Biophysical Reviews

Mechanosensitive pathways are regulated by mechanosensitive miRNA clusters in endothelial cells derived from intact blood vessels

--Manuscript Draft--

Manuscript Number:	
Full Title:	Mechanosensitive pathways are regulated by mechanosensitive miRNA clusters in endothelial cells derived from intact blood vessels
Article Type:	Review
Corresponding Author:	Rob Krams, PhD, MD Queen Mary University of London Barts and The London School of Medicine and Dentistry London, UNITED KINGDOM
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Queen Mary University of London Barts and The London School of Medicine and Dentistry
Corresponding Author's Secondary Institution:	
First Author:	Sean Herault
First Author Secondary Information:	
Order of Authors:	Sean Herault
	Jarka Naser, drs
	Daniele Carasitti, dr.
	K Yean Chooi, doctor
	Rosa Nikolopoulou, doctor
	Marti Llopart Font, drs
	iten Patel, doctor
	Ryan Pedrigi, doctor
	Rob Krams, PhD, MD
Order of Authors Secondary Information:	
Funding Information:	british heart foundation (RG/11/12/29055) — Professor Rob Krams
	british heart foundation (PG/15/49/31595) — Professor Rob Krams
Abstract:	Shear stress is known to affect many processes in (patho -) physiology through a complex, multi-molecular mechanism, termed mechanotransduction. Here, we comprehensively evaluate the role of small non-coding miRNA in the regulation of mechanotransduction in intact blood vessels. Murine carotid arteries were instrumented with a flow modifying cuff for 7 days to induce calibrated reductions in shear stress to infer causal mechanosensitive genetic patterns. After 7 days, the vessels were snap-frozen, cross sectioned and endothelial cells were obtained with an optimised Laser Capture (LCM) protocol and their total RNA processed for deep sequencing. A novel ultrasensitive bioinformatics pipeline identified 8083 mRNA and 215 miRNAs differentially regulated by the 7-day reduction of shear stress, of which ~150 were new mechanosensitive miRNA. Ultrafast, and sensitive machine learning algorithms were developed which revealed that ~45% of differently regulated mRNA (3950 mRNA) were controlled by the 215 mechanosensitive miRNA. Interestingly, the mechanosensitive miRNAs were organised in 43 miRNA-clusters, of

	which 35% consisted of homo-seed (miRNA-families) and 65% of hetero-seed structures and these 43 clusters controlled 2270 mRNA. The mechanosensitive mRNA's were distributed over 41 signaling pathways, with the mechanosensitive miRNA-clusters regulating the majority (65%) of these mechanosensitive signaling pathways. Important and well-known mechanosensitive pathways, like KLF-eNOS, MAPK, ROS and inflammation pathways were regulated by the mechanosensitive miRNA clusters. In conclusion, a new and important mode of regulation of mechanotransduction was discovered, based on miRNA clusters. This finding implicates new avenues for treatment of mechanotransduction and atherosclerosis.
Suggested Reviewers:	

Mechanosensitive pathways are regulated by mechanosensitive miRNA clusters in endothelial cells derived from intact blood vessels

Sean Herault[1], Jarka Naser[4],Daniele Carassiti[1], K Yean Chooi[1], Rosa Nikolopoulou[2], Marti Llopart Font[1], Miten Patel[4], Ryan Pedrigi[3] and Rob Krams[1]

[1] School of Engineering and Materials Science, Queen Mary University of London, London, United Kingdom

[2]Department of Bioengineering, Imperial College London, London, United Kingdom

[3] College of Engineering, Mechanical & Materials Engineering, University of Nebraska-Lincoln, Lincoln, Nebraska, USA

[4]NHLI, Imperial College London, London, United Kingdom

Corresponding Author:

Professor Rob Krams
Room 2.14
Bioengineering
Queen Mary University
Tel: 02078827940
Mobile: +447972413211
Email: r.krams@qmul.ac.uk

Abstract

Shear stress is known to affect many processes in (patho -) physiology through a complex, multi-molecular mechanism, termed mechanotransduction. Here, we comprehensively evaluate the role of small non-coding miRNA in the regulation of mechanotransduction in intact blood vessels.

Murine carotid arteries were instrumented with a flow modifying cuff for 7 days to induce calibrated reductions in shear stress to infer causal mechanosensitive genetic patterns. After 7 days, the vessels were snap-frozen, cross sectioned and endothelial cells were obtained with an optimised Laser Capture (LCM) protocol and their total RNA processed for deep sequencing. A novel ultrasensitive bioinformatics pipeline identified 8083 mRNA and 215 miRNAs differentially regulated by the 7-day reduction of shear stress, of which ~150 were new mechanosensitive miRNA. Ultrafast, and sensitive machine learning algorithms were developed which revealed that ~45% of differently regulated mRNA (3950 mRNA) were controlled by the 215 mechanosensitive miRNA.

Interestingly, the mechanosensitive miRNAs were organised in 43 miRNA-clusters, of which 35% consisted of homo-seed (miRNA-families) and 65% of hetero-seed structures and these 43 clusters controlled 2270 mRNA.

The mechanosensitive mRNA's were distributed over 41 signaling pathways, with the mechanosensitive miRNA-clusters regulating the majority (65%) of these mechanosensitive signaling pathways. Important and well-known mechanosensitive

pathways, like KLF-eNOS, MAPK, ROS and inflammation pathways were regulated by the mechanosensitive miRNA clusters.

In conclusion, a new and important mode of regulation of mechanotransduction was discovered, based on miRNA clusters. This finding implicates new avenues for treatment of mechanotransduction and atherosclerosis.

Keywords: shear stress, laser capture, deep sequencing, miRNA, miRNA families, signaling pathways, mechanotransduction.

INTRODUCTION It is well known that the shape and size of blood vessels is determined by mechanical factors, like shear stress [1-3]. Shear stress is the friction force imposed onto the stationary endothelial cells by the movement of blood through the vessel. The biomechanical environment of endothelial cells are sensed through a complex process called mechanotransduction [4-6]. This process consists of ~5,000-7,000 genes that are organised into >40 signalling cascades which are regulated by >8 mechano-sensors and >50 transcription factors [7-9].

The sheer complexity of mechanotransduction makes one wonder how it is regulated. MicroRNAs (miRNAs) are small (~22 nucleotides) non-coding RNA molecules that are essential regulators of gene expression [10-14]. Binding of miRNAs to the target sequence in messenger RNAs (mRNAs) leads to translational repression and/or mRNA destruction [10-15]. Repression of mRNA by miRNA is a rather complex process that depends upon the level of expression of an miRNA, the abundance of the target mRNA, the number of target mRNAs per miRNA, the number and composition of miRNA binding sites per mRNA, and the cooperativity of individual miRNA, either in families or clusters [16-18]. Interestingly, these factors balance each other so that an individual miRNA only moderately represses individual mRNA. In the last few years, a clear role for overarching structures in miRNA control, like miRNA families and miRNA clusters, have emerged as regulators of signaling pathways, or phenotypic changes that encompass groups of pathways[19-21]. However, their role in mechanotransduction has been not investigated. Here we present an overview and a mixture of our own work providing evidence for the importance of miRNA clusters regulating mechanosensitive signaling pathways.

Evidence for an emerging role of families and clusters in signaling

Families of miRNA's have been defined as miRNA sharing a common ancestor, or a common structural similarity like the seed region[22-24]. They have been Increasingly implicated in a variety of processes in a variety of fields in Embryology, Neurology, Cardiac Development and Oncology. As they act on similar mRNA targets, the recruitment of miRNA family members leads to a gradual repression of their target mRNA [ref]. Their role in mechanotransduction is unknown and we will further discuss their roles below.

Clusters of miRNA are more recently been identified in mammalian genomes. MirBase [v22] identifies 100 clusters in the mouse genome and 156 in human genome and their numbers are increasing. Clusters are considered overarching structures consisting both of members of families and structurally unrelated miRNA's[ref]. Interestingly, they are often regulated as a functional unit through polycystronic mechanism, consisting of either direct transcriptional control, silencing by epigenetic mechanism or directly through coding proteins[ref].

At present, it is not clear how individual miRNA members and entire cluster interact. Several studies indicate miRNA within a cluster regulate each other, probably increasing the homogeneity in their response to stimuli. Other studies have emerged, indicating that miRNA clusters regulate one or more signaling pathways (Table 1). It is clear from Table 1 that either one cluster may regulate one pathway, or multiple clusters may regulate a single pathway, or one cluster may regulate multiple pathways (Table 1). In the latter observation miRNA are involved in processes, like the Inflassome, lipid handling, and immune responses.

Development of a bespoke pipeline for studies on mechanotransduction in intact blood vessels

We have developed a method to induce reductions in shear stress aiming to discover *causal* gene patterns induced by changes in shear strss[ref]. To that end, we have developed a method to laser captured endothelial cells from instrumented (n=5) and control carotid arteries (n=5) using a protocol optimised to reduce RNA loss during the procedure which occurred by laser heating and drying. The instrumentation reduced shear stress by ~60%. After optimising, ~1,000 endothelial cells from an equivalent of 10-15 cross sections of 20 µm were captured to generate sufficient (>1µg/µl), high quality (RIN>7.0) total RNA. Subsequently, we developed a bespoke, ultra-sensitive bioinformatics pipeline for the detection of differentially expressed mRNA and miRNA in the freely available R programming environment. We expanded DESEQ2, which successfully implemented a negative binomial distribution with a weighting factor per gene as described in [25], to further increase power of the statistical tests. The seven-day reduction in shear stress differently expressed 8,083 mRNA (FDR<0.05), of which 4,653 were up-regulated and 3,430 down-regulated, a finding much higher than reported in endothelial cells in culture [26]. In addition, a high number of miRNA were detected (~1500) of which 215 were affected by the 7 day reduction in blood flow, which is a value much higher than the currently reported mechanosensitive miRNA (~60 [11,27]).

In order to analyse these complex data sets we i) developed machine learning software to identify old and new miRNA-mRNA interactions, and ii) to identify signaling pathways on basis of GSEA analysis (Figure 1, upper panel). In parallel, we

developed software to identify overarching structures in the mechanosensitive miRNA (miRNA-Families and miRNA-Clusters, Figure 1, lower panel) to identify how these structures control mechanosensitive signaling pathways.

miRNA Families and Clusters regulate the majority of mechano-sensitive mRNA in vivo.

Approximately 45% of the 1500 miRNA could be allocated to 224 Families (miRbase-v22), of which 187 miRNA-families were downregulated and 37 Families were upregulated by the 7-day reduction of shear stress (Figure 2A).

Interestingly, a large fraction (~65%) of miRNA depended mRNA were regulated by miRNA-family's (Figure 2C). Bootstrapping identified 10 miRNA-families comprising only ~20% of all miRNA (Figure 2B), which regulated ~40% of the 65% of miRNA regulated mechanosensitive mRNA (Figure 2C). These influential miRNA families exerted their influence, not by having more miRNA, but by having more "influential miRNA members (Figure 2D, $p<0.05$ bootstrapping).

We selected clusters on the basis of a strict criterion of < 3,000kB proximity[28] and identified that 35% of mechanosensitive miRNA's are organised in 43 clusters (miRBase v22) which is slightly higher than reported for the entire murine genome (28% of the miRNA [28]). We subsequently confirmed that mechanosensitive clusters are regulated by a polycistronic mechanism[28] – e.g. their variance in expression was lower in a cluster then between cluster ($p<0.05$) - indicating they are functionally controlled as well.

Interestingly, a very large fraction (~60%) of mechanosensitive mRNA controlled by miRNA were regulated by the clustered miRNA (35% of all miRNA, Figure 3A). Similarly as for families, clusters are enriched with influential miRNA ($p<0.05$) and

influential miRNA-Families ($p<0.05$, Figure 3B) providing a basis for this large number of mRNA.

A central role for clusters in mechanosensitive pathway coordination

The seven-day reduction in shear stress induced 8,083 mechanosensitive genes and 215 miRNA (FDR<0.05), the largest number of differentially expressed mRNA and miRNA to date. Gene set Enrichment analysis (GSEA) [29-34] identified >100 mechanosensitive pathways, of which the most prominent were i) metabolism of genes and proteins, ii) extracellular matrix genes, iii) programmed cell death, and iv) signal transduction. A further, focussed analysis of the signal transduction pathways revealed that 41 signaling pathways were affected by the reduction in shear stress. These included well-known shear stress sensitive pathways such as eNOS and MAPK [7,35-37], recently established mechanosensitive pathways such as NOTCH and WnT [7,38-48] and currently unknown mechano-sensitive pathways like Insulin. (Table 2).

A further analysis of our miRNA-clusters revealed that twenty-six (26) out of 43 differentially expressed miRNA-clusters regulated 30 out of 41 (65%, $p<0.05$) signaling pathways (Table 2). We found single clusters affecting a single pathway (clusters 2, 5), but the majority of clusters affected multiple pathways (Table 2). The latter finding has been identified before (see above) in embryology where affected pathways were related to processes, like development of organs[22,49-53]. In addition, multiple clusters could affect a single pathway (clusters 4,7, 26, 46 affecting metabolism), groups of clusters affecting physiological processes like vasodilation (clusters 9, 37, 88 affecting eNOS and prostacyclin, Table 2) while clusters regulating aspects of inflammation appeared more distributed (Cluster 21, MAPK, and JAK-

STAT pathways, Cluster 40, the Inflammasome, and Cluster 73, the Cytokine pathways).

Discussion

Our major findings are that 65% of endothelial mechanotransductive pathways are controlled by Clusters, which is to the best of our knowledge a new finding. The important role of such a small number of clusters on so many signaling pathways was due to an enrichment of influential miRNA (e.g miRNA affecting many mRNA) and influential miRNA-Families in the mechanosensitive clusters. Furthermore, the coordination of multiple clusters leads a subtle and graded regulation on individual signaling pathways. As miRNA are amenable to synthesis this may offer a novel way to control individual signaling pathways.

It is becoming clear that miRNA exert subtle control over mRNA through modification of transcription factors, noise and signaling pathways[54,55] and cooperation in overarching structures might be a currently overlooked mechanism for miRNA regulated repression.

It has been appreciated that miRNA's are not randomly distributed over the genome but are enriched in clusters. These clusters develop evolutionarily and the expression of miRNA in clusters have been shown to be coordinated by polycistronic mechanisms to coordinate processes in embryology and inflammation. Interestingly, 41 out of 100 murine clusters were mechanosensitive and coordinated 65% of the 41 mechanosensitive signaling pathways. Major known atherosclerotic signaling pathways were under control of miRNA clusters challenging the idea of transcription factor control of mechanotransduction.

In conclusion, we used modern machine tools to evaluate mRNA-miRNA interaction of endothelial cells exposed to reduced shear stress in vivo. Our analysis was focussed on finding overarching structures in miRNA, and we identified mechanosensitive miRNA-families and miRNA-clusters that coordinated control of the majority of mechanosensitive signaling pathways.

Pathway	Families	Clusters
TGF-β signalling, Hedgehog, RB pathway, mTORC1 signalling	miR-17/92	7
BH3-only protein Bim	miR-106b/25	25
p21/cyclinD1	miR-212/132	40
KIT/ETV1	miR-221/222	65
PTEN/Akt	miR-144/451	52
SMAD2	miR-212/132	40
Wnt/β-catenin	miR-17/92	7
TGF-β signalling	miR-17/92; miR106b/25	7, 25
Rho/ROCK	miR-200b/429	24
p21/Bim	miR-106b/25	25
KIT/ETV1	miR-221/222; miR-17/92;	7, 65

Table 1: Family is the miRbase-v22 defined family of miRNA, CLUSTER numbering as obtained from miRbase-v22, miRNA is the differentially expressed miRNA of that cluster, the pathway obtained, and size stands for the number of mRNA regulated by the cluster(s). AKT, AKT serine/threonine kinase; BH3, Bcl-2 homology 3 domain; BIM, Bcl-2-like protein 11; EP300, E1A-associated protein p300; ETV1, Ets variant gene 1; KIT, proto-oncogene tyrosine-protein kinase; MET, MET proto-oncogene receptor tyrosine kinase; mTORC1, mammalian target of rapamycin complex 1; P21, cyclin dependent kinase inhibitor 1A; PLCG1, phospholipase c gamma 1; PSAP, prosaposin; P53, tumor protein P53; PTEN, phosphatase and tensin homolog; RB1, RB transcriptional corepressor 1; RHO, ROCK Rho-associated protein kinase; SLU7, pre-mRNA splicing factor SLU7; SMAD2, mothers against dpp homolog 2; ß-TRCP2 (also known as FBXW11), F-box and WD repeat domain containing 11; TGF-ß, transforming

growth factor beta; WEE1, Wee1A kinase; Wnt, wingless-type mmtv integration site family.

FAMILY	CLUSTER	MIRNA'S	PATHWAYS
MIR-154 MIR-329 MIR-368 MIR-379	2	mmu-miR-329-3p mmu-miR-376a-3p mmu-miR-376c-3p mmu-miR-380-5p	Prostacyclin PPAR
MIR-154 MIR-329 MIR-368 MIR-379	2	mmu-miR-329-3p mmu-miR-376a-3p mmu-miR-376c-3p mmu-miR-380-5p	Ubiquitin Proteasome
MIR-471 MIR-742 MIR-743 MIR-881 MIR-883	3	mmu-miR-471-3p mmu-miR-742-5p mmu-miR-743a-3p mmu-miR-881-5p mmu-miR-883a-3p	G-protein signaling
MIR-431	4	mmu-miR-3071-3p mmu-miR-3071-5p mmu-miR-431-5p	Metabolism
MIR-290	5	mmu-miR-291a-5p mmu-miR-292b-5p mmu-miR-293-3p mmu-miR-294-3p	Nima kinases Protein breakdown
MIR-19	7	mmu-miR-19b-3p	Metabolism WnT

MIR-302	9	mmu-miR-302b-3p	Prostacyclin eNOS Drug metabolism
MIR-344	12	mmu-miR-344e-5p/mmu-miR-344h-5p mmu-miR-344f-3p mmu-miR-344i	Mechanosensors
LET-7	18	mmu-let-7d-3p mmu-let-7f-2-3p	G-protein signaling
LET-7 MIR-10	20	mmu-let-7e-5p mmu-miR-125a-5p	RAF-MAPK
MIR-133 MIR-1	21	mmu-miR-133a-5p mmu-miR-1a-3p	RAF-MAPK
MIR-133 MIR-1	21	mmu-miR-133a-5p mmu-miR-1a-3p	JAK-STAT Cytokine-to Cytokine
MIR-8	24	mmu-miR-200a-3p mmu-miR-200c-3p	Scavenger receptors PPAR pathway
MIR-17	25	mmu-miR-106b-3p	GAG and Carbon Metabolism
MIR-182	26	mmu-miR-182-3p	Metabolism
MIR-133 MIR-1	28	mmu-miR-133b-3p mmu-miR-206-3p mmu-miR-206-5p	G-protein Cytokine-to-cytokine

MIR-214	31	mmu-miR-214-5p	G-protein signaling WnT
MIR-216	37	mmu-miR-216b-3p mmu-miR-216c-3p	Prostacycline eNOS calcium- metabolism pyrimidine- metabolism
MIR-132	40	mmu-miR-132-3p mmu-miR-132-5p	ILP3- Inflammasome ROS
MIR-15	46	mmu-miR-15a-5p mmu-miR-15b-3p	Metabolism
LET-7	50	mmu-let-7c-5p	G0-G1 division
MIR-122	64	mmu-miR-122-3p	Chemokine binding receptors
MIR-296 MIR-298	73	mmu-miR-296-5p mmu-miR-298-3p	Cell division Cytokine pathway
MIR-1199	88	mmu-miR-1199-3p	Prostacyclin eNOS PAF
MIR-34	92	mmu-miR-34a-5p	Osteoclast differentiation WnT
MIR-767	98	mmu-miR-767	G-protein, calmodulin
LET-7	100	mmu-miR-98-3p mmu-miR-98-5p	Neurotrophin signalling Protein breakdown WnT

Table 2: Family is the miRbase-v22 defined family of miRNA, CLUSTER numbering as obtained from miRbase-v22, miRNA is the differentially expressed miRNA of that cluster, the pathway obtained, and size stands for the number of mRNA regulated by the cluster(s). AKT, AKT serine/threonine kinase; BH3, Bcl-

2 homology 3 domain; BIM, Bcl-2-like protein 11; EP300, E1A-associated protein p300; ETV1, Ets variant gene 1; KIT, proto-oncogene tyrosine-protein kinase; MET, MET proto-oncogene receptor tyrosine kinase; mTORC1, mammalian target of rapamycin complex 1; P21, cyclin dependent kinase inhibitor 1A; PLCG1, phospholipase c gamma 1; PSAP, prosaposin; P53, tumor protein P53; PTEN, phosphatase and tensin homolog; RB1, RB transcriptional corepressor 1; RHO, ROCK Rho-associated protein kinase; SLU7, pre-mRNA splicing factor SLU7; SMAD2, mothers against dpp homolog 2; ß-TRCP2 (also known as FBXW11), F-box and WD repeat domain containing 11; TGF-ß, transforming growth factor beta; WEE1, Wee1A kinase; Wnt, wingless-type mmtv integration site family.

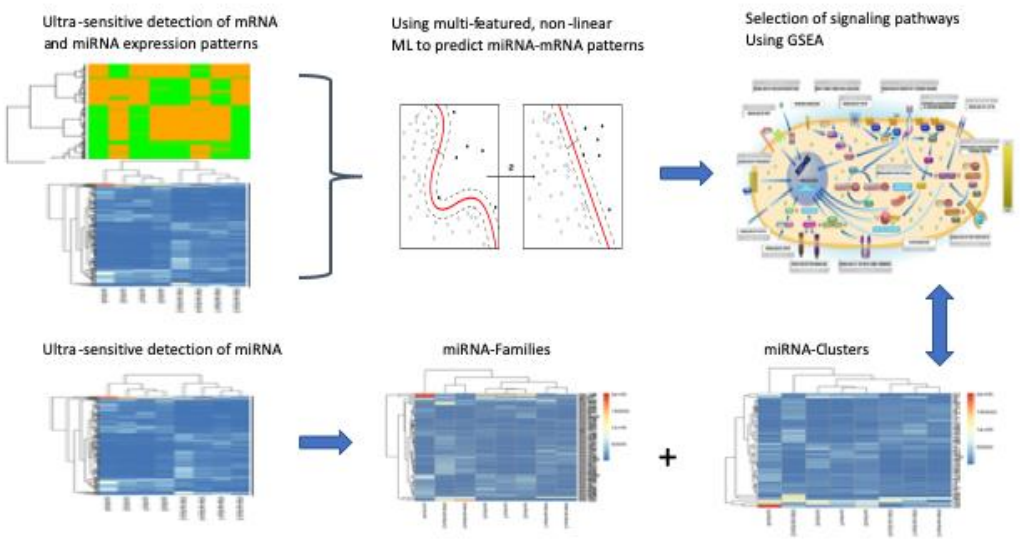


Figure 1 -Legend

Schematic presentation of the proposed analysis of our data obtained from the laser captured endothelial cells. In the upper row on the left side is displayed the map of the top 100 genes and 215 microRNA. In the top row, middle panel is displayed the Support Vector Classifier scheme used to predict miRNA/mRNA interactions and the upper row, right panel shows one of the signaling pathways derived from our analysis. The lower panel shows the distribution of the miRNA, and the miRNA-families and miRNA-Clusters derived from the differentially expressed miRNA. Not that all maps are derived from real data

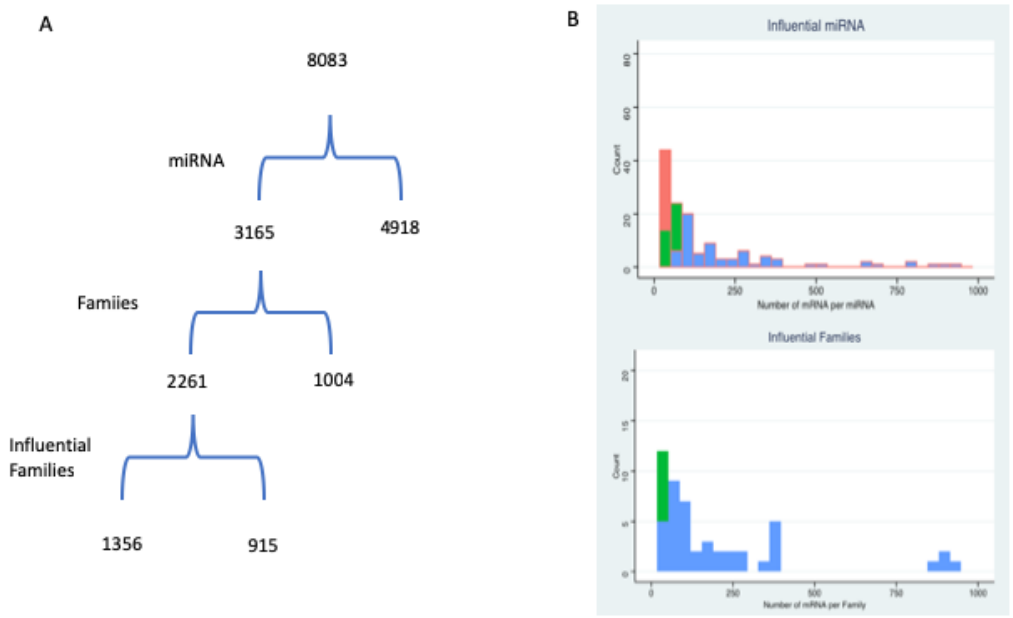


Figure 2-Legend

A detailed analysis of mechanosensitive miRNA Families. Panel A. The number of differentially expressed mechanosensitive mRNA controlled by miRNA (3165), controlled by Families (2261) and controlled by Influential Families (1365). Panel B displays the distribution of mRNA regulated either by miRNA In colour is displayed the lowest one third (red), middle one third (green) and upper third (blue) of mRNA per miRNA for the miRNA and for miRNA families. Note that influential miRNA families contain more influential miRNA. Panel C shows the distribution of Families per mRNA regulated per Family. The colour displays the number of influential miRNA per Family.

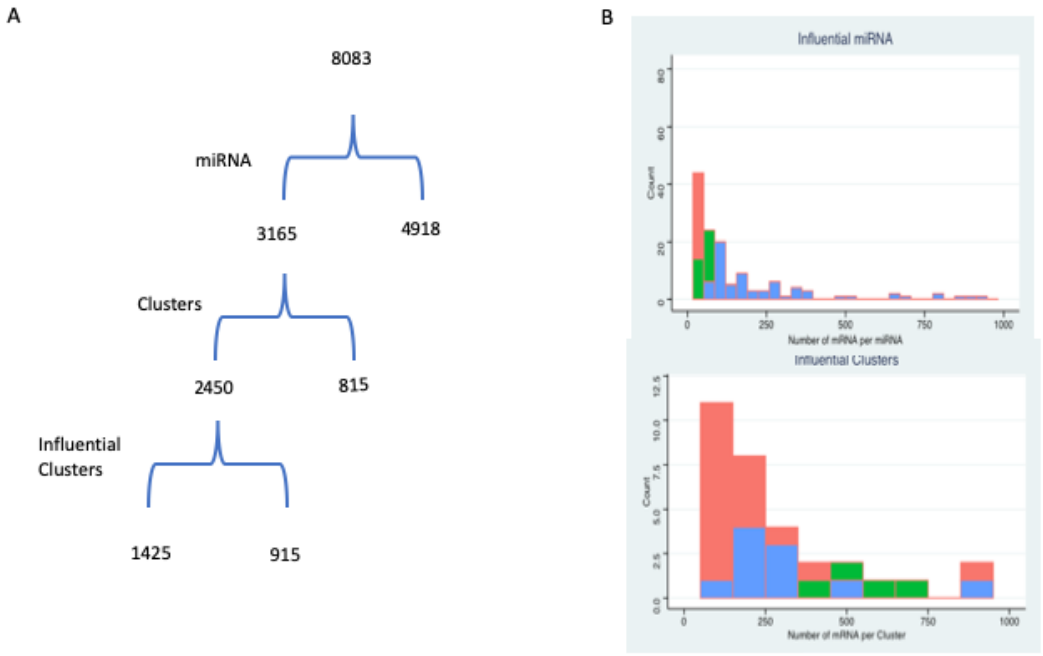


Figure 3-Legend

A detailed analysis of mechanosensitive miRNA Clusters. Panel A displays the distribution of mRNA regulated by miRNA (3165) and by Clusters (2450) and by Influential Clusters (1425). In panel B the distribution of individual miRNA per mRNA is displayed (upper row), while the lower row shows the similar distribution with colour coding related to low, medium and highly influential miRNA. In panel C, the influential Clusters are displayed, with in the lower row shows the distribution of low, medium, and highly influential mRNA. It is clear that the influential Clusters contain more Influential miRNA and influential Families.

Funding

The funding of the BHF (PL3012) is greatly acknowledged

Data availability request

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1 Ghaffari, S., Leask, R. L. & Jones, E. A. Simultaneous imaging of blood flow dynamics and vascular remodelling during development. *Development* **142**, 4158-4167, doi:10.1242/dev.127019 (2015).

2 Agrotou, S. *et al.* Effects of chronic anaerobic training on markers of sub-clinical atherosclerosis. *Hellenic J Cardiol* **54**, 178-185 (2013).

3 Lu, D. & Kassab, G. S. Role of shear stress and stretch in vascular mechanobiology. *J R Soc Interface* **8**, 1379-1385, doi:10.1098/rsif.2011.0177 (2011).

4 Krizaj, D. *et al.* From mechanosensitivity to inflammatory responses: new players in the pathology of glaucoma. *Curr Eye Res* **39**, 105-119, doi:10.3109/02713683.2013.836541 (2014).

5 Kshitiz, Afzal, J., Kim, D. H. & Levchenko, A. Concise review: Mechanotransduction via p190RhoGAP regulates a switch between cardiomyogenic and endothelial lineages in adult cardiac progenitors. *Stem Cells* **32**, 1999-2007, doi:10.1002/stem.1700 (2014).

6 Liu, Y. S. & Lee, O. K. In search of the pivot point of mechanotransduction: mechanosensing of stem cells. *Cell Transplant* **23**, 1-11, doi:10.3727/096368912X659925 (2014).

7 Kunnen, S. J. *et al.* Comparative transcriptomics of shear stress treated Pkd1(-/-) cells and pre-cystic kidneys reveals pathways involved in early polycystic kidney disease. *Biomed Pharmacother* **108**, 1123-1134, doi:10.1016/j.biopha.2018.07.178 (2018).

8 Rajendran, S. *et al.* The expression dynamics of mechanosensitive genes in extra-embryonic vasculature after heart starts to beat in chick embryo. *Biorheology* **53**, 33-47, doi:10.3233/BIR-15075 (2016).

9 Qiao, C. *et al.* Deep transcriptomic profiling reveals the similarity between endothelial cells cultured under static and oscillatory shear stress conditions. *Physiol Genomics* **48**, 660-666, doi:10.1152/physiolgenomics.00025.2016 (2016).

10 Pastorkova, Z., Skarda, J. & Andel, J. The role of microRNA in metastatic processes of non-small cell lung carcinoma. A review. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*, doi:10.5507/bp.2016.021 (2016).

11 Feinberg, M. W. & Moore, K. J. MicroRNA Regulation of Atherosclerosis. *Circ Res* **118**, 703-720, doi:10.1161/CIRCRESAHA.115.306300 (2016).

12 Ballantyne, M. D., McDonald, R. A. & Baker, A. H. lncRNA/MicroRNA interactions in the vasculature. *Clin Pharmacol Ther* **99**, 494-501, doi:10.1002/cpt.355 (2016).

13 Tao, L., Bei, Y., Zhou, Y., Xiao, J. & Li, X. Non-coding RNAs in cardiac regeneration. *Oncotarget* **6**, 42613-42622, doi:10.18632/oncotarget.6073 (2015).

14 Liangju, C., Ming, Y., Yan, C., Huaqin, S. & Wenming, X. [Roles of miR-15b in endothelial cell function and their relevance to vascular diseases]. *Yi Chuan* **37**, 121-127, doi:10.16288/j.yczz.14-288 (2015).

15 Donaldson, C. J., Lao, K. H. & Zeng, L. The salient role of microRNAs in atherogenesis. *J Mol Cell Cardiol* **122**, 98-113, doi:10.1016/j.yjmcc.2018.08.004 (2018).

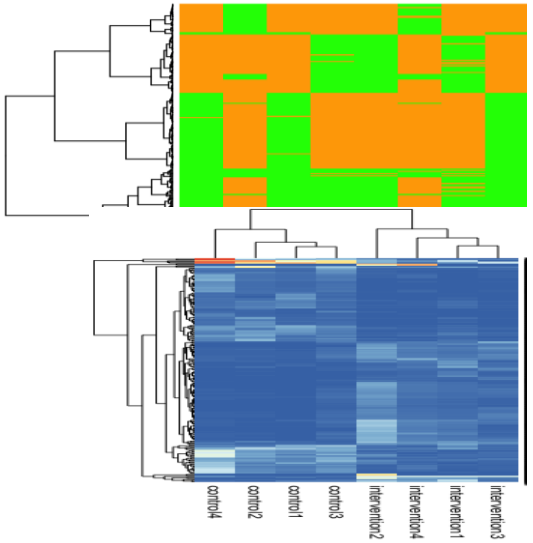
16 Rissland, O. S. *et al.* The influence of microRNAs and poly(A) tail length on endogenous mRNA-protein complexes. *Genome Biol* **18**, 211, doi:10.1186/s13059-017-1330-z (2017).
17 Denzler, R. *et al.* Impact of MicroRNA Levels, Target-Site Complementarity, and Cooperativity on Competing Endogenous RNA-Regulated Gene Expression. *Mol Cell* **64**, 565-579, doi:10.1016/j.molcel.2016.09.027 (2016).
18 Hoffman, K., Aschengrau, A., Webster, T. F., Bartell, S. M. & Vieira, V. M. Associations between residence at birth and mental health disorders: a spatial analysis of retrospective cohort data. *BMC Public Health* **15**, 688, doi:10.1186/s12889-015-2011-z (2015).
19 Singh, A. K. *et al.* Identification and evolutionary analysis of polycistronic miRNA clusters in domesticated and wild wheat. *Genomics* **112**, 2334-2348, doi:10.1016/j.ygeno.2020.01.005 (2020).
20 Rui, T. *et al.* The mir-767-105 cluster: a crucial factor related to the poor prognosis of hepatocellular carcinoma. *Biomark Res* **8**, 7, doi:10.1186/s40364-020-0186-7 (2020).
21 Przygrodzka, E., Sokolowska, G., Myszczynski, K., Krawczynski, K. & Kaczmarek, M. M. Clustered microRNAs: The molecular mechanism supporting the maintenance of luteal function during early pregnancy. *FASEB J* **34**, 6582-6597, doi:10.1096/fj.201903007RR (2020).
22 Cantini, L. *et al.* Identification of microRNA clusters cooperatively acting on epithelial to mesenchymal transition in triple negative breast cancer. *Nucleic Acids Res* **47**, 2205-2215, doi:10.1093/nar/gkz016 (2019).
23 Moi, L., Braaten, T., Al-Shibli, K., Lund, E. & Busund, L. R. Differential expression of the miR-17-92 cluster and miR-17 family in breast cancer according to tumor type; results from the Norwegian Women and Cancer (NOWAC) study. *J Transl Med* **17**, 334, doi:10.1186/s12967-019-2086-x (2019).
24 Srivastava, S. P., Hedayat, A. F., Kanasaki, K. & Goodwin, J. E. microRNA Crosstalk Influences Epithelial-to-Mesenchymal, Endothelial-to-Mesenchymal, and Macrophage-to-Mesenchymal Transitions in the Kidney. *Front Pharmacol* **10**, 904, doi:10.3389/fphar.2019.00904 (2019).
25 Ignatiadis, N., Klaus, B., Zaugg, J. B. & Huber, W. Data-driven hypothesis weighting increases detection power in genome-scale multiple testing. *Nat Methods* **13**, 577-580, doi:10.1038/nmeth.3885 (2016).
26 Ajami, N. E. *et al.* Systems biology analysis of longitudinal functional response of endothelial cells to shear stress. *Proc Natl Acad Sci U S A* **114**, 10990-10995, doi:10.1073/pnas.1707517114 (2017).
27 Hosin, A. A., Prasad, A., Viiri, L. E., Davies, A. H. & Shalhoub, J. MicroRNAs in atherosclerosis. *J Vasc Res* **51**, 338-349, doi:10.1159/000368193 (2014).
28 Wang, Y., Zhang, H. & Lu, J. Response to Comment on "microRNAs in the Same Clusters Evolve to Coordinately Regulate Functionally Related Genes". *Mol Biol Evol* **36**, 1844-1845, doi:10.1093/molbev/msz121 (2019).
29 Alsagaby, S. A. Transcriptomics-based validation of the relatedness of heterogeneous nuclear ribonucleoproteins to chronic lymphocytic leukemia as potential biomarkers of the disease aggressiveness. *Saudi Med J* **40**, 328-338, doi:10.15537/smj.2019.4.23380 (2019).
30 Chung, N. C. *et al.* Unsupervised classification of multi-omics data during cardiac remodeling using deep learning. *Methods*, doi:10.1016/j.ymeth.2019.03.004 (2019).

31 Ding, M. *et al.* Integrative analysis of genome-wide association study and brain region related enhancer maps identifies biological pathways for insomnia. *Prog Neuropsychopharmacol Biol Psychiatry* **86**, 180-185, doi:10.1016/j.pnpbp.2018.05.026 (2018).
32 Kowsar, R., Kowsar, Z. & Miyamoto, A. Up-regulated mRNA expression of some anti-inflammatory mediators in bovine oviduct epithelial cells by urea in vitro: Cellular pathways by Reactome analysis. *Reprod Biol* **19**, 75-82, doi:10.1016/j.repbio.2019.01.001 (2019).
33 Xiong, D. D. *et al.* A circRNA-miRNA-mRNA network identification for exploring underlying pathogenesis and therapy strategy of hepatocellular carcinoma. *J Transl Med* **16**, 220, doi:10.1186/s12967-018-1593-5 (2018).
34 Zhu, K., Zheng, T., Chen, X. & Wang, H. Bioinformatic Analyses of Renal Ischaemia-Reperfusion Injury Models: Identification of Key Genes Involved in the Development of Kidney Disease. *Kidney Blood Press Res* **43**, 1898-1907, doi:10.1159/000496001 (2018).
35 Lee, E. S., Boldo, L. S., Fernandez, B. O., Feelisch, M. & Harmsen, M. C. Suppression of TAK1 pathway by shear stress counteracts the inflammatory endothelial cell phenotype induced by oxidative stress and TGF-beta1. *Sci Rep* **7**, 42487, doi:10.1038/srep42487 (2017).
36 Riquelme, M. A., Burra, S., Kar, R., Lampe, P. D. & Jiang, J. X. MAPK Activated by Prostaglandin E2 Phosphorylates Connexin 43 and Closes Osteocytic Hemichannels in Response to Continuous Flow Shear Stress. *J Biol Chem*, doi:10.1074/jbc.M115.683417 (2015).
37 Wang, Z., Zhang, J., Li, B., Mao, W. & Chen, S. MAPK signaling mediates low shear stress-induced oxidative damage in human umbilical vein endothelial cells in vitro. *Nan Fang Yi Ke Da Xue Xue Bao* **34**, 603-608 (2014).
38 Gater, R. *et al.* Investigation of Conjunctival Fibrosis Response Using a 3D Glaucoma Tenon's Capsule + Conjunctival Model. *Invest Ophthalmol Vis Sci* **60**, 605-614, doi:10.1167/iovs.18-25335 (2019).
39 Han, X. *et al.* Influence of TGF-beta1 expression in endothelial cells on smooth muscle cell phenotypes and MMP production under shear stress in a co-culture model. *Cytotechnology* **71**, 489-496, doi:10.1007/s10616-018-0268-7 (2019).
40 Jia, L. *et al.* Transforming Growth Factor Beta-1 Promotes Smooth Muscle Cell Proliferation and Migration in an Arteriovenous Fistulae: The Role of Wall Shear Stress. *Ther Apher Dial*, doi:10.1111/1744-9987.12781 (2018).
41 Kouzbari, K. *et al.* Oscillatory shear potentiates latent TGF-beta1 activation more than steady shear as demonstrated by a novel force generator. *Sci Rep* **9**, 6065, doi:10.1038/s41598-019-42302-x (2019).
42 Varshney, R. *et al.* Inactivation of platelet-derived TGF-beta1 attenuates aortic stenosis progression in a robust murine model. *Blood Adv* **3**, 777-788, doi:10.1182/bloodadvances.2018025817 (2019).
43 Xu, N. *et al.* Stent-Jailing Technique Reduces Aneurysm Recurrence More Than Stent-Jack Technique by Causing Less Mechanical Forces and Angiogenesis and Inhibiting TGF-beta/Smad2,3,4 Signaling Pathway in Intracranial Aneurysm Patients. *Front Physiol* **9**, 1862, doi:10.3389/fphys.2018.01862 (2018).
44 Yang, T. L. *et al.* Differential regulations of fibronectin and laminin in Smad2 activation in vascular endothelial cells in response to disturbed flow. *J Biomed Sci* **25**, 1, doi:10.1186/s12929-017-0402-4 (2018).

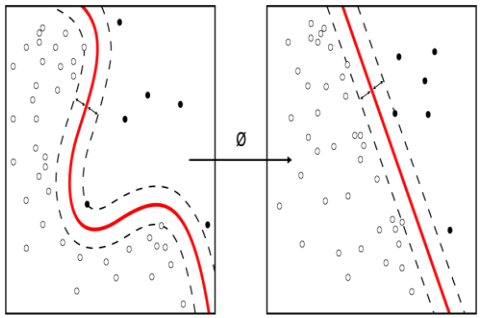
45 Yue, H. *et al.* CD36 Enhances Vascular Smooth Muscle Cell Proliferation and Development of Neointimal Hyperplasia. *Arterioscler Thromb Vasc Biol* **39**, 263-275, doi:10.1161/ATVBAHA.118.312186 (2019).
46 Bondareva, O. *et al.* Identification of atheroprone shear stress responsive regulatory elements in endothelial cells. *Cardiovasc Res*, doi:10.1093/cvr/cvz027 (2019).
47 Kuo, Y. C. *et al.* Oscillatory shear stress mediates directional reorganization of actin cytoskeleton and alters differentiation propensity of mesenchymal stem cells. *Stem Cells* **33**, 429-442, doi:10.1002/stem.1860 (2015).
48 Kuo, Y. C. *et al.* Oscillatory shear stress mediates directional reorganization of actin cytoskeleton and alters differentiation propensity of mesenchymal stem cells. *Stem Cells* **33**, 429-442, doi:10.1002/stem.1860 (2015).
49 Hutter, K. *et al.* Differential roles of miR-15a/16-1 and miR-497/195 clusters in immune cell development and homeostasis. *FEBS J*, doi:10.1111/febs.15493 (2020).
50 Kandettu, A., Radhakrishnan, R., Chakrabarty, S., Sriharikrishnaa, S. & Kabekkodu, S. P. The emerging role of miRNA clusters in breast cancer progression. *Biochim Biophys Acta Rev Cancer* **1874**, 188413, doi:10.1016/j.bbcan.2020.188413 (2020).
51 Liu, H. & Wang, Y. B. Systematic large-scale meta-analysis identifies miRNA-429/200a/b and miRNA-141/200c clusters as biomarkers for necrotizing enterocolitis in newborn. *Biosci Rep* **39**, doi:10.1042/BSR20191503 (2019).
52 Shukla, V. *et al.* Role of miRNA clusters in epithelial to mesenchymal transition in cancer. *Front Biosci (Elite Ed)* **12**, 48-78 (2020).
53 Zhang, X. *et al.* Three paralogous clusters of the miR-17~92 family of microRNAs restrain IL-12-mediated immune defense. *Cell Mol Immunol*, doi:10.1038/s41423-020-0363-5 (2020).
54 Martirosyan, A. *et al.* Kinetic Modelling of Competition and Depletion of Shared miRNAs by Competing Endogenous RNAs. *Methods Mol Biol* **1912**, 367-409, doi:10.1007/978-1-4939-8982-9_15 (2019).
55 Martirosyan, A., Figliuzzi, M., Marinari, E. & De Martino, A. Probing the Limits to MicroRNA-Mediated Control of Gene Expression. *PLoS Comput Biol* **12**, e1004715, doi:10.1371/journal.pcbi.1004715 (2016).

Figure

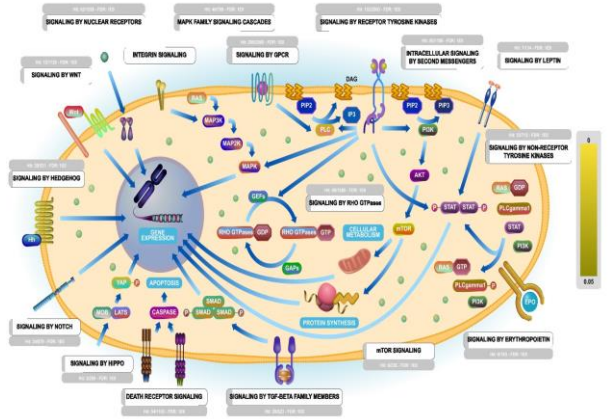
Ultra-sensitive detection of mRNA and miRNA expression patterns



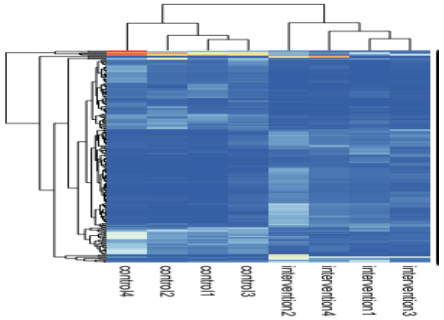
Using multi-featured, non-linear ML to predict miRNA-mRNA patterns



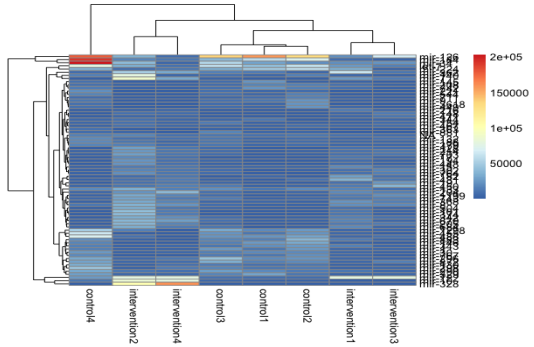
Selection of signaling pathways Using GSEA



Ultra-sensitive detection of miRNA

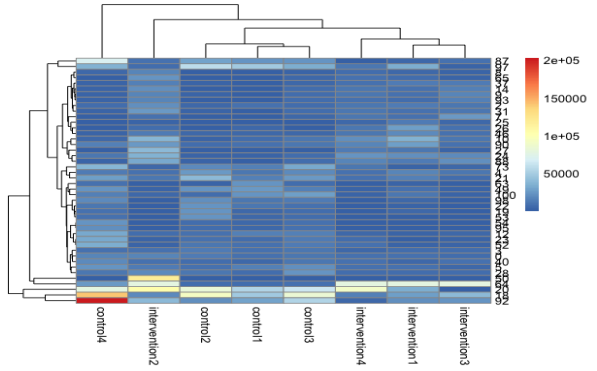


miRNA-Families

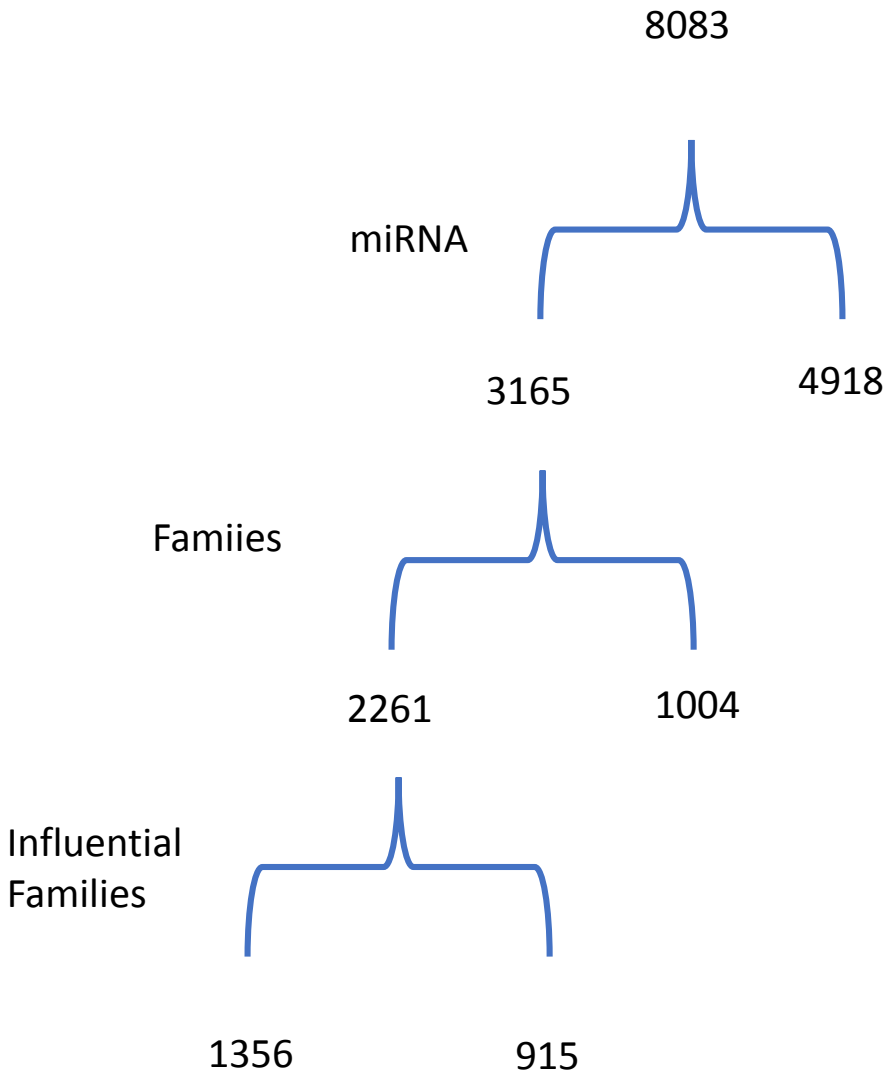


+

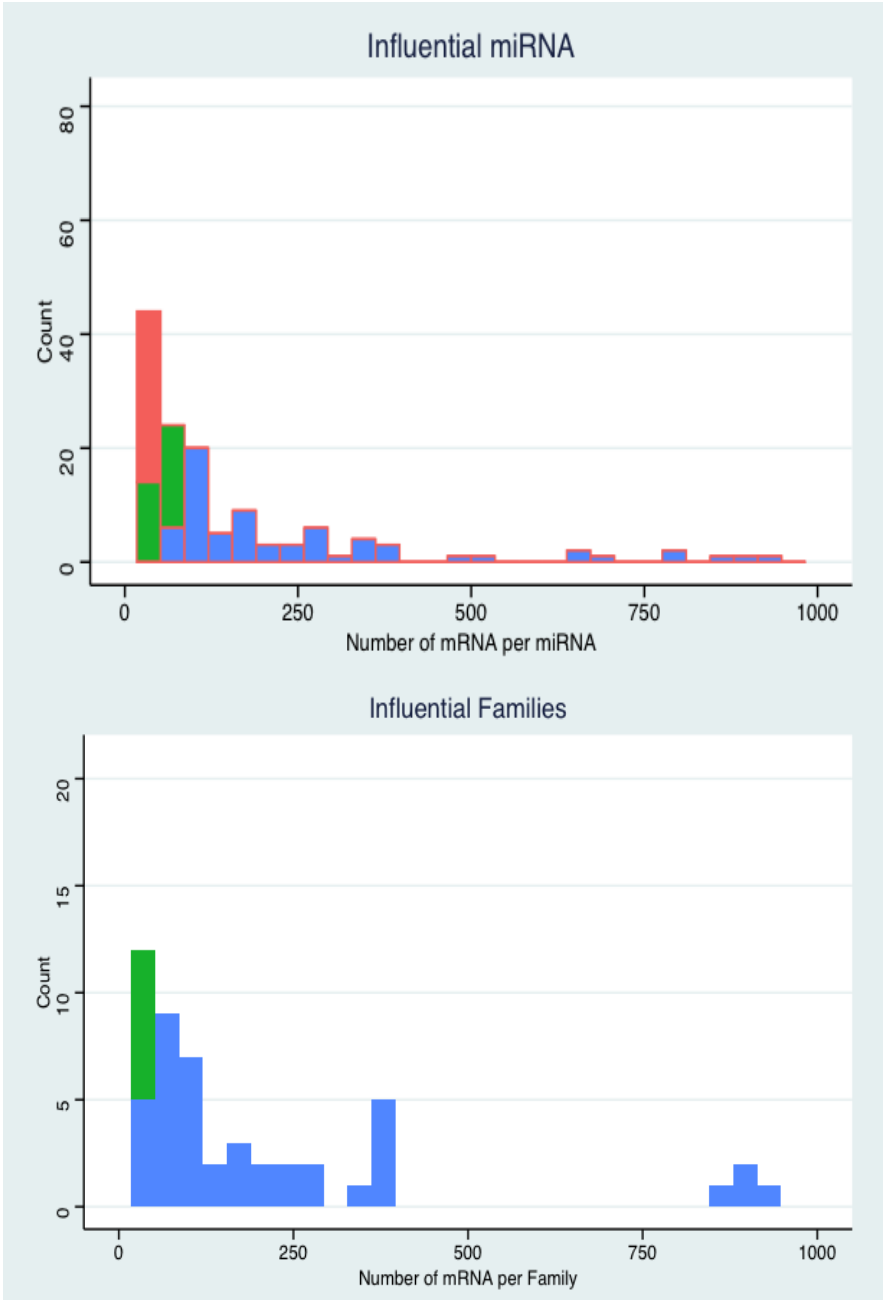
miRNA-Clusters



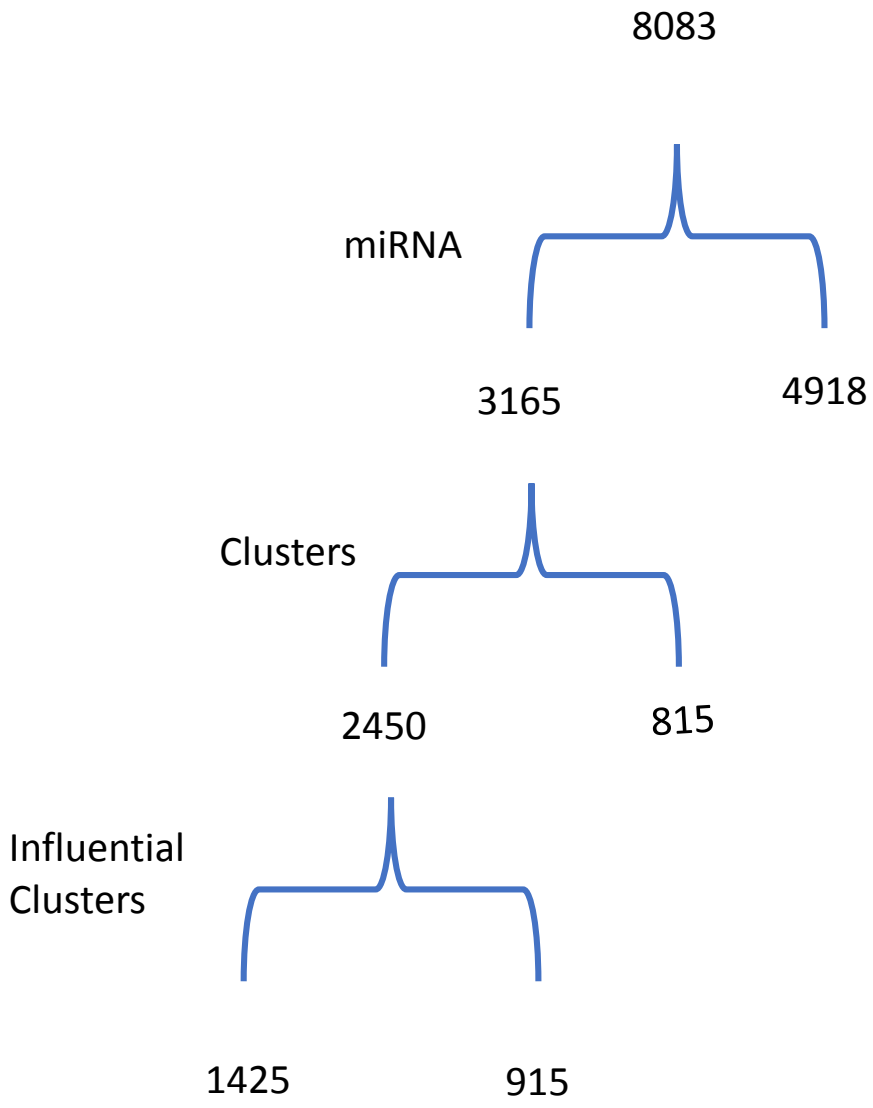
A


8083
miRNA
3165
4918
Famiies
2261
1004
Influential
Families
1356
915

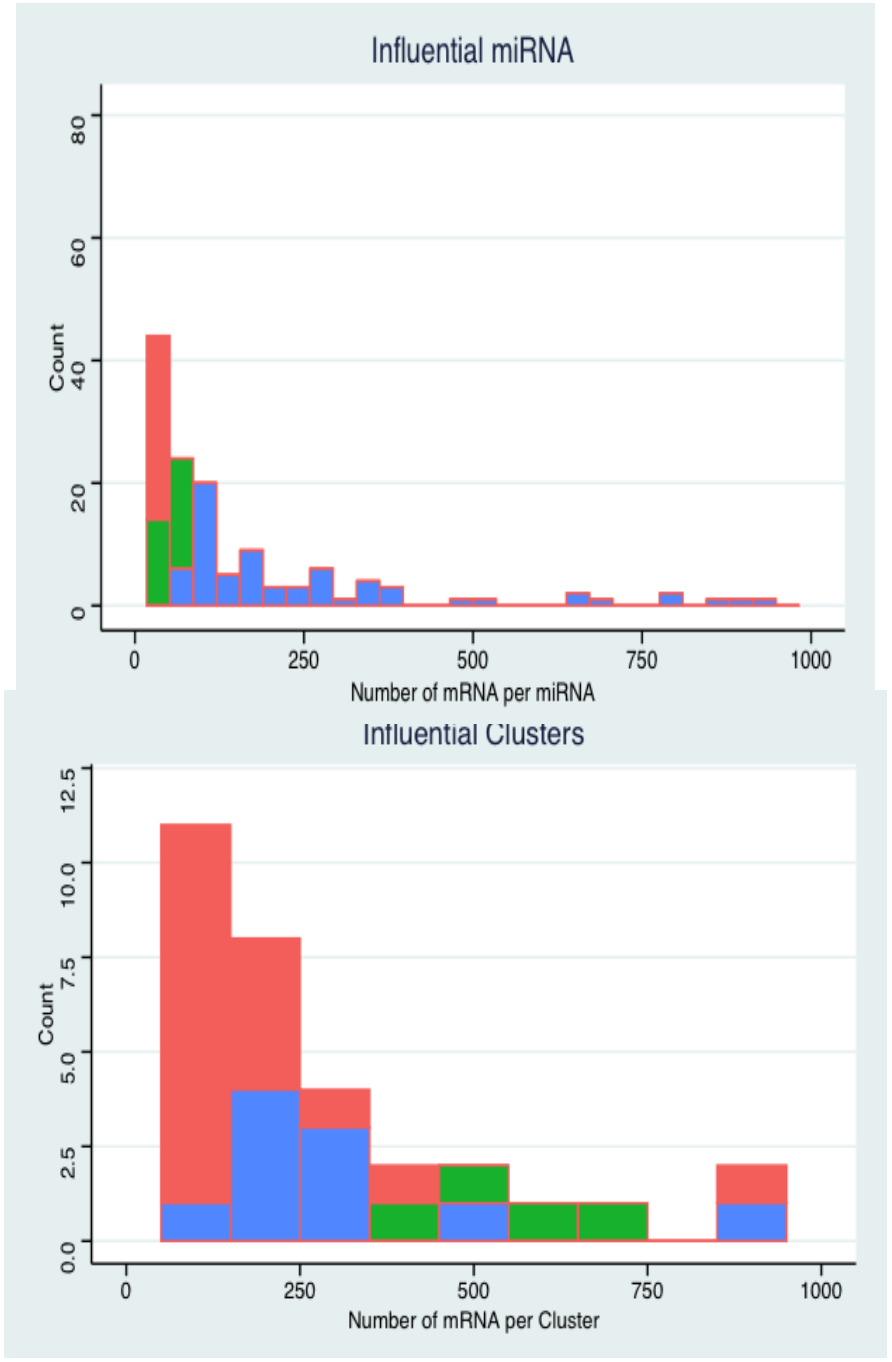
B


Influential miRNA
Count
0
20
40
60
80
0
250
500
750
1000
Number of mRNA per miRNA
Influential Families
Count
0
5
10
15
20
0
250
500
750
1000
Number of mRNA per Family

A


8083
miRNA
3165
4918
Clusters
2450
815
Influential Clusters
1425
915

B


Influential miRNA
Count
0
20
40
60
80
0
250
500
750
1000
Number of mRNA per miRNA
Influential Clusters
Count
0.0
2.5
5.0
7.5
10.0
12.5
0
250
500
750
1000
Number of mRNA per Cluster