Cardiac miRNAs were involved in the regulation of pathophysiological mechanisms underlying HF in pediatric patients after ventricular assist device implantation

R. Ragusa[1], A. Di Molfetta[2], S. Del Turco[1], G. Basta[1], M. Rizzo[3], A. Mercatanti[1], L. Pitto[1], A. Amodeo[2], M.G. Trivella[1], C. Caselli[4]

[1]National Council of Research, Pisa, Italy; [2]Bambino Gesu Children's Hospital, Department of Cardiothoracic surgery, Rome, Italy; [3]Institute of Clinical Physiology, CNR, Tuscan Tumour Institute, Florence, Italy, Pisa, Italy; [4]National Council of Research, Pisa, Italy. Fondazione Toscana Gabriele Monasterio, Pisa, Italy, Pisa, Italy

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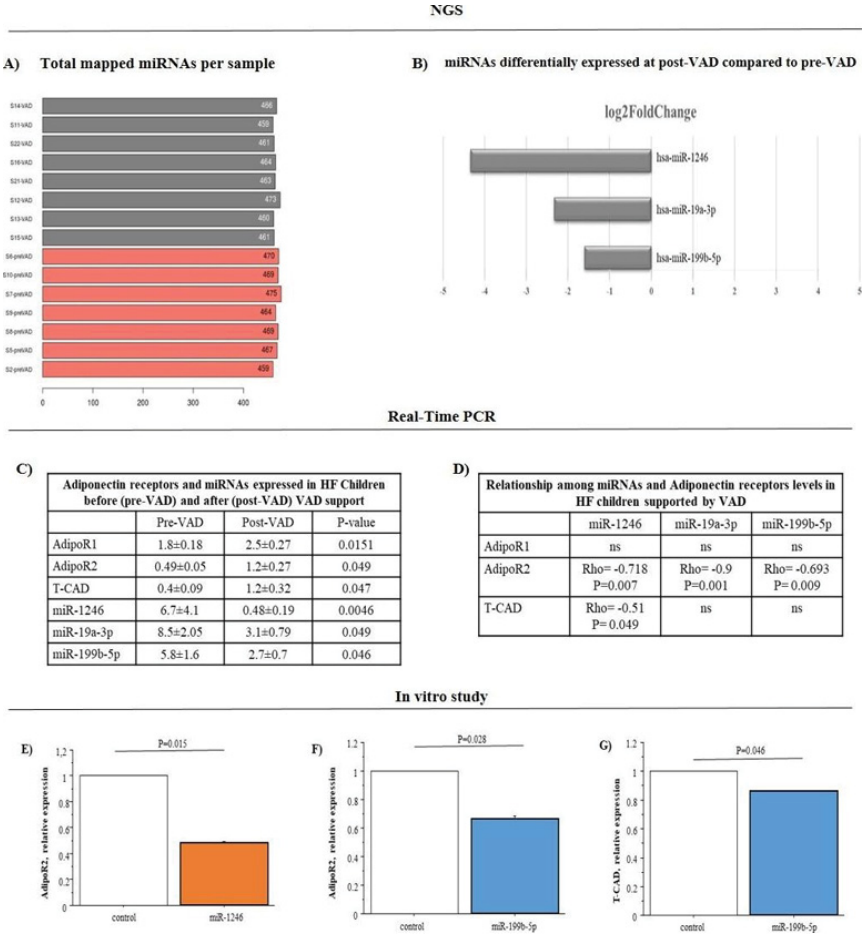
Background: VAD use in heart failure (HF) children have undergone rapid progress in the last three decades through pump technological innovation and improvement of perioperative care. Studies in HF adults showed that VAD put native heart at rest and lead to molecular changes in cardiac muscle, including at microRNA (miRNA) level. However, little is known on changes induced by VAD implant in cardiac miRNA expression and their putative targets in HF children.

Purpose: The aims of this study were to evaluate: 1) modification of miRNA expression in cardiac muscle from HF children after VAD support; 2) the putative targets of selected miRNAs by in silico analysis; 2) the role of the identify miRNAs on putative targets by in vitro study.

Methods: Cardiac biopsies were collected from HF children at the moment of VAD implant [n=8; 20 (7.5–64.5) months, 2 males; 19 (15.75–32.25) LVEF%] and at the time of heart transplant after VAD support [n=5; 32 (5–204) months; 4 males; 13.5 (10–18) LVEF%]. Cardiac miRNA expression was evaluated by NGS. The potential miRNA targets were identified by bioinformatics analyses and their cardiac expression by real-time PCR was evaluated. HL-1 cell line was used for testing the regulatory role of selected miRNA on predicted targets by miRNA mimic transfection study.

Results: At NGS, 465 miRNA were found on average in each sample and the cardiac expression levels of miR19a-3p, miR-1246 and miR-199b-5p decreased in HF children after VAD support compared to pre-implant (Fig. 1A-B). In silico analysis showed that more than 5000 potential gene targets regulated by miR-19a-3p, miR-1246 and miR-199b-5p. Among them, adiponectin receptors (AdipoR1, AdipoR2, T-CAD) were identified as common targets for 3 miRNAs. Real-time PCR data showed that levels of all adiponectin receptors increased significantly whilst the expression of 3 miRNAs decreased after VAD support (Fig. 1C). Moreover, AdipoR2 and T-CAD were inversely related to miRNA levels (Fig. 1D). In vitro studies confirmed the regulatory role of miR-1246 and miR-199b-5p on AdipoR2 (Fig. 1E-F), whilst only miR-199b-5p reduced the expression of T-CAD (Fig. 1G). Finally, AdipoR1 expression levels are not modified compared to control by miRNAs mimic transfection (data not shown).

Conclusion: In HF children the use of VAD could modify the expression of several miRNAs potentially involved in the regulation of several pathophysiological mechanisms underlying HF. Specifically, the reductions of miR-1246, mir-19a-3p, miR-199b-5p were associated with an increase of the adiponectin receptors AdipoR2 and T-CAD mRNA, suggesting the existence of a miRNAs related fine tuning of the adiponectin system at cardiac tissue level by VAD implant, able to favour the protective effect of adiponectin in HF cardiac muscle.



C)

Adiponectin receptors and miRNAs expressed in HF Children before (pre-VAD) and after (post-VAD) VAD support			
	Pre-VAD	Post-VAD	P-value
AdipoR1	1.8±0.18	2.5±0.27	0.0151
AdipoR2	0.49±0.05	1.2±0.27	0.049
T-CAD	0.4±0.09	1.2±0.32	0.047
miR-1246	6.7±4.1	0.48±0.19	0.0046
miR-19a-3p	8.5±2.05	3.1±0.79	0.049
miR-199b-5p	5.8±1.6	2.7±0.7	0.046

D)

Relationship among miRNAs and Adiponectin receptors levels in HF children supported by VAD			
	miR-1246	miR-19a-3p	miR-199b-5p
AdipoR1	ns	ns	ns
AdipoR2	Rho= -0.718 P=0.007	Rho= -0.9 P=0.001	Rho= -0.693 P= 0.009
T-CAD	Rho= -0.51 P= 0.049	ns	ns

Figure 1