Microscopy-based fibrosis phenotypic analysis of rodent and adult NASH cohorts reveal translational traits of fibrosis progression and severity

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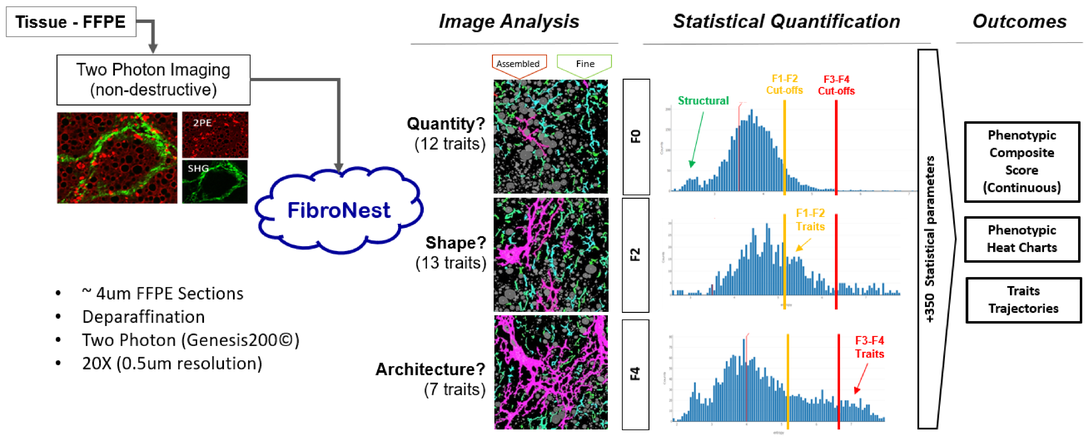
BACKGROUND

To date, no single rodent model can display the whole disease spectrum and metabolic features associated with human NASH but can only imitate characteristics. In this study, we focus on the phenotypic traits of fibrosis severity from three rodent fibrosis progression models and an adult human NASH progression cohort. In each model and for each progression stage, we quantify 32 different traits of the fibrosis phenotypes, and identified the traits of fibrosis severity that are common to all the species.

METHOD

TISSUE PREPARATION, INSTRUMENTATION, AND WORKFLOW



- ~ 4um FFPE Sections
- Deparaffination
- Two Photon (Genesis200©)
- 20X (0.5um resolution)

This retrospective study comprised three Fibrosis and NASH rodent cohorts and one clinical cohort (N=98) of patients with NASH with fibrosis stages 0-4

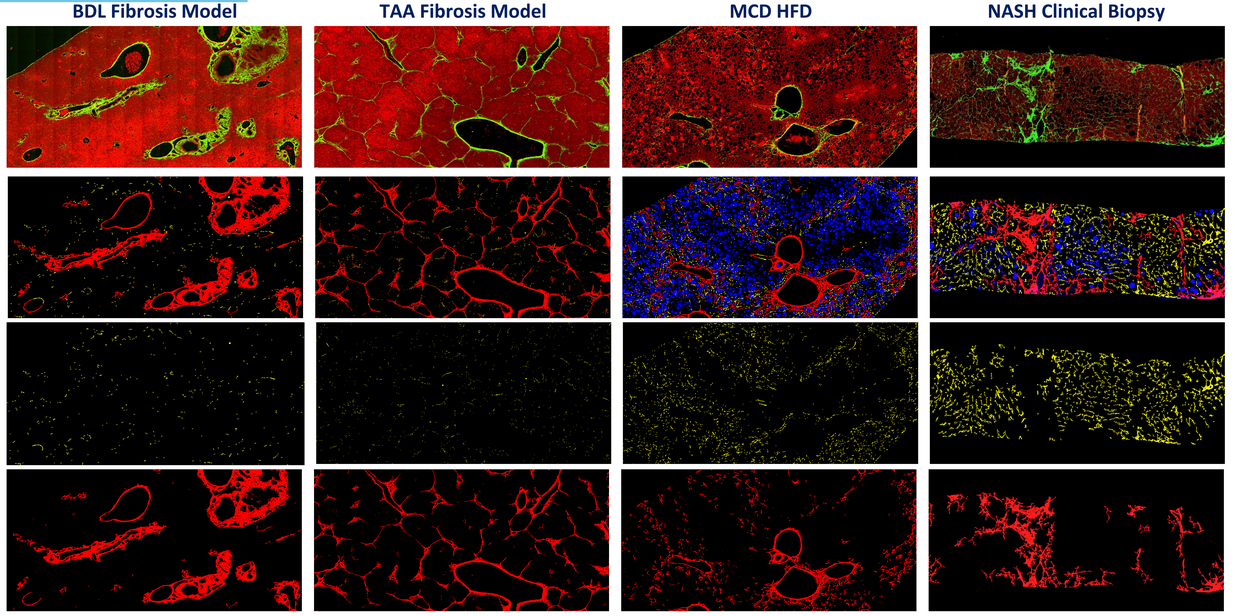
Cohort	Model	Groups	Total N
BDL	A Bile Duct Ligation (BDL) mouse fibrosis progression cohort	Sham, Day 5 – 10 – 14 – 20	N=2 (N=5 / group)
TAA	A thioacetamide (TAA) induced fibrosis in mouse progression cohort	Week 4 and Week 8)	N=10 (N=5 / group)
MCD-HFD	A mouse methionine and choline-deficient (MCD) fat diet	Control (N=4) Week 8 & Week 12 (N=10)	N=24
NASH	A clinical cohort of patients with NASH diagnosed by histologic assessment of liver biopsy according to NASH CRN criteria by pathologists	F0 (N=24) F1 (N=24) F2 (N=25) F3 (N=20) F4(N=5)	N=98

- FFPE sections (3-4 microns) of tissues and biopsies were deparaffinized and imaged using the Two Photon microscope Genesis200® at 20X.
- Using FibroNest© for image analysis of both the Two Photon and WSI images, the fibrosis phenotype is described for its collagen content and structure (12 traits), the morphometric traits of the collagen fibers (13 traits), and fibrosis architecture / texture traits (7). In each image, each morphometric and texture trait is represented by a histogram distribution which is quantified with 7 statistical parameters (qFPs).
- For each group, the Mean qFPs are normalized to their initial value in the fibrosis progression range (e.g. F0 for NASH) and displayed on the progression charts.

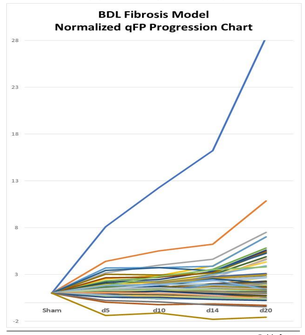
CONCLUSIONS

- Out of the three pre-clinical models studies, the fibrosis progression observed in the Bile Duct Ligation model recapitulates the most the histological traits observed in the F0 to F4 progression
- None of the fibrosis progression mouse model recapitulates the inflexion observed in the Adult NASH qFp progression charts in theF2 region, possibly driven by a metabolic change not existent in the mouse model.
- The Fibrosis Texture (Architecture) and the Skeleton, Area and Tortuosity morphometric features of the collagen fibers quantified individually and collectively play a large role to describe the progression of fibrosis, often more than high-level Collagen Area ratio % metric
- These insights have already started to improve the quality of translational drug discovery and development process and to yield specific biopsy-based diagnostic tools for fibrosis.

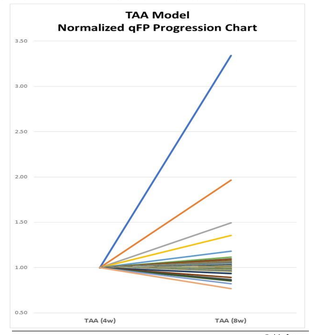
RESULTS



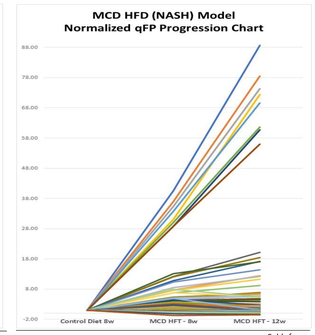
Representative images from each model including : (A) Two Photon Image - Green: Second Harmonic for Collagens, Red: Two Photon Fluorescence for tissue (B) Image Analysis results: Red: Assembled Collagens Yellow: Fine Collagens, Bleu: Steatosis (C) Fine Collagens fibers/ phenotypes only (D) Assembled Collagens / Phenotypes only



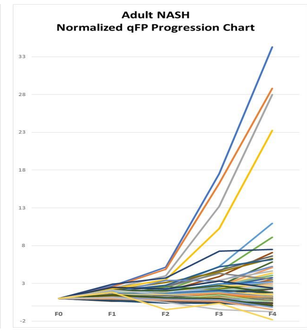
Top20 Normalized qFPS (BDL)	Folds from Baseline
Normalized count of Homogeneous Regions (Texture)	28.4
Skeleton Nodes Normalized Count	10.9
Normalized Count of Fibers	7.5
Assembled Collagen Area Ratio (%)	7.0
STDEV - Total Skeleton Length (all Phenotypes)	7.0
Collagen Area Ratio (%)	5.8
STDEV - Fiber Area (all Phenotypes)	5.5
STDEV - Fiber Perimeter (all Phenotypes)	5.4
STDEV - Fiber Filled Area (all Phenotypes)	4.8
Total Collagen Density Area Ratio (%)	4.7
Collagen Fibers Density	4.6
Normalized count of Contrasted Regions (Texture)	4.3
Kurtosis of the Fiber Width distribution (All Phenotypes)	3.9
Assembled Collagens Normalized Count	3.8
Normalized count of Rounded Fibers (all Phenotypes)	3.1
Mean of the Fiber Filled Area distribution (all Phenotypes)	3.0
Normalized count of Long Total Skeleton fibers (all Phenotypes)	3.0
Normalized count of Tortuous Fibers (All Phenotypes)	2.9
Normalized count of Thick fibers (All Phenotypes)	2.8
Mean of the Fibers Area Distribution - (all Phenotypes)	2.7



Top20 Normalized qFPS (TAA)	Folds from Baseline
Normalized count of Resolved Regions - Texture	3.3
Kurtosis for Fiber Tortuosity (All phenotypes)	2.0
Normalized count of Organized Regions - Texture	1.5
Skewnes of Fiber Tortuosity (All Phenotypes)	1.4
STDEV - Fiber Tortuosity (all Phenotypes)	1.2
Kurtosis for Texture Kurtosis	1.1
Skenwess of Texture Homogeneity	1.1
Skewness of Fiber Orientation (All Phenotypes)	1.1
STDEV for Energy Regions (Texture)	1.1
Normalized count of Dull Regions (texture)	1.1
Median for Energy - (Texture)	1.1
Normalized count of Tortuous Fibers (All Phenotypes)	1.1
Kurtosis for Skewness (Texture)	1.1
Assembled Collagens Normalized Count	1.1
Mean for Energy (Texture)	1.1
Kurtosis for Fibers Filled Area (All Phenotypes)	1.1
Normalized count of Rounded Fibers (all Phenotypes)	1.1
STDEV of Homogeneity (Texture)	1.1
Median of Kurtosis (Texture)	1.1
Skewness of Kurtosis (Texture)	1.1



Top20 Normalized qFPS (MCD HFD)	Folds from Baseline
Normalized Count of Long Perimeter Fibers (all Phenotypes)	88.7
Normalized Count of Large Fibers (Area) - All Phenotypes	78.5
Normalized count of Blurry Regions (Texture)	74.4
Normalized count of Long fibers (all Phenotypes)	72.4
Normalized Count of Large Fibers (Filled Area) - All Phenotypes	69.6
Normalized count of High A2P fibers (all Phenotypes)	61.6
Normalized count of Porous Regions (Texture)	60.7
Normalized count of Long Total Skeleton fibers (all Phenotypes)	56.0
Normalized count of Thick Fibers (all Phenotypes)	20.2
Normalized count of Rough Regions (Texture)	18.4
Skeleton Nodes Normalized Count	17.1
Assembled Collagens Normalized Count	17.0
Normalized count of Tortuous fibers (all Phenotypes)	14.4
Normalized count of Rounded Fibers (all Phenotypes)	12.4
Normalized Count of Skeleton Branches	12.2
Assembled Collagen Area Ratio (%)	11.2
Total Collagen Area Ratio (%)	9.2
Normalized count of faint fibers (All Phenotypes)	6.86
Kurtosis of the Fibers Area distribution (All Phenotypes)	6.6
Total Collagen Density Area Ratio (%)	1.0



Top20 Normalized qFPS (NASH Adult)	Folds from Baseline
STDEV - Fiber Filled Area (all Phenotypes)	34.3
STDEV - Fiber Area (all Phenotypes)	28.8
STDEV - Total Skeleton Length (all Phenotypes)	28.0
STDEV - Fiber Perimeter (all Phenotypes)	23.2
Assembled CAR / Fine CAR Ratio	11.0
Mean for Fibers Filled Area Distribution (All Phenotypes)	9.1
Kurtosis of the fibers Length distribution (all Phenotypes)	7.5
Mean of the Fibers Area Distribution (all Phenotypes)	7.1
Assembled Collagen Area Ratio (%)	6.6
Normalized count of Dull Regions (texture)	6.3
Total Collagen Density Area Ratio (%)	6.2
Collagen Structure Index	5.9
Normalized count of Homogeneous Regions (Texture)	5.3
Mean - Total Skeleton Length Distribution (all Phenotypes)	5.1
Total Collagen Area Ratio (%) for -(Tissue)	4.6
Skeleton Nodes Normalized Count	4.4
Median for Inertia (Texture)	4.1
STDEV of the Fibers Length Distribution (all Phenotypes)	4.1
Mean for Inertia (Texture)	3.8
Mean of the Fiber Perimeter distribution (all Phenotypes)	3.5

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