



Circulating miRNAs in Atherosclerosis and Thrombosis

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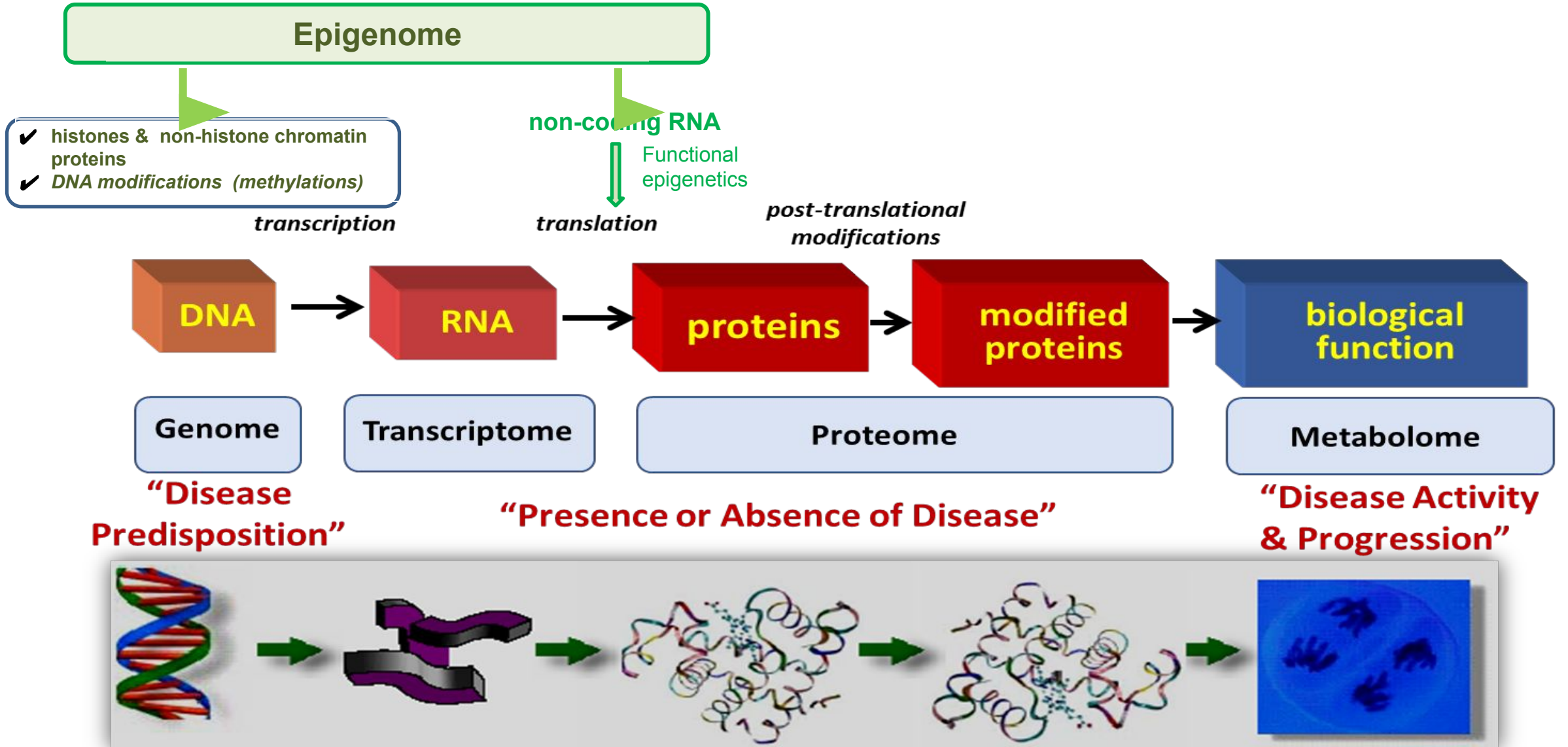




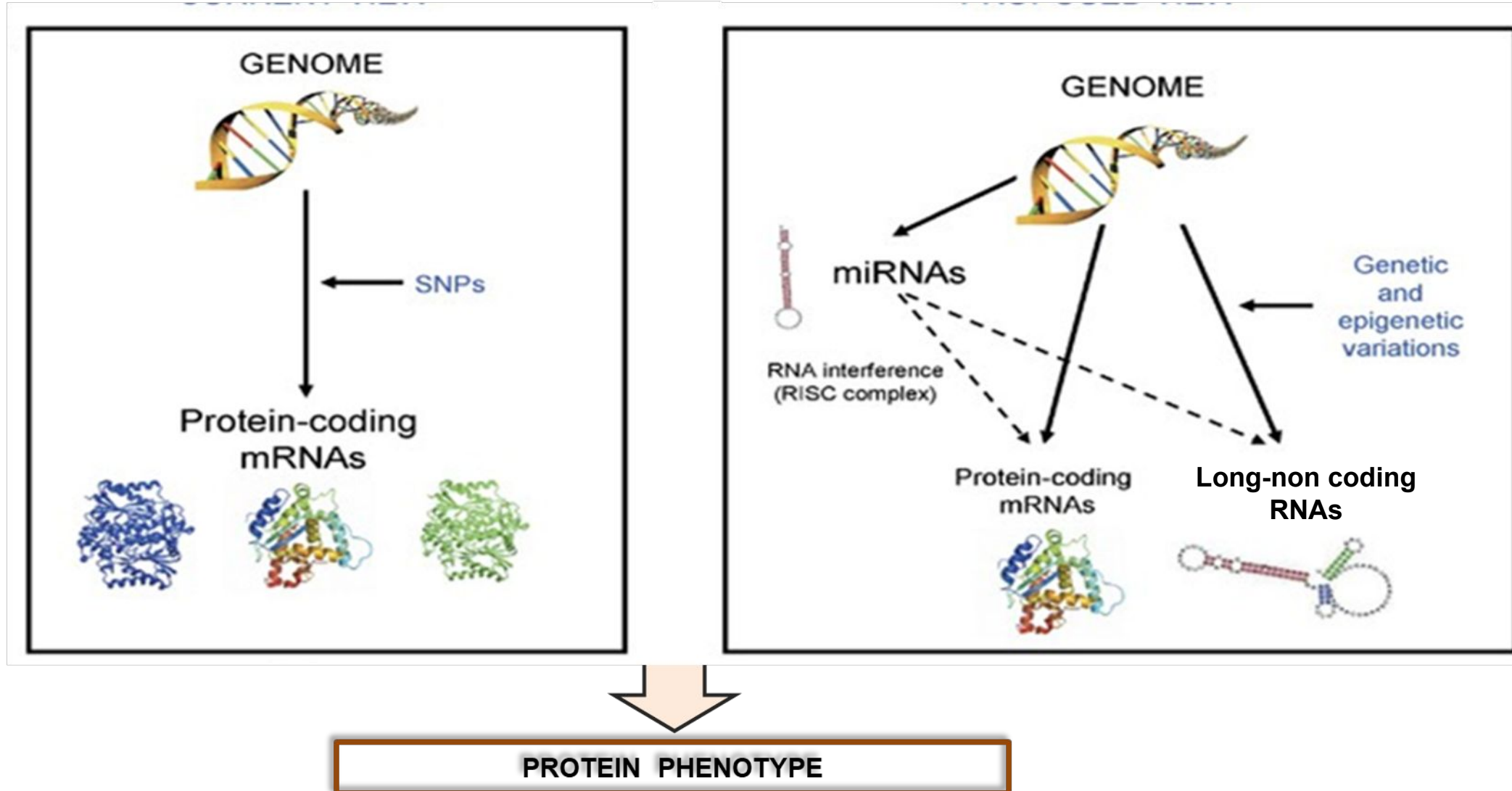
Declaration of Conflict Of Interest

I have no potential conflict of interest to report

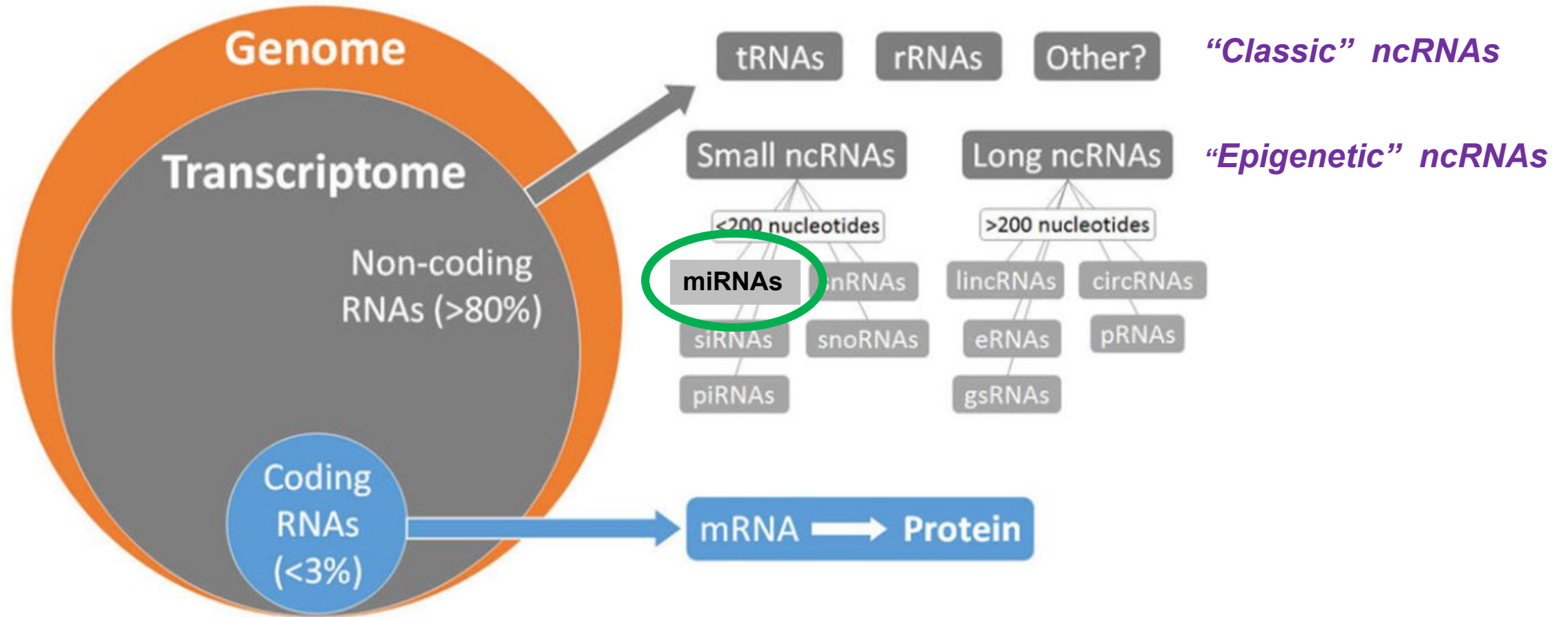
From genetic print to biological function: epigenetic regulation



Genetic and Epigenetic Regulation



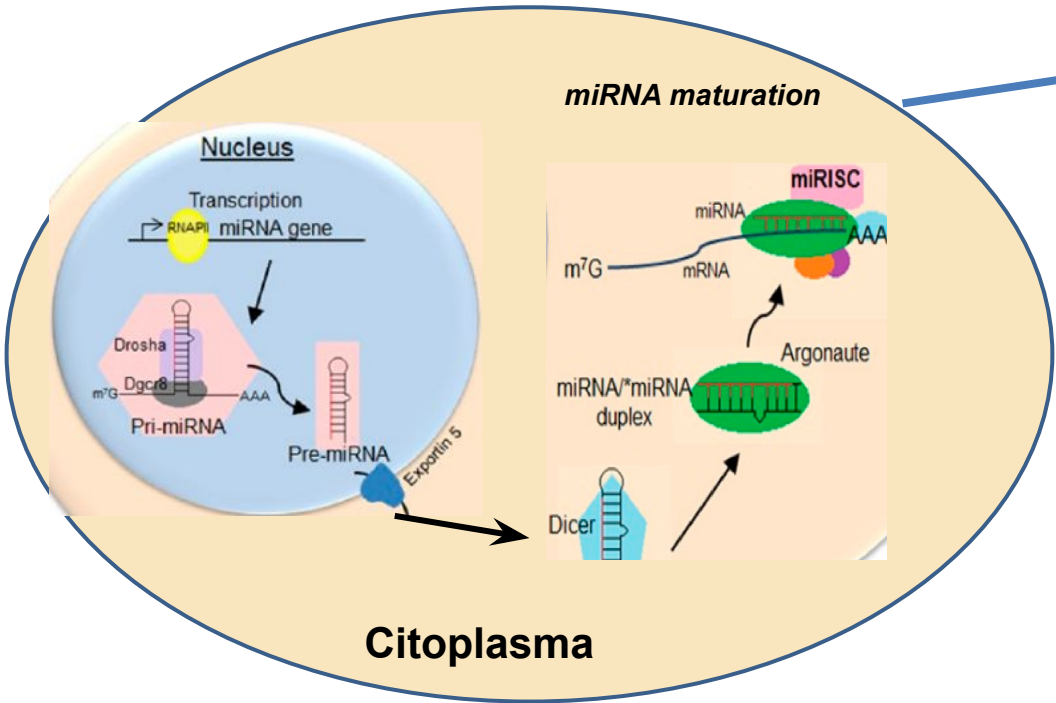
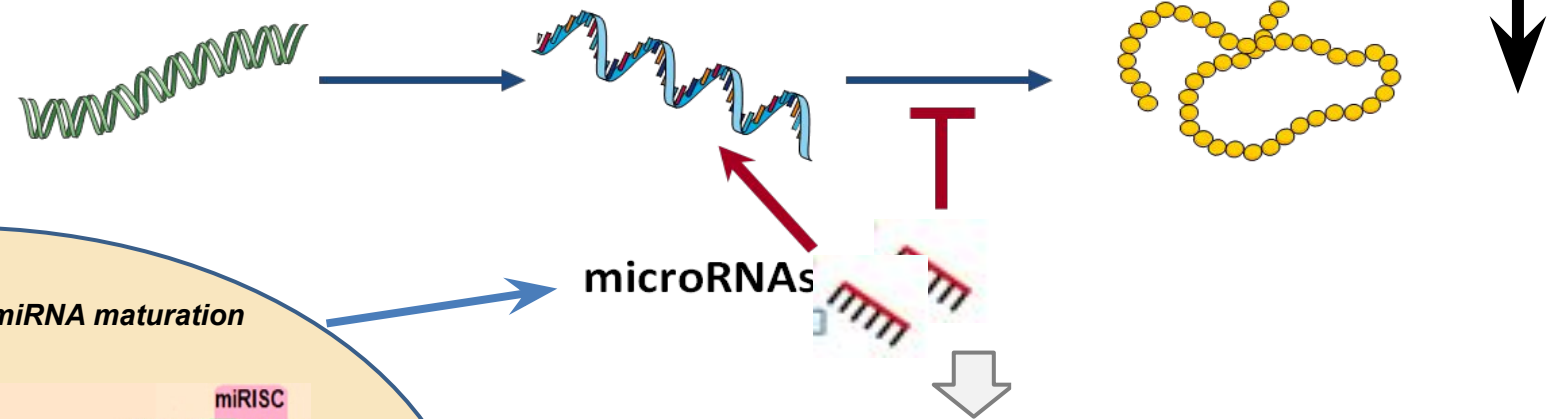
From genetic print to biological function: epigenetic regulation



Non-coding RNA: microRNA

- “micro” ncRNAs (miRNAs)**
- ✓ < 20-30 nucleotides
 - ✓ Well conserved
 - ✓ Cytoplasm

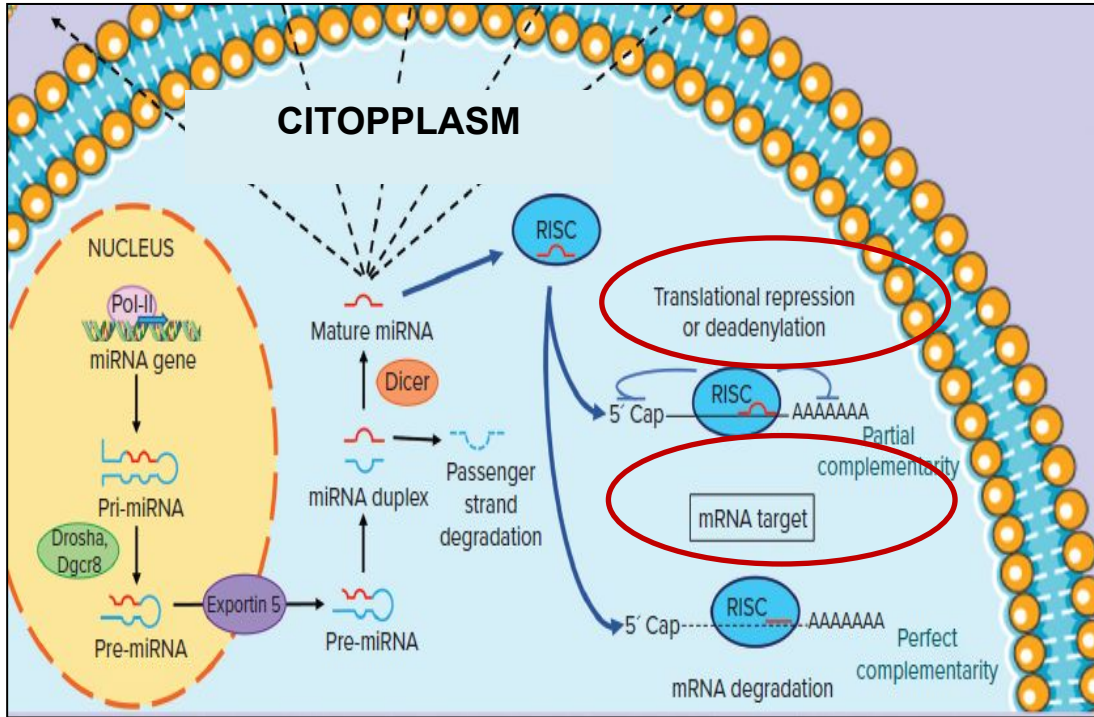
DNA > transcription > RNA > translation > PROTEIN



- **Translational Regulation**
- Target mRNA complementary sequence**
 - block protein translation.
 - mRNA cleavage, or degradation

*>1000 miRNA have been identified
miRNA regulate > 60% coding Genes*

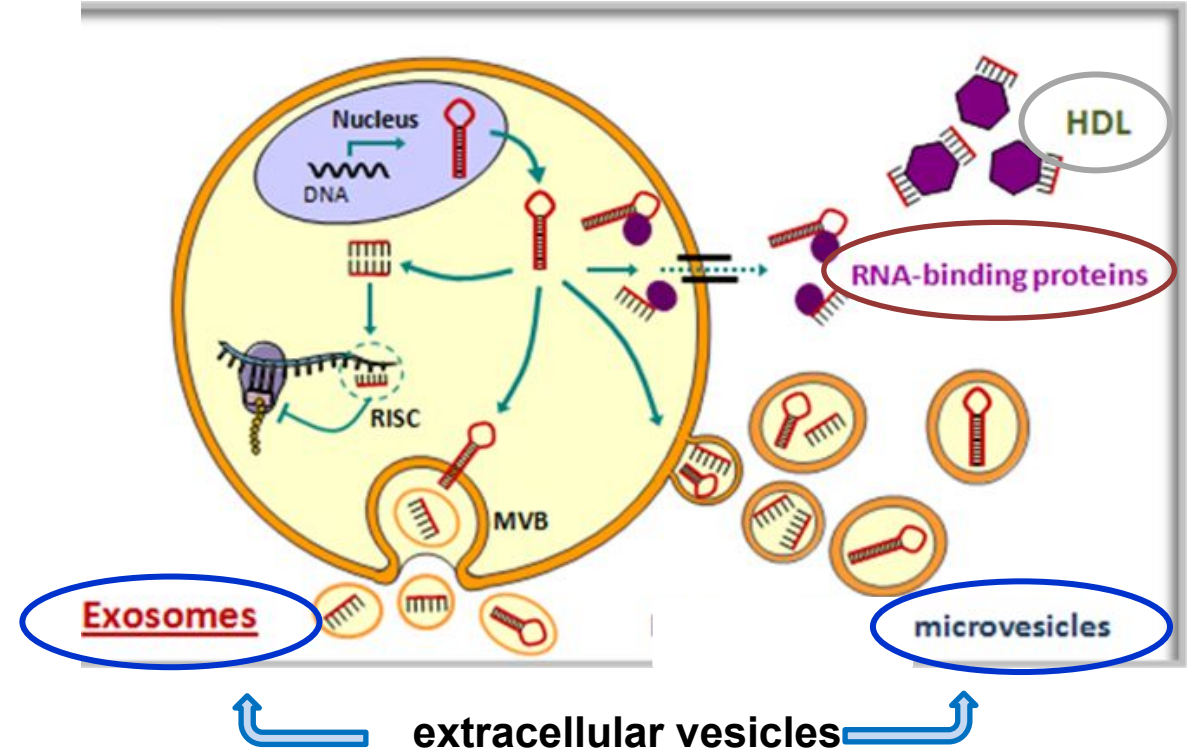
Intracellular miRNAs



In-cell

Protein expression regulation

Extracellular miRNAs



**Paracrine mediated Interactions -
Protein expression regulation**

miRNA dysregulation in Thrombosis

Related to prethrombotic status:

Gao J et al. (2017) The role of microRNAs in prethrombotic status associated with coronary artery disease. Thromb Haemost; 117:429–436

Thrombotic events

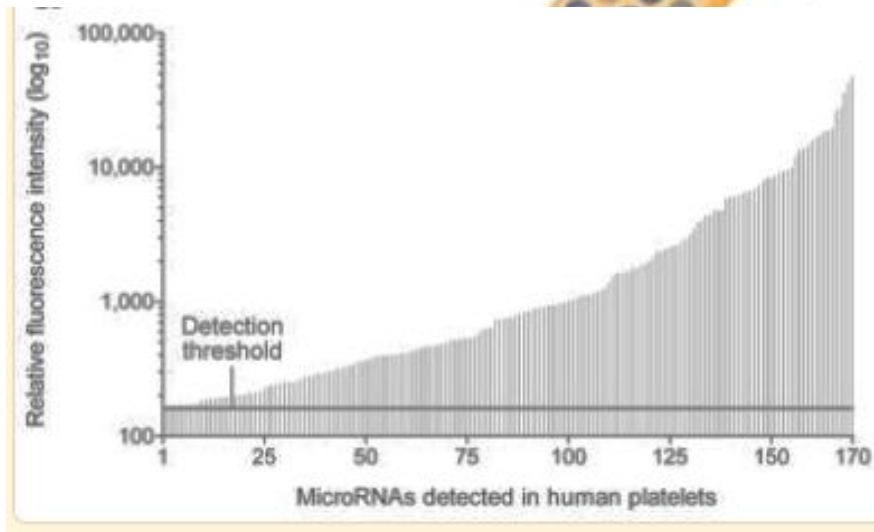
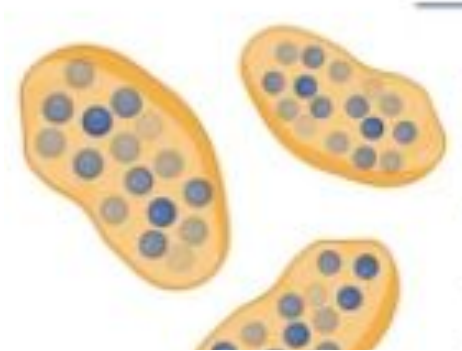
Hembrom AA, et al. (2020) MicroRNAs in venous thrombo-embolism. Clin Chim Acta 504:66–72

Secondary hemostasis

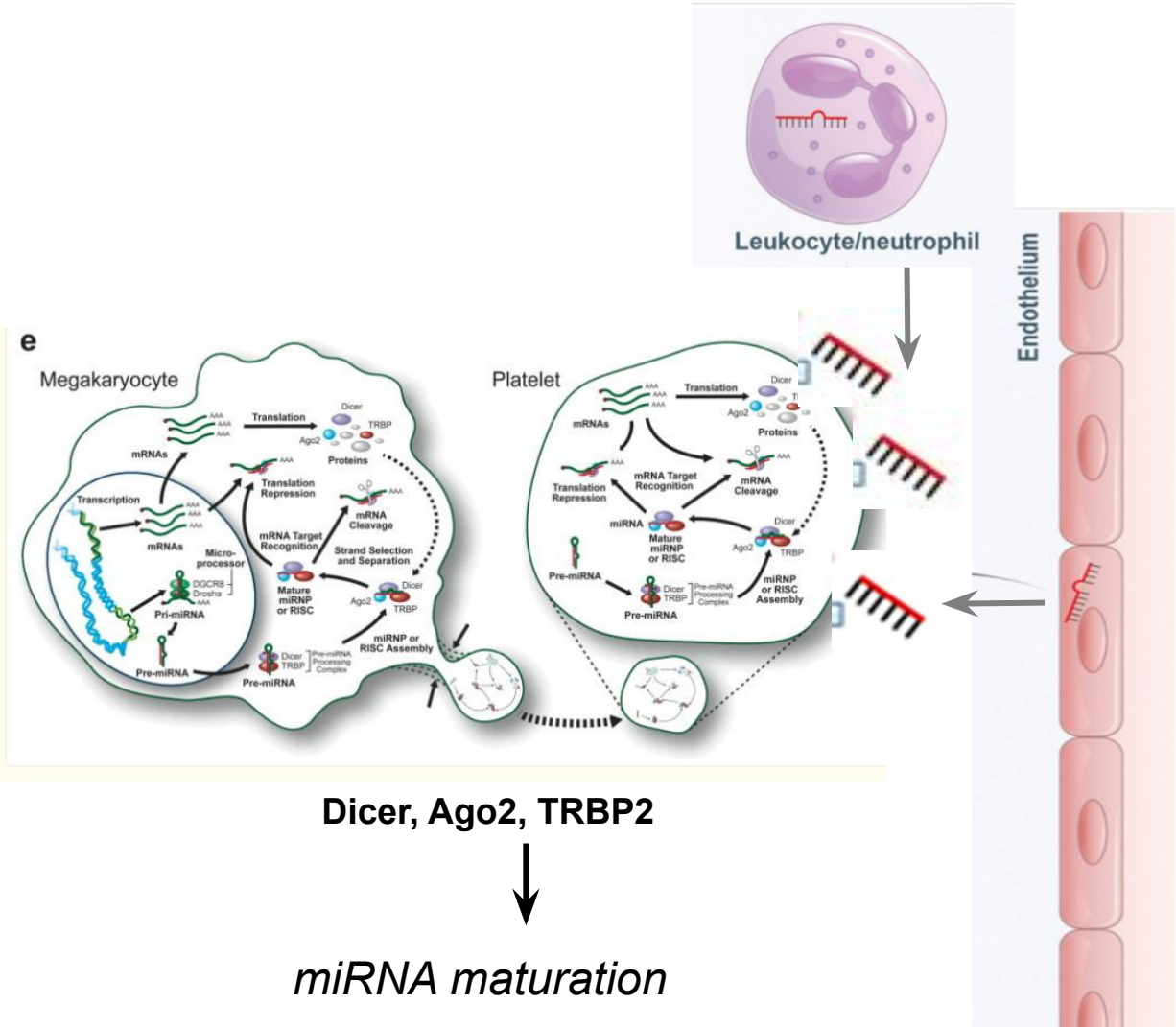
Jankowska KI, et al.(2020) Role of microRNAs in hemophilia and thrombosis in humans Int J Mol Sci 21:3598

miRNAs are highly present in anucleated platelets

219 different miRNAs
RT-PCR
>500 different miRNAs
NGS



Landry P et al. 2009



Landry P et al. 2009
Leng Q et al 2017

miRNAS are promising biomarkers of platelet activity

- MiRNA expression profile differs between activated and resting platelets
Osman and Fälker, Platelets 2011
- Correlations between specific miRNAs and the expression of proteins participating in platelet activation and aggregation
Kaudewitz et al., Circ Res 2016; Liu et al., Thromb Haemost 2019).

Thrombin activation

miR-15a
miR-339.3p
miR-365
miR-495
miR-98
miR-361-3p

mTOR signalling Pathway

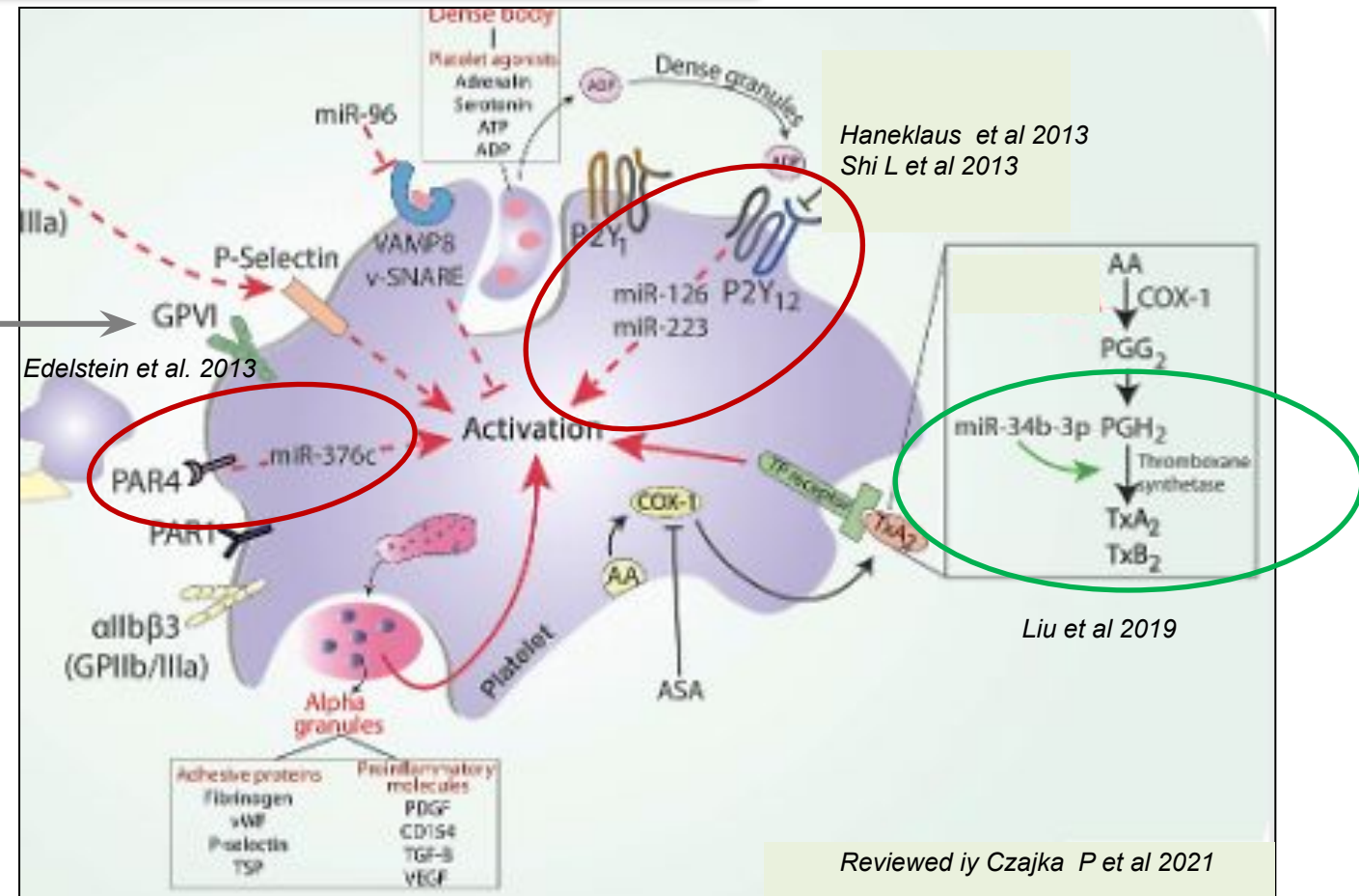
Wang L. et al. J Transl Med. 2021

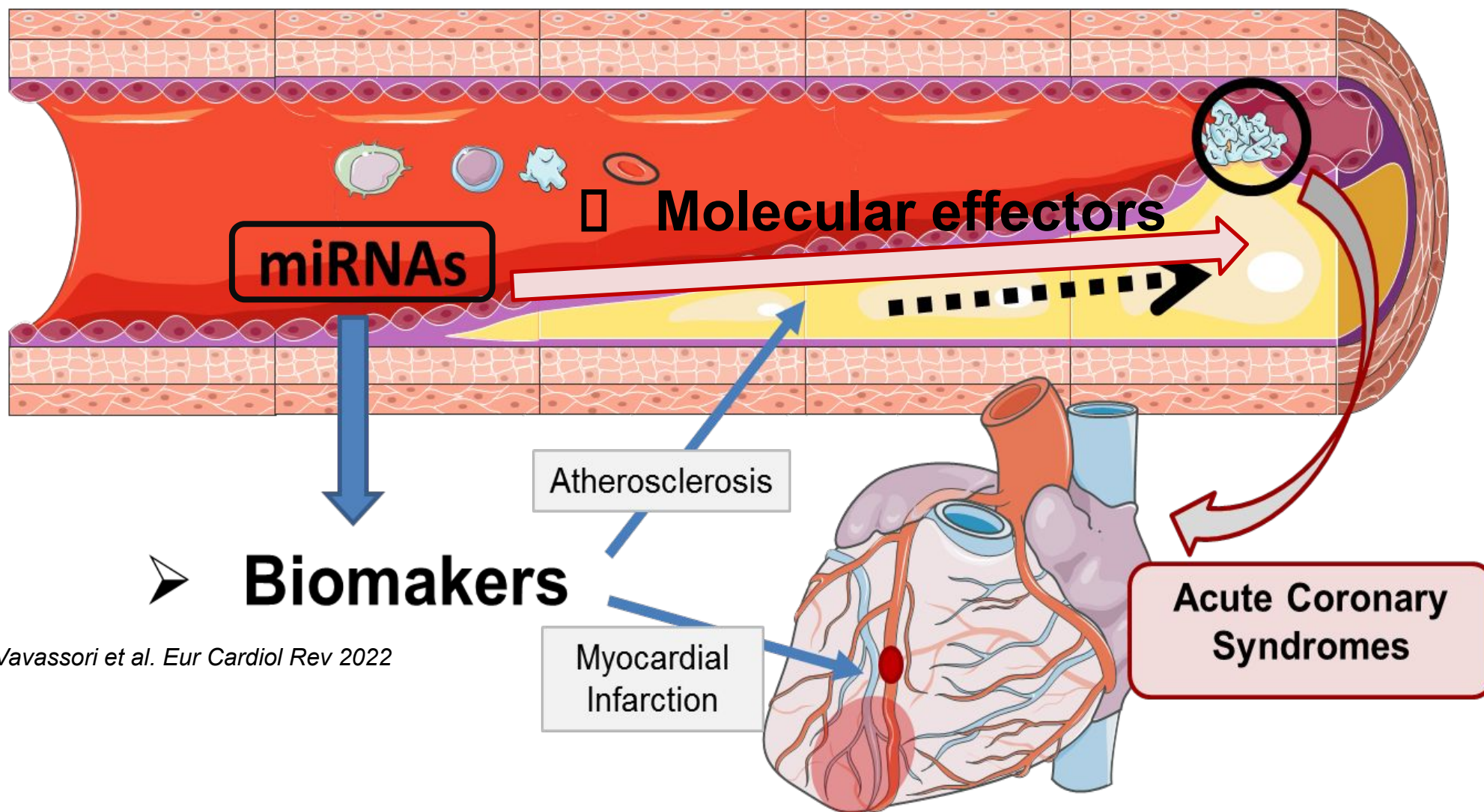
Osman and Fälker. Platelets 2011

miRNA studies linked with antiplatelet treatments

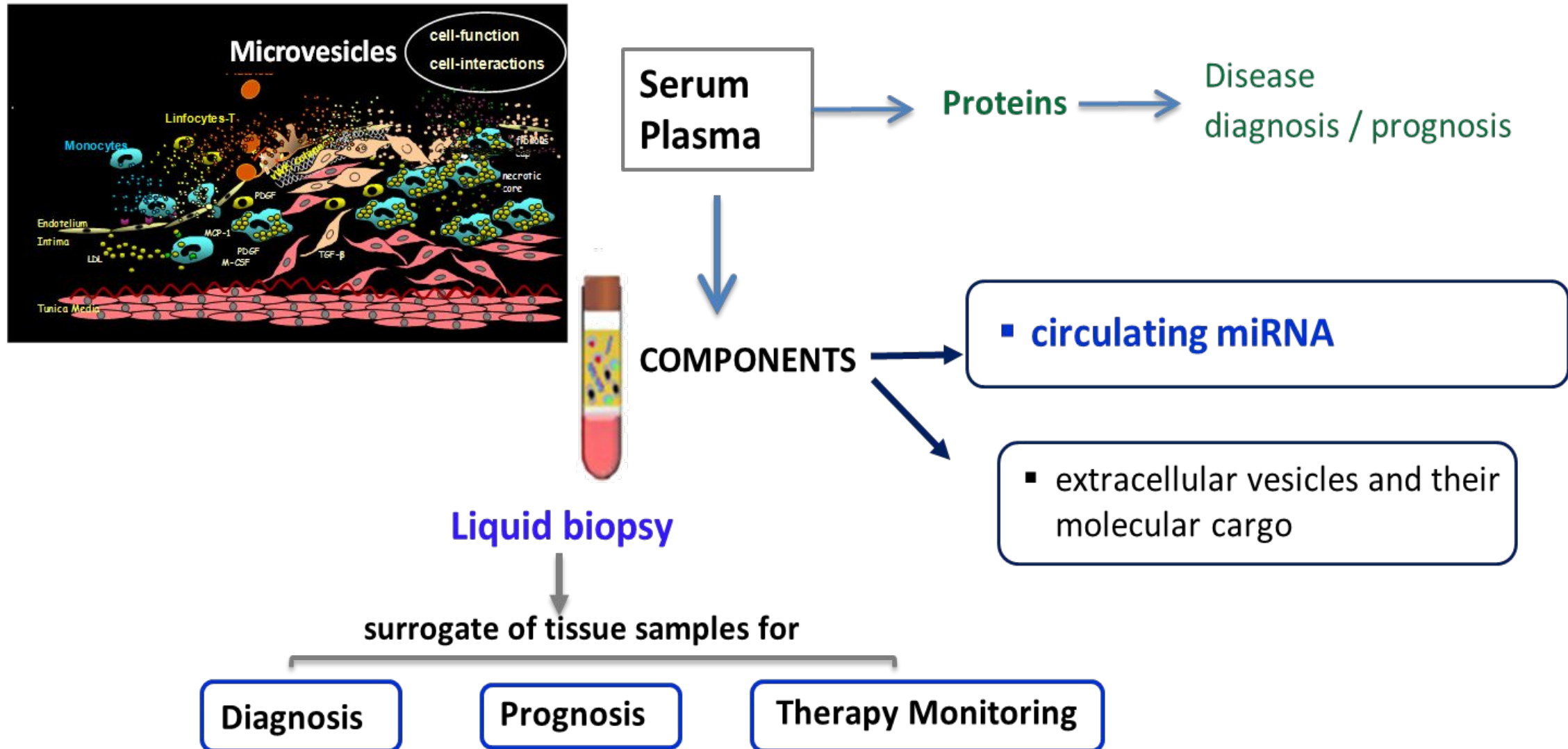
Aspirin, Clopidogrel, Ticagrelor

Czajka P et al Front Physiol 2021

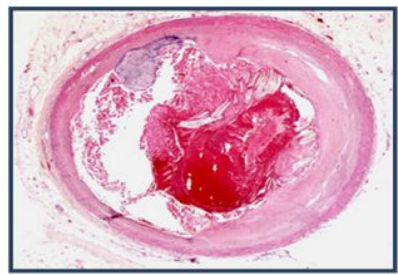
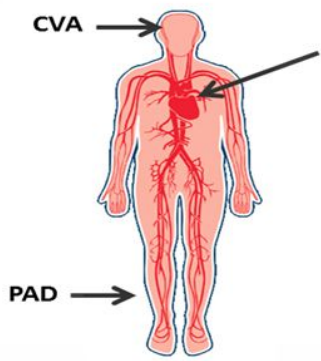
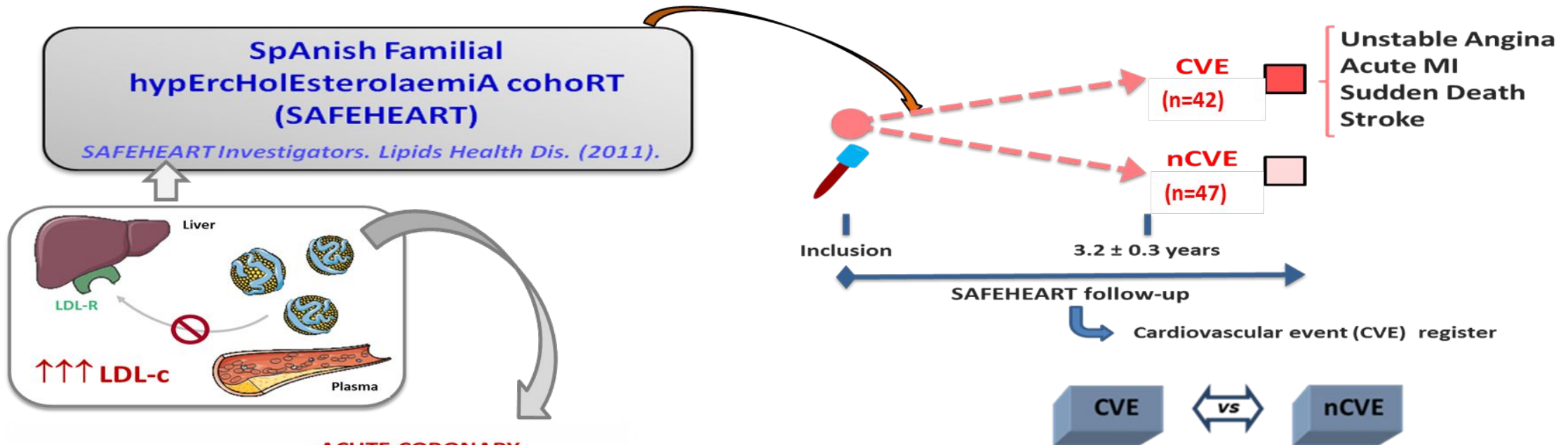




Liquid Biopsies & circulating microRNAs

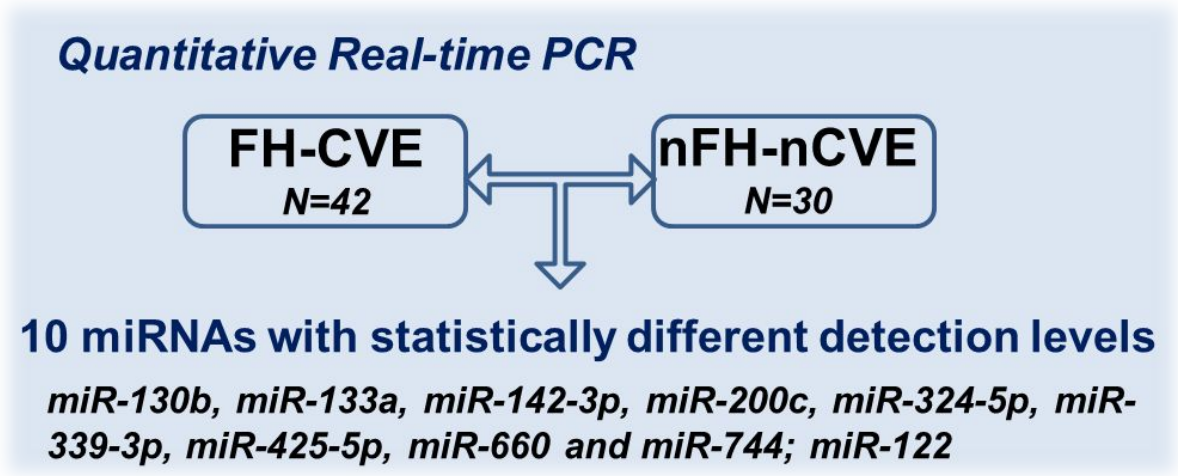
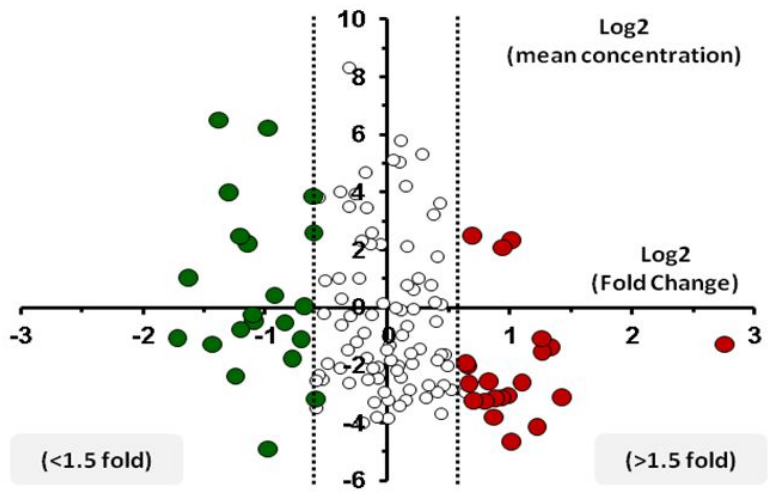
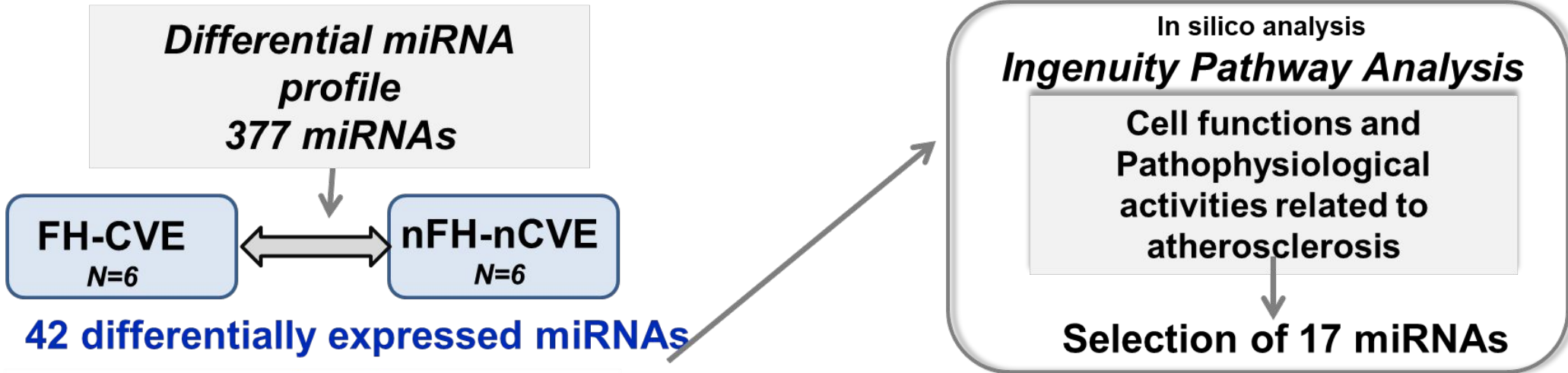


miRNAs as Biomarkers in the progression of atherosclerosis to clinical event presentation

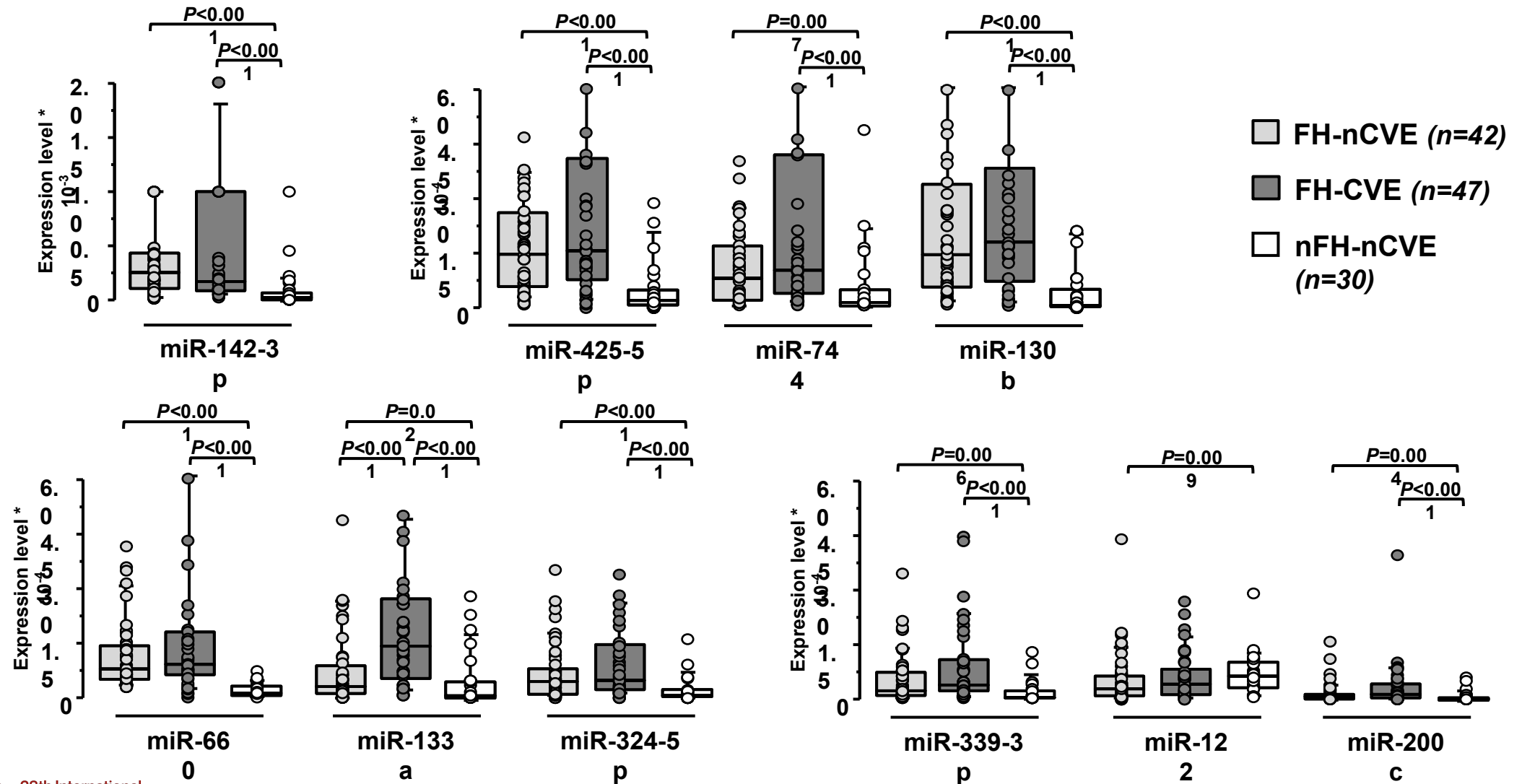


	CVE	nCVE	Statistics
Age (years)	58.11 ± 1.49	59.85 ± 2.14	<i>P</i> = 0.50
Body mass index (Kg/m ²)	28.43 ± 0.40	27.73 ± 0.60	<i>P</i> = 0.33
LDL-cholesterol (mg/dL)	192.12 ± 7.14	175.29 ± 7.42	<i>P</i> = 0.14
Diabetes mellitus (n, %)	9 (9 %)	6 (13 %)	<i>P</i> = 0.58
Arterial hypertension (n, %)	33 (35 %)	17 (35 %)	<i>P</i> = 0.94
Obesity (n, %)	21 (22 %)	10 (21 %)	<i>P</i> = 0.86
Lipid-lowering therapy (years)	13.98 ± 0.75	14.70 ± 1.11	<i>P</i> = 0.58
Framingham Risk Score (FRS)	15.58 ± 0.96	13.18 ± 1.33	<i>P</i> = 0.15

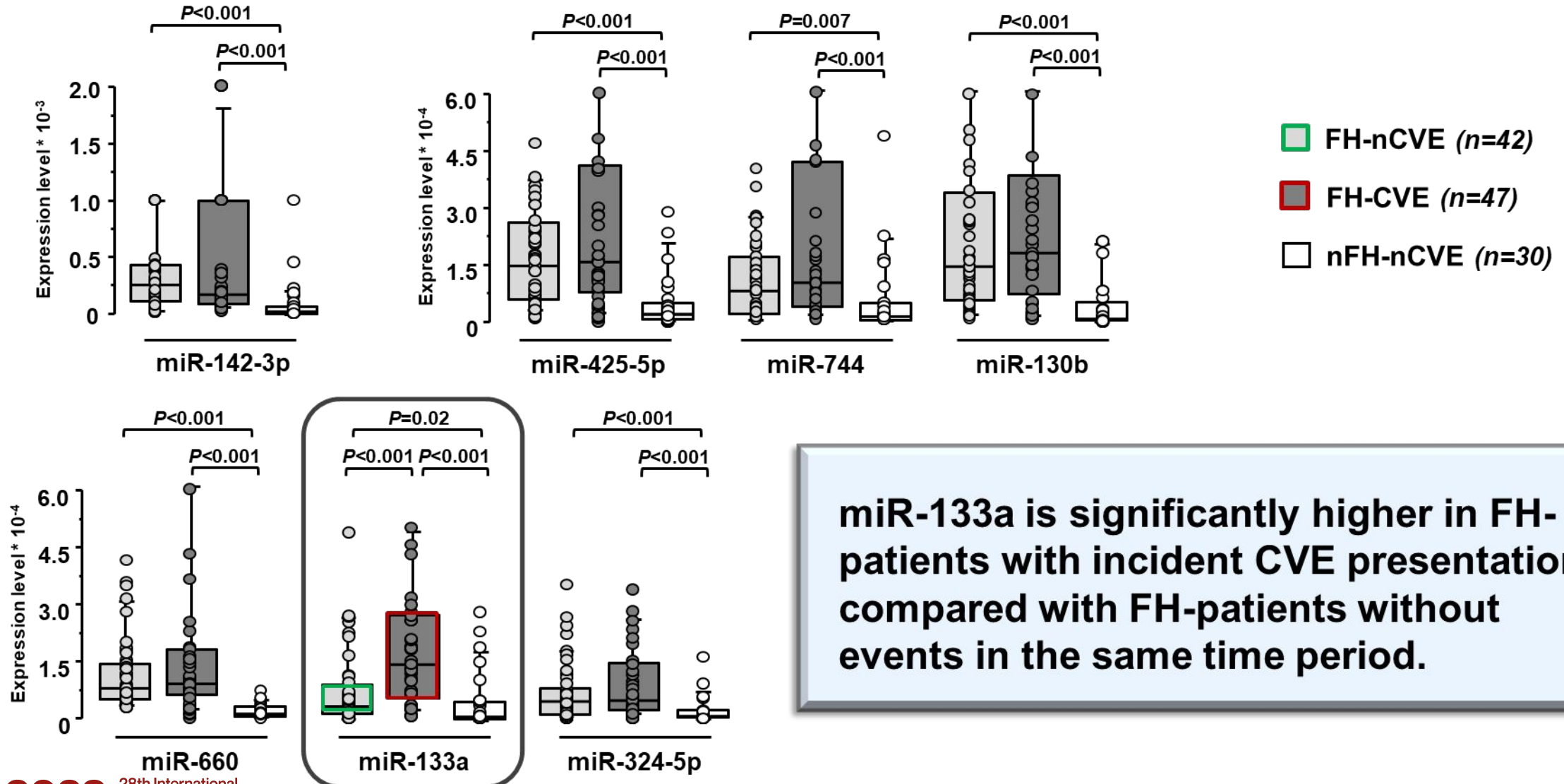
Discovery Studies: Differential miRNA signature associated to incident CVE-presentation in hFH



Validation Studies: Plasma miRNAs levels associated to incident CVE-presentation in hFH

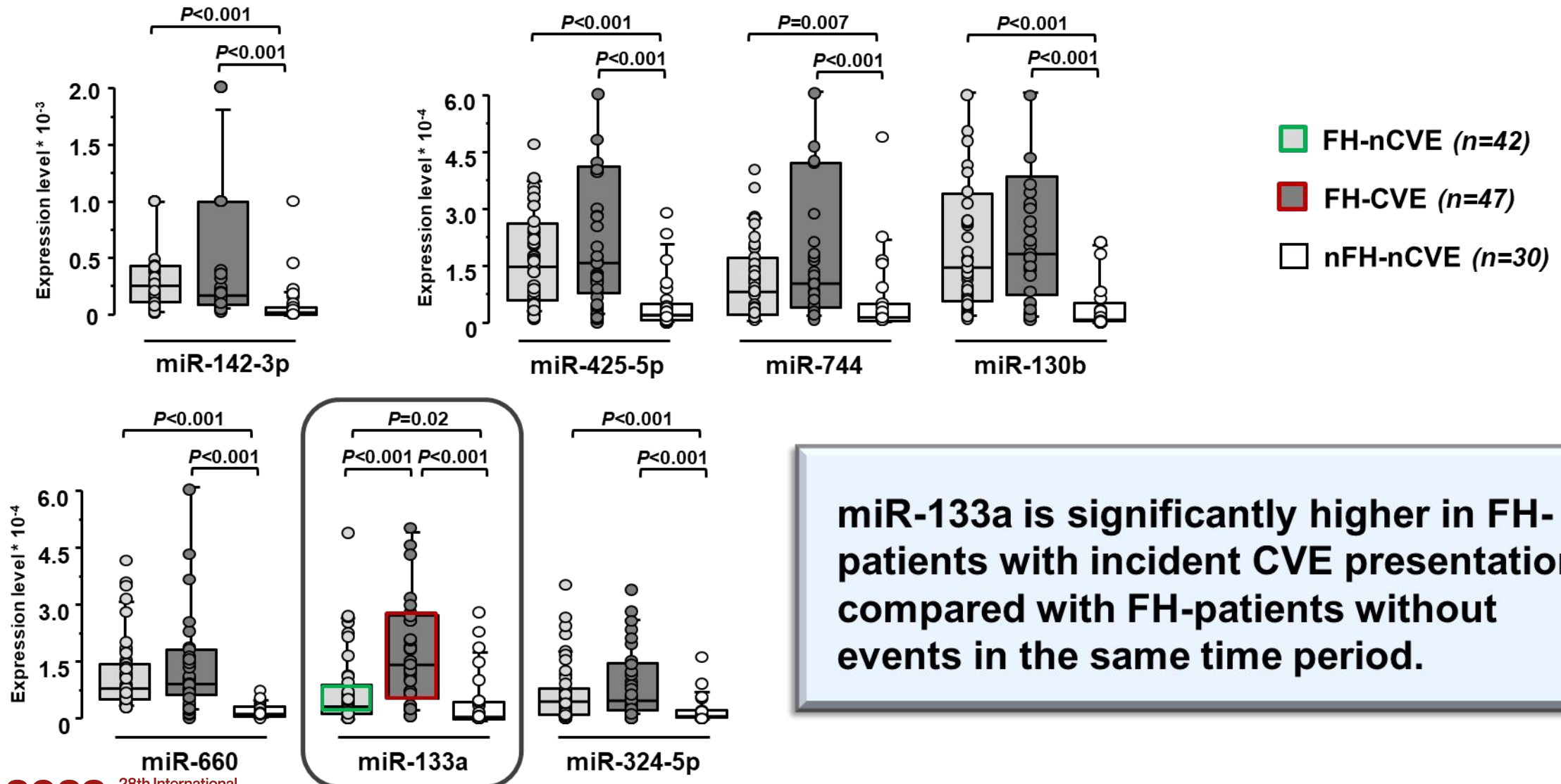


Validation Studies: Plasma miRNAs levels associated to incident CVE-presentation in hFH



miR-133a is significantly higher in FH-patients with incident CVE presentation compared with FH-patients without events in the same time period.

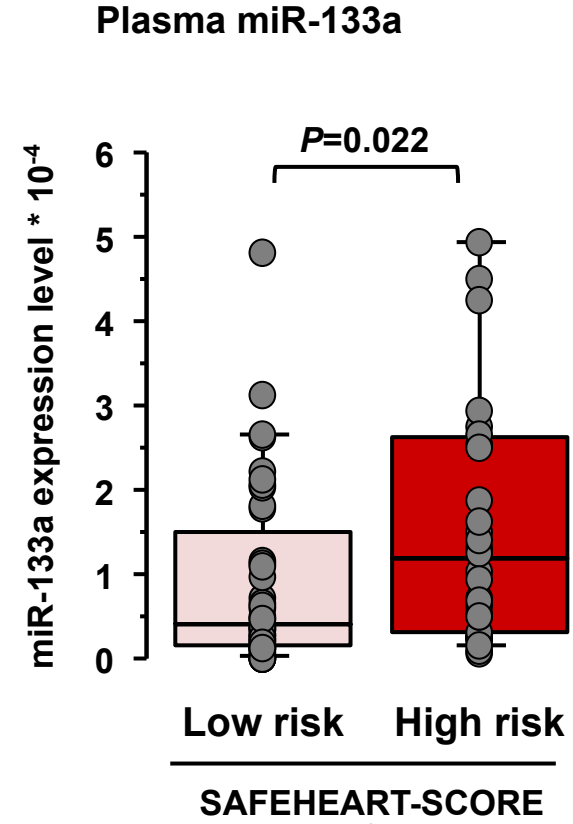
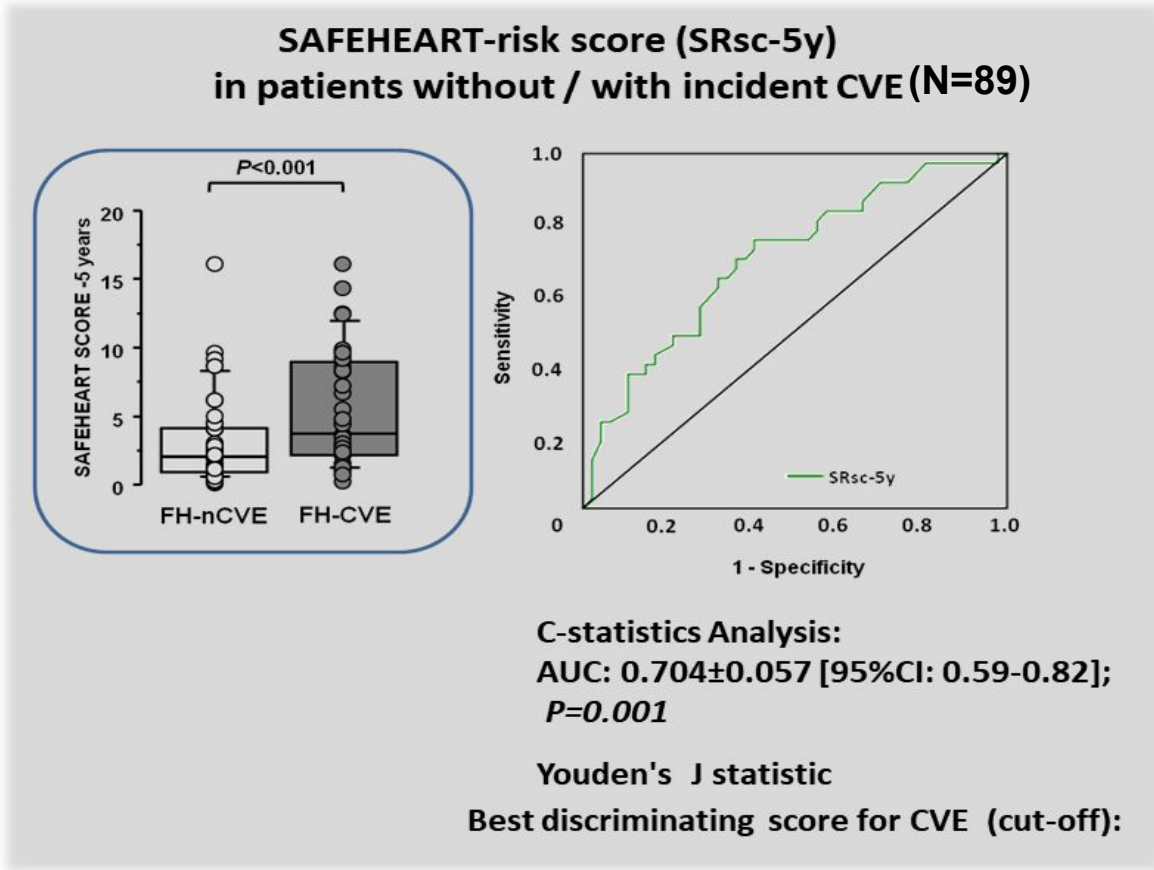
Validation Studies: Plasma miRNAs levels associated to incident CVE-presentation in hFH



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SAFEHEART-Risk Equation to estimate risk of incident CVD in patients with FH

Circulation 2017;135:2133-2144.

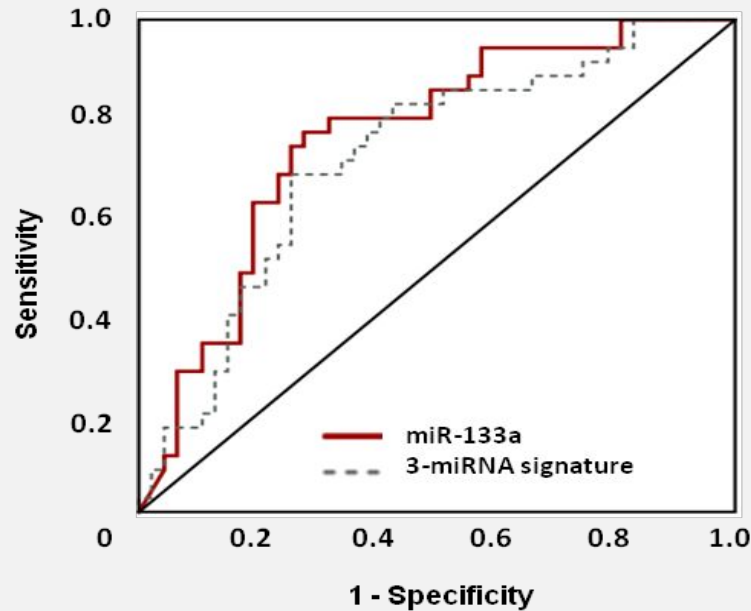


Discriminatory Power of miR133 for incident CV events in FH-patients

Receiver operating characteristic (ROC) Curve

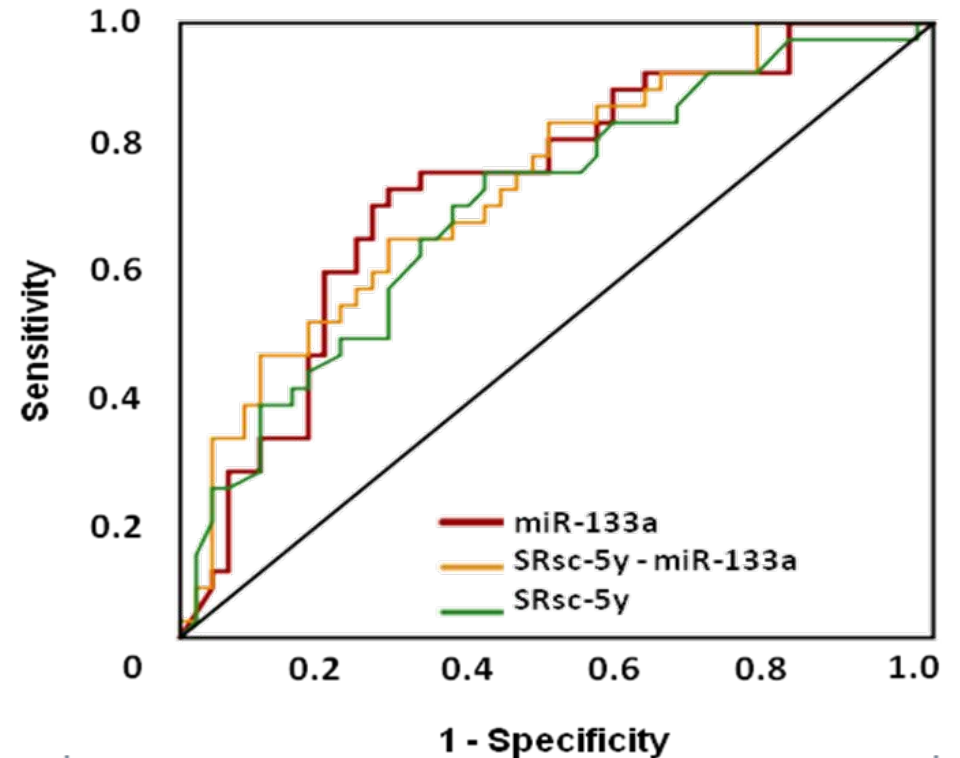
Discrimination of CVE in FH patients

miRNAs	AUC ± SE (95% CI)	P-value
miR-133a	0.76 ± 0.054 (0.66 - 0.87)	<0.001
miR-339-3p	0.65 ± 0.062 (0.52 - 0.77)	0.025
miR-200c	0.63 ± 0.064 (0.5 - 0.75)	0.050
miR-744	0.62 ± 0.063 (0.5 - 0.75)	0.06
miR-130b	0.6 ± 0.064 (0.48 - 0.73)	0.115
miR-425-5p	0.6 ± 0.065 (0.47 - 0.73)	0.133
miR-324-5p	0.59 ± 0.064 (0.46 - 0.71)	0.190
miR-660	0.58 ± 0.066 (0.45 - 0.71)	0.247
miR-122	0.53 ± 0.067 (0.4 - 0.66)	0.653
miR-142-3p	0.52 ± 0.067 (0.39 - 0.65)	0.789
3-miRNA signature	0.72 ± 0.057 (0.61 - 0.83)	0.001



ROC-analysis confirms miR-133a as the best microRNA for predicting CVE in FH-patients

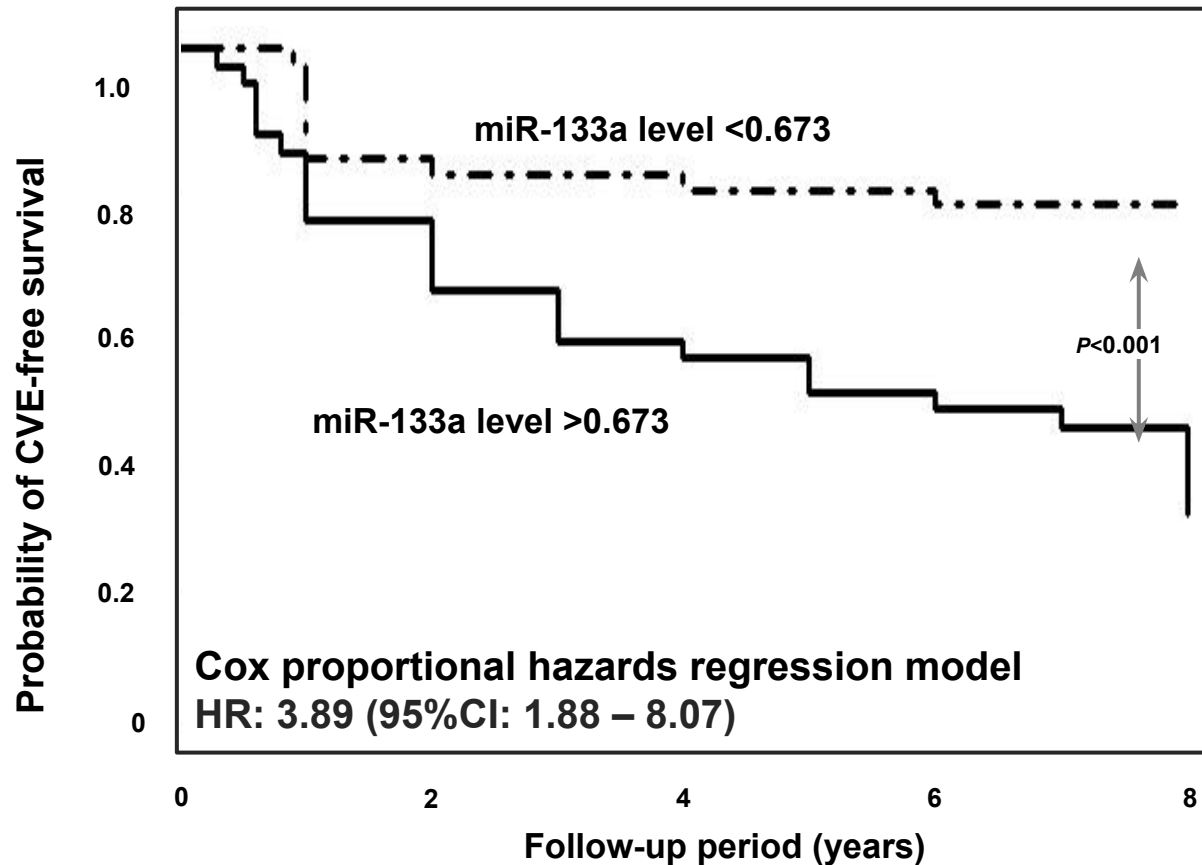
ROC analysis: Combination of miR-133 and SRsc-5y



miRNAs	AUC ± SE (95% CI)	P-value
SRsc-5y - miR-133a	0.74 ± 0.055 (0.63 - 0.84)	<0.001

Prediction Power of miR133 for incident CVE events in FH-patients

Kaplan-Meier curves of freedom from cardiovascular event



Plasma miR-133a levels associated to the higher risk of presenting a CVE within the next 8 years

Conditional logistic regression analysis:

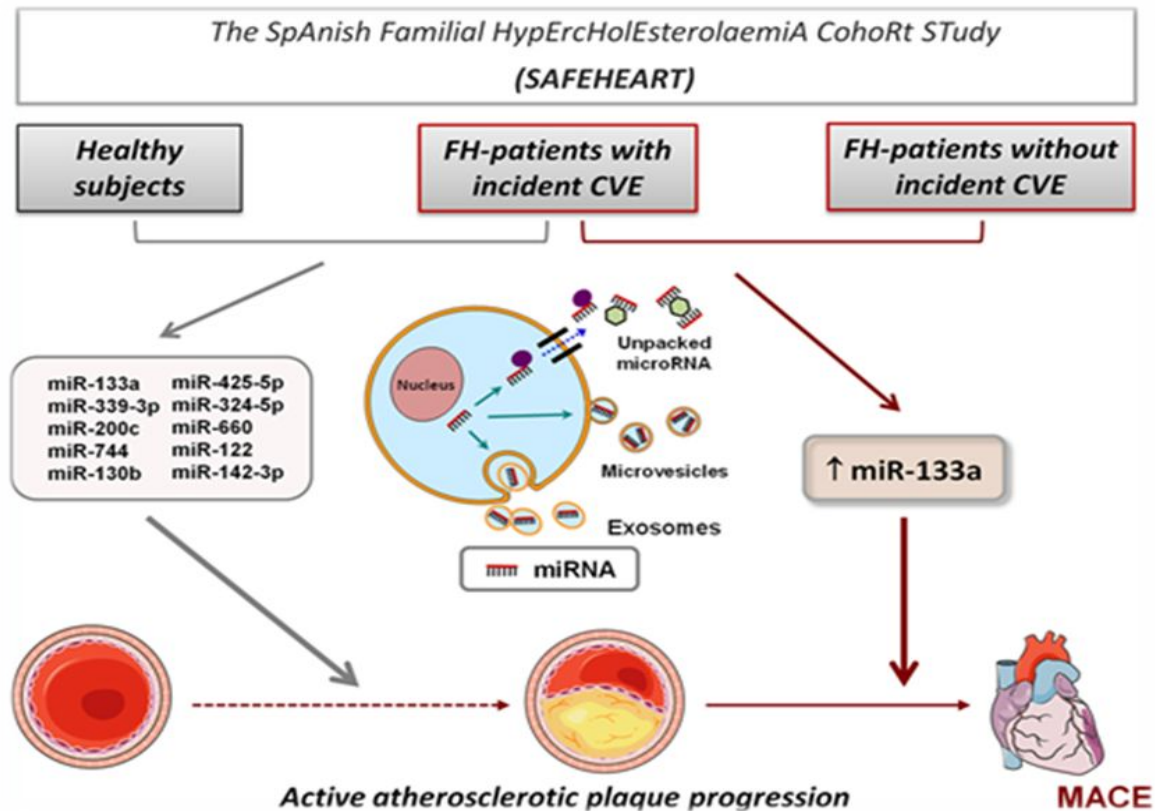
Cox regression model with balanced groups (propensity score matching for the SAFEHEART risk score)

HR of 17 (95%CI: 2.26-127.7; $P=0.006$)

miR133a cut-off value for discriminating MACE-presentation: 0.673 (73% sensitivity; 72% specificity)

In Summary,

miRNAs that circulate in the bloodstream can be a valuable addition to the currently used biomarkers to fine-tune cardiovascular risk stratification in patients with genetic diagnostic of FH.



- FH-patients with active atherosclerosis leading to the presentation of an acute event have increased plasma levels of miR-133a years before the onset of the event
- Plasma levels of miR-133a have prognostic value to predict incident cardiovascular events in FH patients treated as per guidelines.
- Through miR-133a, FH patients at risk have an exacerbated inflammatory system.

To take home.....

- **Circulating microRNAs** may serve as a **non-invasive tool** to detect and **monitor disease activity and progression**
- **miRNAs** could be the basis for potential **therapeutic targets**.



However,

- **Technology to use miRNAs as a diagnostic clinical tool requires optimization.**
- **Methods by which miRNAs are explored, still largely differ between published studies and standardized procedures are still lacking.**
- **Bedside tests need to be developed to allow broad utilization of miRNAs as biomarkers.**

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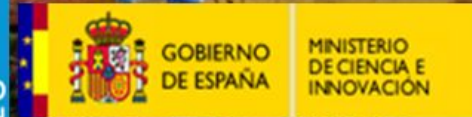
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FUNDACIÓN
HIPERCOLESTEROLEMIA
FAMILIAR



FH patients and their families
"SAFEHEART"