

# Feasibility Study of Assessing Progression of Chronic Liver Disease with MR Elastography in an In Vivo Animal Model

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## Introduction:

At present, the gold standard for diagnosing progressive hepatic inflammation and fibrosis is liver biopsy, which is an invasive procedure with risks of complications, potential sampling errors, and known problems with subjective histology grading. MR Elastography (MRE) is a phase-contrast MRI technique for quantitatively assessing the mechanical properties of soft tissues by visualizing propagating shear waves in soft tissues. Several investigations using MRE for the early differentiation of steatohepatitis from steatosis in an animal model and human studies (1, 2) have agreed with the clinical experience of hepatic MRE that patients with a higher inflammation grade tend to have a higher liver stiffness than patients with lower inflammation grade (3). Investigations using transient elastography have also shown dynamic liver stiffness changes during acute hepatitis progression (4, 5) and antiviral therapy (6). Thus, mechanical properties of the liver may provide independent noninvasive indicators to distinguish hepatic inflammation and fibrosis. However, testing and modeling of the mechanical properties of biological tissues presents a unique set of challenges since biological soft tissues are usually nonlinear, porous, viscoelastic and anisotropic. Many groups have published findings on these mechanical properties of soft tissues, and the methods vary as widely as the values reported (7, 8, 9). In general, a large number of theoretical models can be applied to the data to extract tissue parameters, yet the relationship remains poorly understood, in part because no published study has tracked all of these potential parameters and factors simultaneously in any *in vivo* animal model. The purpose of this study was to perform a feasibility study of a longitudinal liver MRE protocol for small animals which can be used to perform global shear stiffness measurements to quickly identify whether a selected animal model merits further study in monitoring progressive hepatic inflammation and fibrosis in chronic liver disease.

## Methods and Materials:

We used a mouse model of autosomal recessive polycystic kidney disease (ARPKD), which is an inherited disorder of the kidneys and liver caused by mutations in the *Pkhd1* gene and an important cause of congenital hepatic fibrosis (CHF) (10). A loxP flanked puromycin N-acetyltransferase (*pac*) and SV-40 derived transcriptional STOP cassette was inserted into Intron-2 of the *Pkhd1*, LSL. This terminated all *Pkhd1* transcripts after this point (11). The animals are viable to at least 12 months and known to develop progressive hepatic inflammation and fibrosis with age. We imaged 10 wild-type mice (5 male, 5 female) and 10 infected mice (5 male, 5 female) respectively. Evidence of early changes due to inflammation and/or fibrosis were examined at 3 weeks of age to evaluate the practicality and diagnostic potential for the detection of varying extents of hepatic inflammation before and after the onset of fibrosis.

All experiments were implemented on a 3.0-T whole-body GE imager (HDx, GE Medical System, Milwaukee, WI), using a 3-cm receive-only single-channel birdcage coil (BC3 coil). Fig. 1A and B demonstrate our experimental setup, which produced a torsional vibration of the birdcage coil to generate shear waves throughout the mouse body wrapped within a silicon gel blanket. The mice were anesthetized with 1.5% isoflurane. Wave images were acquired with a three-slices 2-D spin-echo MRE sequence with three motion-encoding directions using 400-Hz harmonic vibrations. The MRE wave images were then processed with a direct inversion (DI) algorithm to calculate the complex shear modulus  $G^*$  of the liver tissue. The shear stiffness and attenuation were derived for testing our hypothesis. Statistical analysis was performed with a two-sided paired t-test and 5% significance for this pilot study.

## Results:

From the histological analysis, 3-week-old *Pkhd1* mice had not developed significant hepatic fibrosis yet. However, since they are born with disease, inflammation could persist before the onset of fibrosis. Fig. 1C shows two MRE results obtained from a 3-week-old wild-type mouse and a 3-week-old knockout *Pkhd1* mouse. Assuming homogeneous, isotropic viscoelastic response, the *Pkhd1* mice demonstrated significantly higher global liver stiffness than the control mice (♂: 2.17±0.12 vs. 1.92±0.14 kPa; ♀: 2.12±0.15 vs. 1.81±0.12 kPa;  $p < 0.05$ ), as illustrated in Fig. 2A. The attenuation data did not show a significant difference between the two groups. Fig. 2B shows histologic images from a diseased mouse and a wild-type mouse. Liver histology shows inflammatory infiltrate, mild fibrosis and cyst formation in the *Pkhd1* mice. Therefore, the increased liver stiffness may correlate with both increased interstitial fluid volume caused by inflammation and the slight deposition of collagen from fibrosis.

## Discussion and Conclusion:

The results of this study demonstrated that increased liver stiffness is an early marker of inflammation and fibrosis matrix deposition. If the diseased mice can be imaged at a younger age, the increase in hepatic stiffness could be linked to inflammation and fibrogenesis activation in the early development of liver disease before the development of fibrosis. If these results can be further confirmed with more sophisticated mechanical models (e.g., poroviscoelastic models) to separate the fluid-related effects of inflammation and interstitial fluid from the matrix/structure-related alterations of fibrosis, MRE may be used to discriminate and monitor progressively developed inflammation and fibrosis in the liver. By performing MRE at more time points during the lifespan of mice such as these *Pkhd1* mice, it may be possible to develop mechanical models for hepatic inflammation as the disease progresses.

## References:

- Salameh N, Radiology 2009 253(1): 90-7;
- Chen J, Radiology 2011 259(3): 749-56;
- Yin M, ISMRM 2011, 390;
- Vigano M, Eur J Gastroenterol Hepatol. 2010 22(2): 180-4;
- Seo YS, Scand. J. Gastroenterol. 2010 45(4): 449-56;
- Lim SG, Hepatogastroenterology 2011 58(106): 539-45;
- Asbach P, Radiology 2010 257(1):80-6;
- Holm S, Sinkus R, J. Acoust. Soc. Am. 2010 127(1) 542-59;
- Perrinez PR, IEEE Trans. Biomed. Eng. 2009 56(3): 598-608;
- Ward CJ, Nat Genet 2002 30:259-69.
- Bakeberg JL, J Am Soc Nephrol 2011(22)

