Circulating Long Non-coding RNA ENST00000507296 Is a Prognostic Indicator in Patients with Dilated Cardiomyopathy

Xudong Zhang,1 Xiang Nie,1 Shuai Yuan,1 Huaping Li,1 Jiahui Fan,1 Chenze Li,1 Yang Sun,1 Yanru Zhao,1 Huiying Hou,1 Dao Wen Wang,1 and Chen Chen1

1Division of Cardiology, Department of Internal Medicine, Tongji Hospital, Tongji Medical College and Hubei Key Laboratory of Genetics and Molecular Mechanisms of Cardiologic Disorders, Huazhong University of Science and Technology, Wuhan, China

Background: Long non-coding RNAs (lncRNAs) participate in the pathogenesis of cardiovascular diseases. However, whether circulating lncRNAs serve as dilated cardiomyopathy (DCM) biomarkers remains unclear. Methods: Totally, 266 controls and 818 patients were enrolled. First, microarray-based circulating lncRNA profiling was performed in 10 normal controls and 10 patients with DCM. Second, the top 20 differentially expressed lncRNAs were validated by real-time qPCR in 64 controls and 64 DCM patients. Moreover, lncRNA sequencing was performed in three human heart-derived cell types, and the correlation between circulating lncRNA levels and the severity of heart failure was evaluated in the validated population. The validated two lncRNAs were assessed in 198 DCM patients and 198 matched controls. Finally, the sensitivity and specificity of circulating lncRNA expression in DCM diagnosis were evaluated using receiver-operating characteristic curve analysis, while Cox regression and Kaplan-Meier curve analysis were further performed in 552 DCM patients. Results: Eight candidate lncRNA biomarkers were obtained after microarray screening and real-time PCR validation. Among them, five were validated in the second cohort. However, only the levels of circulating lncRNA ENST00000507296 and ENST00000532365 were significantly correlated with the cardiac function, as well as detectable in at least one of the human heart-derived cell types by lncRNA-seq. Importantly, low circulating ENST00000507296 level was associated with high event-free survival in patients with DCM. Conclusions: Circulating lncRNA ENST00000507296 was a prognostic biomarker in patients with DCM.

INTRODUCTION

Dilated cardiomyopathy (DCM) is conventionally defined by the primary presence of left ventricular (LV) dilation and reduced systolic function, which is not caused by abnormal loading conditions or coronary artery disease.1 DCM is the most common indicator for cardiac transplantation.1 The mortality of 3-year-treated DCM patients remains from 12% to 20%, and the major death causes are heart failure (HF) and ventricular arrhythmias. Compared with those with ischemic heart diseases, patients with DCM are usually younger.3 Moreover, the onset of DCM can be occult (asymptomatic), and the period of diagnosis and treatment may be significantly delayed.4 The pharmacological treatment of established DCM is in accordance with the HF guidelines, such as angiotensin-converting enzyme (ACE) inhibitors and β-blockers.5 Currently, heart transplantation remains the only therapeutic option in patients with end-stage DCM.6 Novel biomarkers may offer the potential for earlier disease diagnosis, with the opportunity to attenuate disease progression.2 Therefore, identification of novel biomarkers for diagnosis and predicting prognosis for DCM is critical.

Long non-coding RNAs (lncRNAs) are characterized by transcripts longer than 200 nt in length and incapable of coding protein.7 Recently, lncRNAs have been reported to participate in cell signaling regulation by chromatin modification,8 structural scaffolds,9 and RNA processing.10 Currently, an increasing number of studies have revealed that lncRNAs played important roles in the heart. The lncRNA Fendrr bound to both Polycomb repressive complexes 2 (PRC2) and the Trithorax group/MLL protein complexes (TrxG/MLL) to modify chromatin signatures in the rodent cardiac mesoderm differentiation, and loss of Fendrr transcripts generated embryonic lethality.11 The lncRNA Braveheart (Bvht) played pivotal roles in cardiovascular lineage commitment by activating epithelial-to-mesenchymal transition (EMT) genes (e.g., Twist) and cardiac transcription factors (e.g., MesP1, Nkx2.5, and Tbx5); also Bvht could interact with SUZ12, a core component of the Polycomb repressive complex 1 (PRC1) and 2 (PRC2).12 Finally, Bvht could promote intercalated disk formation, which is an essential step for cardiac development.13 The lncRNA lncWnt5a is involved in the Wnt signaling axis and plays a key role in cardiac regeneration and ischemia preconditioning.14 The lncRNA linc-RARRAT is associated with atrial fibrillation.15 The lncRNA UCA1 is essential for the growth and development of the atria.16 Finally, the lncRNA linc-Prkarf promotes in vitro heart development by interacting with the transcriptional coactivator PRKARF.17 The lncRNA流水线 were extensively studied and found to be involved in the cardiomyopathy development.

Similarly, the lncRNA ENST00000507296 and ENST00000532365 were identified in the previous study, and the two lncRNAs were validated in the present study. The circulating lncRNA ENST00000507296 was found to be significantly correlated with the cardiac function, and it was detectable in at least one of the human heart-derived cell types by lncRNA-seq. Importantly, low circulating ENST00000507296 level was associated with high event-free survival in patients with DCM. The present study provides evidence for the potential of lncRNA ENST00000507296 as a novel prognostic indicator in DCM patients.
Recent studies have indicated that some circulating microRNAs (miRNAs) can act as biomarkers for various cardiovascular disorders. For example, the levels of circulating miRNAs miR-1, -133a, -133b, and -499-5p were upregulated, while circulating miR-122 and -375 levels were downregulated in ST-segment elevation myocardial infarction (STEMI) patients, compared with controls. In HF patients with dyssynchro-ny, the circulating miR-30d level was reported to be an indicator of cardiac resynchronization therapy (CRT). Similarly to small non-coding RNAs, lncRNAs were stably detectable in the plasma. Circulating lncRNAs could be packaged into microparticles, including microvesicles, exosomes, apoptotic microparticles, and apoptotic bodies, to avoid being degraded. Recently, several studies have indicated that circulating lncRNAs can be regarded as biomarkers for various diseases. For instance, circulating long intergenic noncoding RNA-predicting cardiac remodeling, uc022bq.1 (LIPCAR) levels were independently associated with future cardiovascular deaths in chronic HF, and higher LIPCAR levels predicted a higher risk of cardiac death. The lncRNA CoroMarker could differentiate coronary artery disease patients from control subjects with an area under the curve (AUC) of 0.920. Circulating lncRNAs NRON and MHRT were novel biomarkers of HF, with AUCs of 0.865 and 0.702, respectively. Meanwhile, the expression level of lncRNA PCA3 in urine samples has been found to be more sensitive and specific in diagnosing prostate cancer than the universally used prostate-specific antigen (PSA) serum level. Syndecan-4 was recently reported to be an indicator for adverse LV remodeling in patients with DCM. Meanwhile, some studies suggested that the first, second, and third Fourier transform infrared spectroscopy (FTIR spectroscopy) indicators might be useful biophysical markers of cardiac remodeling in DCM induced by tachycardia.

In our previous study, we provided a global profile of lncRNA changes in failing hearts from patients with DCM. Meanwhile, we found that cardiac-specific lncRNA RP11-544D21.2 was crucial in tube formation and migration in endothelial cells. In the present study, we further explored the expression profile of circulating lncRNAs in DCM-related HF and their associations with the outcomes.

RESULTS

Study Workflow
The flowchart of this study was performed in five phases (Figure 1). The detailed procedures are presented in Materials and Methods.

Profiling of Circulating lncRNAs in the Screening Cohort
Plasma samples from 10 chronic HF patients diagnosed with DCM and 10 control subjects were recruited in the original microarray cohort. Figure 2A shows a volcano plot of plasma lncRNA profiles from control subjects and DCM patients. The scatterplot of plasma lncRNA profiles is shown in Figure 2B. lncRNA expression profiles were distinguishable between controls and DCM patients according to the hierarchical clustering, as shown in Figure 2C. Compared with the control subjects, there were 2,007 upregulated lncRNAs and 1,250 downregulated lncRNAs in chronic HF patients diagnosed with DCM (Figure 2C). The top 100 upregulated and top 100 downregulated lncRNAs based on fold change in DCM patients compared with control subjects are shown in Tables S1 and S2, respectively.

Biomarker Validation by qRT-PCR in the Validation Cohort
Based on the fold changes, we selected the top 20 (10 up- and 10 downregulated) lncRNAs to further detect their expression levels in the plasma of 64 controls and 64 DCM patients. Among these 20 differentially expressed lncRNAs, 8 lncRNAs were detectable by qPCR in plasma. These lncRNAs are highlighted in red in Tables S1 and S2. The relative expression of each lncRNA is shown in Figure 3. Consistent with the microarray assays, compared with the control subjects, there was a 2.98-fold increase of circulating ENST00000507296 in DCM patients (Figure 3A). Meanwhile, circulating ENST00000442293 and ENST00000545794 increased 1.49-fold and 1.76-fold in DCM patients, respectively (Figures 3B and 3C). On the other hand, there was a 0.54-fold decrease of circulating ENST00000532365 and a 0.60-fold decrease of HMLincRNA1548 in DCM patients (Figures 3D and 3E). The raw data of lncRNA ENST00000532365 is shown in Data S1. Another three lncRNAs showed no significant difference between the two groups.
Therefore, five of the eight lncRNAs were potentially biomarkers for DCM and were selected for further investigation.

Expression Patterns of lncRNAs in Three Cardiac-Derived Cell Types
To further analyze the cell sources of the successfully validated 5 lncRNAs, three kinds of human-derived primary cardiomyocyte, fibroblast, and endothelial cells were subjected to lncRNA sequencing (lncRNA-seq) (Figure 4A). As a result, only 2 (ENST00000507296 and ENST00000532365) of the 5 lncRNAs were detectable in human-derived cardiac cells (Figure 4B). Specifically, ENST00000507296 was only expressed in cardiomyocytes, compared with fibroblast and endothelial cells, while ENST00000532365 was wildly expressed in all three types of cardiac-derived cells (Figure 4B). The other 3 lncRNAs were undetectable in three kinds of human-derived cardiac cells (Figure 4B). Therefore, these data indicated that the expression levels of circulating lncRNAs ENST00000507296 and ENST00000532365 might be correlated with heart function.

Association of Circulating lncRNA Levels with the Severity of HF
The 5 successfully validated circulating lncRNA biomarker candidates were further analyzed by detecting their association with the severity of HF, which was indicated by the echocardiographic parameters left ventricular ejection fraction (LVEF) and left ventricular end diastolic diameter (LVEDD) and the plasma levels of N-terminal pro-brain natriuretic peptide (NT-proBNP), in the validation cohort by Spearman correlation analyses. Among them, ENST00000507296 and ENST00000532365, the only 2 lncRNAs expressed in human cardiac-derived cells, were significantly correlated with cardiac function. As shown in Figures 5A–5C, the circulating lncRNA ENST00000507296 level showed significant correlations with all the three parameters, and its expression level was negatively associated with the cardiac function. Meanwhile, the circulating lncRNA ENST00000532365 level also significantly correlated with all the three parameters, but its expression level was positively associated with the cardiac function (Figures 5D–5F). The other 3 lncRNAs, which were undetectable in the three kinds of human-derived cardiac cells, were not correlated with these cardiac function parameters (Figure S1).

Validation of Circulating lncRNA Expression in a Larger Replication Cohort
To further verify whether the increased expression of plasma lncRNA ENST00000507296 and the decreased expression of plasma ENST00000532365 were indicators of HF severity, we used another cohort of 198 DCM patients and 198 control subjects. As shown in Figure 6A and Figure 6B, a 4.60-fold increase of lncRNA ENST00000507296 and a 0.63-fold decrease of lncRNA ENST00000532365 were found in DCM patients, respectively, in comparison with the control subjects. Moreover, we evaluated whether the plasma contents of these lncRNAs could discriminate DCM patients from controls by applying ROC curve analyses. The lncRNA ENST00000507296 yielded a higher AUC of 0.78 than the lncRNA ENST00000532365 with an AUC of 0.61. The combination of the two lncRNAs would generate a higher AUC of 0.81 (Figure 6C). Moreover, the AUC of NT-proBNP was 0.98, since the inclusion of DCM covered many HF patients (Figure 6C).

Prediction of Events in Follow-Up Study
To further identify the associations between the selected plasma lncRNA expression levels and the risk of DCM, the final cohort study of 552 patients with DCM was conducted. In this study, five patients were lost to follow-up, 136 patients (24.6%) died from cardiac diseases, and 150 patients (27.2%) were re-hospitalized due to heart
diseases. The median follow-up time was 21 months, and the maximum period reached up to 60 months. The relative lncRNA expression levels were dichotomized into low and high expression by the optimal cutoff point from the ROC curve. Altogether, 522 DCM patients were analyzed using the lncRNA ENST00000507296, while 508 DCM patients were analyzed using the lncRNA ENST00000532365 (undetectable in some patients). The Kaplan-Meier survival curves showed that high lncRNA ENST00000507296 expression and low lncRNA ENST00000532365 expression were significantly associated with the endpoints of DCM (Figures 7A and 7B). After multivariable adjustments for age, sex, New York Heart Association functional classification, NT-proBNP, LVEF, LVEDD, hypertension, and diabetes mellitus, the low expression level of circulating lncRNA ENST00000507296 (HR = 0.729, 95% confidence interval [CI] = 0.537–0.989, p = 0.042) was significantly associated with the endpoints; however, the expression level of the lncRNA ENST00000532365 (HR = 1.169, 95% CI = 0.856–1.597, p = 0.325) was not significantly correlated with the events (Table S3). However, the Kaplan-Meier survival curves of NT-proBNP could not present a significant association with the primary event or total event (Figure S2).

**DISCUSSION**

DCM may be diagnosed at any age but most usually occurs by ages 30 to 40. In DCM patients, increasing age acts as an independent risk factor for death. Most importantly, due to the hidden symptoms and delay of diagnosis, the prognosis for DCM is not well.

Several studies implied that, in the DCM patients with recent new onset of clinical symptoms of HF, about 25% would have spontaneous improvement, but patients might have less chance of recovery if the symptoms last more than 3 months. DCM accounts for about one third of the HF cases. The presenting symptoms of DCM include arrhythmias, thromboembolic events, and circulatory collapse, while...
the most common symptoms are related to congestive HF. In children with DCM, a BNP concentration greater than 300 pg/mL indicates a possibility of hospital admission because of HF, heart transplantation, or even death. Nowadays, no specific treatment is available for patients with diagnosed DCM, except for drugs against HF and arrhythmia complications.

In the present study, we found that lncRNAs ENST00000507296 and ENST00000532365 were able to act as biomarkers for the diagnosis of DCM-related HF. Higher circulating lncRNA ENST00000507296 levels presented a higher risk of cardiovascular events in DCM patients. Importantly, compared to other circulating lncRNAs, ENST00000507296 and ENST00000532365 were abundant in human-derived cardiac cells, which suggested a possibility of heart origin. However, in adjusted Cox proportional-hazards models including terms for HF risk factors, lower circulating lncRNA ENST00000532365 could not show the significant statistical difference due to the follow-up time and the scale of this cohort. Accumulating evidence has demonstrated that circulating lncRNAs could act as prognostic indicators in variable types of diseases. Circulating lncRNA AK098656, which is human specific and dominant in vascular smooth muscle cells, facilitates hypertension. Circulating RP11-230G5.2 and XLOC_014172 may serve as indicators of macrophage outcome in gestational diabetes mellitus patients. Meanwhile, circulating lncRNA TrAnscript could predict survival in acute kidney injury. Several studies discovered that circulating exosomal small RNAs would be significant diagnostic and prognostic biomarkers in the pathogenesis of diseases, such as multiple myeloma and castration-resistant prostate cancer. Recently, activated circulating myeloid-derived suppressor cells were reported to play important immunomodulatory roles in DCM. The miRNAs miR-155, -636, -646, and -639 were found to have the potential to perform risk stratification in children with DCM. On the other hand, miR-548c in circulating peripheral blood mononuclear cells (PBMCs) was associated with risk in DCM patients. A set of CpGIs as novel epigenetic biomarkers for DCM inducing HF has been identified by a multi-omics approach. Studies have discovered improved cardiac function in children diagnosed with DCM. The incidence of cardiac ameliorating varies from 16% to 63%, relying on the description of improvement and the etiology of DCM.

Nevertheless, there are some limitations in the present study. First, it is not clear whether lncRNAs ENST00000507296 and ENST00000532365 indicate cellular damage or other pathological processes in heart. Additionally, the successfully determined two lncRNAs were not highly significantly dysregulated in the heart tissues compared with the published data, which prompted a more intricate regulation mechanism for DCM. Alternatively, some false-positive signals may exist in the microarray probes, and the abundance of detected lncRNAs may be inaccurate due to the limitation of microarray. Moreover, the plasma samples were from victims of accidents, not from the healthy volunteers who were controls in the screening cohort. We anticipate that a series of lncRNAs is regulated in DCM and may have a prognostic value. Next-generation sequencing technologies should be considered to provide an alternative approach, while a TaqMan probe could be applied, which may increase the specificity of this assay. In addition, expanding follow-up studies is necessary to test the utility of the lncRNA as a predictor of cardiovascular incident. Moreover, it is still not known whether the dysregulation of ENST00000507296 and ENST00000532365 could be a self-compensatory protective mechanism against DCM or not. Therefore, the function studies are crucial to identify the detailed role of dysregulated lncRNAs in DCM as well as in other cardiovascular diseases.
MATERIALS AND METHODS

Study Population
This study was approved by the Ethics Committee of Tongji Hospital. Written informed consent was granted by the subjects recruited in the study or by the immediate family members in accordance with the Declaration of Helsinki. Between January 2010 and October 2014, patients diagnosed with DCM or HF and control subjects were enrolled in Tongji Hospital (Wuhan, China). Diagnoses of HF and DCM were on the basis of the final diagnosis at discharge, according to the American College of Cardiology and American Heart Association (ACC/AHA) guidelines.

In the screening cohort, using a test tube containing EDTA, blood samples (5 mL) were collected from 10 control donors (victims of accidents) and 10 chronic HF patients diagnosed with DCM during heart transplantation surgery. Then, plasma was obtained by centrifugation at 2,000 \( \times g \) at 4°C for 10 min, and the supernatant was carefully transferred to a new RNase and DNase-free 1.5-mL microtube and stored at \(-80^\circ C\) until use. The inclusion criteria for the DCM group were as follows: (1) New York Heart Association (NYHA) classes II–IV; (2) echocardiography LVEF < 50%; (3) echocardiography LVEDD > 55 mm. Exclusion criteria were coronary angiography showing the presence of more than 50% stenosis in the right coronary artery, left anterior descending artery, or left main stem. The clinical characteristics of the patients and controls are shown in Table S4.

In the validation case-control study, plasma samples were obtained from 64 DCM patients and 64 control subjects. The inclusion and exclusion criteria for the DCM group were the same as for the screening cohort, and all the control subjects met the following conditions: (1) no signs or symptoms of HF, or NYHA classes I and II; and (2) echocardiography LVEF > 50% and normal LVEDD. Their clinical and demographic characteristics are provided in Table S5.

In the replication case-control study, 198 DCM patients and 198 control individuals were enrolled. Inclusion and exclusion criteria of the DCM group were in accordance with the validation population. The patient characteristics are listed in Table S6.

In the follow-up cohort study, DCM patients were included according to the inclusion criteria of the validation study. The exclusion criteria of patients were as following: (1) patients with other severe systemic diseases (e.g., renal failure or hepatic diseases) and malignant tumors; (2) congenital heart diseases or significant valvular diseases; and (3) unwillingness to provide informed consent. The endpoints of the study covered cardiovascular death, heart transplantation, implantable cardioverter-defibrillator (ICD) implantation, and hospitalization due to worsening of HF. If the patient had several events, the time of the first event was regarded as the outcome. The median follow-up time was 21 months, and the maximum period reached up to 60 months. The patient characteristics are listed in Table S7.

Study Design
The procedure of this study was indicated as the following five main steps: (1) obtaining IncRNA profiles in the screening population; (2) IncRNA testing in the validation cohort; (3) RNA sequencing (RNA-seq) of selected IncRNAs in different human-derived cardiac cell types and correlation analysis between plasma levels of validated IncRNAs and severity of HF; (4) confirmation of the selected IncRNAs in the replication population; and (5) prognosis association of IncRNAs with patients with DCM in the follow-up population.

RNA Extraction
Total RNA was extracted from 250 \( \mu L \) plasma with TRIzol LS Reagent (Life Technologies, Carlsbad, CA, USA) following the manufacturer’s instructions. 50 pmol/L Caenorhabditis elegans miR-39 (cel-miR-39) was added as the spike-in control after TRIzol LS Reagent was added. The purity and quality of the total RNA were checked using the NanoDrop 2000 (Thermo Scientific, Waltham, MA, USA). The ratio of the
absorbance at 260 and 280 nm (optical density [OD] 260/280) of isolated RNA was between 1.8 and 1.9.

**Microarray and Real-Time qPCR**

RNA was pre-amplified and then underwent microarray analysis (Arraystar, Human IncRNA Array, v3.0). About 30,586 IncRNAs and 26,109 coding transcripts can be detected by Kangcheng Biotechnology (Shanghai, China). The microarray data gathered in this study were deposited in the NCBI Gene Expression Omnibus database under accession number GEO: GSE124401 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE124401).

C. elegans miR-39 (Riobio, Guangzhou, China) was applied as a normalization control. For each sample, the Ct values obtained for the three spiked-in cel-miR-39 samples and targeted IncRNAs were averaged to generate the relative expression levels in each plate. For different plates, cel-miR-39 of the samples were detected again to be a normalization control in this plate. The results from each plate were normalized against normalization controls. Real-time qPCR assays were conducted by the SYBR Select Master Mix (Life Technologies, Carlsbad, CA, USA) on a 7900HT Fast Real-Time PCR System (Life Technologies, Carlsbad, CA, USA). Cycling parameters were as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Relative expression levels between groups were calculated by the $2^{-\Delta\Delta C_T}$ method, as previously described. When Ct value was more than 37, the IncRNA was considered as undetectable. Gel electrophoresis, melt curve analysis, and product sequencing were used to confirm the specificity of primers. The primers are listed in Table S8.

**Cell Culture**

Human cardiac myocytes (HCMs; ScienCell Research Laboratories, Carlsbad, CA, USA) were from a single fetal donor, not pooled, and were maintained in cardiac myocyte medium (CMM; ScienCell Research Laboratories, catalog #6201), according to the instruction manual. CMM consists of basal medium, fetal bovine serum (FBS), cardiac myocyte growth supplement, and penicillin and streptomycin solution. Human cardiac fibroblasts-adult ventricular (HCF-av; ScienCell Research Laboratories, Carlsbad, CA, USA) were maintained in fibroblast medium-2 (FM-2; ScienCell Research Laboratories, catalog #2331), which contained fibroblast growth supplement-2 (FGS-2; Catalog #2382) and FBS. Human cardiac microvascular endothelial cells (HCMECs; ScienCell Research Laboratories, Carlsbad, CA, USA) were maintained in endothelial cell medium (ECM, catalog #1001). ECM consists of basal medium, FBS, endothelial cell growth supplement, and penicillin and streptomycin solution. All these cells were cultured and utilized as described previously.

**IncRNA Sequencing**

IncRNA sequencing and data analyses were performed by Personal Biotechnology (Shanghai, China). The sequencing data analyzed in this study were deposited in the NCBI Gene Expression Omnibus database under accession number GEO: GSE124402 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE124402). $p < 0.05$ was considered statistically significant.

**Statistical Analysis**

All data were analyzed by SPSS (v20.0; Chicago, IL, USA). Gaussian distribution was evaluated by Shapiro-Wilk and Kolmogorov-Smirnov tests. Continuous variables are described as...
means ± SD. For normal distribution data, the two-tailed Student’s t test was used, while the Mann-Whitney U test was used for skewed distribution. As the distribution of plasma IncRNA expression levels was skewed, horizontal lines indicate the median. The chi-square test was used to compare categorical variables. Statistical significance was set at p < 0.05. Correlation analysis was assessed by Spearman correlation for non-normally distributed variables. The receiver operating characteristic (ROC) curves were used to assess IncRNAs as diagnostic tools for distinguishing DCM. The IncRNA expression values were divided into high-expression and low-expression groups based on ROC curves with Youden’s index correction. The Kaplan-Meier survival curve indicated survival probabilities, and the significance of differences was tested by the log-rank test. Cox proportional hazards models were used to determine IncRNAs associated with incident cases.

SUPPLEMENTAL INFORMATION
Supplemental Information includes three figures, eight tables, and one data file and can be found with this article online at https://doi.org/10.1016/j.omtn.2019.02.004.

AUTHOR CONTRIBUTIONS
X.Z. designed the study, analyzed and interpreted the data, and drafted the paper; X.N., S.Y., H.L., J.F., C.L., Y.S., Y.Z., and H.H. participated in acquiring the data; D.W.W. and C.C. designed the work and drafted the paper.

CONFLICTS OF INTEREST
The authors declare no competing interests.

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REFERENCES
1. Elliott, P. (2000). Diagnosis and management of dilated cardiomyopathy. Heart 84, 106–112.
2. Japp, A.G., Gulati, A., Cook, S.A., Cowie, M.R., and Prasad, S.K. (2016). The diagnosis and evaluation of dilated cardiomyopathy. J. Am. Coll. Cardiol. 67, 2996–3010.
3. Halliday, B.F., Cleland, J.G.F., Goldberger, J.J., and Prasad, S.K. (2017). Personalizing risk stratification for sudden death in dilated cardiomyopathy: the past, present, and future. Circulation J. 81, 215–231.
4. Bokurt, B., Colvin, M., Cook, J., Cooper, L.T., Deswal, A., Fonarow, G.C., Francis, G.S., Lenihan, D., Lewis, E.F., McNamara, D.M., et al. (2016). Current diagnostic and treatment strategies for specific dilated cardiomyopathies: a scientific statement from the American heart association. Circulation 134, e579–e646.
5. Jeffries, J.L., and Towbin, J.A. (2010). Dilated cardiomyopathy. Lancet 375, 752–762.
6. Pietra, B.A., Kantor, P.F., Bartlett, H.L., Chin, C., Canter, C.E., Larsen, R.L., Edens, R.E., Colan, S.D., Towbin, J.A., Lipshultz, S.E., et al. (2012). Early predictors of survival to and after heart transplantation in children with dilated cardiomyopathy. Circulation 126, 1079–1086.
7. Kung, J.T., Colognori, D., and Lee, J.T. (2013). Long noncoding RNAs: past, present, and future. Genetics 193, 651–669.
8. Randuri, C. (2011). Kenq1ot1: a chromatin regulatory RNA. Semin. Cell Dev. Biol. 22, 343–350.
9. Clemson, C.M., Hutchinson, J.N., Sara, S.A., Ensminger, A.W., Fox, A.H., Chess, A., and Lawrence, J.B. (2009). An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles. Mol. Cell 33, 717–726.
10. Gong, C., and Maquat, L.E. (2011). IncRNAs transactivate STAU1-mediated mRNA decay by duplexing with 3’ UTRs via Alu elements. Nature 470, 284–288.
11. Grote, P., Wittler, L., Hendrix, D., Koch, F., Währisch, S., Bessaw, A., Macura, K., Blas, G., Kellis, M., Werber, M., and Herrmann, B.G. (2013). The tissue-specific IncRNA Fendrr is an essential regulator of heart and body wall development in the mouse. Dev. Cell 24, 206–214.
12. Klattenhoff, C.A., Scheurmann, J.C., Surface, L.E., Bradley, R.K., Fields, P.A., Steinhauser, M.L., Ding, H., Butty, V.L., Torrey, L., Haas, S., et al. (2013). Braveheart, a long noncoding RNA required for cardiovascular lineage commitment. Cell 152, 570–583.
13. D’Alessandra, Y., Devanna, P., Limana, F., Straino, S., Di Carlo, A., Brambilla, P.G., Rubino, M., Carena, M.C., Spazza, L., De Simone, M., et al. (2010). Circulating microRNAs are new and sensitive biomarkers of myocardial infarction. Eur. Heart J. 31, 2765–2773.
24. Steimle, A.E., Stevenson, L.W., Fonarow, G.C., Hamilton, M.A., and Moriguchi, J.D.

21. Bielecka-Dabrowa, A., von Haehling, S., Aronow, W.S., Ahmed, M.I., Rysz, J., and de Kok, J.B., Verhaegh, G.W., Roelofs, R.W., Hessels, D., Kiemeney, L.A., Aalders, et al. (2016). Circulating long non-coding RNA, LIPCAR, predicts survival in patients with heart failure. J. Cell. Mol. Med. 20(6), 1147–1159.

20. de Kok, J.B., Verhaegh, G.W., Roelofs, R.W., Hessels, D., Kiemeney, L.A., Aalders, et al. (2016). Circulating long non-coding RNAs NRON and MHRT as novel biomarker for coronary artery disease. Cardiovasc. Res. 112, 714–724.

19. Xuan, L., Sun, L., Zhang, Y., Huang, Y., Hou, Y., Guo, Y., Li, Q., Guo, Y., Feng, B., Cui, L., et al. (2016). Circulating 'lncRNA OTTHUMT0000387022' from monocytes as a novel biomarker for coronary artery disease. Cardiovasc. Res. 112, 714–724.

18. Lu, J., Wu, J., Zhao, Z., Wang, J., and Chen, Z. (2018). Circulating lncRNA serve as novel biomarker for coronary artery disease. Cardiovasc. Res. 112, 714–724.

17. Kumaraswamy, R., Bauters, C., Volkman, I., Maury, F., Fetsch, J., Holmman, A., Lemesle, G., de Groote, P., Pinet, F., and Thun, T. (2014). Circulating long noncoding RNA, LIPCAR, predicts survival in patients with heart failure. Circ. Res. 114, 1569–1575.

16. Orosco, A.F., and Lewis, D.E. (2010). Flow cytometric analysis of circulating microparticles in plasma. Cytometry A 77, 502–514.

15. Reis, E.M., and Verjovski-Almeida, S. (2012). Perspectives of long non-coding RNAs in cancer diagnostics. Front. Genet. 3, 32.

14. Melman, Y.F., Shah, R., Danielson, K., Xiao, J., Simonson, B., Barth, A., Chakir, K., Lewis, G.D., Lavender, Z., Truong, Q.A., et al. (2015). Circulating microRNA-30d is associated with response to cardiac resynchronization therapy in heart failure and regulates cardiomyocyte apoptosis: a translational pilot study. Circulation 131, 2202–2216.

13. Reis, E.M., and Verjovski-Almeida, S. (2012). Perspectives of long non-coding RNAs in cancer diagnostics. Front. Genet. 3, 32.

12. Oro, Z., and Lewis, D.E. (2010). Flow cytometric analysis of circulating microparticles in plasma. Cytometry A 77, 502–514.

11. Banach, M. (2013). Heart failure biomarkers in patients with dilated cardiomyopathy. Cardiovasc. Res. 90, 254–262.

10. Banach, M. (2013). Heart failure biomarkers in patients with dilated cardiomyopathy. Cardiovasc. Res. 90, 254–262.

9. Anfossi, S., Babayan, A., Pantel, K., and Calin, G.A. (2018). Clinical utility of circulating microRNA-199a and -210 in heart transplantation. J. Heart Lung Transplant. 37, 1171–1179.

8. Daubeny, P., Negent, A.W., Chondros, P., Carlin, J.B., Colan, S.D., Cheung, M., Davis, A.M., Chow, C.W., and Weintraub, R.G.; National Australian Childhood Cardiomyopathy Study (2006). Clinical features and outcomes of childhood dilated cardiomyopathy: results from a national population-based study. Circulation 114, 2671–2678.

7. O’Sullivan, J.J., Roche, S.L., Crossland, D.S., Chaudhari, M.P., Kirk, R.C., and Asif, H. (2008). Recovery of heart function in children with acute severe heart failure. Transplantation 85, 975–979.

6. Shaddy, R.E., Boucek, M.M., Hsu, D.T., Boucek, R.J., Canter, C.E., Mahony, L., Ross, R.D., Pahl, E., Blume, E.D., Dodd, D.A., et al. (2007). Carvedilol for children and adolescents with heart failure: a randomized controlled trial. JAMA 298, 1171–1179.

5. Xiang, M., Zeng, Y., Yang, R., Xu, H., Chen, Z., Zhong, J., Xie, H., Xu, Y., and Zeng, X. (2014). U6 is not a suitable endogenous control for the quantification of circulating microRNAs. Biochem. Biophys. Res. Commun. 454, 210–214.

4. Anfossi, S., Babayan, A., Pantel, K., and Calin, G.A. (2018). Clinical utility of circulating non-coding RNAs – an update. Nat. Rev. Clin. Oncol. 15, 541–563.

3. Mestdagh, P., Van Vlierberghe, P., De Weer, A., Muth, D., Westermann, F., Speelman, F., and Vandesompele, J. (2009). A novel and universal method for microRNA RT-qPCR data normalization. Genome Biol. 10, R64.

2. Shah, R.V., Rong, J., Larson, M.G., Yeri, A., Ziegler, O., Tanriverdi, K., Murthy, V., Liu, X., Xiao, C., Pico, A.R., et al. (2018). Associations of circulating extracellular microRNAs with myocardial remodeling and heart failure. JAMA Cardiol. 3, 871–876.

1. Hunt, S.A., Abraham, W.T., Chin, M.H., Feldman, A.M., Francis, G.S., Ganiats, T.G., et al. (2006). Guidelines for the Evaluation and Management of Heart Failure: developed in collaboration with the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure): endorsed by the Heart Rhythm Society; Society for Heart and Lung Transplantation: endorsed by the Heart Rhythm Society. Circulation 114, 1101–1179.