A Micro-Ribonucleic Acid Signature Associated With Recovery From Assist Device Support in 2 Groups of Patients With Severe Heart Failure

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Objectives
This study was conducted to test the hypothesis that cardiac micro-ribonucleic acid (miR) profiling in severe heart failure patients at the time of ventricular assist device (VAD) placement would differentiate those who remained VAD-dependent from those with subsequent left ventricular (LV) recovery.

Background
The relationship of myocardial miR expression to ventricular recovery is unknown.

Methods
We studied 28 patients with nonischemic cardiomyopathy requiring VAD support consisting of test and validation cohorts from 2 institutions: 14 with subsequent LV recovery and VAD removal and 14 clinically matched VAD-dependent patients. Apical core myocardium was studied for expression of 376 miRs by polymerase chain reaction (PCR) array and real-time-PCR methods. Samples from 7 nonfailing hearts were used in confirmatory studies.

Results
By PCR array, 10 miRs were differentially expressed between LV recovery and VAD-dependent patients in the test cohort. The real-time PCR confirmed lower expression in LV recovery patients for 4 miRs (15b, −1.5-fold; 23a, −2.2-fold; 26a, −1.4-fold; and 195, −1.8-fold; all p < 0.04 vs. VAD dependent). The validation cohort similarly showed lower miRs expression in LV recovery patients (23a, −1.8-fold; and 195, −1.5-fold; both p < 0.03). Furthermore, miR 23a and 195 expression in nonfailing hearts was similar to LV recovery patients (both p < 0.04 vs. VAD dependent). The LV recovery patients also had significantly smaller cardiomyocytes by quantitative histology in both cohorts.

Conclusions
Lower cardiac expression of miRs 23a and 195 and smaller cardiomyocyte size at the time of VAD placement were associated with subsequent LV functional recovery. Differential expression of miRs at VAD placement may provide markers to assess recovery potential. (J Am Coll Cardiol 2011;58:2270–8) © 2011 by the American College of Cardiology Foundation

Despite the beneficial effects of mechanical unloading achieved after ventricular assist device (VAD) placement on molecular markers and cardiomyocyte structure and function in the failing heart (1–7), only a small number of VAD-supported patients recover sufficient function to allow permanent removal of the VAD (8–10). Current data suggest that an unappreciated percentage of VAD-supported patients have a potential for recovery but may not be adequately tested for the ability to permanently and successfully remove mechanical support (9,10). Clinical variables may assist in the prediction of recovery

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(9–12) but there are few studies that identify biochemical markers associated with functional recovery sufficient to allow device removal (13,14).

Micro-ribonucleic acids (miRs) are short (21 to 23 nucleotides long) noncoding ribonucleic acids (RNAs) that may play potent and widespread roles in post-transcriptional regulation of gene expression. These miRs affect diverse pathways including apoptosis, cell growth and proliferation, oncogene suppression and activation, and stem cell activation (15). Recent human and animal studies have shown significant alterations in cardiac miR expression patterns with heart failure, while others demonstrated miR involvement in key heart failure pathways (16–24).

In this study, we investigated patterns of cardiac miR expression at the time of VAD placement to identify patterns associated with functional recovery from severe heart failure in VAD-supported patients. We also investigated whether this “recovery potential” expression profile was induced in failing hearts by introduction of VAD support. Finally, we assessed this miR profile in nonfailing control hearts.

**Methods**

**Patient selection and tissue collection.** All studies were performed under protocols approved by the University of Pittsburgh, Texas Heart Institute, and Cleveland Clinic Foundation institutional review boards. **Test cohort.** The test cohort consisted of 14 heart failure patients with LV tissue samples (cores) obtained and banked at the time of VAD placement. The recovered group (n = 7) consisted of patients who recovered sufficient cardiac function to allow VAD removal. Recovered patients were compared with VAD-implanted patients who remained VAD dependent (dependent group, n = 7), and who were retrospectively matched for clinical variables associated with recovery so as to closely approximate those variables in the recovered group patients. Patients with ischemic heart disease were excluded from all groups reported in this study. Of the 7 explanted and discharged patients, 1 had unexplained death ~1.5 months after discharge and 1 died ~3 months post-explantation due to *Staphylococcus aureus* mediastinitis with preserved cardiac function. The remaining 5 have survived free of heart failure >6 months. **Validation cohort.** The validation cohort tissue provided by the Texas Heart Institute arose from 7 recovered patients and 7 dependent patients (as defined for the test cohort, and matched for clinical variables). All 7 explanted patients survived and were free of heart failure symptoms for >6 months. **Patient selection pre- and post-VAD.** Paired LV samples, collected at the time of VAD implantation (pre-VAD) and cardiac transplantation (post-VAD), were obtained from an independent third group of 6 patients (University of Pittsburgh) with severe heart failure from nonischemic cardiomyopathy. **Nonfailing hearts.** Nonfailing human heart tissue (non-failing) was obtained from the Cleveland Clinic Foundation.

**Statistical analyses.** The miR expression data are presented as fold up or down regulation in the dependent group versus recovered (26). A negative value indicates lower expression in the recovered group. For confirmatory PCR, data are expressed as fold up or down expression in dependent versus recovered, or between pre-VAD and post-VAD samples, or normalized to the mean of nonfailing samples for nonfailing versus recovered or dependent. Results were compared between groups by a nonparametric 1-way analysis of variance (Kruskal-Wallis test). Upon detection of overall significance, limited hypothesis driven post-hoc analyses were performed using Mann-Whitney *U* test. For pre-VAD versus post-VAD, a paired *t* test was employed. All data are reported as mean ± SD. Significance was accepted at *p* < 0.05.

**Results**

**Patient characteristics.** Within each cohort (Table 1), parameters at the time of VAD implantation were not significantly different between recovered and dependent groups. However, there were significant differences between the cohorts: the validation cohort had more male patients, greater LV diameters, a longer duration of heart failure before VAD implantation, longer time on VAD support, and more patients on rotary VAD support. Table 1 also lists clinical parameters while on VAD support, and follow-up LV ejection fraction 6 months after VAD explantation in the recovered groups of both cohorts. Test and validation cohorts differed in the duration of support and EF at 6 months post-explantation. **Screening real-time PCR array.** In the test cohort, 6 of 7 patients in each group were first analyzed by real-time PCR array for miR expression. Of 376 miRs studied in the test cohort, 141 were expressed at a level of ≤35 cycle threshold (Ct) in at least 1 VAD core sample; 108 were detected in
every VAD core sample at a level of ≤35 Ct. Ten miRs were differentially expressed between the recovered and dependent groups (p < 0.05) (Fig. 1, Table 2). The miRs previously reported to play roles in heart failure relevant pathways were not differentially expressed (miRs 1, 21, 23a, 195, and 10th miR (181b) could not be confirmed in the test cohort because of limitation of RNA availability but was tested in the validation cohort. In the seventh patient from each group of the test cohort, Taqman PCR was used to measure the expression of 8 miRs (1, 15b, 26a, 133a, 133b, 195), and the reference small RNA RNU48.

**Validation cohort.** The 4 miRs confirmed to be differentially expressed in the test cohort, along with miR 181b, were similarly measured in the validation cohort. The miRs 23a and 195 had significant differential expression (Table 2, Fig. 3). Expression of miRs 15b, 26a, and 181b was not significantly different, although the direction of change was the same as in the test cohort. Combining the 2 datasets demonstrated that miR 15b was also differentially expressed (p < 0.03).

**Comparison with nonfailing heart tissue.** The 2 miRs, 23a and 195, found differentially expressed in both test and validation cohorts were measured in nonfailing LV tissue. Expression of both miR 23a and 195 was significantly increased in the dependent groups compared with nonfailing or recovered samples, but was similar between nonfailing and recovered group in both cohorts (Fig. 4).

**Cardiomyocyte size determination.** Fluorescent microscopy of wheat germ agglutinin stained cardiomyocytes from the test cohort demonstrated that the recovered group had a significantly smaller cardiomyocyte cross-sectional areas when compared with the dependent group (Fig. 5A). Independent examination by light microscopy of hematoxylin and eosin stained samples show that the recovered group had significantly smaller cardiomyocyte diameters when compared with the dependent group (Fig. 5B).

**Effects of VAD support on selected miRs.** To determine whether miRs that were differentially expressed between

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**Table 1** Characteristics of Patients Demonstrating Clinical Similarity Between Recovered and Dependent Groups Before VAD Implantation in Both Test and Validation Cohorts

<table>
<thead>
<tr>
<th>Clinical Characteristics at Baseline</th>
<th>Test Cohort</th>
<th>Validation Cohort</th>
<th>p Value</th>
<th>Test vs. Validation Cohorts</th>
<th>All VAD vs. Nonfailing Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recovered</td>
<td>Dependent</td>
<td>p Value</td>
<td>Recovered</td>
<td>Dependent</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>7</td>
<td>NS</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>40 ± 12</td>
<td>43 ± 15</td>
<td>NS</td>
<td>27 ± 8</td>
<td>33 ± 9</td>
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<tr>
<td>Percent ischemic</td>
<td>0</td>
<td>0</td>
<td>NS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Inotropic support</td>
<td>100%</td>
<td>100%</td>
<td>NS</td>
<td>85%</td>
<td>100%</td>
</tr>
<tr>
<td>Intra-aortic balloon pump</td>
<td>43%</td>
<td>57%</td>
<td>NS</td>
<td>43%</td>
<td>14%</td>
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<tr>
<td>Other mechanical support</td>
<td>0%</td>
<td>0%</td>
<td>NS</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>50%</td>
<td>50%</td>
<td>NS</td>
<td>71%</td>
<td>71%</td>
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<td>EF before VAD, %</td>
<td>18 ± 5</td>
<td>15 ± 5</td>
<td>NS</td>
<td>18 ± 5</td>
<td>17 ± 5</td>
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<td>LVEDD before VAD, cm</td>
<td>6.0 ± 0.8</td>
<td>6.2 ± 0.8</td>
<td>NS</td>
<td>7.0 ± 1.0</td>
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<td>PCWP before VAD, mm Hg</td>
<td>26 ± 9</td>
<td>27 ± 10</td>
<td>NS</td>
<td>27 ± 5</td>
<td>27 ± 11</td>
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<td>Cardiac index before VAD, l/min/m² s</td>
<td>1.9 ± 0.89</td>
<td>2.0 ± 0.73</td>
<td>NS</td>
<td>1.6 ± 0.24</td>
<td>1.8 ± 0.41</td>
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<td>Duration of CHF, days</td>
<td>62 ± 49</td>
<td>75 ± 58</td>
<td>NS</td>
<td>680 ± 1117</td>
<td>771 ± 802</td>
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<tr>
<td>Type of VAD, % rotary</td>
<td>14%</td>
<td>14%</td>
<td>NS</td>
<td>71%</td>
<td>42%</td>
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<tr>
<td>Days on VAD</td>
<td>53 ± 31</td>
<td>61 ± 29</td>
<td>NS</td>
<td>433 ± 250</td>
<td>369 ± 167</td>
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**Clinical Characteristics at 6-Month Follow-Up Post-VAD Explantation**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Beta-blocker use while on VAD</th>
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<th>7</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>67%</td>
<td>83%</td>
<td>100%</td>
<td>85%</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>67%</td>
<td>83%</td>
<td>100%</td>
<td>85%</td>
</tr>
</tbody>
</table>

Values are n, mean ± SD, or n (%). Duration of congestive heart failure was defined as the time in days from original presentation with heart failure until ventricular assist device (VAD) placement. Days on VAD was defined as time to VAD explant (recovered group) or transplantation (dependent group). All patients on pulsatile VAD support were on Thoratec (Pleasanton, California) extracorporeal devices. *Pulmonary capillary wedge pressure and cardiac index measured immediately before VAD implantation, while the patient was receiving inotropes. **ACE = angiotensin-converting enzyme; CHF = congestive heart failure; EF = ejection fraction; LVEDD = left ventricular end-diastolic dimension; NA = not applicable; NS = not significant; PCWP = pulmonary capillary wedge pressure.
recovered and dependent core samples were also responsive to mechanical unloading, the expression of select miRs was measured in a separate set of 6 paired cardiac samples obtained at the time of VAD implantation and subsequent cardiac transplantation. Patient characteristics are listed in Table 3.

The VAD support did not alter expression of any of the miRs found to be differentially expressed between the recovered and dependent groups (Table 4). To assess whether mechanical unloading alters expression of other miRs, additional miRs previously shown to play a role in heart failure (15–24) were analyzed by real-time PCR. Whereas miRs 208 and 21 showed altered expression in response to mechanical support (Table 4), miRs 1, 133a, and 133b did not. None of these miRs, however, was differentially altered between recovered and dependent groups.

**Discussion**

We report, for 2 separate patient cohorts, a pattern of myocardial miR expression at the time of VAD placement that is associated with subsequent LV functional recovery. The main findings in this study are as follows. 1) In patients with advanced nonischemic heart failure requiring VAD support, cardiac expression of miR 23a and 195 before VAD implantation differs between hearts with eventual recovery of ventricular function and hearts with persistent VAD dependence. 2) This differential expression is associated with remission of heart failure independent of clinical...
parameters previously associated with recovery, such as left ventricle size, duration of heart failure, and duration of VAD support. 3) Remission of heart failure is associated with a smaller cardiomyocyte size at the time of VAD placement. The experimental protocol and main findings are summarized in Figure 6.

### Figure 2
**Recovered Patients in Test Cohort Have Decreased Expression of Selected miRs**

Individual micro-ribonucleic acids (miRs) found differentially expressed in the screening array were tested using individual polymerase chain reaction (n = 7 per group). MiRs 15b, 23a, 26a, and 195 were confirmed to be differentially expressed. *p < 0.04.

### Figure 3
**Recovered Patients in Validation Cohort Have Decreased Expression of miRs 23a and 195**

Micro-ribonucleic acids (miRs) confirmed to be differentially expressed in the test cohort were tested in the validation cohort (n = 7 per group). MiRs 23a and 195, but not 15b and 26a, were differentially expressed. *p < 0.03.
As myocardial transcriptomic biomarkers can associate with outcomes in heart failure (27), we hypothesized that biomarkers (such as miR expression patterns) exist at the time of VAD implantation that are associated with the potential for recovery. We analyzed cardiac miR expression in patients selected to match clinical variables between the recovered and dependent groups within each cohort (test and validation). However, the 2 cohorts differed significantly in clinical features typically associated with the development of VAD independence. These differences between the test and validation cohorts make the observation of a conserved relationship between miR expression and the development of VAD independence particularly noteworthy.

**Figure 4** Expression of miRs 23a and 195 Is Similar in Nonfailing Hearts Compared With Recovered But Significantly Increased in Dependent Versus Nonfailing

(A and B) Data from the test cohort. (C and D) Data from the validation cohort. *p < 0.04 dependent versus recovered; †p < 0.03 dependent versus nonfailing control hearts. miR = micro-ribonucleic acid.

**Figure 5** Recovered Patients Have Smaller Cardiomyocytes at the Time of VAD Implantation

(A) Representative images (×20) of wheat germ agglutinin stained (green [nuclei blue]) ventricular assist device (VAD) core samples from the test cohort recovered and dependent samples, with calculated cross-sectional area in μM² (n = 3 per group, *p = 0.01). (B) Representative images (hematoxylin and eosin) and measurements of cardiomyocyte diameter (μM) in the validation cohort recovered and dependent samples (n = 7 per group, *p = 0.01).
Patients who recovered may have had less severe heart failure, a different disease etiology that allows greater likelihood of recovery, or genetic variants that underlie decreased expression of particular miRs that mediate pathophysiologic processes of heart failure, or the response to mechanical unloading. The observation that recovered patients had miRs 23a and 195 levels that resembled that of nonfailing hearts and a smaller cardiomyocyte size than dependent hearts raises the important possibility that the recovered patients had less severe heart failure. Although we cannot exclude this possibility, we think that this is unlikely given that the standard clinical measures of heart failure (Table 1) were identical in recovered and dependent groups from both cohorts. Regarding the role of disease etiology, cardiac miR expression profiles differ according to heart failure etiologies (24). However, we carefully matched the recovered and dependent groups for clinical parameters and etiology so as to prevent bias with diseases (such as myocarditis, peripartum cardiomyopathy) having a higher capacity for recovery (Online Table 1). Finally, as the recovery population is only a small percentage of all VAD-supported patients, it is plausible that these patients possess infrequent miR gene variants whereby decreased miR expression is associated with recovery from heart failure, or beneficial response to mechanical unloading. Indeed, expression quantitative trait loci have been found for ~20% of miRs expressed in fibroblasts (34), and miR single-nucleotide polymorphism have been associated with congenital heart diseases susceptibility (35) and dilated cardiomyopathy incidence (36).

**Study limitations.** A major limitation of this observational study is that the list of differentially expressed miRs does not provide insight into the mechanistic processes associated

<table>
<thead>
<tr>
<th>miR ID</th>
<th>Selection Criteria</th>
<th>Fold Change Pre-/Post-VAD</th>
<th>p Value</th>
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<tbody>
<tr>
<td>miR-181b</td>
<td>†</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>miR-424</td>
<td>†</td>
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<td>NS</td>
</tr>
<tr>
<td>miR-376a</td>
<td>†</td>
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<td>NS</td>
</tr>
<tr>
<td>miR-195</td>
<td>†</td>
<td>No difference</td>
<td>NS</td>
</tr>
<tr>
<td>miR-27a</td>
<td>†</td>
<td>No difference</td>
<td>NS</td>
</tr>
<tr>
<td>miR-23a</td>
<td>†</td>
<td>No difference</td>
<td>NS</td>
</tr>
<tr>
<td>miR-103</td>
<td>†</td>
<td>No difference</td>
<td>NS</td>
</tr>
<tr>
<td>miR-26a</td>
<td>†</td>
<td>No difference</td>
<td>NS</td>
</tr>
<tr>
<td>miR-142-3p</td>
<td>†</td>
<td>No difference</td>
<td>NS</td>
</tr>
<tr>
<td>miR-15b</td>
<td>†</td>
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<td>NS</td>
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<tr>
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<td>NS</td>
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<tr>
<td>miR-21*</td>
<td>Literature</td>
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<td>0.04</td>
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<tr>
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<td>NS</td>
</tr>
<tr>
<td>miR-133b*</td>
<td>Literature</td>
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<td>NS</td>
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<tr>
<td>miR-208*</td>
<td>Literature</td>
<td>1.9</td>
<td>0.02</td>
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</table>

*Micro-ribonucleic acids (miRs) reported as playing key roles in heart failure (15–24) were tested for differential expression in a separate group with paired samples obtained before ventricular assist device (VAD) implantation and at the time of transplantation. †MIrs differentially expressed in recovered hearts by screening array were not altered after VAD placement in this group. NS = not significant.
with recovery. One approach to find insight into mechanistic processes would be to use target prediction algorithms (e.g., Targetscan or Pictar) to develop a list of possible messenger RNA targets. However, when analyzing miRs 23a and 195, we found little congruency in either the list of potential messenger RNA targets identified by the 2 target prediction programs, or the biologic pathways/processes identified through multiple pathway analysis programs (data not shown). The lack of convergent results may reflect either the current limitations of these bioinformatic methods, or the need to identify additional miRs that are differentially expressed between the recovered and dependent groups. Indeed, because of the rarity of VAD-supported patients who recover cardiac function (8–10), the number of patients studied is small, and likely underpowered for detection of all differentially expressed miRs.

**Conclusions**

In summary, we report cardiac miR expression differences that may identify which patients have the greatest potential for recovery after VAD support. Such patients may have less severe disease, unrecognized disease etiologies that allow for recovery, or genetic variants that relate miR expression to functional recovery. This “recovery” expression profile is not induced by mechanical unloading, suggesting that the patients’ potential for recovery is already established at the time of VAD placement. If confirmed in a larger cohort, such information could help guide clinical decisions regarding utilization of mechanical support devices.

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REFERENCES


Key Words: cardiomyopathy • heart assist device • hypertrophy • microRNA.