MicroRNA-574-5p: A Circulating Marker of Thoracic Aortic Aneurysm

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On behalf of the Cardiolinc™ network (www.cardiolinc.org)

Background & Aim

- Ascendant thoracic aortic aneurysm (TAA) is an asymptomatic enlargement of the aorta above the heart. It can be associated with fatal complications such as aortic dissection. However, aneurysm dimension only poorly predicts dissection risk.
- MicroRNAs (miRNAs) are small non-coding RNAs which may be useful for diagnosis or risk prediction in cardiovascular disease.

Aim → Identify miRNAs associated with TAA pathogenesis and with the potential to improve TAA diagnosis

Results

1. DISCOVERY AXIS - Human samples

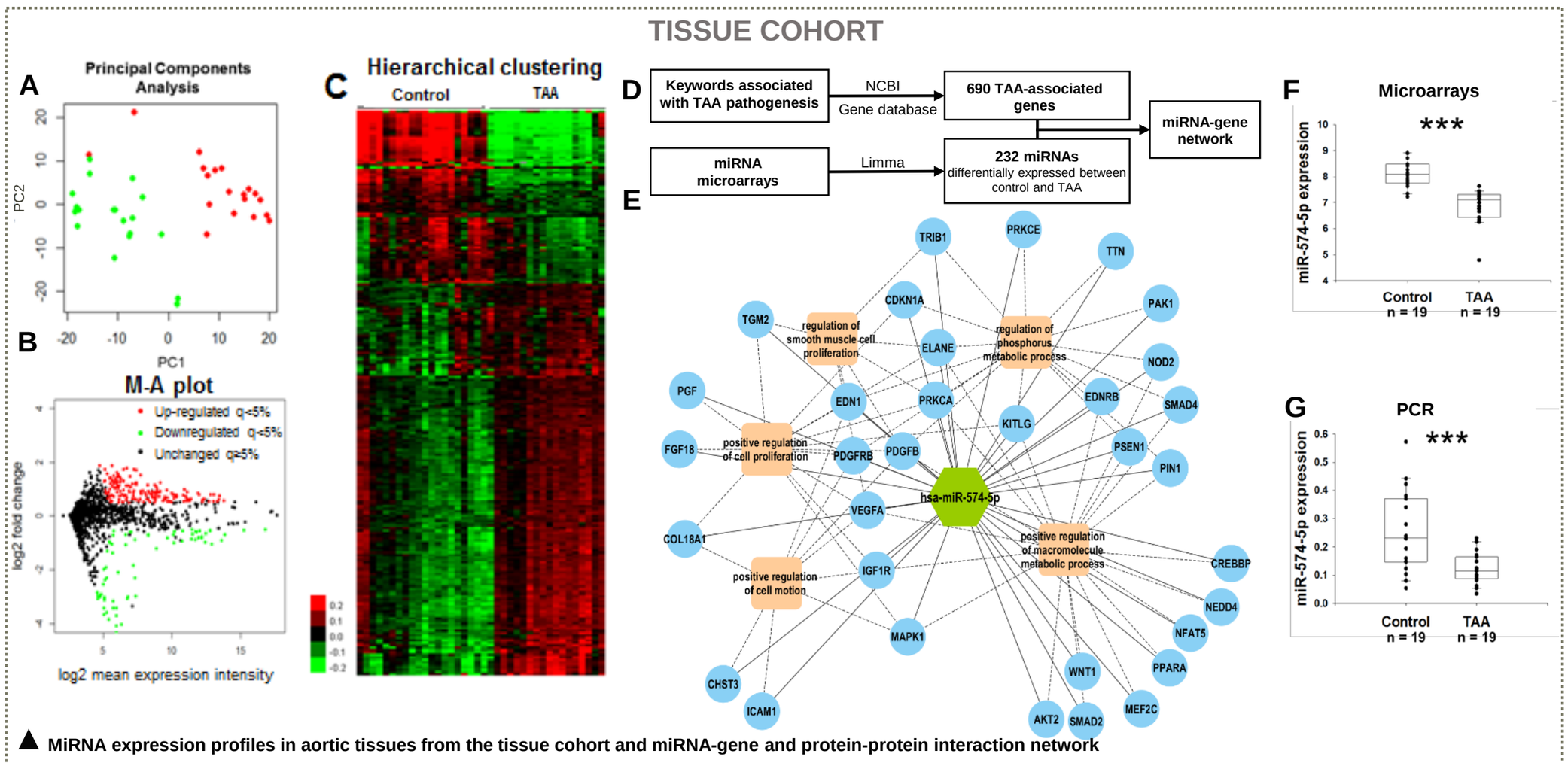
TISSUE COHORT

miRNA microarrays 19 control vs 19 TAA → Differentially expressed miRNAs → Genes involved in TAA pathogenesis → miRNA-gene interaction prediction → miR-574-5p selection → Replication of miR-574-5p by PCR 19 control vs 19 TAA

SERUM COHORT

Measurement of miR-574-5p by PCR 20 control vs 28 TAA → Association with aortic diameter

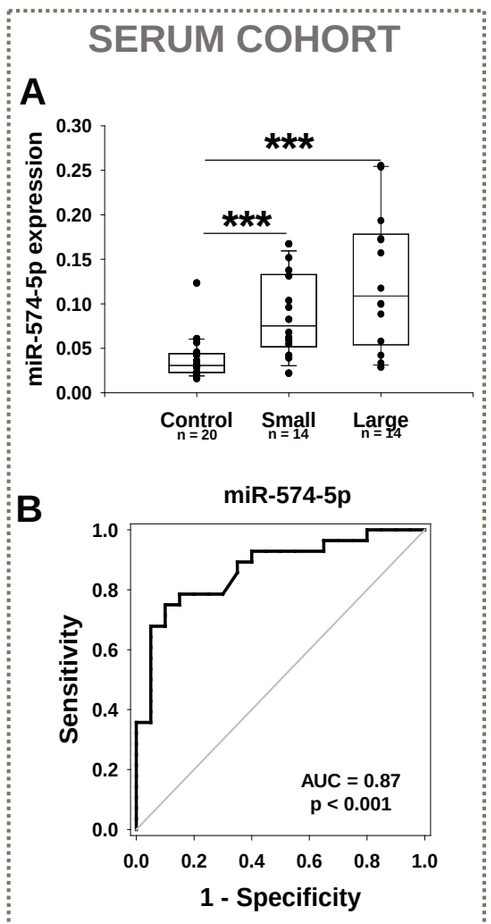
TISSUE COHORT



▲ **MiRNA expression profiles in aortic tissues from the tissue cohort and miRNA-gene and protein-protein interaction network**

A-C. Microarrays were conducted on RNA samples isolated from the aortic media of 19 TAA patients and 19 non-aneurysmal control donors. **A.** Principal component analysis showing the separation of TAA and control samples according to miRNA expression. **B.** M-A plot (M= intensity log-ratio vs A= mean log intensity) showing the distribution of miRNAs in TAA vs control samples. Log2 transformed-fold change is shown in the y axis and the average signal of each miRNA is shown in the x axis **C.** Heat-map displaying regulated miRNAs between TAA and control samples. Log2-transformed fold change > 0.5 or < -0.5, q-value <5% and relative expression level > 5 were used as cut-offs. **D.** Procedure to create the interaction network displayed in E. 690 TAA-associated genes were identified using NCBI Gene database and literature search. 232 miRNAs were differentially expressed between aneurysmal and non-aneurysmal aortic tissue in microarrays. Interactions between TAA-associated genes and differentially expressed miRNAs were predicted using targetScan, miRDB and DIANA microT-CDS databases. **E.** MiR-574-5p-gene interaction network showing the association between-miR-574-5p, its predicted gene targets, and the top 5 biological processes enriched by these targets. Solid line: interaction between miRNA and target gene. Dash line: association between gene targets and biological processes. **F-G.** Expression levels of miR-574-5p by microarrays and quantitative PCR. *** p<0.001.

SERUM COHORT



▲ **Association between serum levels of miR-574-5p and TAA in serum cohort**

A. Expression of miR-574-5p according to the size of the aneurysm. Small indicates an aneurysm < 49mm and large indicates an aneurysm > 49mm. *** p<0.001. **B.** ROC (receiver operating characteristic) curve analysis showing the association between serum levels of miR-574-5p and TAA. AUC: area under the curve,

2. CHARACTERIZATION AXIS - Mice samples

TISSUE GROUP

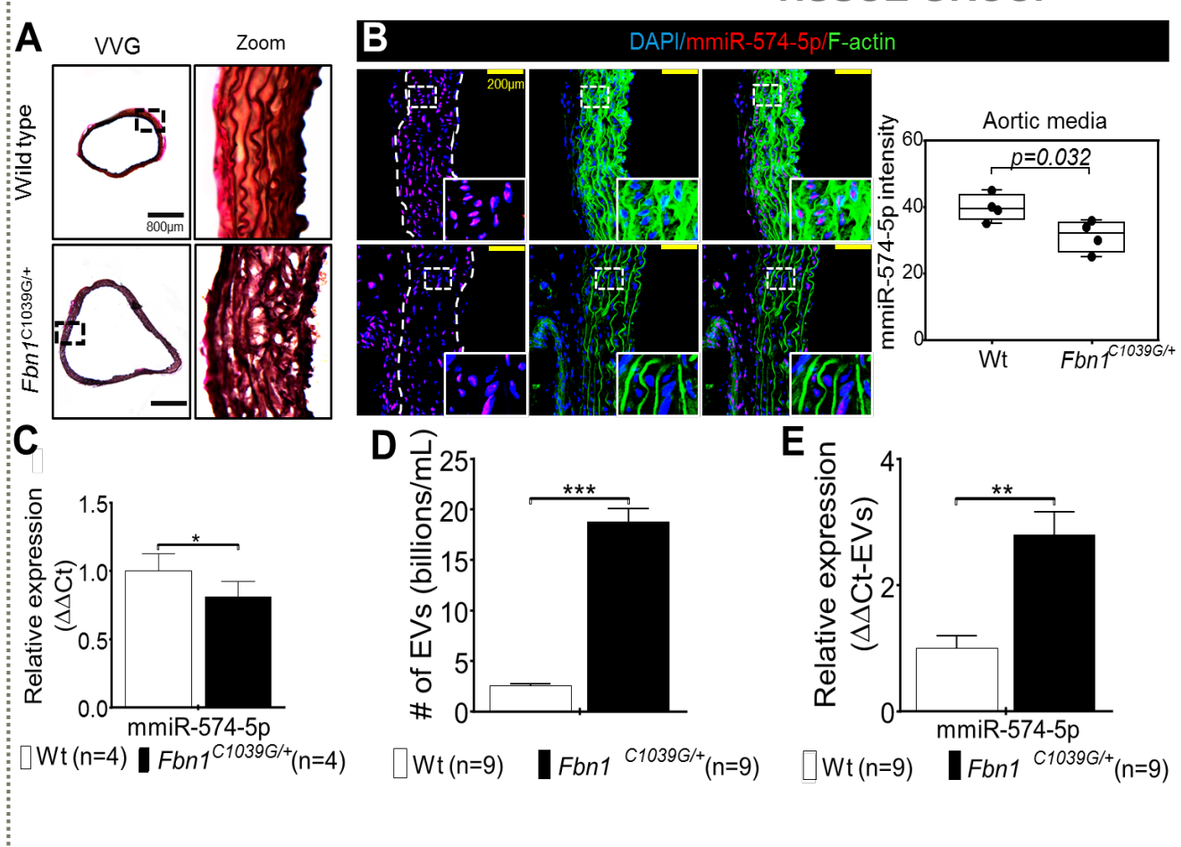
miR-574-5p labelling in aortic tissue 4 wild type vs 4 Fbn1$^{C1039G/+}$ → Measurement of miR-574-5p by PCR in aortic tissue 4 wild type vs 4 Fbn1$^{C1039G/+}$ → Measurement of miR-574-5p by PCR in extracellular vesicles from plasma 9 wild type vs 9 Fbn1$^{C1039G/+}$ → miR-574-5p labelling in several organs 3 wild type vs 3 Fbn1$^{C1039G/+}$

PLASMA GROUP

Measurement of miR-574-5p by PCR 6 wild type vs 10 Fbn1$^{C1039G/+}$ → Association with aortic diameter

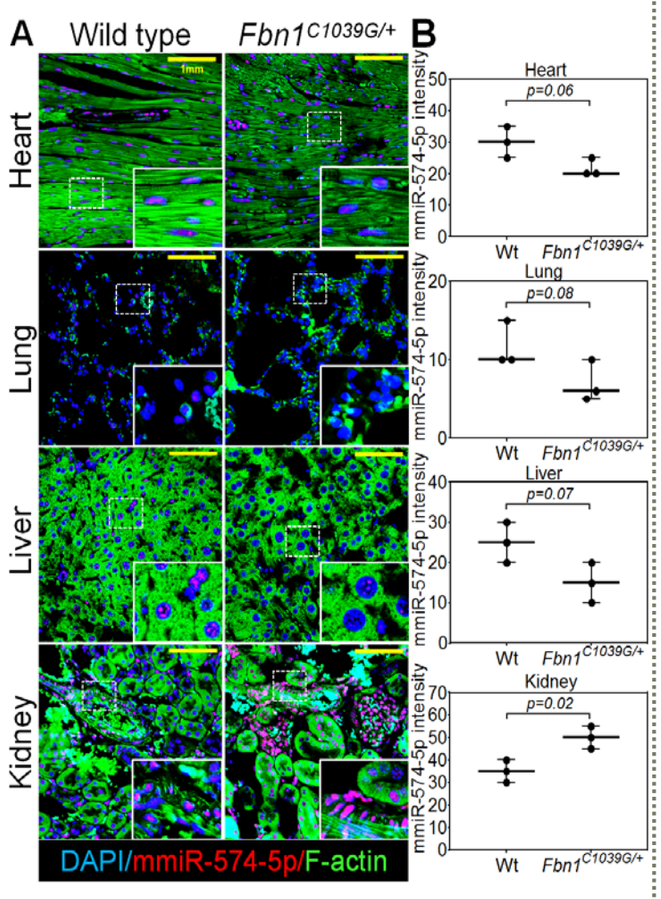
VSMC: Vascular smooth muscle cell; Fbn1: fibrilin-1; EV: extracellular vesicles

TISSUE GROUP



▲ **Expression of miR-574-5p in aorta of Fbn1C1039G/+ and wild-type (Wt) mice.**

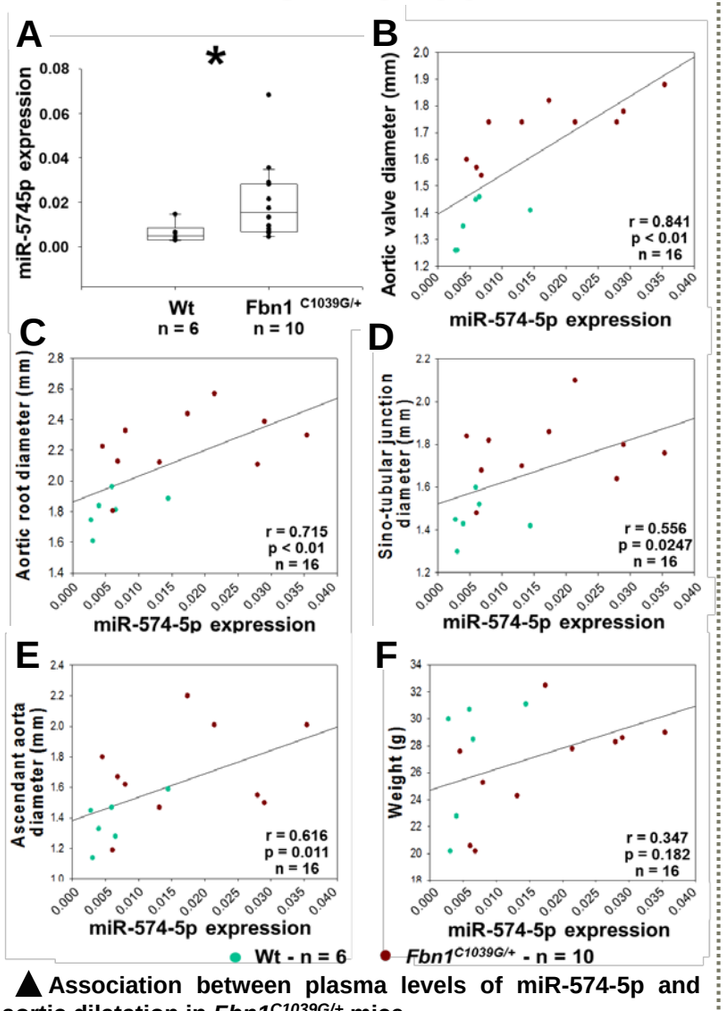
A. Staining of elastin fibers (VVG) in the aorta of Wt and *Fbn1*$^{C1039G/+}$ mice. **B**. **Left:** Immunofluorescence and fluorescent in-situ hybridization (FISH) staining of miR-574-5p (red), F-actin (green) and nucleus (blue) in section of ascending aortic tissue from Wt (n=4) and *Fbn1*$^{C1039G/+}$ (n=4) mice. **Right:** Relative fluorescence intensity quantification of miR-574-5p in the aortic media from Wt (n=4) and *Fbn1*$^{C1039G/+}$ (n=4) mice. **C.** Relative expression of miR-574-5p measured using PCR in aortic tissue from Wt (n=4) and *Fbn1*$^{C1039G/+}$ (n=4) mice. **D**. Quantification of circulating EVs from Wt (n=9) and *Fbn1*$^{C1039G/+}$ (n=9) mice. **E.** Relative expression of miR-574-5p measured in EVs from Wt (n=9) and *Fbn1*$^{C1039G/+}$ (n=9) mice using PCR. *p<0.05; **p<0.01 ; ***p < 0.001.



▲ **Expression of miR-574-5p in different organs in wild-type (Wt) and *Fbn1*$^{C1039G/+}$ mice.**

A. Immunofluorescence and FISH of miR-574-5p (red), F-actin (green) and nucleus (blue) from Wt and *Fbn1*$^{C1039G/+}$ mice. **B.** Relative fluorescence intensity quantification from Wt (n=3) and *Fbn1*$^{C1039G/+}$ (n=3) mice.

PLASMA GROUP



▲ **Association between plasma levels of miR-574-5p and aortic dilatation in *Fbn1*$^{C1039G/+}$ mice.**

A. Expression of miR-574-5p in the plasma of *Fbn1*$^{C1039G/+}$ and wild-type (Wt) mice. * p<0.05. **B-F**. Spearman correlation between miR-574-5p and aortic valve diameter **(B)**, aortic root diameter **(C)**, sino-tubular junction diameter **(D)**, ascendant aorta diameter **(E)** and weight **(F)**. r= correlation coefficient.

Conclusion & Perspectives

- miR-574-5p is associated with TAA pathogenesis and may help in diagnosing this life-threatening disease
- miR-574-5p may act as paracrine mediator in TAA pathogenesis
- Further studies and investigations are needed to confirm the role of miR-574-5p in TAA pathogenesis

No conflict of interest to disclose