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1 Review

## 2 Integrative system biology analyses identify seven 3 microRNAs to predict heart failure

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24 **Abstract:** Heart failure (HF) has several etiologies including myocardial infarction (MI) and left  
25 ventricular remodeling (LVR), but its progression remains difficult to predict in clinical practice.  
26 Systems biology analyses of LVR after MI predict molecular insights of this event such as  
27 modulation of microRNA (miRNA) that could be used as a signature of HF progression. To define  
28 a miRNA signature of LVR after MI, we use 2 systems biology approaches integrating either  
29 proteomic data generated from LV of post-MI rat induced by left coronary artery ligation or  
30 multi-omics data (proteins and non-coding RNAs) generated from plasma of post-MI patients from  
31 the REVE-2 study. The first approach predicts 13 miRNAs and 3 of these miRNAs were validated  
32 to be associated with LVR *in vivo*: miR-21-5p, miR-23a-3p and miR-222-3p. The second approach  
33 predicts 24 miRNAs among 1310 molecules and 6 of these miRNAs were selected to be associated  
34 with LVR *in silico*: miR-17-5p, miR-21-5p, miR-26b-5p, miR-222-3p, miR-335-5p and miR-375. We  
35 identified a signature of 7 microRNAs associated with LVR after MI that support the interest of  
36 integrative systems biology analyses to define a miRNA signature of HF progression.

37 **Keywords:** biomarkers; miRNAs; heart failure; system biology

38

### 39 1. Introduction

40 Heart failure (HF) is a major cause of mortality in occidental countries that is difficult to predict  
41 in clinical practice [1]. HF can be the consequence of left ventricle remodeling (LVR) induced by a  
42 myocardial infarction (MI) [2,3]. LVR is characterized by cardiac hypertrophy and reduction of LV  
43 wall. Although, LVR is an adaptive response early after MI, it is becoming deleterious in a long term  
44 [4]. Deciphering molecular events underlying LVR may offer new opportunities in the identification  
45 of early predictive biomarkers of LVR and HF. Omics approaches including transcriptomics,

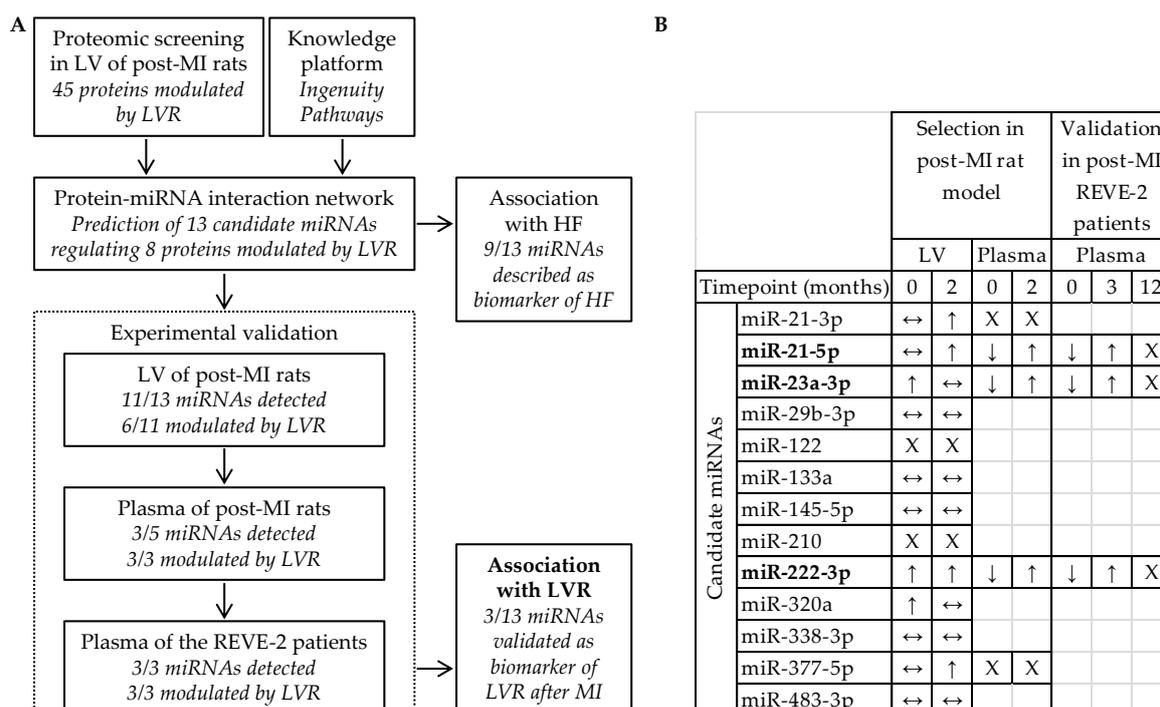
46 proteomics, and metabolomics have been extensively used to explore these mechanisms but the  
 47 amount of complex generated data prevents from their comprehensive analysis.

48 Recently, systems biology opened new opportunities to understand molecular networks and  
 49 identify new targets involved in HF [5,6]. Among those potential targets, microRNAs (miRNAs) are  
 50 non-coding RNAs of 19 to 23 nucleotides that regulate gene expression by targeting messenger  
 51 RNAs [7]. MiRNAs are involved in many processes like cardiomyocyte hypertrophy, fibroblast to  
 52 myofibroblast transformation and cell to cell communication [8–10]. The modulation of expression  
 53 of a small set of miRNAs associated with LVR may define a miRNA signature to detect this process.  
 54 The present study aims to define a signature of miRNAs associated with LVR after MI to predict HF.  
 55 We analyzed two systems biology approaches that we previously developed from post MI rat model  
 56 [10-13] and post-MI patients from the REVE-2 study [14].

57 **2. Results**

58 *2.1. Analysis of the protein-miRNA network derived from post-MI rats identified circulating miR-21-5p,*  
 59 *miR-23a-3p and miR-222-3p to be associated with LVR after MI*

60 The proteomic screening in LV of post-MI male rats [11], previously published [12,13], allowed  
 61 the identification of 45 proteins modulated by LVR. Using the Qiagen’s Ingenuity Pathway  
 62 knowledge base, we built a protein-miRNA interaction network highlighting 13 candidate miRNAs  
 63 which were prioritized to identify candidate miRNAs to detect LVR after MI (**Figure 1**).

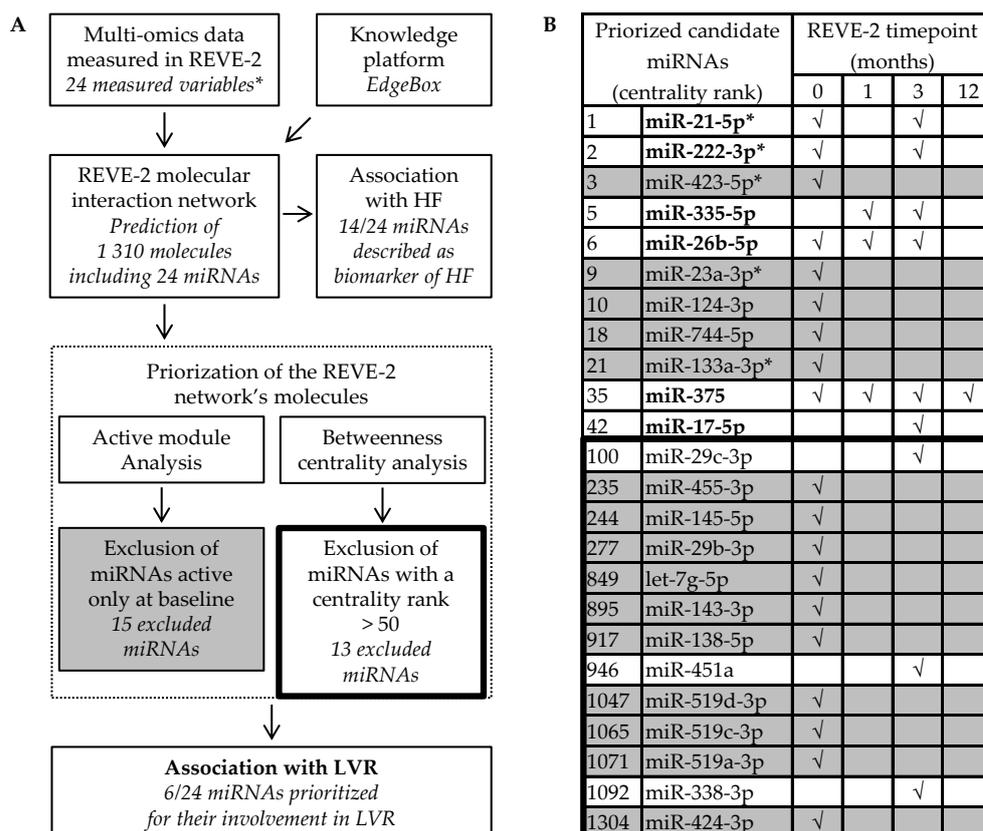


64 **Figure 1.** Identification of miR-21-5p, miR-23a-3p and miR-222-3p to detect left ventricular  
 65 remodeling (LVR) after myocardial infarction (MI) and to predict heart failure (HF). (A) Design and  
 66 (B) experimental selection and validation of the 13 candidate miRNAs predicted from the proteomic  
 67 data obtained in LV of post-MI rats by the Ingenuity Pathway knowledge platform. Quantification of  
 68 candidate miRNAs in LV and in plasma of post-MI rats at 7 days (Timepoint 0) and at 2 months and  
 69 in plasma of REVE-2 patients at baseline (Timepoint 0), 3 month and 12 months after MI was  
 70 published elsewhere [10]. ↑ and ↓ indicates respectively a significant increase and decrease of  
 71 miRNAs (p < 0.05) detected between sham- and post-MI rats / between patients with no and high  
 72 LVR, ↔ indicates no modulation, X indicates a lack of detection. MiRNAs remaining after the  
 73 validation process are in bold.  
 74

75 The 13 candidate miRNAs are predicted to interact with 8 out of the 45 proteins modulated by  
76 LVR [10], testifying that they are involved in LVR after MI. Moreover, 9 out of the 13 candidate  
77 miRNAs have been described as biomarkers of HF: miR-21-3p, miR-21-5p, miR-23a-3p, miR-29b-3p,  
78 miR-122, miR-133a, miR-145-5p, miR-222-3p and miR-320a [10,15-20], confirming their potential as  
79 targets to predict HF (**Figure 1A**). To prioritize candidate miRNAs with high relation specificity with  
80 LVR, we evaluated *in vivo* the association of the 13 candidate miRNAs with LVR after MI (**Figure**  
81 **1B**). In LV of post-MI rats, we selected 6 candidate miRNAs up-regulated by LVR at 7 days  
82 (miR-23a-3p, miR-222-3p and miR-320a) and at 2 months after MI (miR-21-3p, miR-21-5p,  
83 miR-222-3p and miR-377-5p) [10] and we excluded the 7 candidate miRNAs which were not  
84 detected (miR-122 and miR-210) or not modulated by LVR (miR-29b-3p, miR-133a, miR-145-5p,  
85 miR-338-3p and miR-483-3p). In plasma of post-MI rats, we selected 3 candidate miRNAs  
86 down-regulated by LVR at 7 days and up-regulated by LVR at 2 months (miR-21-5p, miR-23a-3p  
87 and miR-222-3p) [10] and we excluded the 3 candidate miRNAs which were not detected (miR-21-3p  
88 and miR-377-5p) or not measurable (miR-320a). In plasma of post-MI patients from the REVE-2  
89 study, we show that the 3 selected candidate miRNAs: miR-21-5p, miR-23a-3p and miR-222-3p are  
90 down-regulated by LVR at baseline and are up-regulated by LVR after 3 months, especially in men,  
91 validating their potential as circulating biomarkers of adverse LVR after MI to predict HF as  
92 previously published [10].

93 *2.2. Analysis of the REVE-2 network identified miR-21-5p, miR-222-3p, miR-335-5p, miR-26b-5p, miR-375*  
94 *and miR-17-5p to detect LVR after MI*

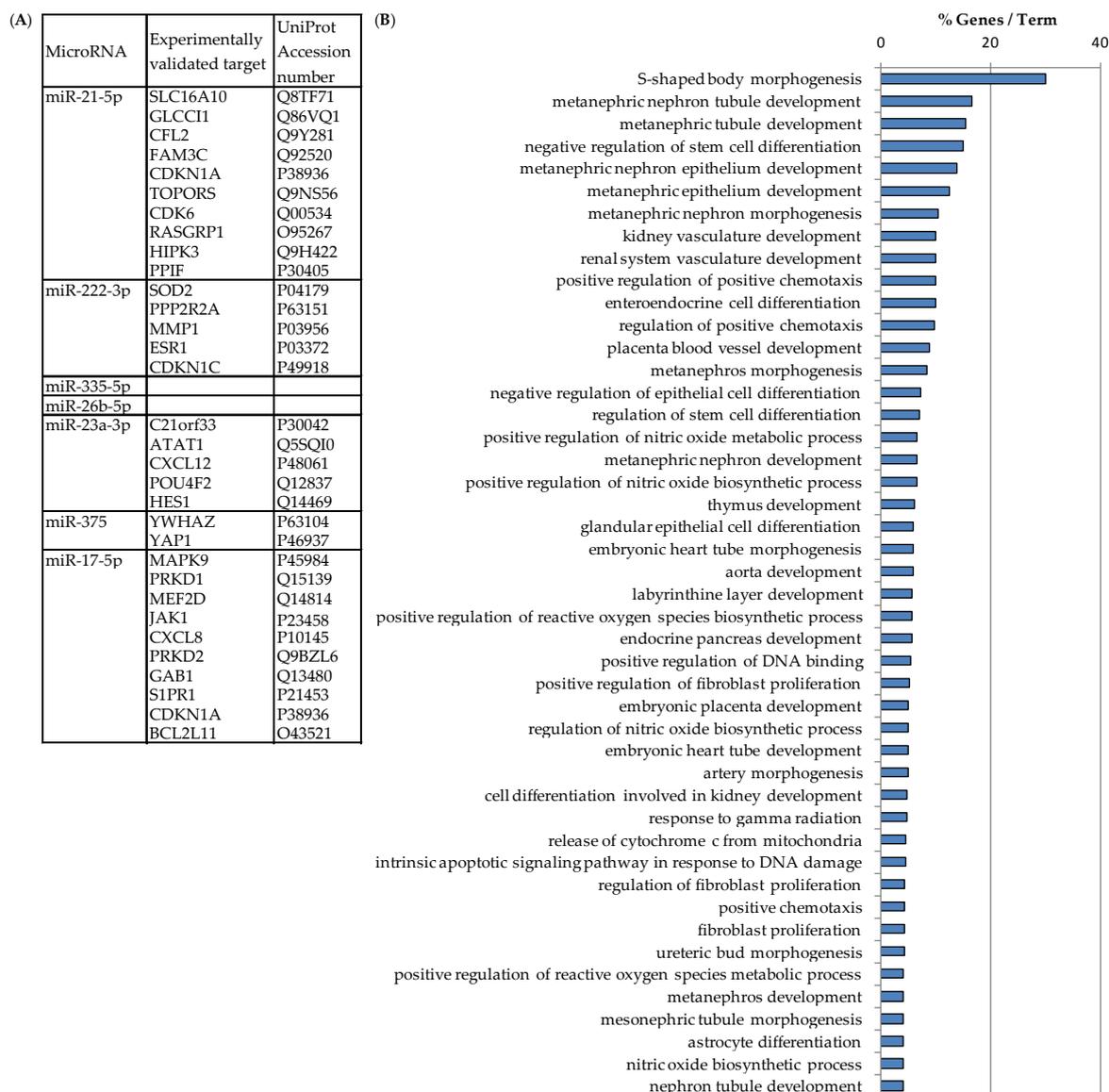
95 The REVE-2 molecular data generated by the measurement of 24 variables in the plasma of the  
96 patients from the REVE-2 study (including miR-21-5p, miR-23a-3p and miR-222-3p) and the  
97 EdgeLeap's knowledge platform EdgeBox were used to build the REVE-2 molecular interaction  
98 network described in detail elsewhere [14]. The REVE-2 network contains 1310 molecules, including  
99 24 miRNAs which were prioritized to identify candidate miRNAs to detect LVR after MI (**Figure 2**).  
100 Fourteen out of the 24 candidate miRNAs are described to be associated with HF: miR-21-5p,  
101 miR-222-3p, miR-423-5p, miR-26b-5p, miR-23a-3p, miR-744-5p, miR-133a-3p, miR-17-5p,  
102 miR-29c-3p, miR-145-5p, miR-29b-3p, let-7g-5p, miR-143-3p and miR-451a [10,15,17,18,21-24],  
103 confirming they are interesting targets to predict HF (**Figure 2A-B**). To prioritize candidate miRNAs  
104 with high relation specificity with LVR, we evaluated *in silico* the association of the 24 candidate  
105 miRNAs with LVR after MI through 2 criteria: active modules and betweenness centrality (**Figure**  
106 **2A**). To avoid the selection of miRNAs associated with mechanisms not specific of LVR such as  
107 inflammation [14], we excluded the 15 candidate miRNAs active only at baseline. However, it could  
108 be interesting to analyze the miRNAs only active at baseline as potential biomarker of early LVR. To  
109 avoid the selection of miRNAs less significant in LVR, we excluded 13 miRNAs that were not in the  
110 top 50 molecules with the highest centrality. When combining these 2 criteria, among the 24  
111 candidate miRNAs, only 6 remained: miR-21-5p, miR-222-3p, miR-335-5p, miR-26b-5p, miR-375 and  
112 miR-17-5p (**Figure 2B**). Interestingly, the 6 remaining candidate miRNAs are predicted to be active  
113 at least at 3 months after MI, when only specific mechanisms of LVR seems to be effective [14]. To  
114 date, only miR-21-5p and miR-222-3p, also identified by the first approach, were validated *in vivo*  
115 to detect LVR after MI [10], testifying that the signature defined by the 6 last candidate miRNAs may  
116 be used as circulating biomarker of adverse LVR after MI to predict HF.



**Figure 2.** Identification of miR-21-5p, miR-222-3p, miR-335-5p, miR-26ba-3p, miR-375 and miR-17-5p to detect left ventricular remodeling (LVR) after myocardial infarction (MI) and to predict heart failure (HF). (A) Selection process and (B) prioritization analysis of the 24 miRNAs predicted from the multi-omic data obtained in the 226 patients from the REVE-2 study by the EdgeBox knowledge platform. √ indicates that miRNA is predicted to be active at the corresponding timepoint: baseline (0), 1 month, 3 month and 12 months after MI. MiRNAs only active at baseline (grey) and with a betweenness centrality rank lower than 50 (inside the thick line) were excluded from further investigation because they are not expected to be highly involved in LVR after MI. \* indicates REVE-2 variables. MiRNAs remaining after the selection process are in bold.

### 2.3. Gene ontology analysis of the 7 miRNAs targets predicted with a high relation specificity with processes involved in LVR after MI

An analysis of the 7 miRNAs targets was performed using the ClueGO [25] and CluePedia [26] applications of Cytoscape (version 3.4.0). The applications used the miRecords database to identify the experimentally validated targets of each miRNA which were submitted to Gene Ontology enrichment (Figure 3).



133  
 134 **Figure 3.** Functional annotation enrichment analysis of the 7 miRNA’s targets. (A) Targets of  
 135 miRNAs were predicted by miRecords database. No targets have been described for miR-335-5p and  
 136 miR-26b-5p. (Bb) Biological processes of miRNA’s target genes were predicted by Cytoscape plugin  
 137 ClueGO and Cluepedia applications (p < 0.05).

138 Thirty-one targets were predicted to interact with 5 out of 7 miRNAs (miR-21-5p, miR-222-3p,  
 139 miR-23a-3p, miR-375 and miR-17-5p) (**Figure 3A**). The 2 remaining miRNAs (miR-335-5p and  
 140 miR-26b-5p) had no validated target in miRecords. We can observe that the miRNA’s targets are  
 141 involved in pathways involved in LVR development such as fibroblast proliferation, regulation of  
 142 reactive oxygen species metabolism and intrinsic apoptotic signaling pathways, but also embryonic  
 143 heart development as embryonic heart tube development and aorta development (**Figure 3B**). These  
 144 results testify of the involvement of the 7 miRNAs in cardiac development and LVR processes.

145 **3. Discussion and Perspectives**

146 Systems biology approaches have been shown to improve the biomarker discovery in  
 147 comparison with traditional omics approaches by allowing the selection of biomarkers with a  
 148 biological relevance in the pathology [5,6]. In this study, we described two systems biology analyses  
 149 integrating both omics data to identify candidate miRNAs associated with LVR after MI and to  
 150 predict HF. As interaction networks rely on the use of public databases, prediction of miRNA  
 151 interactions are not always experimentally validated and may give an approximate vision of the

152 complex mechanisms underlying LVR [27,28]. To avoid this issue, predicted miRNA interactions  
153 were either experimentally validated or predicted by at least 3 different databases in this study.  
154 Moreover, while most systems biology approaches often integrate a single timepoint, we used  
155 different timepoints to integrate the molecular events underlying LVR progression. Finally, both  
156 approaches include a selection of candidate miRNAs associated with LVR after MI either by  
157 experimental validation *in vivo* or by *in silico* prioritization before validation in post-MI patients.

158 The first approach, derived from post-MI rat model, predicted 13 candidate miRNAs and 3 of  
159 these candidate miRNAs were selected to be associated with LVR *in vivo* both in LV and in plasma of  
160 post-MI rats: miR-21-5p, miR-23a-3p and miR-222-3p. In this first approach, candidate miRNAs  
161 were selected based on the hypothesized behavior of circulating miRNAs as endocrine signal, but  
162 our results show that candidate miRNAs does not have necessarily the same profile in LV and in  
163 plasma, testifying that miRNAs secreted by LV are quantitatively and qualitatively different from  
164 their intracellular profile in LV. Moreover, although circulating miRNA profile in HF rat models  
165 may not reflect human profile [29], we validated the association of miR-21-5p, miR-23a-3p and  
166 miR-222-3p with LVR after MI both in plasma of HF rat model and HF patients, testifying that these  
167 3 selected miRNAs define a conserved miRNA signature of LVR. The second approach predicted 24  
168 candidate miRNAs among 1310 molecules and 6 of these miRNAs were selected to be associated  
169 with LVR *in silico*: miR-17-5p, miR-21-5p, miR-26b-5p, miR-222-3p, miR-335-5p and miR-375.  
170 Although miR-21-5p and miR-222-3p were validated by the first approach, miR-335-5p, miR-26b-5p,  
171 miR-375 and miR-17-5p remain to be validated. Altogether, these two integrative systems biology  
172 analyses identified a signature of 7 microRNAs associated with LVR after MI and more especially  
173 with mechanisms underlying LVR, such as apoptosis, oxidative stress and fibroblasts proliferation.

174 MiR-21-5p is one of the most studied miRNAs in cardiovascular diseases. Indeed, it was shown  
175 that the failing heart was able to release miR-21-5p into the circulation [18]. MiR-23a-3p is less  
176 studied. However, a correlation was described between miR-23a and pulmonary function of patients  
177 with idiopathic pulmonary hypertension [30]. MiR-23a was also shown to regulate cardiomyocyte  
178 apoptosis by targeting SOD2 mRNA [31]. MiR-222-3p is known to regulate SOD2 expression in HF  
179 patients [10] and to be involved in the inhibition of myocardial fibrosis by targeting TGF $\beta$  [32].  
180 Recently, we showed that miR-21-5p, miR-23a-3p and miR-222-3p were decreased in the plasma of  
181 patients with high LVR at baseline and increased at one year [10]. MiR-335-5p has not been yet  
182 linked with cardiovascular diseases and it has been described as a potential biomarker of  
183 osteosarcoma in children [33] and of osteoporosis [34]. MiR-375 has been shown to be mainly  
184 expressed in the developing heart and to a lower extent in the adult heart [35] and it is also increased  
185 in the plasma of pregnant women with fetal congenital heart defects [36]. MiR-26b-5p and miR-17-5p  
186 are well known to be involved in the cardiovascular system. MiR-26b-5p has been shown to be  
187 modulated in plasma of patients presenting major cardiovascular events [23]. MiR-17-5p was shown  
188 to be increased in the plasma of patients with hypertrophic cardiomyopathy and diffuse myocardial  
189 fibrosis [24]. These results testify that miR-21-5p, miR-23a-3p, miR-222-3p, miR-320a, miR-335-5p,  
190 miR-26b-5p, miR-375 and miR-17-5p may be interesting targets to predict HF.

191 In conclusion, we highlighted the interest of integrative systems biology analyses to define a  
192 miRNA signature of LVR to predict HF. However, even though the building of molecular networks  
193 relies on experimental data, we showed that miRNA signature still need to be experimentally  
194 validated to be relevant *in vivo*.

195 Our study has some limitations: further experiments should be done in order to understand  
196 why there is no correlation for some miRNAs between the intracellular (LV) and extracellular  
197 (plasma) miRNA profiles. It would be important to determine the cellular origin (fibroblasts,  
198 cardiomyocytes, endothelial cells,..) of miRNAs and the characterization of their transporters from  
199 the intracellular to extracellular medium.

## 200 4. Methods

### 201 4.1. Experimental model of HF in rats

202 All animal experiments were performed according to the Guide for the Care and Use of  
203 Laboratory Animals published by the US National Institutes of Health (NIH publication  
204 NO1-OD-4-2-139, revised in 2011). Experimental protocols were performed under the supervision of  
205 a person authorized to perform experiments on live animals (F. Pinet: 59-350126 and E.  
206 Dubois-Deruy: 59-350253). Approval was granted by the institutional ethics review board (CEEA  
207 Nord Pas-de-Calais N°242011, January 2012). MI was induced as previously described [10, 11] by  
208 permanent left anterior descending coronary artery ligation in 10 weeks old Wistar male rats.  
209 Hemodynamic and echocardiographic measurements were performed to detect LVR, at 7 days (n=8  
210 in sham- and MI) and 2 months (n=8 in sham- and MI) after surgery, followed by non-infarcted area  
211 of LV sampling and plasma sampling.

#### 212 4.2. The REVE-2 study

213 The REVE-2 study is a prospective multicenter study to analyze the association between  
214 circulating biomarkers and LVR and has been previously detailed [3]. Briefly, REVE-2 study  
215 included 226 patients with a first anterior wall Q-wave MI between February 2006 and September  
216 2008. The research protocol was approved by the Ethics Committee of the Centre Hospitalier et  
217 Universitaire de Lille, and each patient provided written informed consent. Serial echocardiographic  
218 studies were performed at hospital discharge (baseline), 3 months and one year after MI and were  
219 used to assess LVR. LVR was defined as a >20% increase in end diastolic volume from baseline to 1  
220 year. Serial blood samples were taken at 4 timepoint: baseline, 1 month, 3 months and 12 months.  
221 Twenty-four molecular variables were measured in the REVE-2 plasma at 1 to 4 timepoints.

#### 222 4.3. Quantification of candidate miRNAs

223 RNAs were extracted from non-infarcted area of LV of rats and from plasma of rats and REVE-2  
224 patients as described elsewhere [10]. Candidate miRNAs were quantified and normalized with  
225 miR-423-3p in LV and with *Caenorhabditis elegans* Cel-39 in plasma as described elsewhere [10].

#### 226 4.4. Functional analysis of miRNAs targets

227 MiRNA target prediction was performed using the ClueGO (version 2.5.2  
228 <http://apps.cytoscape.org/apps/cluego>) [25] and CluePedia (version 1.5.2  
229 <http://apps.cytoscape.org/apps/cluepedia>) applications [26] of Cytoscape software (version 3.4.0  
230 <http://www.cytoscape.org/>). Only validated miRNAs targets from the miRecords database were  
231 selected. A Gene Ontology (GO) enrichment analysis for Biological Processes was performed for all  
232 the miRNAs targets using the ClueGO application of Cytoscape software. *P*-value was set at 0.05,  
233 and corrected for multiple testing using Benjamini-Hochberg adjustment.

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237 substitute of COST action cardioRNA (CA1229).

238 **Author Contributions:** F.P. and C.B. conceived and designed the experiments; H.C., M.C., E.D.D. and P.M.  
239 performed the experiments; H.C., M.C., E.D.D., P.M., V.R., C.B. and F.P. analyzed the data; H.C., M.C., E.D.D.,  
240 C.B. and F.P. wrote the manuscript.

241 **Conflicts of Interest:** The authors declare no conflict of interest.

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