Common and distinct signatures of interstitial and perivascular fibrosis in mouse models of hypertensive heart disease

Authors: Louise Thisted[1], Claudia Correia[2], Karin Jennbacken[2], Maria Wågberg[2], Franziska Wichern[1], Martin Madsen[1], Chen Zhang[1], Jacob Lercke Skytte[1], Henrik H Hansen[1], Nora E. Zois[1], Niels Vrang[1], Jacob Jelsing[1], Urmas Roostalu[1], Qing-Dong Wang[2]

[1]Gubra, Hørsholm, Denmark; [2]Bioscience Cardiovascular, Research and Early Development, Cardiovascular, Renal and Metabolism (CVRM), BioPharmaceuticals R&D, AstraZeneca. Gothenburg, Sweden

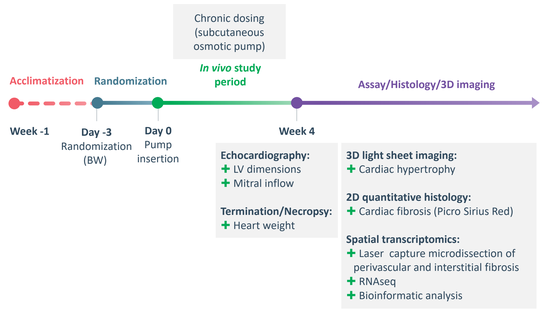
Corresponding author: Urmas Roostalu - uro@gubra.dk - Gubra ApS - Hørsholm, Denmark

Background & Aim

Fibrosis has fundamental significance in the pathogenesis of nearly every type of heart disease, including hypertensive heart disease, diabetic cardiomyopathy and heart failure with preserved ejection fraction (HFpEF). Whether the two primary types of cardiac fibrosis, perivascular and interstitial, differ is largely unknown. Here we aimed at providing in-depth functional and histological characterization of mouse models of cardiac fibrosis and show how the two fibrosis types differ at gene expression level.

Methods and Study Outline

Male C57BL6/J mice were dosed over 4 weeks using subcutaneous osmotic minipumps (Alzet 2004) with saline (Control group), angiotensin II (Ang II) and combination of AngII and phenylephrine (PE). 50% of samples in each group were allocated for light sheet imaging and histology and 50% for gene expression analysis.



Group name	Number of animals	Dose
Control	16	Saline: NA
AngII	24	AngII: 2.88 mg/kg/day
AngII/PE	24	AngII: 1.5 mg/kg/day PE: 50 mg/kg/day

Cardiac hypertrophy and dysfunction

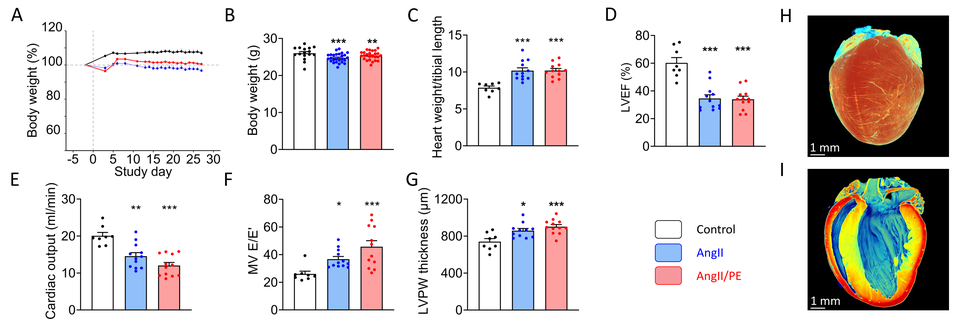


Figure 1. Cardiac hypertrophy and dysfunction in AngII and AngII/PE mouse models
(A) Relative body weight change throughout the study and **(B)** terminal absolute body weight. **(C)** Relative heart weight in AngII and AngII/PE groups compared to Control. **(D)** Systolic heart failure illustrated by reduced left ventricle (LV) ejection fraction (EF) in AngII and AngII/PE. **(E)** Cardiac output decreased in AngII and further in AngII/PE group in comparison to Control. **(E)** Diastolic dysfunction characterized by mitral peak early filling velocity (E) to mitral early diastolic annular velocity (E') measured by pulsed wave Doppler ultrasound. **(G-I)** Light sheet imaging enables high resolution analysis of cardiac hypertrophy. **(G)** LV posterior wall (PW) thickness increased in AngII and AngII/PE groups in comparison to Control. Significance: *p<0.05, **p<0.01, ***p<0.001, compared to Control. Standard error of the mean is shown.

Quantitative histology for perivascular and interstitial fibrosis

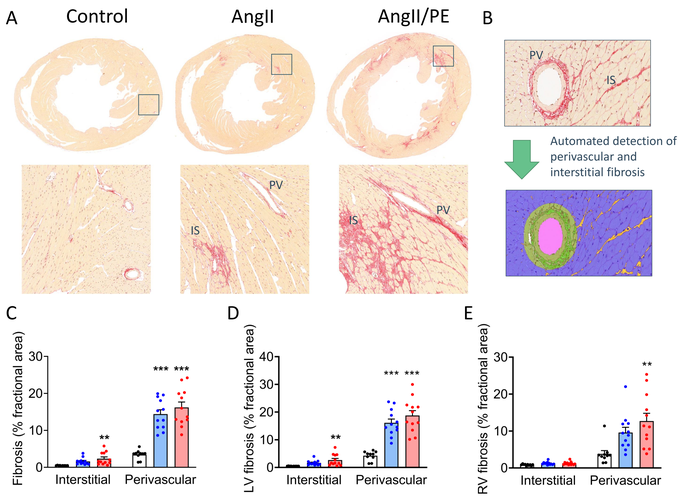


Figure 2. Automated analysis of cardiac fibrosis
(A) Picro Sirius Red (PSR) stained sections from representative samples. Fibrosis is visualized in red and boxed area is magnified on the lower panel. (PV) Perivascular; (IS) interstitial. **(B)** Automated image analysis was utilized to quantify PSR staining in perivascular (green) and interstitial (yellow) areas. **(C)** Quantification of PSR staining in the entire heart, **(D)** in the left ventricle (LV) and **(E)** in the right ventricle (RV). Interstitial fibrosis is more prevalent in the AngII/PE model in comparison to AngII. Perivascular fibrosis extends into the right ventricle in the AngII/PE model. Significance: **p<0.01, ***p<0.001, compared to Control. Standard error of the mean is shown.

Transcriptomic profile for perivascular and interstitial fibrosis

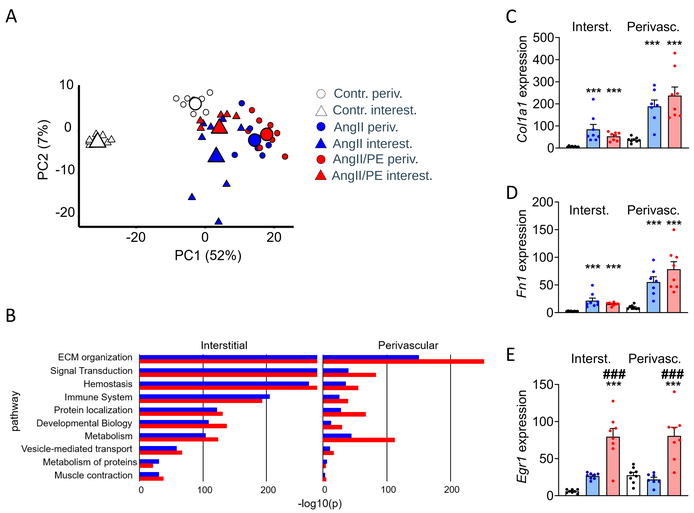


Figure 3. Laser-capture microdissection and RNA-seq demonstrate gene expression changes in perivascular and interstitial fibrosis areas. **(A)** Principal component analysis. Large symbols represent the mean of each group. Perivascular fibrosis samples from AngII and AngII/PE cluster together and share similarities with interstitial tissue from AngII/PE group. There is large variability in the interstitial tissue transcriptome from AngII. **(B)** Reactome pathway enrichment analysis shows differential regulation of extracellular matrix (ECM) organization. **(C-E)** Normalized expression of collagen 1a1 (*Col1a1*), fibronectin 1 (*Fn1*) and early growth response factor-1 (*Egr1*). Significance: *** p<0.001 compared to Control, ### p<0.001 compared to AngII. Standard error of the mean is shown.

Unique molecular signature of perivascular fibrosis

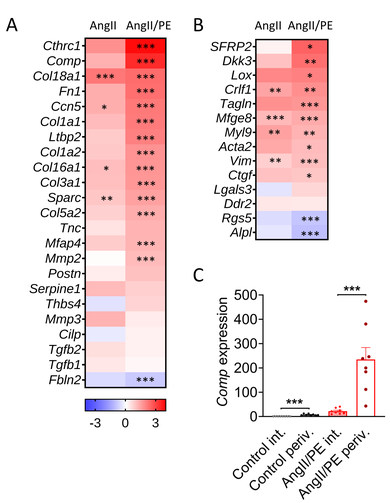


Figure 4. Comparison of perivascular and interstitial fibrosis transcriptome.
(A) Heatmap overview of transcriptional changes in genes encoding for extracellular matrix components. **(B)** Heatmap overview of fibroblast markers. Red colour indicates higher and blue colour lower expression in perivascular fibrosis in comparison to interstitial fibrosis. Colour denotes log2 fold change. **(C)** Cartilage oligomeric matrix protein (*Comp*) shows high expression in the perivascular fibrotic compartment and low level in interstitial fibrosis. Significance: *p<0.05, **p<0.01, ***p<0.001, perivascular compared to interstitial fibrosis.

CONCLUSION

+ AngII/PE infusion mouse model develops both systolic and diastolic dysfunction and more advanced cardiac pathology than AngII single-dosed mice.
+ Perivascular and interstitial fibrosis are prevalent in AngII/PE infusion model and are coupled with pro-fibrotic gene expression changes
+ Comparison of interstitial and perivascular cardiac fibrosis transcriptomes revealed unique transcriptional signatures and upregulation of osteochondrogenic pathways in the perivascular tissue.