequence due to advanced kidney function disorder (Figure 3A–C, PCK rats). Morphological results of kidney imaging among all animal groups could be well correlated with histological findings.

The microscopic assessment of renal medulla and cortex on the histological slides allowed detailed evaluation of the cyst-fibrosis ratio in the PCK and the PKD/Mhm rats (Table 2). Renal morphology and histology were intact in the SD rats (Figure 4A–B, SD rats), not exceeding a mean kidney weight of 1.6 g. PKD/Mhm and PCK rats presented with a mean single kidney weight of 2.5 and 7 g, respectively (Table 2). In both animal groups, the cystic changes were more obvious in the renal cortex than in the medullary region (Table 2). The cyst score did not exceed Grade 4 in the PDKDMhm rats (Figure 4A–B, PKD/Mhm rats). The PCK rats demonstrated single kidney cyst scores between Grades 4 and 5 (Figure 4A–B, PCK rats). In both, PCK and PKD/Mhm rats, massive cortex fibrosis with interstitial inflammation was present which was detected by histological workup (Table 2).

Discussion

Disturbed kidney function, irrespective of the underlying cause, can induce end-stage renal disease, depriving patients of appropriate therapy, when diagnosed at an advanced stage [27]. Especially in hereditary renal disorders like PCK and PKD disease, kidney function deteriorates due to fluid-filled polycystic renal enlargement and interstitial fibrosis. This condition affects children and adults equally, resulting in high morbidity and mortality rates if diagnosed too late [3, 14].

An increasing implementation of MR-based evaluation of renal morphology and function is documented [1, 28–30]. PMI analysis, based on a two-compartment model, has been established for GFR assessment in humans and small animals [13, 19, 31]. To our knowledge, GFR evaluation in a small animal model of polycystic kidney disease, applying MR–GFR analysis in comparison with standard GFR estimation methods, like inulin clearance, has not been described so far. Mendichovszky et al. [32] evaluated the literature with regard to absolute MRI–GFR quantification in comparison to gold standard methods and came to the conclusion that also here, only limited patient data exist on GFR estimation in MRI with direct correlation to blood plasma sampling of GFR. Although a relative correlation with laboratory findings of kidney function in healthy volunteers and patients with renal artery stenosis exist [23, 25, 31], there is neither data available on the performance of PMI–GFR analysis in animal models nor in humans suffering from polycystic kidney disease compared to a gold standard. In contrast, the approach with optical imaging was previously established against simultaneous sinistrin (inulin analogue) bolus clearance in various rat models, including UNX-PKD/Mhm and UNX-PCK rats [20].
The aim of our study was to evaluate GFR analysis on small animal models of polycystic kidney disease with emphasis on non-invasive imaging tools: the recently published morphologic and dynamic MRI and the previously established functional approach with optical imaging. The results showed a tremendous intra-method bias of overall GFR values, making a direct comparison of the assessed overall GFR values impossible (Figure 2).

In optical imaging, healthy SD rats showed higher overall GFR values than the PKD/Mhm and PCK rats. GFR values of these two rat strains were decreased to a similar extent (Figures 1 and 2). Although urea and creatinine are only surrogate markers of kidney function and are known to be imprecise, both plasma parameters showed remarkably lower concentrations in the SD rats compared to the animal models with polycystic kidney disease (Figure 1).

Table 1. Kidney function perfusion and filtration parameters, assessed for each study group on PMI-based GFR analysis in MRI

<table>
<thead>
<tr>
<th>Rat</th>
<th>PF(mL/min/100 mL)</th>
<th>Plasma volume(mL/100 mL)</th>
<th>Plasma mean transit time(s)</th>
<th>Extraction fraction(%)</th>
<th>ROI GFR(mL/min/100 mL)</th>
<th>TF(mL/min/100 mL)</th>
<th>ROI volume(mL)</th>
<th>Tubular mean transit time(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>440.2 ± 86.3</td>
<td>8.4 ± 2.47</td>
<td>1.5 ± 0.6</td>
<td>13.1 ± 3.6</td>
<td>0.39 ± 0.09</td>
<td>55.1 ± 12.2</td>
<td>0.7 ± 0.1</td>
<td>57.7 ± 19.6</td>
</tr>
<tr>
<td>PKD Mhm</td>
<td>55.5 ± 40.2</td>
<td>23.1 ± 8.35</td>
<td>9.3 ± 3.53</td>
<td>13.75 ± 3.3</td>
<td>0.17 ± 0.06</td>
<td>21.2 ± 6.4</td>
<td>0.8 ± 0.1</td>
<td>Not assessable</td>
</tr>
<tr>
<td>PCK</td>
<td>69.0 ± 22.77</td>
<td>15.3 ± 13.21</td>
<td>14.5 ± 4.2</td>
<td>6.7 ± 1.41</td>
<td>0.03 ± 0.02</td>
<td>4.6 ± 1.8</td>
<td>0.7 ± 0.4</td>
<td>Not assessable</td>
</tr>
</tbody>
</table>

*Deparadur mean transit time could not be assessed in the PKD/Mhm and PCK rats.

Fig. 1. Kidney function surrogate markers plasma creatinine (mg/dL) and plasma urea plotted against optical GFR (mL/min/100 g b.w.) (A and C) and MRT GFR (mL/min/100 g b.w.) (B and D). Optical GFR shows comparable GFR and plasma surrogate marker concentrations for PCK and PKD/Mhm rats. In contrast, the MR–GFR technique shows lower GFR values for PCK rats compared to PKD-Mhm rats, although plasma surrogate marker concentrations are comparable in the two animal models. Both methods show highest GFR values and lowest surrogate marker concentrations for the healthy SD rats.
The results of urea and creatinine were consistent with the GFR estimation results of optical imaging. Results of MR–GFR analysis demonstrated reduced GFR values in the PKD/Mhm rats compared to the SD rats. The PCK rats showed significantly lower MR–GFR data than the PKD/Mhm rats (Figures 1 and 2). These findings were neither concordant with the optical imaging approach nor with the plasma surrogate marker analysis (Figures 1 and 2).

The underlying one-compartment model of the optical imaging GFR method is definitely a drawback of this technique, as one-compartment models tend to overestimate GFR. Also, all animals required anaesthesia for optical imaging and MRI. Previous experience with small animal imaging clearly demonstrated that anaesthesia influences GFR estimation to a certain extent [20]. However, these effects cannot explain the tremendous bias between MRT and optical imaging GFR analysis. PMI analysis based on a two-compartment model is frequently used for GFR-assessment in MRI [13, 19, 25, 31], but overall GFR values were never compared to gold standard exogenous GFR determination methods, like inulin or iothalamate clearance. Most probably, a normalization factor or function is required to normalize PMI–GFR to inulin-clearance GFR. Still, this does not explain the discrepancy in the data obtained for the PCK rats. One possible explanation might be the massive appearance of cysts in the PCK model (Table 2; Figure 3), which made adequate separation of healthy from diseased renal cortex difficult. This possible inadequate ROI definition may have resulted in incorrect kidney perfusion and filtration parameters assessed by PMI method (Table 1). Besides, in cases of renal cysts communicating with the collecting systems or the parenchyma, the underlying principal of the PMI-two compartment model may not be appropriate for GFR assessment and therefore might be an explanation for poor correlation between optical and MR–GFR. Another limitation of the study is that the two different methods were not performed at the same day, so day to day variability of GFR or disease progression may also play a role regarding the unsatisfactory correlation of the

Table 2. Cyst-fibrosis score and kidney weight in the SD, PKD/Mhm and PCK rats a

<table>
<thead>
<tr>
<th>Rat strain</th>
<th>Right kidney</th>
<th>Left kidney</th>
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<tbody>
<tr>
<td></td>
<td>Weight (g)</td>
<td>Cyst score</td>
</tr>
<tr>
<td>SD</td>
<td>Mean ± SD</td>
<td>cortex</td>
</tr>
<tr>
<td>n = 7</td>
<td>Median</td>
<td>1.5</td>
</tr>
<tr>
<td>PKD/Mhm</td>
<td>Mean ± SD</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>n = 8</td>
<td>Median</td>
<td>1.5</td>
</tr>
<tr>
<td>PCK</td>
<td>Mean ± SD</td>
<td>6.8 ± 0.8</td>
</tr>
<tr>
<td>n = 8</td>
<td>Median</td>
<td>6.8</td>
</tr>
</tbody>
</table>

aCyst formation was scored with a five-grade scale (Grade 0, no cysts; Grade 1, very few small/middle-sized cysts; Grade 2, few middle-sized/large cysts; Grade 3, multiple middle-sized/large cysts; Grade 4, vast amount of middle-sized/large cysts; Grade 5, formation of cystic mesh). Fibrosis was scored with a four-grade scale (Grade 0, no fibrosis; Grade 1, mild fibrosis; Grade 2, moderate fibrosis; Grade 3, massive fibrosis; Grade 4, massive fibrosis with interstitial inflammation).
two methods. GFR was assessed only once in each animal with both methods. However, the optical imaging GFR values of the healthy SD rats were in the same range as measured in a previous study [20]. As studied and described by the Consortium for Radiologic Imaging for the Study of Polycystic Kidney Disease (CRISP) [6, 14], a long initial asymptomatic phase is followed by a late onset of renal dysfunction in polycystic kidney disease. This means that an increase in kidney volume and total kidney weight due to cyst growth does not correlate with kidney function and especially GFR in the initial stage of the disease. Inspite of histologically proven highest cyst and fibrosis score in the PCK rats, their optical imaging GFR and plasma concentrations of urea and creatinine were not altered significantly compared to the PKD/Mhm rats (Figure 1). This seems to reflect the findings of the CRISP study where a long compensatory glomerular hyperfiltration takes place in association with cystic renal enlargement and nephron loss during disease progression.

This compensatory effect seems not to be adequately reflected by the MR–GFR assessment method, using the described settings in small animals. Even though the MR-based analysis of GFR allowed to distinguish between healthy rats and animals with reduced kidney function, the limited minimum possible slice thickness of 2.5 mm on a clinical 3T MR scanner might be insufficient for small animal investigation. Especially in animal models of polycystic kidney disease, this makes an adequate separation of renal tissue structure and cysts impossible, thus creating partial volume effects, which finally negatively influence functional parameter quantification. Moreover, as during PMI-based GFR analysis in MRI, the cortex is defined manually in one representative coronal slice position, the real 3D kidney volume is not taken into consideration, further affecting measurements negatively. Moreover, the PMI model used was originally optimized for human application, in order to optimize MR–GFR analysis in small animal models of polycystic kidney disease, the use of a dedicated 9 T MR scanner with the gain of further slice reduction might be a solution. On the other hand, the integration of dedicated MR protocols with the capability of cyst/fibrosis segmentation and 3D volume calculation of healthy renal tissue would support improved GFR assessment in polycystic kidney disease like described by Kobayashi et al. [33].

The considerable advantage of MRI on the clinical 3.0 Tesla scanner compared to Optical Imaging was the display of kidney morphology in all three study groups. The T2-w 2D HASTE sequence provided excellent image quality for diagnosis of intact renal structure in the rats. Future preclinical studies require dedicated software for cyst segmentation and threshold-based tissue quantification in order to distinguish between total renal enlargement and cyst volume increase during disease progression. Histological analysis of the kidneys confirmed cystic changes and also demonstrated massive kidney fibrosis with interstitial inflammation, not visible on MR imaging (Table 2). This fact emphasizes the need for further dedicated MR protocols, which will support disease monitoring.

In conclusion, our study compares the performance of two non-invasive methods of GFR estimation in established rat models of polycystic kidney disease and healthy rats. A major finding is that both estimation methods, MR–GFR analysis and optical imaging, can differentiate between healthy rats and animals with limited kidney function, even if GFR results cannot be directly correlated with each other. Furthermore, optical imaging, unlike MRI, seems to consider that disease progression with increase of renal polycystic deterioration does not correlate with
decrease of GFR in the initial stage of compensatory hyperfiltration. Due to the limitations of a clinical 3T MR scanner for small animal imaging, there is a demand for dedicated small animal MR scanners and integrated MR protocols for further studies and integrated cyst/fibrosis segmentation algorithms allowing to separate cysts from healthy kidney tissue and to calculate the 3D volume of the latter.

Conflict of interest statement. None declared.

References


Fig. 4. Homogenous histological appearance of the kidneys in a SD rat. Histologically, intact renal cortex and medulla (A), also in the enlarged view of the cortex (B). Very few middle-sized cysts in a PKD/Mhm rat with Grade 2–3 cyst score in the renal cortex (C and D). Enlarged polycystic kidneys in a PCK rat with macroscopic irregular surface of the kidneys (E) and cortical polycystic disruption visible on the enlarged longitudinal section (F).
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