Articles in PresS. Physiol Genomics (August 21, 2007). doi:10.1152/physiolgenomics.00144.2007

Altered microRNA expression in human heart disease

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Abstract

MicroRNAs are recently discovered regulators of gene expression, and are becoming increasingly recognized as important regulators of heart function. Genome-wide profiling of microRNAs in human heart failure has not been reported previously. We measured expression of 428 microRNAs in 67 human left ventricular samples belonging to control (n=10), ischemic cardiomyopathy (ICM, n=19), dilated cardiomyopathy (DCM, n=25), or aortic stenosis (AS, n=13) diagnostic groups. miRNA expression between disease and control groups was compared by ANOVA with Dunnett's post hoc test. We controlled for multiple testing by estimating the false discovery rate. Out of 428 microRNAs measured, 87 were confidently detected. 43 were differentially expressed in at least one disease group. In supervised clustering, microRNA expression profiles correctly grouped samples by their clinical diagnosis, indicating that microRNA expression profiles are distinct between diagnostic groups. This was further supported by class prediction approaches, in which the class (control, ICM, DCM, AS) predicted by a microRNA-based classifier matched the clinical diagnosis 69% of the time ($p < 0.001$). These data show that expression of many microRNAs is altered in heart disease, and that different types of heart disease are associated with distinct changes in microRNA expression. These data will guide further studies of the contribution of microRNAs to heart disease pathogenesis.

Keywords: heart failure; gene expression; expression profiling

Introduction

Pathological changes in cardiomyocyte gene expression lead to cardiomyocyte hypertrophy and impaired cardiomyocyte survival and contraction, ultimately resulting in heart failure (12, 14). However, the molecular mechanisms that regulate gene expression in cardiac hypertrophy and failure remain incompletely understood.

MicroRNAs (miRNAs) are recently discovered, post-transcriptional regulators of gene expression (reviewed in refs. 1, 2). These ~22 nucleotide RNAs make complementary base-pairing interactions with the 3' untranslated regions (3' UTRs) of target genes, negatively regulating target gene mRNA stability or translation into protein. Each miRNA is estimated to influence expression of hundreds of target genes, thereby regulating key cellular processes including proliferation, survival, and differentiation. Altered miRNA expression has been implicated in oncogenesis and neural disease (1, 2, 7). Out of 475 currently described human miRNAs (9), three (miR-1, miR-133, and miR-208) are highly enriched in the heart (3, 11) and are important regulators of heart development and myocyte differentiation (5, 20, 23, 24). Altered expression of miR-1 and miR-133 were recently reported in human heart failure (4, 22). However, global measurement of microRNA expression in human heart disease has not been previously reported.

We performed genome-wide miRNA expression profiling in left ventricular myocardium of 67 patients belonging to four diagnostic groups (ischemic cardiomyopathy (ICM), dilated cardiomyopathy (DCM), aortic stenosis (AS), and non-failing controls). We found that miRNA expression profiles were significantly altered in heart disease, and that the pattern of miRNA expression was distinct in different forms of heart disease.

Methods

Patients

Human left ventricle samples belonged to four diagnostic groups (control, ICM, DCM, and AS). End-stage ICM and DCM samples were from explanted hearts of transplant recipients. ICM and DCM patients on mechanical assist devices or with ejection fraction (EF) greater than 45% were excluded. Control samples were from unused transplant donor hearts, with a maximal time between cardiectomy and sample collection of two hours. Aortic stenosis (AS) samples were obtained at the time of aortic valve replacement. Myocardial samples were snap frozen in liquid nitrogen. Areas of fibrosis visible on gross inspection were excluded from the collected myocardial samples. Samples were from Brigham and Women's Hospital (Boston, MA) and Georg August University (Göttingen, Germany), and collected under protocols approved by the respective Institutional Review Boards.

miRNA measurement

RNA was isolated from myocardial samples by homogenization in Trizol (Invitrogen, Carlsbad, CA). miRNA profiling was performed using a high-throughput platform based on hybridization to optically addressed beads, as previously described (13). Quantitative reverse transcription PCR (qRTPCR) was performed on an ABI7300 Real-Time PCR System using Sybr Green chemistry and commercial primers (Applied Biosystems, Foster City, CA).

Bioinformatics and statistical analysis

Expression threshold was set at average signal intensity detected in samples without input miRNA. miRNA expression data by bead-based assay was normalized by the locally weighted smooth spline (LOWESS) method on log-scaled raw data (21). After normalization, all expression values were transformed to linear scale for statistical comparisons. The miRNA expression heat map was constructed by unsupervised hierarchical clustering of miRNAs.

Oneway Analysis of Variance (ANOVA) with Dunnett's post hoc test was performed for signal intensity of each miRNA. We used Significance Analysis of Microarray software (18) to estimate the false discovery rate for each pairwise comparison between disease group and control. Supervised clustering by miRNA expression profiles was performed using Fisher's linear discriminant analysis (21). Class prediction was performed using a classifier derived by a supervised machine learning technique (support vector machine, SVM) implemented for the R statistical language in CRAN package e1071(6).

Statistical analysis was performed using JMP IN version 5 statistical software (SAS Institute, Cary, NC). Values are reported as mean ± standard deviation.

Results

Patient Characteristics

We purified total RNA from left ventricular myocardium of 67 patients belonging to four diagnostic groups (control, n=10; ICM, n=19; DCM, n=25; and AS, n=13). Patient characteristics are summarized in Table 1. ICM and DCM patients had severely depressed EF and elevated pulmonary capillary wedge pressures. 10 out of 13 AS patients had preserved EF (EF > 40%). ICM patients were more likely to be male than controls. AS patients were significantly older than controls. ICM, DCM, and AS patients were more likely to be treated with medications and to have comorbid conditions than controls.

Differential expression of miRNAs in human heart disease

We profiled expression of 428 miRNAs using a high throughput bead-based platform (13). This platform was previously validated using Northern blotting (13). We further confirmed the reliability of this platform by measuring expression of nine miRNAs in 46 samples using qRTPCR. The nine miRNAs were selected to span the range of high, medium, and low intensity

signals. There was strong correlation between the bead-based and qRTPCR measurements in eight out of nine miRNAs (Supplementary Table 1). Within these 46 samples, seven miRNAs were differentially expressed in disease compared to control by bead-based measurements. This was supported by qRTPCR measurement in six of the seven cases.

Eighty-seven miRNAs were expressed above detection threshold in greater than 75% of samples (Table 2). Figure 1 displays an overview of these data in a heat map and a dendrogram, with samples grouped horizontally by diagnosis, and miRNAs arranged vertically by similarity of expression to one another. We focused our attention on these confidently detected miRNAs so that the downstream analysis was based on the most reliable expression data. The entire miRNA expression dataset is available in Supplementary Table 2.

To identify individual miRNAs with altered expression in heart disease, we compared miRNA expression between each disease group and the control group, using ANOVA with Dunnett's post-hoc test (significance threshold $P < 0.05$). To address multiple concurrent testing, we also required the estimated false discovery rate to be less than 5%. Out of 87 miRNAs that were confidently detected, 43 were differentially expressed in at least one disease group (Table 2), suggesting that expression of many miRNAs is altered in heart disease. Differential expression of these miRNAs persisted after multiple regression to control for sex and body mass index. Likewise, correction for age did not influence differential expression between ICM or DCM and control. AS patients were significantly older than controls, and the age distributions did not permit controlling for this confounding variable by multiple regression (see Discussion).

Among the miRNAs with known cardiac-enriched expression (miRNA-1, -133, and -208), miR-1 was downregulated in DCM and AS, and tended to be downregulated in ICM ($P = 0.054$). Expression of miR-133 and miR-208 were not significantly changed. The most strongly

upregulated miRNA was miR-214, which increased 2-2.8 fold in all three disease groups (Table 2). Upregulation of miR-214 may contribute to cardiac hypertrophy, as cardiomyocyte overexpression of miR-214 induced cardiomyocyte hypertrophy (19). The most strongly downregulated miRNA family was miR-19. The two miR-19 family members miR-19a and miR-19b were downregulated 2-2.7 fold in DCM and AS, but not in ICM (Table 2).

miRNA expression profiles are distinct between diagnostic classes

The pattern of altered miRNA expression in each disease group was distinct (Figure 2a). Differential expression of 13 miRNAs was specific to AS, while 8 miRNAs were differentially expressed in cardiomyopathy groups (ICM + DCM) and did not overlap with those altered in AS (Figure 2a; Table 2). This suggests that altered expression of some miRNAs reflects distinct disease mechanisms or disease stage in AS compared to cardiomyopathy samples.

To further assess whether miRNA expression profiles were distinct between diagnostic groups, we performed supervised clustering of samples. Using Fisher's linear discriminant analysis (21), miRNA expression profiles segregated the samples by etiological diagnosis with 100% accuracy (Figure 2b). These results indicate that each form of heart disease is characterized by a miRNA expression profile that is sufficiently distinctive to allow construction of a discriminator that can accurately cluster samples by diagnostic group.

To further investigate the association of heart disease classes with distinct miRNA expression profiles, we asked if the expression profiles could predict clinical diagnosis. We used a supervised learning technique, SVM, to develop a miRNA-based classifier. After training on the set of 67 samples, the SVM-derived classifier assigned class labels that matched the clinical diagnosis in all cases. Next, we performed cross-validation studies in which 45 randomly chosen samples were used for SVM training, and the resulting classifier was applied to the remaining 22

samples. This procedure was repeated 20,000 times (Figure 3a). The classes assigned by the SVM-generated classifier matched the clinical diagnosis 69.2% ± 3.8% of the time (Figure 3b). The likelihood of achieving this performance by chance was less than 0.001, estimated by SVM training on datasets in which the sample labels were randomly permuted (20,000 datasets with randomly permuted sample labels, each with 20,000 cross-validation studies). These results suggest that miRNA expression profiles are sufficiently distinct between disease classes to predict clinical diagnosis with moderate success.

Discussion

In this work, we report the first extensive genome-wide profiling of miRNA expression in human heart disease. We found that expression of many miRNAs changed significantly in diseased myocardium. Multiple independent lines of evidence corroborate our profiling data. First, miRNA expression measurements correlated closely between bead-based and qRTPCR platforms (Supplementary Table 1). Second, our study yielded results largely concordant with previously reported findings. Olson and colleagues used northern blotting to compare miRNA expression in six DCM samples to four controls (19). They reported on 11 miRNAs, 10 miRNAs that were detectably expressed on our platform. The two studies were in agreement for 9 of the 10 miRNAs. Northern analysis suggested that miR-208 expression was not altered in human ICM (20), consistent with our data (Table 2). miR-1 was recently reported to be downregulated in four different murine models of cardiac hypertrophy or failure (4, 15), consistent with our finding of miR-1 downregulation in AS and DCM.

However, not all studies are in agreement. While miR-133 was not significantly changed in our study, it was reported to be downregulated in hypertrophic cardiomyopathy and in dilated atrial myocardium (4). We found that miR-1 was downregulated in ICM, while Yang and

colleagues recently reported it was upregulated in ICM (22). An oligonucleotide microarray study of a small number of samples (DCM, n = 6; control, n = 4) was recently published, and overall there was low concordance between data sets (16). These divergent findings may reflect differences in tissues sampled (endocardial versus transmural; atrial versus ventricular), diagnostic groups studied, heterogeneity in human myocardial samples, systematic differences in the manner in which control or diseased samples are collected, and sample size differences that can lead to false discovery as well as false negatives (17). Additional miRNA profiling studies with larger sample numbers and careful attention to patient characteristics and details of tissue procurement will be necessary to resolve these differences.

miRNAs are emerging as important post-transcriptional regulators of gene expression, with each miRNA predicted to regulate hundreds of target genes (1, 2). A growing body of data indicates that miRNAs are key regulators of cardiac development, contraction, and conduction (4, 15, 19, 20, 22-24). In this study, we found that expression of many miRNAs was altered in human heart disease, albeit the magnitude of expression changes was generally small. These changes are not a simple epiphenomenon of end-stage heart disease, because AS patients had at the same time the most distinctive miRNA expression profile and largely compensated ventricular function. Rather, these miRNA changes likely contribute to heart disease pathogenesis by mediating pathological changes in gene expression. The distinctive pattern of miRNA expression changes between heart disease etiologies further suggests that miRNAs contribute to etiology-specific gene expression changes. The functional significance of these broad but often subtle changes in miRNA expression will need to be studied in model systems where levels of one or more miRNAs can be finely manipulated.

One long term goal of expression profiling studies is to develop expression signatures that can be used in clinically relevant classification problems, such as prognosis or prediction of drug responsiveness (8, 10). In this study, we showed the miRNA expression profiles can classify samples by etiological diagnosis. This provides proof-of-concept that miRNA expression profiles may be useful in other more challenging and clinically relevant class prediction problems, and supports further studies of miRNAs as potential biomarkers for determining prognosis and response to therapy.

Analysis of human myocardial tissue is complicated by limited availability and by biological variability arising from differences in age, gender, body habitus, medications, co-morbidities, and individual course of disease. Intergroup differences in confounding variables was an important limitation of this study. We were able to control for some of these variables (gender, BMI, and age in DCM and ICM). However, we were unable to control for co-morbidities or medication use. In addition, AS patients were significantly older than cardiomyopathy patients or controls. We cannot exclude the possibility that the age difference contributed to altered miRNA expression in the AS group. However, we found no significant correlation between miRNA expression and age for any of the differentially expressed miRNAs within the control group, suggesting that miRNA expression does not systematically vary with age through adult life.

This study demonstrated that expression of many miRNAs is altered in human heart disease, and that the pattern of alteration differs by underlying disease etiology. This dataset of human miRNA expression in nonfailing and diseased hearts will guide further studies on the contribution of miRNAs to heart disease pathogenesis.

Acknowledgements

W.T.P. was supported by NHLBI P01 HL074734 and by a charitable donation from Edward P. Marram and Karen K. Carpenter. The Cardiogenomics Project (NIH HL66582) provided human samples used in this paper.

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Table 1. Clinical Characteristics of the Study Subjects

	Control	ICM	DCM	AS
Sample number	10	19	25	13
Age -- decades	5.8 ± 1.4	6.6 ± 0.6	6.0 ± 1.5	8.6 ± 0.7
Male sex -- no. (%)	6 (60%)	17 (89%)	17 (68%)	6 (46%)
BMI --kg/m^2	24.2 ± 4.7	25.4 ± 5.1	23.5 ± 2.9	26.9 ± 3.0
Medical History -- no (%)				
Hypertension	6 (60%)	11 (58%)	5 (20%)	7 (50%)
DM	1 (10%)	11 (58%)	5 (20%)	3 (21%)
Atrial fibrilation	0 (0%)	3 (16%)	9 (36%)	3 (21%)
Cardiac function				
LVEF -- %	65.0 ± 5.0†	20.0 ± 7.5	15.9 ± 7.5	55.8 ± 16.9
PCWP -- mmHg	N/A	20.2 ± 8.6	20.5 ± 7.9	29.8 ± 4.3††
Medication -- no.(%)				
ACE inhibitor/ AR blockers	0 (0%)	14 (74%)	20 (80%)	8 (62%)
Beta-blockers	2 (20%)	10 (53%)	15 (60%)	7 (54%)
Diuretics	0 (0%)	17 (90%)	19 (76%)	10 (77%)
Digoxin	0 (0%)	11 (58%)	15 (60%)	3 (23%)

† only available for three patients
†† only available for seven patients
BMI, body mass index; DM, diabetes mellitus; LVEF, left ventricular ejection fraction; PCWP, pulmonary capillary wedge pressure. ACE, angiotensin converting enzyme; AR, angiotensin II receptor.

Table 2. Confidently detected miRNAs.

The miRNAs listed in this table were expressed above detection threshold in more than 75% of samples. Orange boxes indicate significant differences from control ($P < 0.05$, ANOVA with Dunnett's post-hoc testing; and false discovery rate (q) < 5%).

	DCM			ICM			AS		
miRNA	**fold**	**p**	**q (%)**	**fold**	**p**	**q (%)**	**fold**	**p**	**q (%)**
let-7a	1.07	0.192	3.3	1.07	0.158	2.0	1.03	0.709	7.5
let-7b	1.39	0.046	1.7	1.23	0.034	<0.02	1.10	0.593	6.4
let-7c	1.32	0.039	1.7	1.21	0.019	<0.02	1.24	0.011	<0.02
let-7d	1.10	0.217	8.9	1.06	0.356	10.0	1.04	0.727	11.9
let-7d*	4.10	0.576	15.2	1.80	0.929	24.1	0.65	0.994	2.7
let-7e	1.08	0.996	43.8	1.10	0.313	17.1	1.34	<0.001	<0.02
let-7f	0.80	0.053	<0.02	0.97	0.936	34.4	0.85	0.078	<0.02
let-7g	0.89	0.690	26.5	0.99	1.000	36.4	0.87	0.402	4.3
miR-1	0.62	0.023	<0.02	0.82	0.054	<0.02	0.68	<0.001	<0.02
miR-10a	0.83	1.000	43.8	1.23	0.614	15.5	0.24	0.013	<0.02
miR-10b	1.04	0.995	43.1	1.57	0.069	2.0	0.45	0.111	<0.02
miR-15a	0.91	1.000	43.8	1.08	0.767	17.1	0.98	0.996	29.9
miR-15b	1.68	0.010	1.7	1.28	0.242	12.3	1.69	0.001	<0.02
miR-16	0.95	0.996	43.1	1.00	1.000	31.4	0.98	0.933	21.6
miR-17-5p	0.82	0.002	<0.02	0.92	0.266	19.1	0.88	0.072	2.7
miR-19a	0.41	<0.001	<0.02	0.76	0.073	12.9	0.37	<0.001	<0.02
miR-19b	0.50	0.001	<0.02	0.80	0.100	12.9	0.46	<0.001	<0.02
miR-20a	0.75	0.005	<0.02	0.87	0.101	12.3	0.76	0.002	<0.02
miR-20b	0.75	0.007	<0.02	0.82	0.034	12.9	0.72	0.001	<0.02
miR-21	0.75	0.346	6.3	1.03	0.989	31.4	0.71	0.183	0.8
miR-22	1.09	0.997	43.1	1.04	0.906	24.1	1.13	0.204	0.8
miR-23a	1.24	0.001	<0.02	1.14	0.013	<0.02	1.31	<0.001	<0.02
miR-23b	1.08	0.659	15.2	1.00	0.999	31.4	1.24	<0.001	<0.02
miR-24	1.14	0.334	11.8	1.14	0.036	<0.02	1.29	<0.001	<0.02
miR-26a	1.08	0.295	8.9	1.07	0.177	2.0	1.04	0.584	8.8
miR-26b	0.81	0.111	1.8	0.96	0.790	29.3	0.80	0.006	<0.02
miR-27a	1.10	0.196	7.7	1.15	0.045	<0.02	1.21	0.008	<0.02
miR-27b	1.05	0.742	22.7	1.06	0.409	10.0	1.22	<0.001	<0.02
miR-28	0.86	0.001	<0.02	0.89	0.023	5.2	0.91	0.104	2.7
miR-29a	1.01	1.000	47.4	1.08	0.625	12.3	0.98	0.991	28.4
miR-29b	0.76	0.542	19.1	1.03	0.957	26.7	0.81	0.137	<0.02
miR-29c	0.80	0.327	9.3	0.96	0.944	34.5	0.83	0.168	<0.02
miR-30a-3p	0.94	0.850	46.7	0.88	0.256	19.1	0.92	0.636	21.6

miR-30a-5p	0.90	0.560	19.1	0.96	0.657	29.3	0.91	0.190	<0.02
miR-30b	0.95	0.807	26.5	0.93	0.119	12.9	0.94	0.230	2.7
miR-30c	1.03	0.647	12.4	0.97	0.667	29.3	1.01	0.987	27.2
miR-30d	1.12	0.340	8.9	1.01	0.992	31.4	1.11	0.226	0.8
miR-30e-3p	0.99	1.000	43.8	0.91	0.451	29.3	0.98	0.989	29.9
miR-30e-5p	0.54	0.005	<0.02	0.81	0.120	12.9	0.54	<0.001	<0.02
miR-92	0.91	0.218	26.5	0.84	0.314	29.3	0.83	0.327	19.2
miR-93	1.21	0.575	31.9	1.22	0.073	7.3	1.44	<0.001	0.8
miR-98	0.83	0.888	46.7	0.96	0.918	36.4	0.92	0.762	25.3
miR-99a	1.13	0.923	41.0	1.07	0.592	15.5	1.16	0.092	0.8
miR-99b	1.39	0.037	3.3	1.16	0.233	7.3	1.50	<0.001	<0.02
miR-100	1.60	0.013	1.7	1.46	0.002	<0.02	1.74	<0.001	<0.02
miR-101	0.53	0.019	<0.02	0.88	0.499	29.3	0.53	<0.001	<0.02
miR-103	1.44	0.002	1.7	1.27	0.006	<0.02	1.24	0.029	0.8
miR-106a	0.82	0.003	<0.02	0.91	0.231	19.1	0.88	0.093	3.9
miR-106b	0.99	0.997	47.4	1.12	0.431	10.0	1.11	0.548	8.8
miR-107	1.13	0.551	22.7	1.02	0.992	31.4	1.01	0.998	33.4
miR-125a	1.15	0.473	12.4	1.03	0.978	31.4	1.18	0.199	1.5
miR-125b	1.37	0.026	1.7	1.17	0.086	<0.02	1.44	<0.001	<0.02
miR-126	0.89	0.448	19.1	0.96	0.767	29.3	0.82	0.004	<0.02
miR-126*	0.58	<0.001	<0.02	0.80	0.019	<0.02	0.56	<0.001	<0.02
miR-130a	1.27	0.185	8.9	1.16	0.209	2.0	1.20	0.132	1.5
miR-133a	1.02	0.932	41.0	0.97	0.885	33.6	1.02	0.948	24.2
miR-133b	1.02	0.877	34.5	0.96	0.626	29.3	1.01	0.984	27.2
miR-140*	1.38	0.019	1.7	1.24	0.032	<0.02	1.52	<0.001	<0.02
miR-143	1.05	0.951	37.0	1.09	0.507	7.3	1.06	0.771	19.2
miR-145	1.21	0.724	21.1	1.05	0.924	24.1	1.32	0.015	<0.02
miR-146a	0.89	0.969	47.4	0.94	0.910	35.7	0.81	0.298	4.3
miR-146b	0.75	0.562	26.5	0.84	0.565	29.3	0.62	0.069	2.7
miR-150	1.41	0.337	13.7	1.04	0.994	31.4	1.15	0.837	19.2
miR-151*	1.23	0.185	9.6	1.09	0.361	7.3	1.11	0.284	0.8
miR-152	1.03	0.896	41.3	1.11	0.227	15.5	0.93	0.601	21.6
miR-181a	1.70	0.132	13.7	1.32	0.196	10.0	1.73	0.001	<0.02
miR-185	1.01	0.790	46.7	0.88	0.399	32.3	0.98	0.990	33.4
miR-191	1.40	0.034	8.9	1.26	0.022	2.0	1.57	<0.001	<0.02
miR-191*	0.80	0.869	46.7	0.80	0.141	19.1	0.77	0.116	4.8
miR-195	1.14	0.01	<0.02	1.19	0.020	<0.02	1.02	0.991	27.2
miR-199a*	1.70	0.012	1.7	1.65	0.002	<0.02	1.33	0.218	3.3
miR-208	0.51	0.623	24.0	1.21	0.630	17.1	0.57	0.190	<0.02
miR-214	2.83	0.003	1.7	2.07	0.011	2.0	2.11	0.014	<0.02
miR-222	0.59	<0.001	<0.02	0.58	<0.001	<0.02	0.92	0.778	21.6

miR-320	1.44	0.238	12.4	1.36	0.011	<0.02	1.44	0.004	<0.02
miR-335	0.82	0.237	9.3	0.87	0.415	29.3	0.81	0.189	4.3
miR-342	1.46	0.006	1.7	1.15	0.263	7.3	1.30	0.012	0.8
miR-361	1.11	0.925	41.3	1.04	0.910	31.4	1.16	0.137	6.4
miR-365	1.33	0.610	21.1	1.13	0.698	15.5	1.13	0.702	7.9
miR-374	0.65	0.109	1.8	0.95	0.864	32.3	0.67	0.002	<0.02
miR-422b	0.89	0.012	<0.02	0.83	0.014	<0.02	0.98	0.970	28.4
miR-423*	1.41	0.234	9.6	1.20	0.251	3.6	1.37	0.027	<0.02
miR-424	0.75	0.995	47.4	1.26	0.141	3.6	0.67	0.066	<0.02
miR-451	1.10	0.863	22.7	1.30	0.438	7.3	0.92	0.007	<0.02
miR-483*	0.99	1.000	47.4	1.03	0.989	31.4	1.06	0.902	27.2
miR-495	0.65	0.552	27.7	0.67	0.111	12.3	0.88	0.054	1.5
miR-499	0.65	0.075	<0.02	0.67	0.264	0.9	0.88	0.044	<0.02

Figure Legends

Figure 1. miRNA expression in human heart failure. Heat map summarizing miRNA expression. Each column represents one of 67 samples, and each row represents one of 87 detectable miRNAs. Samples were grouped by diagnosis, and miRNAs arranged by unsupervised hierarchical clustering. Red and blue indicate up- and down-regulation, respectively, relative to the overall mean for each miRNA.

Figure 2. miRNA expression profiles accurately segregated samples by diagnosis. a. Venn diagram summarizing differential expression of individual miRNAs in each disease group compared to control, after controlling for age, sex, and BMI. Numerals indicate number of miRNAs falling into the indicated region. **b.** Supervised clustering was performed using linear discriminant analysis. Samples were plotted in the space of two linear discriminants (LD1 and LD2).

Figure 3. Diagnostic class prediction by miRNA expression profiles. a. Schema of cross-validation study. A classifier derived by training a support vector machine on 45 randomly selected samples was tested on the remaining 22 samples. This procedure was repeated for 20,000 permutations, yielding 22/67 * 20,000 ~ 6600 assigned labels per sample. **b.** Summary of results of cross-validation study. The percent of times each of the 67 samples was assigned each of the four diagnostic labels is plotted. Overall diagnostic accuracy (assigned class matched clinical diagnosis) was 69%.

Supplementary Table 1. Correlation between bead-based and qRTPCR platforms

Correlation between platforms in 46 samples representing the four diagnostic groups. miRNAs were chosed to include low, medium, and high expression values, displayed as mean ± sd. Relative miRNA expression values by qRTPCR were normalized to total input RNA.‡

miRNA	Average expression in bead-based assay	Pearson correlation coefficient	p-value	
miR-1	8654 ± 1820	0.497	<0.001	
miR-30b†	1800 ± 170	-0.201	0.203	
miR-103	126 ± 21	0.458	0.003	
miR-126*	685 ± 185	0.720	<0.001	
miR-133a§	1210 ± 141	0.583	<0.001	
miR-140*	196 ± 48	0.575	<0.001	
miR-191	98 ± 25	0.608	<0.001	
miR-199a*	85 ± 29	0.753	<0.001	
miR-208	133 ± 89	0.909	<0.001	

†qRTPCR assay measured both miR-30b and -30c. The assay did not detect miR-30a, -30d, or -30e. Expression levels of miR-30b and miR-30c were quite similar in the bead-based assay (r=0.860, p<0.001, Pearson correlation coefficient).

§qRTPCR did not distinguish miR-133a and miR-133b. Expression levels of miR-133a and miR-133b were quite similar in the bead-based assay (r=0.898, p<0.001, Pearson correlation coefficient).

‡U6 was not used as an internal control because its expression changed significantly in heart disease.

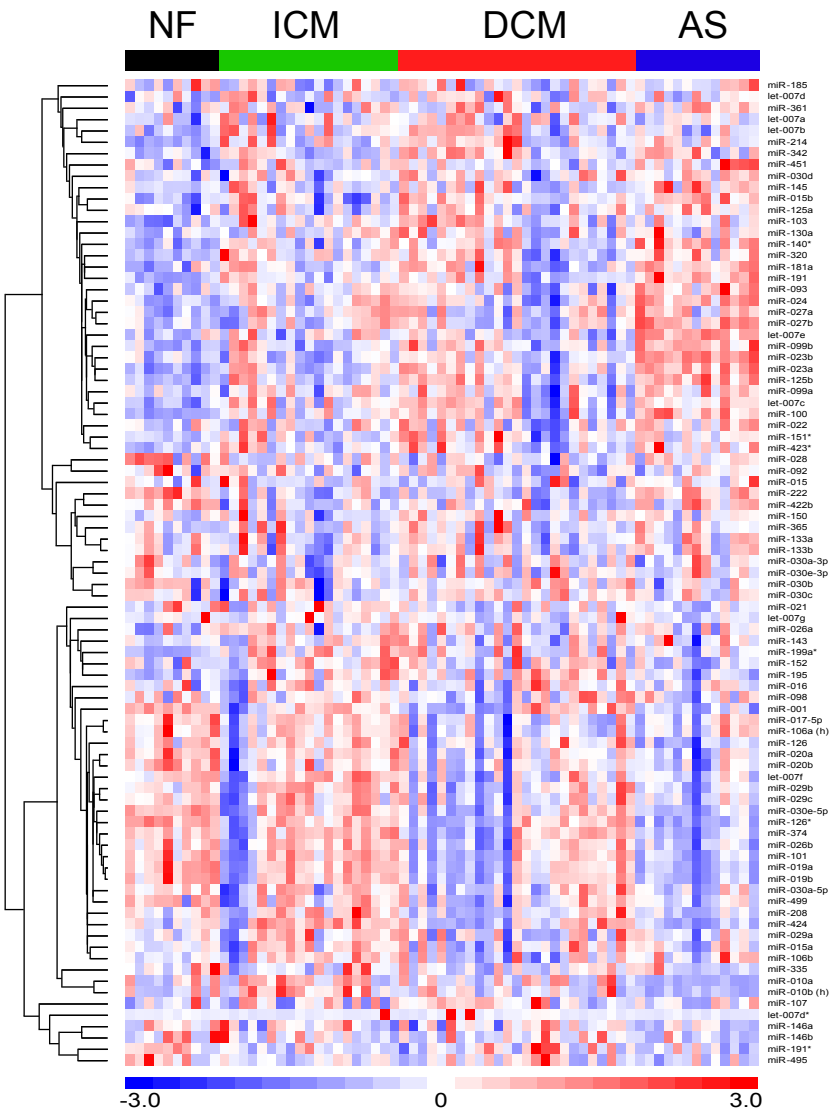

Figure 1
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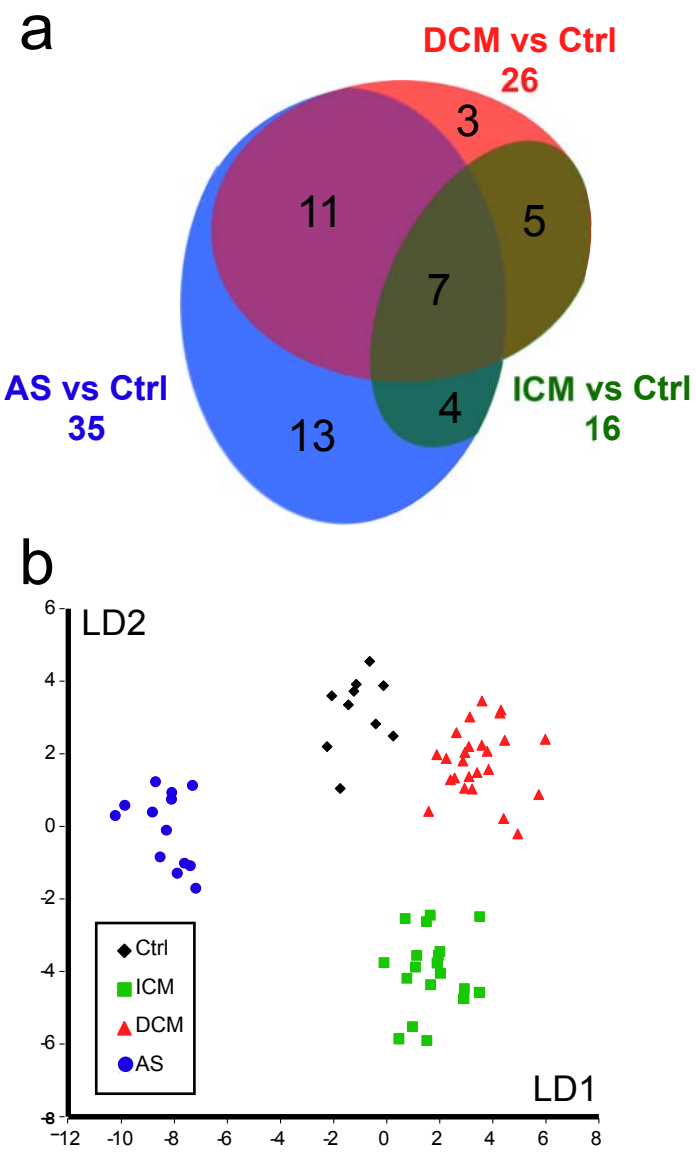

Figure 2
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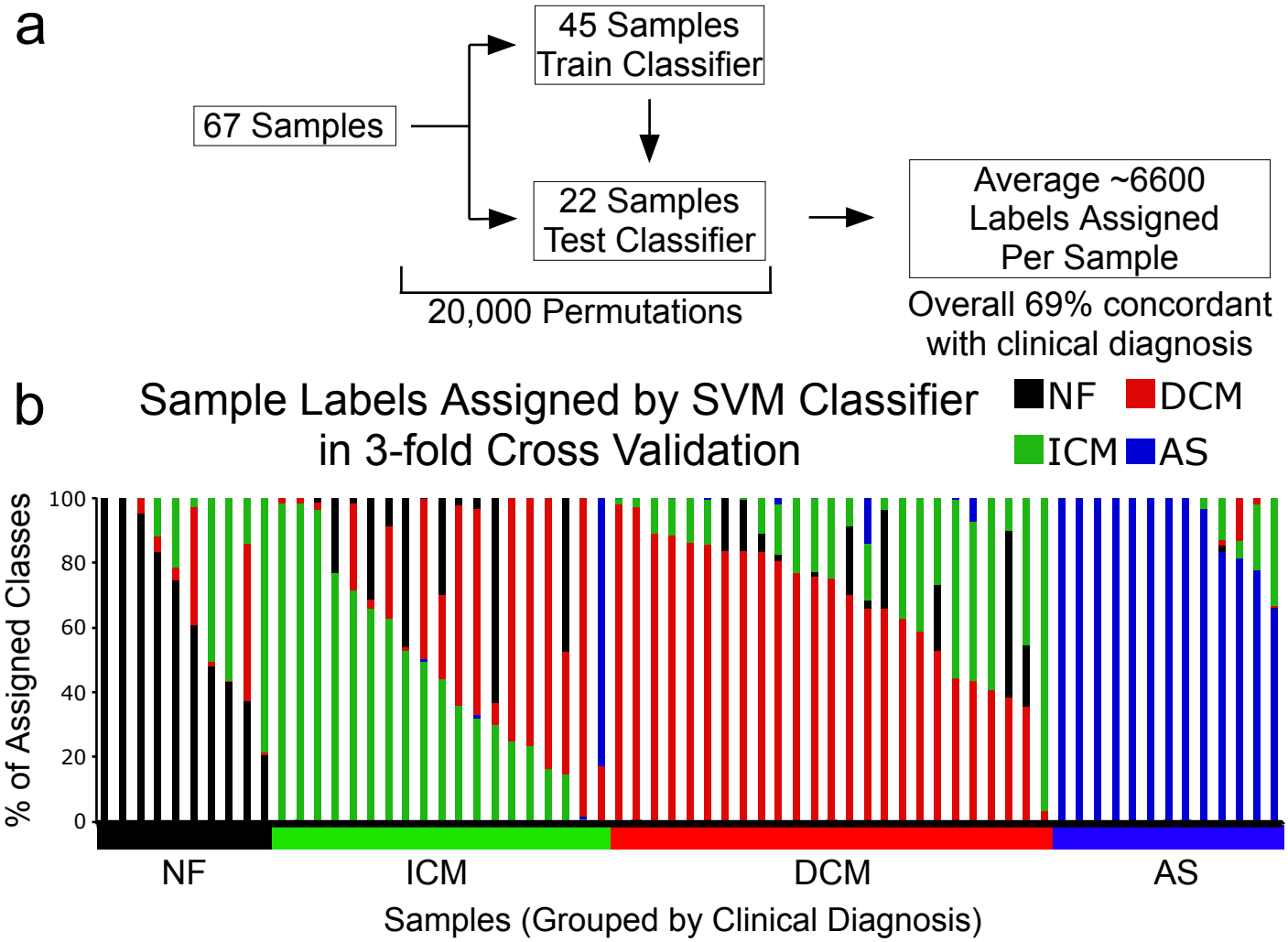

Figure 3
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