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Spin-lock Relaxation Rate Dispersion Reveals Spatiotemporal Changes Associated with Tubulointerstitial Fibrosis in Murine Kidney

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Abstract

Purpose: To develop and evaluate a reliable non-invasive means for assessing the severity and progression of fibrosis in kidneys. We employed spin-lock MR imaging with different locking fields to detect and characterize progressive renal fibrosis in an hHB-EGF^{Tg/Tg} mouse model.

Methods: Male hHB-EGF^{Tg/Tg} mice, a well-established model of progressive fibrosis, and age-matched normal wild type (WT) mice, were imaged at 7T at ages 5–7, 11–13 and 30–40 weeks. Spin-lock relaxation rates $R_{1\rho}$ were measured at different locking fields (frequencies) and the resultant dispersion curves were fit to a model of exchanging water pools. The obtained MRI parameters were evaluated as potential indicators of tubulointerstitial fibrosis in kidney. Histological examinations of renal fibrosis were also carried out post-mortem following MRI.

Results: Histology detected extensive fibrosis in the hHB-EGF^{Tg/Tg} mice, in which collagen deposition and reductions in capillary density were observed in the fibrotic regions of kidneys. R_2 and $R_{1\rho}$ values at different spin-lock powers clearly dropped in the fibrotic region as fibrosis progressed. There was less variation in the asymptotic locking field relaxation rate $R_{1\rho}^\infty$ between the groups. The exchange parameter S_ρ and the inflection frequency ω_{infl} changed by larger factors.

Conclusion: Both S_ρ and ω_{infl} depend primarily on the average exchange rate between water and other chemically shifted resonances such as hydroxyls and amides. Spin-lock relaxation rate dispersion, rather than single measurements of relaxation rates, provides more comprehensive and specific information on spatiotemporal changes associated with tubulointerstitial fibrosis in murine kidney.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Keywords

Spin-lock; $R_{1\rho}$; dispersion; relaxation; renal fibrosis; mouse kidney disease; MRI

1 | INTRODUCTION

Renal fibrosis is a characteristic of chronic kidney diseases, and it induces further kidney injury and ultimately leads to renal failure.(1,2) It is crucial to assess the spatial extent of fibrosis in kidneys and its changes over time in order to guide treatment and track the efficacy of anti-fibrotic agents. However, renal fibrosis often progresses without overt increases in urinary albumin excretion, blood pressure, or serum creatinine until it pervades most of the kidney.(3) Currently, urine and blood markers cannot provide sensitive assessments of renal fibrosis, while renal biopsy is quite limited in assessing fibrosis because it is invasive and subject to sampling bias. There is thus no reliable non-invasive means for assessing the severity and progression of fibrosis in individual kidneys (4,5) and so it would be clinically valuable to develop and validate noninvasive imaging methods that can safely and accurately assess kidney fibrotic burden.

In vivo multi-parametric MRI techniques permit the evaluation of structural, functional, and metabolic aspects of kidney diseases.(6–8) Furthermore, the application of noninvasive MRI methods to mouse models allows evaluation of the spatial and temporal progression of renal disease.(9–11) Recent advances in MRI have raised its role in the diagnostic assessment of renal fibrosis, but novel proposed MRI techniques are still under evaluation.(3) These include blood oxygenation level dependent (BOLD) MRI, diffusion weighted MRI (DWI), magnetic resonance elastography (MRE) and magnetization transfer (MT) imaging.(12–20) These techniques have limitations even though they have shown potential for detecting renal fibrosis.(12–20) Proton relaxation can provide information on the dynamics of water molecules in different biological environments. While dipolar cross-relaxation dominates the longitudinal relaxation in hydrated macromolecules,(21–24) chemical exchange between solvent water and labile protons in various metabolites and mobile macromolecules makes significant contributions to the measured transverse relaxation rates of water in samples of biomolecules and tissues,(25) especially at high fields.

Chemical exchange between water protons and exchangeable protons is a dominant process for transverse relaxation in collagen.(22,26) In principle, estimates of exchange parameters can be obtained from multi-echo (e.g. CPMG) pulse sequences using different pulse rates (27) but when exchange is fast the necessary high pulse rates are not achievable. Spin-lattice relaxation in the rotating frame, $R_{1\rho}$ provides an alternative approach for assessing exchange in the fast exchange regime. In spin-lock imaging, the equilibrium magnetization is rotated into the transverse plane first. Then this magnetization relaxes in the presence of an on-resonant RF locking pulse, and the corresponding relaxation rate with regard to the spin-lock duration is $R_{1\rho}$. While measurements of $R_{1\rho}$ at a single locking pulse amplitude can be informative, they may not provide any more insight than, for example, measurements of R_1 or R_2 . However, the variation of relaxation rates $R_{1\rho}$ with the amplitude of the locking field, which is relaxation rate dispersion, provides a basis for specifically isolating exchange

contributions and for quantitatively estimating the exchange rates and chemical shifts of exchanging protons.(28–31)

$T_{1\rho}(= 1/R_{1\rho})$ mapping at a single moderate spin-lock amplitude has previously been applied to study fibrosis in liver and kidney (32–34) but there have not been previous studies which reported changes in the dispersion or exchange during the progression of fibrosis. This study aimed to evaluate the sensitivity of changes in parameters obtainable from measurements of $R_{1\rho}$ dispersion to corresponding changes in the composition of tissue as fibrosis developed in a mouse model.

Animal models play an important role in studying fibrosis and can be used for evaluating the application of advanced MRI techniques. Epidermal growth factor receptor (EGFR) has been implicated in the pathogenesis of diabetic nephropathy and renal fibrosis. A fibrotic kidney mouse model (35) has become well established with selective activation of EGFR by overexpressing human heparin-binding EGF-like growth factor (hHBEGF^{Tg/Tg}), in renal proximal tubule epithelium. With selective and persistent tubulointerstitial fibrosis induced in the outer stripe of the outer medulla (OSOM), this murine hHB-EGF^{Tg/Tg} model provides a useful and realistic platform for the assessment and treatment of renal fibrosis in chronic kidney disease. In this study, we focused on the potential value of parameters derived from spin-lock relaxation dispersion imaging for detecting tubulointerstitial fibrosis in this renal fibrotic hHB-EGF^{Tg/Tg} mouse model.(35)

2 | METHODS

2.1 | Animals

Male hHB-EGF^{Tg/Tg} mice, a well-established model of progressive fibrosis,(35) and normal wild type (WT) mice, both on C57BL/6 backgrounds, were studied at 5–7, 11–13 and 30–40 weeks of age. All procedures followed NIH guidelines on the care and use of laboratory animals.

2.2 | MRI

MR images were acquired on a 16-cm bore Bruker horizontal 7T magnet, using a Doty 38-mm inner diameter transceiver coil. For imaging, mice were immobilized and anesthetized (isoflurane 1.0–1.5%), and a constant rectal temperature of 37.5 °C was maintained throughout each imaging session using a warm-air feedback system (SA Instruments, Stony Brook, NY, USA). T_1 -weighted images were acquired to guide data collection (Supporting Information Figure S1). A fast spin-echo sequence (TR = 5500 ms, RARE-factor = 4, resolution = $0.25 \times 0.25 \times 1 \text{ mm}^3$, multiple echo times of 14, 42, 70, 98, 126 ms) was used to achieve T_2 contrast and calculate $R_2(= 1/T_2)$ maps.

Spin-lock images were acquired in a transverse plane (Supporting Information Figure S1) using a fast spin echo sequence preceded by a conventional preparatory spin-lock cluster consisting of a 90° excitation pulse followed by a single rectangular locking pulse with duration of τ and a –90° excitation pulse ($90_x - \tau_y - 90_x$). Imaging parameters were TR/TE = 3000/24 msec, RARE factor = 8, resolution = $0.25 \times 0.25 \times 1 \text{ mm}^3$. Sets of images were acquired with different spin-lock amplitudes SLB1 (locking frequencies at 200, 400, 600,

800, 1000, 1500, 2000, 2500 and 3000 Hz). Spin-lock times at each spin-lock amplitude were varied as 1, 5, 15, 25, 35, 55, 75 ms (Supporting Information Figure S1).

2.3 | Histology

Paraffin tissue sections were stained with picosirius red using standard procedure for histological confirmation.(20)

2.4 | Data analysis

MRI data were analyzed using MATLAB (The Mathworks, Natick, MA, US). All intra-session images used in quantification were co-registered using a rigid registration algorithm. R_2 values were calculated pixel-by-pixel by fitting signals to a single exponential decay with echo time. $R_{1\rho}$ values were obtained pixel-by-pixel for each locking field by fitting signals to a single exponential decay with locking time. Then the regional dispersion of $R_{1\rho}$ with locking frequency ω_1 was fit to a model proposed by Chopra et al. (36) as in previous studies.(28)

$$R_{1\rho} = \frac{R_{2fit} + \frac{R_{1\rho}^{\infty} \omega_1^2}{s_{\rho}^2}}{1 + \frac{\omega_1^2}{s_{\rho}^2}} \quad (1)$$

The fits provided values of transverse relaxation rate R_{2fit} , $R_{1\rho}$ at asymptotic spin-lock frequency ($R_{1\rho}^{\infty}$), and an exchange rate-weighted parameter S_{ρ} . In realistic cases,

$$S_{\rho}^2 \approx k_{sw}^2 + \Delta\omega_s^2 \quad (2)$$

where k_{sw} is the chemical exchange rate of protons with resonance frequency offset from water ω_s . When the second derivative of equation 1 with respect to ω_1 is equal to zero, the dispersion curve undergoes an inflection, and the corresponding inflection frequency ω_{infl} of the dispersion curve was calculated (29).

$$\omega_{infl} = \sqrt{S_{\rho}^2/3} \quad (3)$$

T_2 -weighted images were used for manual selection (37) of regions of interest (ROIs), such as cortex (CR), outer stripe of outer medulla (OSOM), inner stripe of outer medulla (ISOM), and inner medulla and papilla (IM+P) for quantification. It is often challenging to distinguish cortex and OSOM from all the kidneys of disease models.(38) Therefore, in this work, the defined CR+OSOM region included both renal cortex and OSOM for regional $R_{1\rho}$ dispersion analyses. Regions suspected to have fibrosis were identified as areas with low R_2 and $R_{1\rho}$ values. The normal R_2 and $R_{1\rho}$ ranges of CR+OSOM region were defined as Mean $\pm$ 1.96SD (standard deviation, 95%) of R_2 and $R_{1\rho}$ respectively from normal WT mouse kidneys, and the regions with significantly lower R_2 and $R_{1\rho}$ were defined as voxels whose respective values were out of this normal range in the CR+OSOM region. Regions with values below the respective lower threshold Mean–1.96SD of CR+OSOM were detected and

percent areas of those regions in the kidney were calculated. The positive areas below the respective R_2 and $R_{1\rho}$ thresholds were quantified as tR_2 or $tR_{1\rho}$.

$$tR_2 = \frac{\text{Area}(R_2 < R_2 \text{ threshold})}{\text{Total Area}} \times 100\% \quad (4)$$

$$tR_{1\rho} = \frac{\text{Area}(R_{1\rho} < R_{1\rho} \text{ threshold})}{\text{Total Area}} \times 100\% \quad (5)$$

The detected abnormal areas were suspected to have fibrosis (positive fibrosis areas), and their averaged R_2 and $R_{1\rho}$ (mR_2 and $mR_{1\rho}$) and parameters derived from the Chopra model fits were then quantified and compared to respective values of normal tissues in WT kidneys.

The histological fibrosis index was evaluated using the same procedure as in our previous work.(17,20)

$$\text{Positive picrosirius red} = \frac{\text{Positive Area}}{\text{Total Area}} \times 100\% \quad (6)$$

The correlations between different measures were evaluated across kidneys, using the Pearson correlation function. Student's t -tests were used for evaluating the differences between groups. It was considered significant when the false discovery rate adjusted $p < 0.05$. Receiver operating characteristic (ROC) curves were also calculated to show true positive rates (sensitivity) and false positive rates (1-specificity) of all the MRI and histological measures.

3 | RESULTS

3.1 | Histological findings of typical features in hHB-EGF^{Tg/Tg} kidney

Extensive fibrosis in the hHB-EGF^{Tg/Tg} mice was revealed by picrosirius red staining (Figure 1), in which collagen deposition and capillary density reduction were observed mainly in the OSOM of kidneys. The accumulation of extracellular matrix proteins showed up in 6-week hHB-EGF^{Tg/Tg} kidney, and the relative volume occupied by abnormal tubulointerstitial matrix became larger when mice aged (Figure 1). However, other common features of kidney diseases, such as tubular dilation and atrophy,(9,11,37–39) were not evident in hHB-EGF^{Tg/Tg} kidney (Figure 1). Previous histologic studies of the hHB-EGF^{Tg/Tg} model also revealed that fibrosis predominated in its renal disease progression. (35)

3.2 | Features of R_2 and $R_{1\rho}$ in normal mouse kidneys

In normal WT mice, T_2 -weighted images differentiated cortex from OM and IM+P with good contrast (Figure 2). However, OSOM had T_2 values closer to cortex than to ISOM. IM+P exhibited highest T_2 values (lowest R_2) among all the regions in kidneys (Figure 2B). Regional $T_{1\rho}$ values showed similar trends as T_2 . OSOM showed quite similar $T_{1\rho}$ as cortex (Figure 2B), much lower than ISOM and IM+P. Figure 3 compares the variability of

averaged R_2 and $R_{1\rho}$ of cortex, OSOM, ISOM, and IM+P regions across WT kidneys (N=16). $R_{1\rho}$ at different spin-lock powers also showed quite similar regional trends as R_2 (Figure 3). No significant differences were observed between cortex and OSOM regions for R_2 and $R_{1\rho}$ across WT mouse kidneys (Figure 3). This was the basis for grouping cortex and OSOM together for assessing normal distributions of R_2 and $R_{1\rho}$ and setting up respective thresholds for detecting abnormal outliers.

3.3 | Relaxation changes in progressive hHB-EGF^{Tg/Tg} fibrosis model

In hHB-EGF^{Tg/Tg} mice, the T_2 and $T_{1\rho}$ contrasts in some derived parametric maps were dissimilar to those found in normal WT mice (Figure 4), consistent with the associated pathologic changes occurring in OSOM of kidneys. The characteristic T_2 and $T_{1\rho}$ maps of hHB-EGF^{Tg/Tg} kidney showed a ring-shaped stripe of very high T_2 and $T_{1\rho}$ values at the final stage of fibrosis progress, located in OSOM (indicated by arrows in Figure 4A). Thus, unusually low R_2 and $R_{1\rho}$ were observed in OSOM. The R_2 and $R_{1\rho}$ maps were also compared between WT and hHB-EGF^{Tg/Tg} kidneys at different fibrosis stages (Figure 5). Longitudinal decreases of R_2 and $R_{1\rho}$ were observed in OSOM regions as fibrosis progressed in hHB-EGF^{Tg/Tg} kidneys from week 7 to week 33 (Figure 5).

Because it was difficult to separate OSOM from cortex in some fibrotic kidneys, longitudinal changes of R_2 and $R_{1\rho}$ were first compared in CR+OSOM regions (Supporting Information Figure S2) across kidneys. While the R_2 and $R_{1\rho}$ measures in fibrotic kidneys all showed significantly lower values than those of WT kidneys, significant differences among different fibrotic stages were not evident. Both R_2 and $R_{1\rho}$ are sensitive to fibrosis at an early stage (5–7 weeks). $R_{1\rho}$ at optimum locking frequencies (1000 Hz) consistently showed most significance in detecting differences between WT and fibrotic kidneys than R_2 and $R_{1\rho}$ measured at other locking frequencies (Supporting Information Figure S2). ROC analyses of observed R_2 and $R_{1\rho}$ measures in CR+OSOM regions at different fibrotic stages are shown in Supporting Information Figure S3 (left column) and Supporting Information Table S1. Among all the R_2 and $R_{1\rho}$ measures, $R_{1\rho}$ obtained with locking fields in the range 600–1500 Hz showed high AROC (area under the curve) for detecting the differences between normal and fibrotic kidneys at different stages (Supporting Information Table S1). $R_{1\rho}$ at optimum locking frequencies showed larger AROC than R_2 . We noticed that $R_{1\rho}$ with locking field 1000 Hz consistently showed large AROC and high combined sensitivity and specificity for detecting fibrosis at different stages. Thus, we selected it for further threshold analysis in this study.

3.4 | R_2 and $R_{1\rho}$ distributions and threshold measurements

The changes of MRI parameters induced by fibrosis can be significantly underestimated when normal tissues in both cortex and OSOM were included in group analyses of fibrotic kidneys. A threshold analysis was conducted to identify abnormally low R_2 and $R_{1\rho}$ areas in CR+OSOM regions that may be associated with fibrosis. Comparisons of R_2 and $R_{1\rho}$ distributions at spin-lock frequency 1000 Hz in CR+OSOM regions in representative normal WT and hHB-EGF^{Tg/Tg} kidneys are shown in Figure 6. The regional Mean and SD of R_2 and $R_{1\rho}$ have been quantified across voxels in the CR+OSOM region in each normal WT kidney, and these values were averaged across normal kidneys to set up the R_2 and $R_{1\rho}$

thresholds for identifying abnormal clusters with lower values. The respective Mean $\pm$ SD values were 26.31 ± 2.63 and 20.30 ± 2.16 Hz for R_2 and $R_{1\rho}$ (locking field 1000 Hz) of CR+OSOM regions (N=16). The normal range was set at Mean $\pm$ 1.96SD based on the averaged values across pixels (indicated by the green dashed lines in Figure 6) of the CR+OSOM region. Then, the voxels with R_2 or $R_{1\rho}$ below the respective lower threshold could be identified and their % area in kidney was quantified (Equations 4–5). With the above mean R_2 and $R_{1\rho}$ and their respective averaged SD values across voxels observed for CR+OSOM regions of normal WT kidneys (N=16), the corresponding thresholds at the lower end for R_2 and $R_{1\rho}$ (locking field 1000 Hz) were 21.16 and 16.05 Hz.

In WT kidney (Figure 6), the fractions of voxels below the respective R_2 and $R_{1\rho}$ thresholds were low (tR_2 and $tR_{1\rho} < 5.0\%$). However, in the hHB-EGF^{Tg/Tg} kidney, the mean R_2 and $R_{1\rho}$ of CR+OSOM region shifted lower (Figure 6), and they were distributed over a broader range of values than those of normal WT kidney (Figure 6), and the probabilities of pixels having values below their respective thresholds were increased. The percentages of the voxels that were below the R_2 and $R_{1\rho}$ thresholds of CR+OSOM regions (tR_2 and $tR_{1\rho}$) in this individual fibrotic hHB-EGF^{Tg/Tg} kidney were 12.63% and 17.58% respectively. For the detected positive fibrosis areas in this selected fibrotic kidney, their averaged R_2 and $R_{1\rho}$ (mR_2 and $mR_{1\rho}$) were also quantified, with 15.70 and 11.13 Hz for mR_2 and $mR_{1\rho}$ (locking field 1000 Hz) respectively. These measures narrowed down the positive fibrotic areas, which mitigates against any partial volume effects from adjacent normal tissues in the CR+OSOM regions.

3.5 | Comparison of regional $R_{1\rho}$ dispersions of normal and fibrotic kidneys

At 7T all kidneys showed considerable dispersion in $R_{1\rho}$ with locking field, with rate values decreasing about 25% over the range 200 – 3000 Hz in CR+OSOM regions (Supporting Information Figure S4 and Figure 7A). $R_{1\rho}$ dispersion curves showed different patterns for CR+OSOM regions in normal and fibrotic kidneys at different stages, with lower $R_{1\rho}$ observed in fibrotic kidneys than in normal kidneys (Figure 7A). Based on the differences between dispersion curves of CR+OSOM regions in normal and fibrotic kidneys at different stages ($R_{1\rho}$), the maximum of $R_{1\rho}$ in the early stage (week 7) occurred around a locking frequency 1500 Hz, and this shifted to lower locking frequencies as fibrosis became more severe (Figure 7B). $R_{1\rho}$ dispersion curves for $tR_{1\rho}$ -detected positive fibrosis areas (Figure 7C) showed lower $R_{1\rho}$ than the respective value of CR+OSOM (Figure 7A) at each locking frequency at different fibrosis stage. The locking frequency for the maximal $R_{1\rho}$ of positive fibrosis areas was lower than respective value of CR+OSOM regions, especially for the early and middle fibrosis stages (Figure 7B&D). The $tR_{1\rho}$ -based regional dispersion analysis reduced partial volume affects from adjacent normal tissues. The locking frequencies for the maximal $R_{1\rho}$ of detected abnormal areas were around 1000 Hz at different stages (Figure 7D), which suggests the optimum locking frequency in spin-lock imaging for detecting fibrosis at 7T.

From the fitting of $R_{1\rho}$ dispersion of detected abnormal regions using the Chopra model (Figure 7C), R_{2fit} , $R_{1\rho}^\infty$, and S_ρ were quantified (Equation 1) and the inflection frequency ω_{infl} was obtained thereafter (Equation 3). Group comparisons between the derived values of

abnormal tissues in fibrotic kidneys at different ages and respective values of normal tissues in WT kidneys are shown in Figure 8A. Except $R_{1\rho}^{\infty}$, the derived R_{2fit} , S_{ρ} and ω_{inff} all showed significant decreases in fibrotic kidneys. R_{2fit} values clearly dropped as fibrosis progressed (Figure 8), but the exchange parameter S_{ρ} and the inflection frequencies ω_{inff} changed by much larger factors (>50%). Supporting Information Figure S5 summarizes the values of the derived parameters of CR+OSOM regions for comparison. While R_{2fit} differences were not significant in early stage fibrosis in CR+OSOM region (Supporting Information Figure S5), S_{ρ} and ω_{inff} showed greater sensitivity for detecting the differences between different fibrosis stages. In addition, progressively lower S_{ρ} and ω_{inff} were also observed as fibrosis progressed when mice aged (Supporting Information Figure S5). There was less variation in the asymptotic value of $R_{1\rho}^{\infty}$ between the groups. Please note that $R_{1\rho}^{\infty}$ derived from dispersion 200–3000 Hz can be very different from R_1 relaxation rate, because additional dispersion for chemicals with fast chemical exchange pools (such as uracil) may occur beyond locking frequency 3000 Hz.

3.6 | MRI measures from threshold analyses and histologic fibrosis indices

Group comparisons between MRI measures of positive fibrosis areas in fibrotic kidneys and respective values of normal tissues in WT kidneys are shown in Figure 8. Among all the measures, the mR_2 and $mR_{1\rho}$ of fibrotic tissues showed most significant decreases compared to respective normal tissues, while S_{ρ} and ω_{inff} decreased by the largest factor. It is of note that comparisons between derived measures of CR+OSOM regions, with fibrotic measures including both normal and abnormal tissues, can significantly underestimate the differences between groups (Figure 8 and Supporting Information Figures S2 and S5). We also compared the changes of tR_2 - and $tR_{1\rho}$ -detected fibrotic areas (%) with histological fibrosis indices between WT and hHB-EGF^{Tg/Tg} kidneys at different stages of progress (Figure 8B). Twelve WT and eight hHB-EGF^{Tg/Tg} kidneys with both MRI and histological results were included in the comparison. The tR_2 and $tR_{1\rho}$ increased drastically in fibrotic kidneys, validated by the histologic fibrosis index. The MRI detected fibrotic areas (%) were comparable to those detected by picrosirius red stains (Figure 8B), even though the estimated mean and SD values of tR_2 and $tR_{1\rho}$ for normal WT (Mean $\pm$ SD: 2.988% $\pm$ 2.711% and 1.686% $\pm$ 0.898%) were larger than those from histological indices (Mean $\pm$ SD: 0.257% $\pm$ 0.134%) across subjects. Parameters tR_2 and $tR_{1\rho}$ showed gradual changes as fibrosis progressed from week 5–7 to week 11–13, and to week 30–40. The tR_2 - and $tR_{1\rho}$ -detected fibrotic areas (%) were highly correlated with the histological fibrosis index measured with picrosirius red stains ($r = 0.804$ and 0.880 , $p < 0.05$) across WT and hHB-EGF^{Tg/Tg} kidneys.

ROC comparisons of these parameters are shown in Table 1. Histological fibrosis indices consistently showed highest sensitivity and specificity in detecting fibrosis at different stages (Table 1). The $tR_{1\rho}$ at locking frequency 1000 Hz showed a larger AROC than tR_2 , with sensitivity and specificity more comparable to histological fibrosis indices. The mR_2 and $mR_{1\rho}$ always showed highest AROC in detecting the differences between normal and fibrotic tissues at different stages. Among all parameters from the Chopra modeling, S_{ρ} and ω_{inff} showed the largest AROC in detecting differences between normal and fibrotic tissues at different stages, while $R_{1\rho}^{\infty}$ showed the lowest AROC. The sensitivity and specificity of

MRI measures for detected abnormal tissues (Table 1) were much higher than those for CR+OSOM regions (Supporting Information Figure S3 and Tables S1–2).

4 | DISCUSSION

Measures obtained from spin-lock $R_{1\rho}$ dispersion imaging are sensitive to alterations in chemical compositions and the physico-chemical environment in tissue during fibrosis in hHB-EGF^{Tg/Tg} kidney. This study demonstrates that *in vivo* spin-lock imaging may be useful for assessing renal fibrosis in progressive kidney disease.

4.1 | MRI parameters and their regional differences in kidney

Our results showed significant regional differences in R_2 and $R_{1\rho}$ among cortex, OSOM, ISOM and IM+P in WT kidneys (Figure 3). R_2 and $R_{1\rho}$ can be sensitive to free water content and capillary density. Low R_2 and $R_{1\rho}$ could result from decreased capillary density and/or increased free water. Relaxation rates are usually highly correlated with concentrations of macromolecules such as proteins. Both R_2 and $R_{1\rho}$ decreased from cortex to IM+P (Figure 3), and the regional trend could be associated with a decrease of microvascular density from the cortex to IM+P. The regional differences in R_2 and $R_{1\rho}$ in kidneys could also be related to variations in free water contents and tubular density in each renal compartment (CR+OSOM < ISOM < IM+P) (20). These observations are consistent with regional kidney structures and functions, including glomerular filtration and tubular absorption in the cortex and urine concentration in the medulla.

4.2 | Sensitivity of spin-lock measures to renal fibrosis

Different MRI measures showed different sensitivities to changes caused by renal fibrosis. The locking frequencies for maximal $R_{1\rho}$ of detected fibrotic areas were observed around 1000 Hz at different fibrosis stages (Figure 7D), and $R_{1\rho}$ with locking frequency 1000 Hz consistently showed large AROC and high combined sensitivity and specificity for detecting fibrosis at different stages (Supporting Information Figure S3 and Table S1). These observations indicate that the optimum spin-lock frequency for detecting renal fibrosis at 7T is about 1000 Hz. In this hHB-EGF^{Tg/Tg} model, renal fibrosis develops by 6 weeks of age and its level progressively increases as the mice age.(35) Threshold analysis improves the sensitivity of each measure for detecting fibrotic areas compared to the respective regional measures of CR+OSOM (Table 1 and Supporting Information Tables S1–2). The hHB-EGF^{Tg/Tg} mice at 5–7 weeks showed significant increments in tR_2 - and $tR_{1\rho}$ -detected regions as compared to WT mice (Figure 8). Spin-lock MRI detected fibrosis in OSOM, and similar results were observed by histology (Figures 1 and 8). The measures of tR_2 and $tR_{1\rho}$ could be used to detect mild structural changes in earlier stages of fibrosis.

It is well known that $R_{1\rho}$ at a single spin-lock power is confounded by many factors, whereas $R_{1\rho}$ dispersion provides a more specific measure of exchange effects by separating relaxation rates, exchange rates and frequency offsets of exchanging pools. Previous works have demonstrated that both R_2 and $R_{1\rho}$ could be very sensitive to fibrosis in organs,(32–34) but it is not clear whether $R_{1\rho}$ measurements at one single spin-lock power has any advantage over R_2 . $R_{1\rho}$ at an optimum spin-lock amplitude showed higher sensitivity and

specificity than R_2 in detecting fibrosis in hHB-EGF^{Tg/Tg} mice (Supporting Information Figure S3 and Table S1). Our data demonstrate that the area exhibiting abnormally low R_2 , $R_{1\rho}$, S_ρ and ω_{infl} values is related to regional histological fibrosis in the OSOM of hHB-EGF^{Tg/Tg} kidney, suggesting these measures could be used for detecting and quantifying renal fibrosis in kidney diseases.

4.3 | Challenges in spin-lock imaging of murine renal fibrosis

The parameters R_{2fit} , $R_{1\rho}^\infty$, S_ρ and ω_{infl} from $R_{1\rho}$ dispersion showed wide ranges for normal WT kidneys (Figure 8). In addition to renal structure and function, the $R_{1\rho}$ dispersion is highly sensitive to the chemical components, including metabolites having exchanging groups such as glucose, creatinine and uracil, etc. Therefore, parameters from $R_{1\rho}$ dispersion could be very sensitive to physiological conditions, anesthesia level and metabolism during *in vivo* imaging. Restriction on food and drink before imaging and stabilization of physiological conditions and anesthesia level during imaging may help reduce the variabilities of these MRI measures for normal tissues and improve their sensitivity to abnormal change. Successful spin-lock mapping was restricted to a prescribed homogeneous B_1 and B_0 region. More advanced spin-lock sequences with additional phase shifts and 180° refocusing pulses can minimize artifacts arising from the B_1 and B_0 inhomogeneities but were not needed for small mouse imaging. High-resolution imaging is critical for the detection of scattered macromolecular accumulations (fibrosis) in small mouse kidneys. Even though high SNR offered at high fields permits relatively high spatial resolution for spin-lock imaging (SNR is ~100 for resolution 0.25×0.25×1 mm³), MRI measures may be still affected by partial volume averaging in small mouse kidneys. Variations of R_2 and $R_{1\rho}$ in each voxel could reflect the net effect of pathological changes. Tubular dilation/urine retention, tubular atrophy, inflammation, increase of extracellular space, and decrease in capillary density could all reduce R_2 and $R_{1\rho}$ in a voxel, while collagen deposition, cell infiltration and formation of myofibroblasts may show opposite effects. If the resolution is not high enough, the changes of R_2 and $R_{1\rho}$ in each voxel could underestimate or overestimate renal fibrosis. Thus, it is critical to apply high-resolution spin-lock MRI for assessing the extent of fibrosis in kidney disease. Overall, measures from positive fibrosis areas showed higher sensitivity than respective measures of CR+OSOM regions in detecting renal fibrosis in this model, indicating that threshold analysis may reduce the influence from confounding factors.

4.4 | Comprehensive and specific measures for assessing multiple features and parallel events during renal fibrosis

Multiple pathologic features present during the progression of fibrosis in kidney diseases.(3) Different kidney diseases such as renal artery stenosis,(18) nephrosclerosis (40) and obstructive kidney disease,(41) can show different characteristics in fibrosis.(20) In addition to deposition of rigid macromolecules and increased matrix cross-linking, progressive capillary loss, reduced microvascular blood flow and diminished oxygen delivery are also common during renal fibrosis.(3) Other parallel events, including inflammation, apoptosis, tubular dilation and polyuria may also accompany fibrosis in kidney disease.(9,37–39,42) Histology detected extensive fibrosis in the hHB-EGF^{Tg/Tg} mice, in which collagen deposition and capillary density reduction were observed in the fibrotic regions of kidneys.

(35) The tubular dilation or polyuria in the hHB-EGF^{Tg/Tg} model is not evident. Thus, the decreases of R_2 and/or $R_{1\rho}$ in the detected positive fibrosis areas in the hHB-EGF^{Tg/Tg} model could be mainly due to events highly specific to fibrosis, such as increase of tubulointerstitial space and matrix, deposition of collagen, increased matrix cross-linking, and capillary loss. However, in a UUO model, advanced, parallel and sequential events such as tubular dilation and polyuria, tubular atrophy, decreases in blood flow and blood volume, could also cause R_2 and/or $R_{1\rho}$ decrease.(32,33,37,38) Even though R_2 and/or $R_{1\rho}$ decreased drastically in the cortical regions of UUO kidneys, fibrosis was very mild.(32,33,37,38) Thus, although R_2 and $R_{1\rho}$ could be correlated with renal fibrosis in the cortex of UUO kidneys, they are not specific enough to renal fibrosis alone. On the other hand, $R_{1\rho}$ dispersion, rather than single measurements of relaxation rates, can provide additional information on exchanging and/or diffusion to study fibrosis. Measures obtained from spin-lock $R_{1\rho}$ dispersion imaging are sensitive to alterations in chemical compositions during fibrosis in hHB-EGF^{Tg/Tg} kidney. Both S_ρ and the inflection frequency depend primarily on the exchange rate between water and other chemically shifted resonances such as hydroxyls, amines and amides.(36) This average exchange rate is sensitive to changes in pH and other physico-chemical aspects of the tissue microenvironment such as increases in fractions of proteins with slower exchanging or smaller chemically shifted protons. Collagen accumulation and cross-linking are the characteristic features when fibrosis occurs. The slow exchange rates k_{sw} of hydroxyl protons in collagen due to crosslink and their small RF offsets ω_s (150–300 Hz at 7T) lower down the averaged S_ρ values observed for fibrotic tissues. In addition, $R_{1\rho}$ dispersion in the range of lower spin-locking powers (locking frequency 0–200 Hz) may provide measurements to quantify aspects of diffusive and intrinsic susceptibility variations in inhomogeneous biological tissues, caused by changes of vessel density and oxygen saturation level during fibrosis progression.(28,29)

5 | CONCLUSIONS

Renal tubulointerstitial fibrosis in kidneys can be assessed by spin-lock MRI and measures obtained from $R_{1\rho}$ dispersion. This technique provides exchange rate information in addition to relaxation rates, which may be used as a novel imaging approach to assess chronic renal diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Comparison of histological results from picrosirius red stains.

Representative WT and hHB-EGF^{Tg/Tg} kidney tissues were compared side by side at different magnification level. The middle and lower panels are magnifications from OSOM. ISOM: inner stripe of outer medulla. OSOM: outer stripe of outer medulla. Intensive fibrosis is shown in red in hHB-EGF^{Tg/Tg} kidney (indicated by arrows in OSOM).

Figure 2. *In vivo* spin-lock mapping of normal WT kidneys.

(A) T₂-weighted, and conventional T₂ and R₂ maps. 1, cortex (CR); 2, outer stripe of outer medulla (OSOM); 3, inner stripe of outer medulla (ISOM); 4, inner medulla and papilla (IM+P). (B) Selected spin-lock maps at different spin-lock powers with locking frequencies at 200, 600, 1000, 1500, and 2500 Hz respectively.

Figure 3. Group comparison of regional MRI measures in normal WT kidneys (N = 16). The regional R_2 and $R_{1\rho}$ at selected locking frequencies (400, 1000, 1500, 2000 and 3000 Hz) were shown for comparison. In the boxplots, middle lines and circles indicate median and mean values across subjects respectively. Crosses indicate outliers in each group. CR, cortex; OSOM, outer stripe of outer medulla; ISOM, inner stripe of outer medulla; IM+P, inner medulla and papilla. Significant parametric differences across regions are indicated by asterisks. * $p < 10^{-8}$, ** $p < 10^{-12}$, *** $p < 10^{-15}$, and **** $p < 10^{-17}$ versus measures of CR regions.

Figure 4. *In vivo* spin-lock mapping of a representative fibrotic hHB-EGF^{Tg/Tg} mouse.

(A) T₂-weighted, and conventional T₂ and R₂ maps. 1, cortex (CR); 2, outer stripe of outer medulla (OSOM); 3, inner stripe of outer medulla (ISOM); 4, inner medulla and papilla (IM + P). Arrows indicate that fibrosis occurs in OSOM regions. (B) Selected spin-lock maps at different spin-lock powers with locking frequencies at 200, 600, 1000, 1500, and 2500 Hz respectively. One subject with severe fibrosis in the OSOM is shown.

Figure 5. Progress of renal fibrosis in hHB-EGF^{Tg/Tg} model.

Comparison of representative T₂-weighted (T2W) images and R_2 and $R_{1\rho}$ maps of normal (week 33) and fibrotic kidneys at different stage (week 7, 12 and 33) during fibrosis progress. $R_{1\rho}$ maps from locking frequency 1000 Hz are shown. Double-headed arrows in red, orange, and green indicate CR+OSOM, ISOM and IM+P regions respectively, and the yellow crosses indicate the thin OSOM stripes.

Figure 6. Comparison of R_2 and $R_{1\rho}$ distributions in CR+OSOM regions of representative WT and fibrotic hHB-EGF^{Tg/Tg} kidneys.

(A) R_2 distributions of individual WT and fibrotic kidneys indicated by gray and red color respectively. Green dashed lines indicate lower and upper R_2 thresholds at 21.15 and 31.47 Hz. (B) $R_{1\rho}$ distributions of individual WT and fibrotic kidneys indicated by gray and red color respectively. Green dashed lines indicate lower and upper $R_{1\rho}$ thresholds at 16.05 and 24.54 Hz. The double-headed arrows in R_2 and $R_{1\rho}$ (locking frequency at 1000 Hz) maps indicate CR+OSOM regions. The green dashed lines in distribution plots indicate the normal range (Mean $\pm$ 1.96SD) of R_2 and $R_{1\rho}$ for CR+OSOM regions in normal WT kidneys (N=16), and asterisks indicate outliers with R_2 and $R_{1\rho}$ values smaller than their respective lower thresholds. The regional Mean and SD values of R_2 and $R_{1\rho}$ across voxels in the CR +OSOM regions of the representative WT and hHB-EGF^{Tg/Tg} kidneys are shown. The selected individual WT and fibrotic kidneys were from mice aged 33 weeks. F, fibrotic.

Figure 7. Comparison of representative regional $R_{1\rho}$ dispersions.

(A) $R_{1\rho}$ dispersions of CR+OSOM regions in normal kidney at week 33 and fibrotic kidneys showing progressive fibrosis at week 7, 12 and 33. The markers indicate the raw $R_{1\rho}$ values at different locking frequencies and the dashed lines show the fitting curves using Chopra model. (B) Difference in $R_{1\rho}$ dispersions of CR+OSOM regions between WT kidney and fibrotic kidney at different stage. (C) $R_{1\rho}$ dispersions of $tR_{1\rho}$ -detected (locking frequency 1000 Hz) positive fibrosis areas in the respective fibrotic kidneys. The markers indicate the raw $R_{1\rho}$ values at different locking frequencies and the dashed lines show the fitting curves using Chopra model. (D) Difference in $R_{1\rho}$ dispersions between normal tissues in WT kidney and $tR_{1\rho}$ -detected fibrosis tissues in fibrotic kidney at different stage.

Figure 8. Group comparison of histological fibrosis index and MRI parameters from positive fibrosis areas.

(A) Comparison of MRI measures of normal tissues of WT kidneys and fibrotic tissues in hHB-EGF^{Tg/Tg} kidneys detected by $tR_{1\rho}$ -based analysis (locking frequency 1000 Hz). In all the boxplots, middle lines and circles indicate median and mean values across subjects respectively. Crosses indicate outliers in each group. N=16, N=12, N=16, and N=16 for normal WT, fibrotic hHB-EGF^{Tg/Tg} at ages 5–7 weeks (F5–7W), 11–13 weeks (F11–13W), and 30–40 weeks (F30–40W), respectively. (B) Comparison of histologic fibrosis index by positive picosirius red (%) and tR_2 and $tR_{1\rho}$ (locking frequency 1000 Hz) detected fibrosis areas. N=12, N=8, N=8, and N=8 for normal WT, fibrotic hHB-EGF^{Tg/Tg} at ages 5–7 weeks (F5–7W), 11–13 weeks (F11–13W), and 30–40 weeks (F30–40W), respectively.

Characteristic map with tR_2 or $tR_{1\rho}$ detected fibrosis areas overlaid on spin-lock anatomical image was shown as an insert. Significant parametric differences between models are indicated by p values. * $p < 0.05$, ** $p < 10^{-3}$, *** $p < 10^{-4}$, **** $p < 10^{-8}$, ***** $p < 10^{-12}$ vs. relative measures of WT.

Table 1.ROC results of selected MRI measures and histological fibrosis index^a

Parameters		Threshold ^b	Sensitivity ^b	Specificity ^b	AROC
F5–7W	tR_2	4.55 %	0.58	0.82	0.71
	$tR_{1\rho}$ (1000Hz)	3.41 %	0.83	1.00	0.97
	mR_2	22.79 Hz	1.00	1.00	1.00
	$mR_{1\rho}$ (1000Hz)	17.02 Hz	1.00	1.00	1.00
	R_{2fit}	19.93 Hz	0.92	0.92	0.93
	$R_{1\rho}^\infty$	15.02 Hz	0.58	1.00	0.72
	S_ρ	1356.49 Hz	1.00	1.00	1.00
	ω_{infl}	783.00 Hz	1.00	1.00	1.00
	HFI	0.84 %	1.00	1.00	1.00
F11–13W	tR_2	6.74 %	0.92	0.91	0.93
	$tR_{1\rho}$ (1000Hz)	5.72 %	1.00	1.00	1.00
	mR_2	22.18 Hz	1.00	1.00	1.00
	$mR_{1\rho}$ (1000Hz)	16.68 Hz	1.00	1.00	1.00
	R_{2fit}	20.08 Hz	0.92	0.92	0.95
	$R_{1\rho}^\infty$	13.40 Hz	0.75	1.00	0.81
	S_ρ	1322.36 Hz	1.00	0.92	0.99
	ω_{infl}	763.50 Hz	1.00	0.92	0.99
	HFI	4.46 %	1.00	1.00	1.00
F30–40W	tR_2	5.58 %	0.92	0.91	0.97
	$tR_{1\rho}$ (1000Hz)	5.96 %	1.00	1.00	1.00
	mR_2	22.32 Hz	1.00	1.00	1.00
	$mR_{1\rho}$ (1000Hz)	16.58 Hz	1.00	1.00	1.00
	R_{2fit}	19.57 Hz	0.92	1.00	0.99
	$R_{1\rho}^\infty$	13.05 Hz	0.75	0.83	0.78
	S_ρ	1227.58 Hz	1.00	0.92	0.97
	ω_{infl}	708.78 Hz	1.00	0.92	0.97
	HFI	9.39 %	1.00	1.00	1.00

^a Comparisons between positive fibrotic tissues detected in hHB-EGF^{Tg/Tg} mice and normal tissues in the CR+OSOM regions of WT mice. Histological fibrosis index (HFI) was measured based on positive picrosirius red (%). AROC, area under ROC curve.

^b Threshold for each parameter shows the optimum value that maximizes the sensitivity and specificity, collocated in the nearest point to (0, 1).