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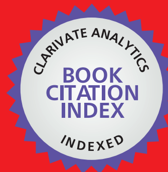

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Altered Expression of mRNA and miRNA during Mechanical Support of the Failing Human Heart

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1. Introduction

Remodeling during heart failure is characterized by structural rearrangement of the cardiac ventricular wall architecture. It involves hypertrophy of cardiomyocytes, fibroblast proliferation, and increased deposition of extracellular matrix (ECM) proteins (Brower et al., 2006). Support of the left ventricle with a Left Ventricular Assist Device (LVAD) in patients with end-stage heart failure results in less neurohormonal activation (Estrada-Quintero et al., 1995; Frazier and Myers, 1999; Bruggink et al., 2006a), improvement of the patient's general condition (De Jonge et al., 2001; Grady et al ., 2003), reduction in ventricular diameter (reverse remodeling), and limited recovery of contractile elements in cardiomyocytes (Muller et al., 1997; De Jonge et al., 2002). Furthermore, reduction of ECM volume (Milting et al., 2008; Goldsmith and Borg , 2002; Bruggink et al., 2006b), diminished production of tumor necrosis factor (Thohan et al., 2005; Bruggink et al., 2008), and reduction in brain natriuretic protein serum levels (Bruggink et al., 2006a; Kemperman et al., 2004) have been described during LVAD support. The changes in ECM during this process of reverse remodeling resulted not only in a time-dependent change of type I and type III collagen protein (Goldsmith and Borg, 2002; Stamenkovic, 2003), but also in considerable changes in composition of the basal membrane. These included amongst others reduced collagen type IV content in the cardiomyocyte basal membrane, as a result of increased matrix metalloproteinase activity (Bruggink et al., 2007; Spinale, 2002; Li et al., 2001; Klotz et al., 2005). So, during LVAD support myocardial architecture and composition change at the level of both the cardiomyocytes and the ECM.

The mechanics of the heart require a close interplay between cardiomyocytes and the ECM (Parker and Ingber, 2007) and therefore, one may anticipate a coordinated change in the molecules responsible for this interaction. These changes may not be the same in all heart failure patients supported by a mechanical support device. Some patients' hearts may improve and may be eligible for removal of the support device without a heart transplantation (bridge to recovery and weaning from the device), whereas others do not

improve on support or may even deteriorate and these patients remain on the device (destination therapy) or will ultimately receive a heart transplant (bridge to transplantation). To make the proper choice of the type of therapy for each patient a good set of (bio)markers is required (De Weger and De Jonge, 2009).
In this chapter, we describe whether mRNA expression patterns could be indicative for the state of heart functionality supported by a LVAD (Heart-Mate I, Thoratec, Pleasanton, CA, USA). The changes in mRNA profiles that are detectable in myocardial biopsies taken from patients with end-stage heart failure due to dilated cardiomyopathy (DCM) or ischaemic heart disease (IHD) before and after LVAD support were analyzed, and compared with biopsies taken from control hearts as a reference (Table 1). Furthermore, the expression of 109 genes is described, which are involved in the process of mechanotransduction in the heart. Their expression was studied by Quantitative(Q)-PCR. The cohort comprised selected genes coding for ECM filaments (such as collagens), transmembrane proteins (molecules that connect cells and matrix components like integrins and sarcoglycans), intracellular molecules, adhesion molecules related to mechanotransduction and signal transduction, ion-channel molecules, and factors involved in pro- and anti-fibrotic processes. The expression of mRNA is not always directly related to protein production, due to post-transcriptional regulation. Recently, it is has been shown that intracellular gene expression is regulated in part by small RNA molecules: microRNAs (miRs). These miRs are highly expressed in heart tissue (Ji et al., 2007; Cheng et al., 2007) and have also been related to heart diseases (Chen, 2007; Van Rooij et al., 2006). The list of regulatory miRs involved in heart disease is constantly increasing (Coutinho et al., 2007; Markham and Hill, 2010). Each miR can regulate various mRNA expressions and which mRNA is

Nr	*Age*	*Diagnosis*	*Gender*	*Days on LVAD*	*Medication during LVAD-support*
1	56	IHD	Male	138	None
2	57	IHD	Male	225	None
3	45	IHD	Male	259	2,5 mg Ramipril
4	57	IHD	Male	263	None
5	36	IHD	Male	325	2x 4 mg Perindopril
6	26	IHD	Male	357	None
7	39	IHD	Male	548	3x 6,25 mg Capoten
8	34	DCM	Female	55	3x 6,25 mg Capoten
9	17	DCM	Male	111	None
10	47	DCM	Male	190	None
11	35	DCM	Male	196	None
12	32	DCM	Female	219	3 mg Captopril
13	25	DCM	Male	263	1x 25 mg Losartan
14	32	DCM	Male	286	4 mg Perindopril
15	25	DCM	Male	330	2x 10 mg Fosinopril
16	46	DCM	Male	484	3x 50 mg Capoten

DCM: dilated cardiomyopathy, IHD: ischemic heart disease, LVAD: left ventricular assist device.

Table 1. Patient characteristics.

regulated by which miR is not determined with certainty for most genes (see www.targetscan.org; Schuldt, 2010).
An additional goal of this study was therefore, to analyse the changes in expression of 4 miRs that are known to be expressed in the myocardium (Chen, 2007; Ikeda et al., 2007): miR-1, miR-133a, miR-133b and miR-208. These miRs have been related to heart failure. The expression of these miRs was measured in the same group of patients used to analyse the mRNA expression after LVAD support. Our purpose was to find out whether the LVAD-induced remodeling of the heart was accompanied by changes in the expression of miRs that could influence the protein expression of the mRNA studied. This could make expression of some mRNAs less suitable as biomarker for the assessment of the functional quality of the supported heart. If this is the case the expression of miRs may serve as better markers either in the myocardium or the serum.

2. Tissue distribution of mRNA and miR in the myocardium

Tissue samples taken form various locations in cross sections of the heart showed that the expression of both miR in the right and left ventricular wall did not show significant variation. Only in the infarcted area the expression of miR was low to absent. Similar data were obtained for mRNA (Figure 1).

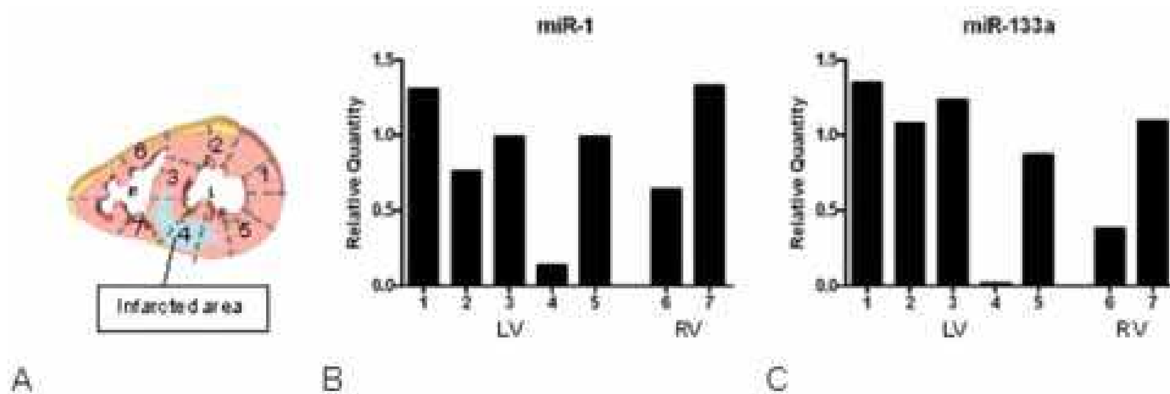

Fig. 1A. Circular cross section of the heart indicating the biopsy areas.
B and C. miRNA expression in and around the infarcted area in the indicated biopsies of a representative case. The miRNA expression in an infarcted area (biopsy 4) is much lower than in the surrounding areas. LV and RV = left and right ventricle.

3. Hierarchical clustering of gene expression in myocardial tissue of IHD and DCM patients

The gene profiles in DCM and IHD heart tissue, detected by Q-PCR, were compared using TIGR software (www.tm4.org). Figure 2 shows the whole data set for all pre-LVAD samples versus the median of control samples. Hierarchical clustering was performed on all 92 detectable genes. The genes that were not detectable (n=14) and house keeping genes (n=3) were excluded (Table 2). The clustering segregated two groups: one group consists of 6 DCM and 1 IHD patients and the other group consists of 5 IHD patients with 2 DCM patients. One DCM and one IHD patient were clustered outside both groups. So, there is a strong tendency of segregation between IHD and DCM. Therefore, the DCM and IHD patient groups were analyzed separately in this study.

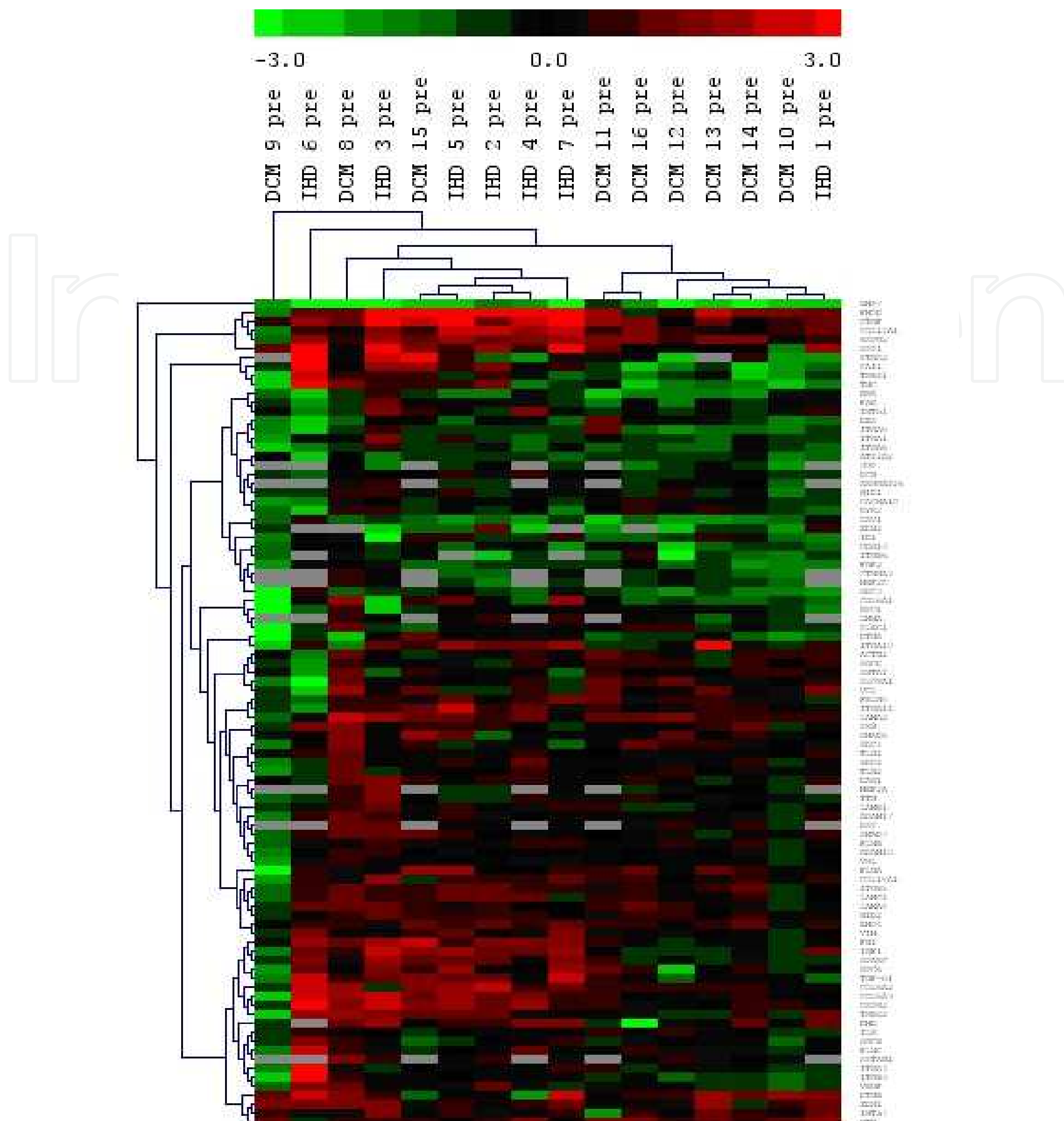

Fig. 2. Unsupervised hierachical clustering of myocardial gene expression profiles pre-LVAD in IHD and DCM patients. Clustering was performed on all 92 detectable genes. Unsupervised hierarchical clustering was performed on normalized data using the multi-experiment viewer (MeV, version 4.3) of the TIGR software (www.tm4.org). The Reletive quantity (RQ) of each sample per gene was normalized:
Normalized signal of sample x = Log2 (RQ sample x / median RQ).
To compare DCM and IHD the median was taken from the RQ (relative quality) of control hearts. To compare pre- and post-LVAD in DCM and IHD samples, the median of all DCM or all IHD samples were taken, respectively. Clustering was performed on the whole dataset, and distance metric selection (Euclidean distance) and linkage metric selection (Complete linkage clustering) were used (www.tm4.org). This segregated two groups; one group consisting of 6 DCM patients with 1 IHD patient, and the other of 5 IHD patients with 2 DCM patients. Two patients (one DCM and one IHD) clustered outside these groups. Red: mRNA expression is higher than the median of control hearts. Green: mRNA expression is lower than the median of control hearts. Grey: not done.

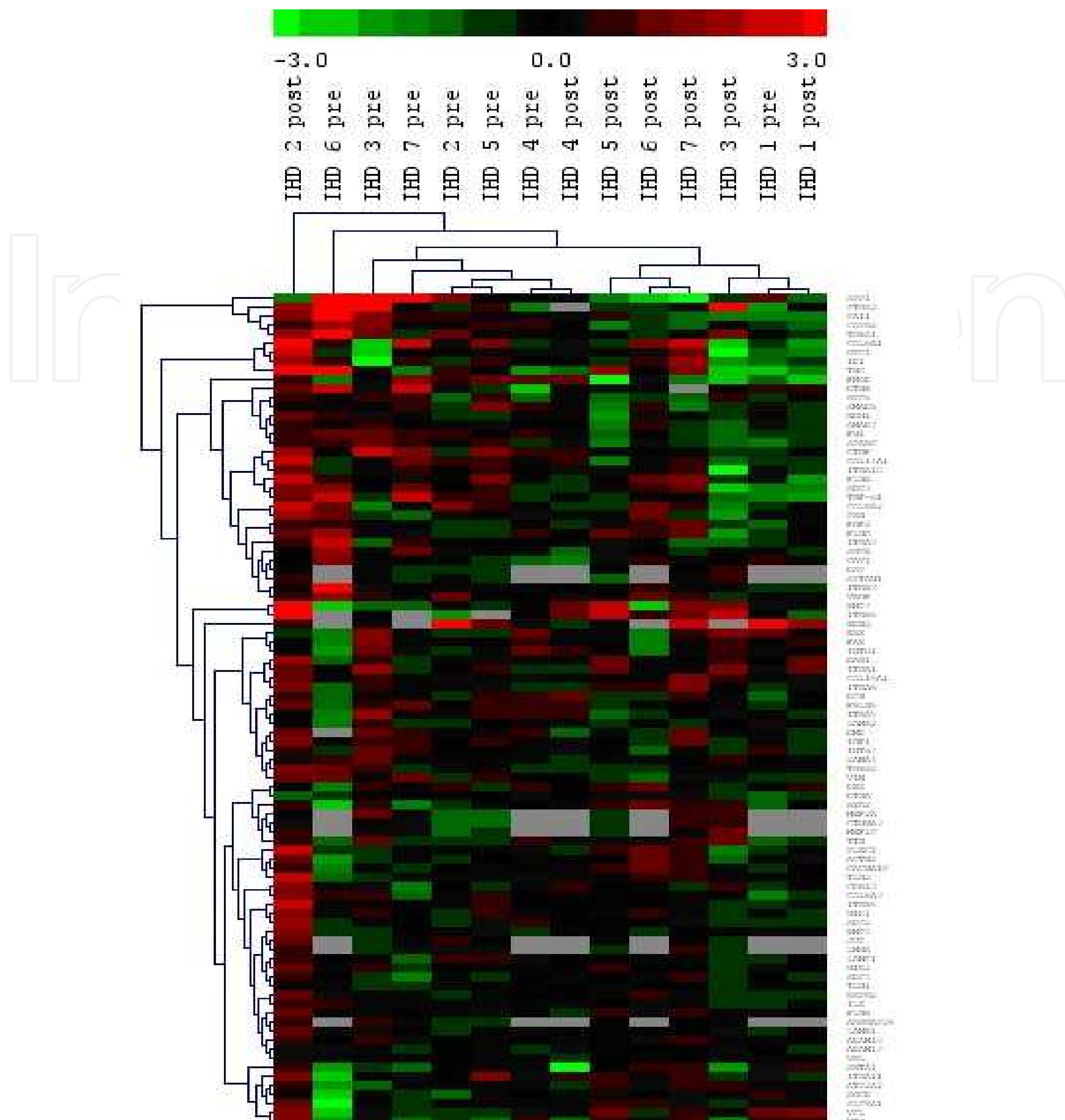

Fig. 3. Unsupervised hierarchical clustering of myocardial gene expression profiles pre- and post-LVAD in IHD patients.
Clustering was performed on all 92 detectable genes and it segregated the patient group into a pre- and post-LVAD group. See for explanation Figure 2. Red: mRNA expression is higher than the median of all IHD samples. Green: mRNA expression is lower than the median of all IHD samples. Grey: not done.

The expression of 14 genes was below level of detection and are therefore not included in this table: ADAM 12 (ADAM metallopeptidase domain 12), ADAM 15 (ADAM metallopeptidase domain 15), AGC1 (aggrecan 1), ANK1 (Ankyrin 1), DSPG3 (dermatan sulfate proteoglycan 3), EDN3 (endothelin 3), LAMC3 (laminin, gamma 3), MMP-7 (matrix metallopeptidase 7), MUC16 (mucin 16), NOS1 (nitric oxide synthase 1), SCN1A (sodium channel, voltage-gated, type I, alpha), SCN2A2 (sodium channel, voltage-gated, type II, alpha 2), SDC1 (syndecan 1), TNXB (tenascin XB).

Category	Gene name	Gene code AB	assay code AB	DCM p-value pre vs post	DCM fold change pre vs post	DCM p-value pre vs control		DCM p-value post vs control		IHD p-value pre vs post	IHD fold change pre vs post	IHD p-value pre vs control		IHD p-value post vs control	
ECM	collagen, type XIV, alpha 1	COL14A1	Hs00385388_m1	0,033	1,75	0,576		0,055	>	0,542		0,014	>	0,197	
	collagen, type XV, alpha 1	COL15A1	Hs00266332_m1	0,003	1,76	0,353		0,131		0,079		0,645		0,161	
	collagen, type VI, alpha 1	COL6A1	Hs00242448_m1	0,104		0,716		0,181		0,374		0,903		0,451	
	collagen, type VI, alpha 2	COL6A2	Hs00242484_m1	0,154		0,129		0,121		0,488		0,061		0,195	
	collagen, type VI, alpha 3	COL6A3	Hs00365098_m1	0,042	1,59	0,842		0,048	>	0,703		0,075		0,081	
	chondroitin sulfate proteoglycan 2 (versican)	CSPG2	Hs00171642_m1	0,990		0,470		0,547		0,078		0,065	>	0,472	
	decorin	DCN	Hs00370385_m1	0,049	0,56	0,051	<	0,903		0,273		0,352		0,964	
	fibulin 5	FBLN5	Hs00197064_m1	0,343		0,490		0,662		0,721		0,481		0,660	
	fibromodulin	FMOD	Hs00157619_m1	0,250		0,097		0,196		0,062	-0,67	0,009	>	0,134	
	fibronectin 1	FN1	Hs00415006_m1	0,445		0,587		0,885		0,024	-0,76	0,023	>	0,589	
	heparan sulfate proteoglycan 2 (perlecan)	HSPG2	Hs00194179_m1	0,024	0,96	0,491		0,067	>	0,805		0,052	>	0,122	
	osteonectin	SPARC	Hs00277762_m1	0,592		0,260		0,236		0,190		0,018	>	0,195	
AFF	bone morphogenetic protein 4	BMP4	Hs00370078_m1	0,029	0,98	0,215		0,023	>	0,120		0,017	>	0,064	>
	bone morphogenetic protein 7	BMP7	Hs00233476_m1	0,034	2,14	0,006	<	0,732		0,072		0,018	<	0,693	
	inhibitor of DNA binding 1	ID1	Hs00357821_g1	0,031	0,87	0,099		0,288		0,323		0,166		0,540	
	prostaglandin-endoperoxide synthase 2/COX2	PTGS2	Hs00153133_m1	0,464		0,911		0,358		0,752		0,445		0,490	
	mothers against DPP homolog 7	SMAD7	Hs00178696_m1	0,380		0,522		0,876		0,296		0,065	>	0,681	
PFF	connective tissue growth factor	CTGF	Hs00170014_m1	0,258		0,318		0,082		0,398		0,048	>	0,112	
	endothelin 1	EDN1	Hs00174961_m1	0,710		0,332		0,442		0,913		0,040	>	0,184	
	endothelin 2	EDN2	Hs00266516_m1	0,431		0,084		0,185		0,419		0,267		0,150	
	fibroblast growth factor 2	FGF2	Hs00266645_m1	0,306		0,022	<	0,369		0,143		0,113		0,849	
	insulin-like growth factor 1	IGF1	Hs00153126_m1	0,084		0,938		0,114		0,903		0,003	>	0,076	
	mothers against DPP homolog 6	SMAD6	Hs00178579_m1	0,089		0,193		0,881		0,125		0,571		0,030	<
	transforming growth factor beta 1	TGF-β1	Hs00171257_m1	0,565		0,728		0,965		0,223		0,318		0,855	
	vascular endothelial growth factor	VEGF	Hs00900054_m1	0,586		0,852		0,134		0,254		0,880		0,538	
BM	glypican 1	GPC1	Hs00157805_m1	0,546		0,738		0,916		0,285		0,330		0,749	
	glypican 6	GPC6	Hs00170677_m1	0,200		1,000		0,373		0,839		0,095		0,104	
	laminin, alpha 2	LAMA2	Hs00166308_m1	0,505		0,072		0,111		0,798		0,078		0,003	>
	laminin, alpha 4	LAMA4	Hs00158588_m1	0,965		0,933		0,949		0,234		0,610		0,935	
	laminin, beta 1	LAMB1	Hs00158620_m1	0,001	0,82	0,338		0,255		0,268		0,696		0,201	
	laminin, gamma 1 (formerly LAMB2)	LAMC1	Hs00267056_m1	0,058	0,91	0,273		0,024	>	0,938		0,026	>	0,019	>
	lamin A/C	LMNA	Hs00153462_m1	0,261		0,287		0,717		0,938		0,055	>	0,112	
	nidogen 1	NID1	Hs00159600_m1	0,264		0,434		0,973		0,763		0,491		0,953	
	nidogen 2 (osteonidogen)	NID2	Hs00201233_m1	0,871		0,950		0,957		0,395		0,718		0,332	
	plasminogen activator inhibitor-2	PAI1	Hs00167155_m1	0,491		0,321		0,476		0,336		0,433		0,657	
	plectin 1	PLEC1	Hs00356977_m1	0,145		0,963		0,138		0,161		0,624		0,294	
	syndecan 4	SDC4	Hs00161617_m1	0,445		0,516		0,903		0,242		0,484		0,905	
	osteopontin	SPP1	Hs00167093_m1	0,001	-1,37	0,805		0,042	<	0,039	-1,82	0,064	>	0,155	
	thrombospondin 1	THBS1	Hs00170236_m1	0,943		0,094		0,118		0,452		0,931		0,322	
	thrombospondin 2	THBS2	Hs00170248_m1	0,656		0,785		0,523		0,764		0,040	>	0,264	
	tenascin C	TNC	Hs00233648_m1	0,654		0,389		0,538		0,890		0,538		0,364	
	vitronectin	VTN	Hs00169863_m1	0,464		0,010	>	0,061	>	0,171		0,366		0,071	
TAM	ADAM metallopeptidase domain 10	ADAM10	Hs00153853_m1	0,960		0,697		0,614		0,378		0,654		0,211	
	ADAM metallopeptidase domain 17	ADAM17	Hs00234224_m1	0,461		0,832		0,294		0,596		0,051	>	0,095	
	ATPase, Ca++ transporting, cardiac muscle	ATP2A2	Hs00155939_m1	0,416		0,096		0,109		0,008	1,02	0,002	<	0,297	
	calcium channel, alpha 1C subunit	CACNA1C	Hs00167681_m1	0,018	0,92	0,296		0,319		0,041		0,125		0,439	
	caveolin 1, caveolae protein, 22kDa	CAV1	Hs00184697_m1	0,267		0,011	<<	0,027	<	0,442		0,291		0,126	
	cadherin 13, H-cadherin (heart)	CDH13	Hs00169908_m1	0,002	0,89	0,118		0,356		0,057	0,81	0,168		0,414	
	dystroglycan 1	DAG1	Hs00189308_m1	0,324		0,599		0,126		0,089		0,244		0,054	>
	integrin, alpha 1	ITGA1	Hs00235030_m1	0,014	0,98	0,064		0,500		0,337		0,998		0,511	
	integrin, alpha 10	ITGA10	Hs00174623_m1	0,023	2,47	0,651		0,060	>	0,832		0,017	>	0,072	
	integrin, alpha 11	ITGA11	Hs00201927_m1	0,150		0,264		0,030	>	0,581		0,258		0,049	>
	integrin, alpha 3	ITGA3	Hs00233722_m1	0,309		0,856		0,525		0,364		0,398		0,818	
	integrin, alpha 5	ITGA5	Hs00233732_m1	0,370		0,206		0,945		0,039	-0,41	0,810		0,367	
	integrin, alpha 6	ITGA6	Hs00173952_m1	0,007	1,40	0,035	<	0,457		0,046	1,23	0,249		0,303	
	integrin, alpha 7	ITGA7	Hs00174397_m1	0,062	-0,07	0,102		0,518		0,401		0,237		0,368	
	integrin, beta 1	ITGB1	Hs00559595_m1	0,231		0,313		0,646		0,377		0,747		0,840	
	integrin, beta 3	ITGB3	Hs00173978_m1	0,908		0,643		0,676		0,325		0,406		0,769	
	integrin, beta 5	ITGB5	Hs00609896_m1	0,510		0,252		0,132		0,410		0,012	>	0,288	
	integrin, beta 6	ITGB6	Hs00168458_m1	0,163		0,138		0,602		0,026	0,34	0,070		0,766	
	syndecan 3	SDC3	Hs00206320_m1	0,059	1,03	0,066	<	0,163		0,825		0,345		0,325	
	sarcoglycan, beta	SGCB	Hs00165095_m1	0,107		0,090		0,045	<	0,047	-0,78	0,333		0,003	
	sarcoglycan, delta	SGCD	Hs00165726_m1	0,538		0,152		0,051	>	0,056	1,06	0,589		0,002	>
	solute carrier family 8, member 1	SLC8A1	Hs00253432_m1	0,587		0,287		0,285		0,110		0,590		0,188	

Category	Gene name	Gene code AB	assay code AB	DCM p-value pre vs post	DCM fold change pre vs post	DCM p-value pre vs control	DCM p-value post vs control	IHD p-value pre vs post	IHD fold change pre vs post	IHD p-value pre vs control	IHD p-value post vs control
IF	actinin, alpha 2	ACTN2	Hs00153809_m1	0,685		0,212	0,569	0,171		0,610	0,073
	Rho GTPase activating protein 26	ARHGAP26	Hs00209396_m1	0,294		0,350	0,876	0,349		0,869	0,445
	desmin	DES	Hs00157258_m1	0,416		0,617	0,156	0,829		0,169	0,136
	dystrophin	DMD	Hs00244243_m1	0,186		0,202	0,203	0,862		0,036 >	0,044 >
	desmoplakin	DSP	Hs00189422_m1	0,559		0,869	0,367	0,015	0,50	0,918	0,120
	dystrobrevin, alpha	DTNA	Hs00263201_m1	0,428		0,375	0,673	0,890		0,981	0,885
	dystrobrevin, beta	DTNB	Hs00222463_m1	0,458		0,041 >	0,074	0,313		0,143	0,263
	filamin A, alpha	FLNA	Hs00155065_m1	0,227		0,131	0,095	0,575		0,155	0,214
	filamin B, beta	FLNB	Hs00181698_m1	0,003	0,92	0,895	0,034 >	0,177		0,161	0,068
	filamin C, gamma	FLNC	Hs00155124_m1	0,118		0,248	0,564	0,705		0,677	0,804
	junction plakoglobin	JUP	Hs00158408_m1	0,505		0,050 <	0,076	0,947		0,052 <	0,093
	paxillin	PXN	Hs00236064_m1	0,077		0,514	0,121	0,393		0,494	0,319
	syntrophin, alpha 1	SNTA1	Hs00162045_m1	0,312		0,112	0,399	0,454		0,372	0,964
	spectrin alpha	SPTAN1	Hs00162203_m1	0,948		0,361	0,353	0,065	0,56	0,848	0,232
	talin 1	TLN1	Hs00196775_m1	0,653		0,210	0,127	0,370		0,113	0,037 >
	talin 2	TLN2	Hs00322257_m1	0,005	1,13	0,936	0,022 >	0,135		0,428	0,298
	titin	TTN	Hs00399225_m1	0,120		0,803	0,401	0,126		0,951	0,195
	vinculin	VCL	Hs00247826_m1	0,093		0,154	0,503	0,078		0,934	0,108
	vimentin	VIM	Hs00185584_m1	0,304		0,833	0,513	0,551		0,160	0,474
STF	catenin (cadherin-associated protein), alpha 3	CTNNA3	Hs00379052_m1	0,377		0,446	0,900	0,002	1,09	0,163	0,492
	integrin-initiated extracellular signal-regulated kinase	ERK	Hs00177066_m1	0,102		0,054 <	0,849	0,433		0,435	0,836
	Focal adhesion kinase	FAK	Hs00178587_m1	0,016	0,57	0,312	0,496	0,235		0,907	0,471
	integrin-linked kinase	ILK	Hs00177914_m1	0,526		0,527	0,292	0,947		0,692	0,720
	myocyte enhancer factor 2A	MEF2A	Hs00271535_m1	0,164		0,239	0,864	0,030	0,94	0,367	0,865
	myocyte enhancer factor 2C	MEF2C	Hs00231149_m1	0,491		0,564	0,260	0,023	0,69	0,302	0,956
	ryanodine receptor 2 (cardiac)	RYR2	Hs00181461_m1	0,045	0,70	0,476	0,007 >	0,023	1,73	0,364	0,046 >
	syndecan 1	SDC1	Hs00174579_m1	0,348		0,838	0,495	1,000		0,890	0,920
	von Hippel-Lindau tumor suppressor	VHL	Hs00184451_m1	0,234		0,762	0,699	0,979		0,762	0,774

Table 2. Statistical analysis of gene expression profiles in DCM and IHD patients. Significant changes are indicated in yellow. The genes are grouped by function/location. Abbreviations: extracellular matrix proteins (ECM), pro- and anti-fibrotic factors (P/AFF), basal membrane proteins (BM), transmembrane and adhesion molecules (TAM), intracellular filaments (IF), and signal transduction factors (STF). Applied Biosystems (AB). > and < indicate whether gene expression is significantly higher or lower compared to control.

4. Hierarchical clustering of gene expression in myocardial tissue pre- and post-LVAD support

Hierarchical clustering of the IHD samples showed a clear segregation into a pre- and a post-LVAD group (Figure 3). In DCM patients a similar segregation into a pre- and a post-LVAD group was not evident (data not shown).

5. Differential expression of genes in myocardial tissue pre- and post LVAD

Changes in gene expression were tested individually using the paired t-test in DCM and IHD separately. Furthermore, these gene profiles were compared with gene profiles of controls to test whether gene profiles normalized or showed a tendency to deviate from normal after LVAD therapy using the unpaired t-test. Table 2 shows all genes investigated, grouped by function/ location: extracellular matrix proteins (ECM), basal membrane proteins (BM), transmembrane and adhesion molecules (TAM), intracellular filaments (IF),

signal transduction factors (STF) and pro- and anti-fibrotic factors (P/AFF) with the p-values and fold changes.

In Table 3 only the genes that show significant changes are indicated for DCM and IHD patients separately. Only a minority of genes showed a significant difference between pre- and post-LVAD: DCM 19/92 genes (21 %) and IHD 12/92 genes (13 %). Most of these genes showed an upregulation post-LVAD (DCM 18/19 genes and IHD 8/12 genes). In DCM pre-LVAD 6 genes and post-LVAD 9 genes were upregulated compared to control. Only one gene, encoding caveolin, showed a decreased expression in both pre- and post-LVAD compared to control. In IHD pre-LVAD 12 genes were upregulated and 2 downregulated compared to control. Post-LVAD 6 were upregulated and 2 downregulated. Among these, two genes (dystrophin and laminin gamma 1) showed an increased expression compared to control in both pre- and post-LVAD samples.

DCM

Gene name	pre vs post	pre vs control	post vs control
osteopontin	▼	=	<
bone morphogenetic protein 4	▲	=	>
collagen, type VI, alpha 3	▲	=	>
filamin B, beta	▲	=	>
laminin, gamma 1	▲	=	>
ryanodine receptor 2 (cardiac)	▲	=	>
talin 2	▲	=	>
cadherin 13, H-cadherin (heart)	▲	=	=
calcium channel, alpha 1C subunit	▲	=	=
collagen, type XIV, alpha 1	▲	=	=
collagen, type XV, alpha 1	▲	=	=
decorin	▲	=	=
focal adhesion kinase	▲	=	=
heparan sulfate proteoglycan 2 (perlecan)	▲	=	=
inhibitor of DNA binding 1	▲	=	=
integrin, alpha 1	▲	=	=
integrin, alpha 10	▲	=	=
laminin, beta 1	▲	=	=
bone morphogenetic protein 7	▲	<	=
integrin, alpha 6	▲	<	=
dystrobrevin beta	=	>	=
vitronectin	=	>	=
integrin, alpha 11	=	=	>
sarcoglycan, beta	=	=	<
fibroblast growth factor 2	=	<	=
junction plakoglobin	=	<	=
caveolin 1	=	<	<

IHD

Gene name	pre vs post	pre vs control	post vs control
fibronectin 1	▼	>	=
integrin, alpha 5	▼	=	=
osteopontin	▼	=	=
sarcoglycan, beta	▼	=	=
ryanodine receptor 2 (cardiac)	▲	=	>
cadherin 13, H-cadherin (heart)	▲	=	=
calcium channel, alpha 1C subunit	▲	=	=
catenin (cadherin-associated protein), alpha 3	▲	=	=
desmoplakin	▲	=	=
integrin, alpha 6	▲	=	=
integrin, beta 6	▲	=	=
myocyte enhancer factor 2A	▲	=	=
myocyte enhancer factor 2C	▲	=	=
spectrin alpha	▲	=	=
ATPase, Ca++ transporting, cardiac muscle	▲	<	=
dystrophin	=	>	>
lamin, gamma 1	=	>	>
bone morphogenetic protein 4	=	>	=
collagen, type XIV, alpha 1	=	>	=
connective tissue growth factor	=	>	=
fibromodulin	=	>	=
insulin-like growth factor 1	=	>	=
integrin, beta 5	=	>	=
integrin, alpha 10	=	>	=
integrin, alpha 6	=	>	=
osteonectin	=	>	=
thrombospondin 2	=	>	=
integrin, alpha 11	=	=	>
laminin, alpha 2	=	=	>
sarcoglycan, delta	=	=	>
talin 1	=	=	>
mothers against DPP homolog 6	=	=	<
bone morphogenetic protein 7	=	<	=

Table 3. Summary of significant alterations in gene expression.
▼: decreased or ▲: increased gene expression after LVAD support, =: no change, >:higher or <:lower expression pre- or post-LVAD compared to control. The shaded (green) genes are significantly altered in both DCM and IHD.

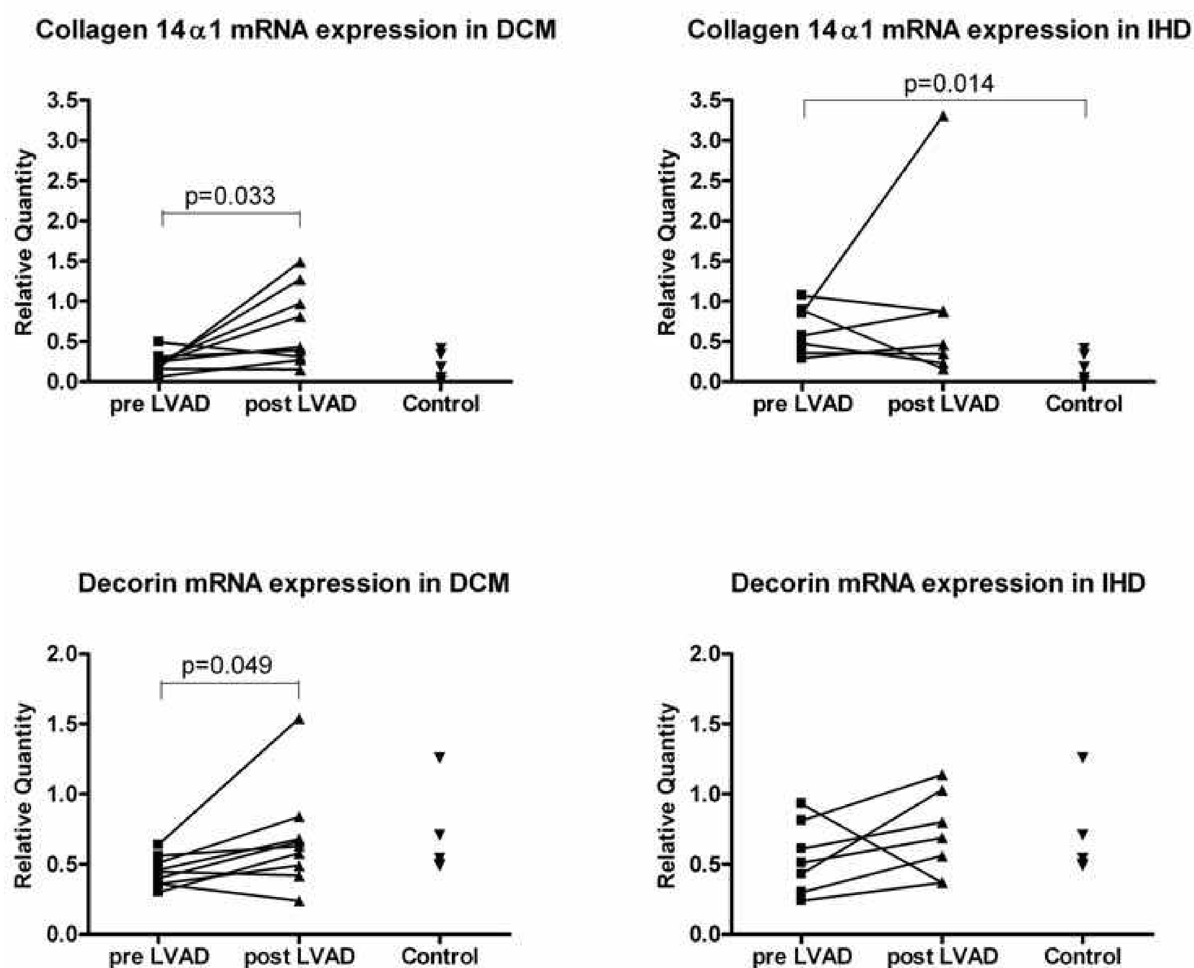

Fig. 4. Relative mRNA expression of genes encoding ECM proteins.

Relative mRNA expression was determined pre- and post-LVAD of DCM and IHD and tested in paired t-test. Increase of collagen 14α1 mRNA expression is significant in DCM but not in IHD. Compared to the control, only the mRNA expression pre-LVAD of IHD patients is significantly higher (unpaired t-test). Decorin is significantly increased post-LVAD in DCM. None of the pre- and post-LVAD samples differed significantly from the control samples.

5.1 Genes encoding extracellular matrix proteins

In DCM, 5 genes encoding ECM proteins were upregulated post-LVAD. However, except for collagen type VI alpha3, these genes did not differ significantly (either pre- or post LVAD) from control. This indicates that the increased expression of these 5 genes induced by the LVAD support is significant but as a group are not different from the control group (Figure 4). In IHD most differences between pre-LVAD and control were observed in genes encoding ECM proteins, but in post-LVAD samples these differences had disappeared, suggesting a high expression of ECM gene activity pre-LVAD (Figure 4).
Relative mRNA expression was determined pre- and post-LVAD of DCM and IHD and tested with the paired t-test. Increase of integrin α6 mRNA expression was significant in both DCM and IHD during LVAD support. Compared to the control, only the mRNA expression pre-LVAD of DCM patients is significantly lower (unpaired t-test). Integrin β6 is

only significantly increased post-LVAD in the IHD group. Compared to the control none of the pre- and post-LVAD samples differed significantly.

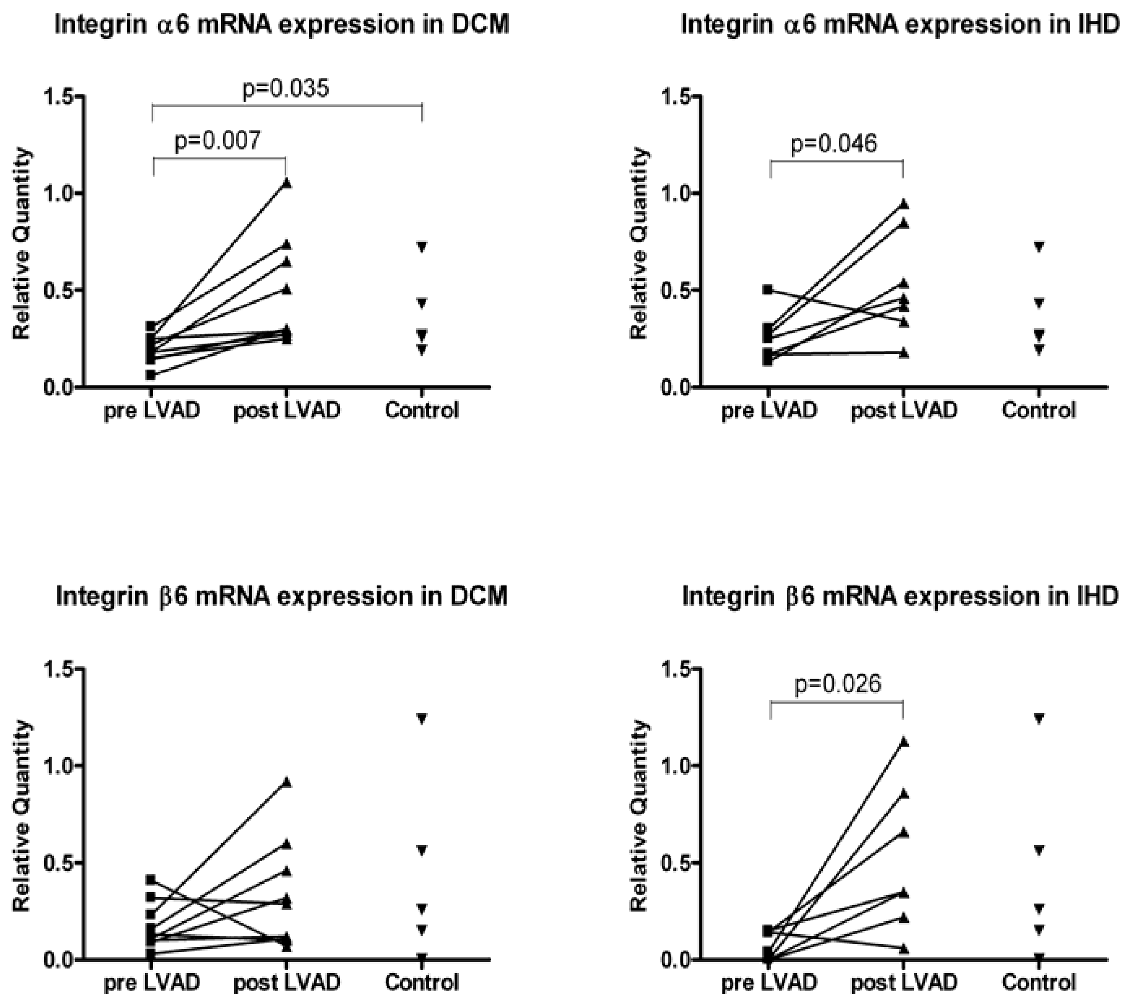

Fig. 5. Relative mRNA expression of genes encoding different integrins.

5.2 Genes involved in the fibrotic pathway

In the fibrotic pathway remarkable differences between DCM and IHD were observed. In DCM patients the expression of genes encoding pro-fibrotic factors (TGFβ1, FGF, IGF, endothelin and CTGF) remained unchanged, whereas the genes encoding anti-fibrotic proteins (BMP-4, BMP-7, decorin and ID1) increased after LVAD support. Pre-LVAD the expression of the pro-fibrotic factor FGF2 and the anti-fibrotic factor BMP-7 was low compared to control. Post-LVAD the expression of the anti-fibrotic factor BMP-4 was increased compared to control. However, in IHD patients these genes showed unchanged expression during LVAD support, but in pre-LVAD samples the pro-fibrotic genes are expressed stronger than in control and the anti-fibrotic gene BMP-7 is expressed less than in control. The post-LVAD expression pattern is comparable to that of control.

5.3 Genes encoding basal membrane proteins

The gene encoding osteopontin is the most remarkable member of the BM group. In both DCM and IHD patients, osteopontin expression is significantly reduced after LVAD

support. Other BM proteins showed hardly any change, apart from laminin, vitronectin and thrombospondin (anchoring proteins).
Several integrins showed differential expression (mostly upregulation) in both DCM and IHD patients. In particular, integrin β6 gene expression showed a strong increase after LVAD in the IHD group (Figure 5). Other membrane molecules, like caveolin, sarcoglycan and ATPase calcium transporting molecule, showed a low expression compared to control either pre- or post-LVAD.

5.4 Genes encoding intracellular proteins

Expression of some intracellular filament genes changed significantly after LVAD support in DCM (2/19: filamin, talin) and in IHD (1/19:desmoplakin), suggesting only a minor intracellular filament involvement. In this group it was remarkable that in IHD the gene encoding dystrophin was upregulated both pre- and post-LVAD.
Relatively many changes in the expression of signal transduction factors were observed after LVAD support both in DCM (2/8: Focal Adhesion Kinase and Ryanodine Receptor 2) and in IHD (4/8: catenin, myocyte enhancer factor 2A and 2C, and Ryanodine Receptor 2).

6. Changes in miR expression during LVAD support

Total RNA was isolated from heart tissue of heart failure patients pre- and post-LVAD. The relative quantities of miRNA1, miRNA133a, miRNA133b and miRNA-208 were established with the Taqman® MicroRNA assay (Applied Biosystems, Foster City, CA, USA). In Figure 6 the expression of miR-1, miR-133a and of miR133b is shown for DCM and IHD patients pre- and post-LVAD. Compared to control levels the miR expression in both heart failure groups was low for all miR tested. These low levels were more significant in IHD than in DCM. After LVAD support the levels did not change significantly, although in IHD there was a tendency that the miR expression levels return to normal. In patients with DCM we observed a tendency of further decrease. The expression of miR-208 showed similar changes (data not shown) as did the other three miRs. However, the expression was too low to make a reliable statistical analysis.

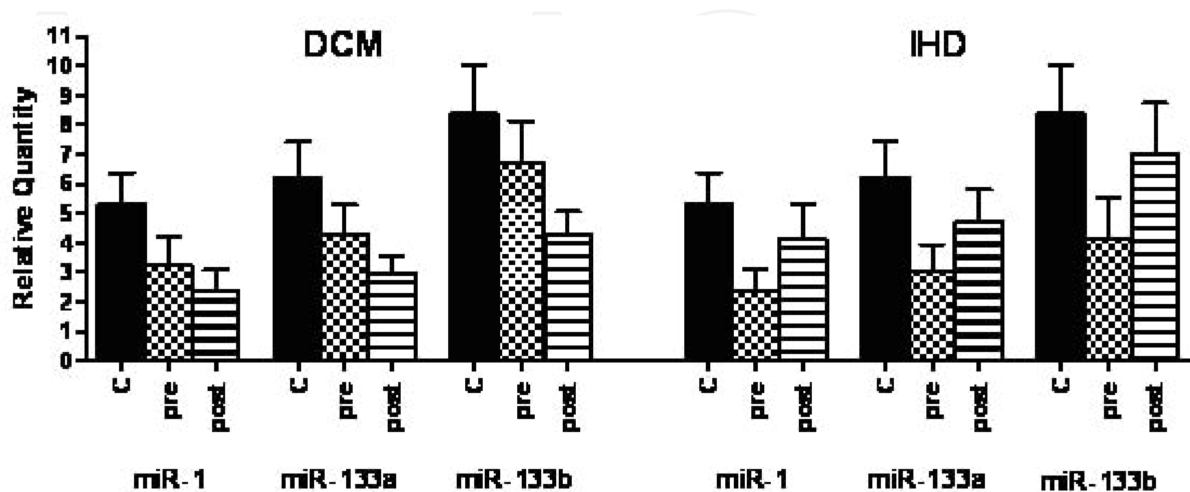

Fig. 6. Changes in miR expression after LVAD support

miR	DCM-patients			IHD-patients		
	1	2	3	1	2	3
miR-1	0.20	0.24	0.06	0.08	**0.05**	0.44
miR-133a	0.73	0.19	**0.04**	0.11	0.07	0.35
miR-133b	**0.02**	0.44	**0.04**	0.11	0.09	0.44

Table 4. Statistical analysis of miR expression changes after LVAD support.
The relative quantities of miR-1, miR-133a and miR-133b measured in heart tissue obtained from patients suffering from IHD (n= 8) or DCM (n=9) or C: controls (n=5; Pre = pre LVAD support ; Post= post LVAD support.
The p-values for the various differences in relative quantitative expression of the miRs in the myocardium obtained from DCM and IHD patients, respectively, before and after LVAD support. P< 0.05 is considered significant.
1: pre-LVAD versus post LVAD; 2: pre-LVAD versus control; 3: post LVAD versus control.

The results of the statistical analyses are presented in Table 4. These data confirm that in DCM patients, LVAD support did not increase the low miR-expression. In patients with IHD the values of expression of the miRs after LVAD support were not significantly different from those in the controls, indicating that there was a tendency of the low levels of miRs to increase after LVAD in IHD patients.

7. Discussion

During unloading, the myocardium of the failing heart shows various changes, both macroscopically and microscopically. Major changes include reduction of cardiomyocyte size, and changes in the volumes of ECM and BM components (Goldsmith and Borg, 202; Bruggink et al., 2006; Parker and Ingber, 2007). In many studies analyzing the effect of mechanical support on heart failure, only marginal differences have been observed between IHD and DCM (Bruggink et al., 2006a; De Jonge et al., 2001, 2002; Grady et al., 2003). However, in the present study hierarchical clustering of all expressed genes in end-stage heart failure showed that DCM and IHD segregated and could be identified as separated entities (Figure 2). For this reason both groups were analyzed separately. In the IHD group pre- and post-LVAD samples did segregate by hierarchical clustering (Figure 3). In the DCM group no such separation of pre- and post-LVAD samples was observed. The explanation for this difference between both groups is unknown. DCM may have a genetic background that leads primarily to hypertrophy and fibrosis, leading to gene expression that differs from controls in several aspects, but is not completely reversed by LVAD support. By contrast, in IHD, the gene expression alterations that are induced by infarction are partly normalized by the unloading of the heart. The differences in mRNA expression between IHD and DCM may give an important clue in finding targets that are informative for the state of the (un)supported hearts.
In pre-LVAD samples of DCM patients, the expression of only 7 genes (2 up- and 5 downregulated) differed from control which increased to 10 genes post-LVAD (7 up- and 3 down-regulated). In IHD pre-LVAD samples, the expression of 15 genes (13 up- and 2 down-regulated) differed from control, which decreased to 8 genes post-LVAD (7 up- and only 1 down-regulated). In both groups most genes that were differentially expressed pre-LVAD normalized to control levels after LVAD support. On the other hand, LVAD support can also induce a down- or upregulation of genes of which the pre-LVAD levels did not differ from

control level (Table 2). Eleven genes showed significant changes pre- and post-LVAD in both DCM and IHD. However, only 3 genes showed changes that were the same in DCM and IHD (calcium channel alpha 1C subunit, integrin-α11 and ryanodine receptor 2).

In DCM, the expression of caveolin remained low (both pre- and post-LVAD) compared to control. This is in contrast to the described up-regulation of caveolin protein after LVAD support (Uray et al., 2003). In IHD the expression of dystrophin and laminin (gamma 1) remained high after LVAD support. Changes in expression of both genes after LVAD have been described by others (Vatta et al., 2004; Birks et al., 2005; Refaat et al., 2008).

LVAD-induced changes in ECM and cardiomyocytes have been described by others as well (Milting et al., 2008; Bruggink et al 2006b; Thohan et al., 2005). In this respect, the total number of genes coding for various structural elements, that were differentially expressed pre- and post-LVAD was surprisingly low. Morphological changes during LVAD support were paralleled by changes in collagen turnover and expression of genes encoding for structural collagens (Type I and III; Bruggink et al, 2006b; 2007). So, the minor changes observed in expression of ECM genes in the present study may imply that most ECM changes are induced post-transcriptionally, either by micro-RNA regulation (Schipper et al., 2008) or in the matrix itself (e.g. by MMP). The latter is supported by significant changes in mRNA expression of MMP during LVAD support (Li et al., 2001; Klotz et al., 2005). Interestingly the anchoring and connecting collagens (types VI, XIV and XV) and molecules involved in ECM assembly like fibulin, fibronectin, osteonectin and proteoglycans (fibromodulin, heparan sulfate and decorin; Pollard et al., 2008) changed upon LVAD, although not similar in DCM and IHD patients. The LVAD-induced changes in the expression of these molecules, including collagen, also observed by others (Jahanyar et al., 2007; Gabrielsen et al., 2007), may contribute to the increased rigidity of the heart after LVAD support (Klotz et al., 2005).

Previously, we have shown that unloading of the left ventricle decreased the immunohistochemical expression of collagen IV in the BM (Bruggink et al., 2007). In contrast, immunoreactivity of laminin did not show substantial changes upon LVAD. Of the 17 tested genes that encode BM proteins only few showed expression changes after LVAD, indicating a dysbalance between mRNA expression and protein expression. The few genes that did show changes upon LVAD, either in DCM or IHD, are involved in cell-adhesion (laminin β1 and γ1, osteopontin). Together with the changes observed in the gene expression of the integrin, cadherin and sarcoglycan family members, these results underline the importance of these specific anchoring or connecting proteins in the structural changes observed (Birks et al.,2005; Gabrielsen et al., 2007; Latif et al., 2007; Kim et al.,1999).

Only minor changes were observed in the expression of genes encoding intracellular cytoskeleton proteins. In DCM, alterations after LVAD support in cytoskeletal filaments (dystrobrevin, filamin, junction plakoglobin, and talin) are more pronounced than in IHD (desmoplakin, dystrophin and talin; Gabrielsen et al., 2007). This could indicate that this class of genes is more affected in DCM than in IHD, which may be explained by the different onset of myocardial damage in both diseases.

In the fibrotic pathway a remarkable difference between DCM and IHD is observed. In DCM the expression of pro-fibrotic factors (TGFβ1, FGF, IGF, endothelin and CTGF) did not change upon LVAD support, but the expression of anti-fibrotic genes (BMP-4, BMP-7, decorin, and Id1) increased. This is paralleled by reduced fibrosis in DCM (Bruggink et al., 2006b). In patients with IHD the expression of both anti- and pro-fibrotic factors remain unchanged upon LVAD support. However, in IHD the pre-LVAD expression levels of the

pro-fibrotic response genes are stronger than in control whereas the expression of the anti-fibrotic gene BMP-7 is lower than in control. This will favour fibrosis in the hearts of patients with IHD. In these patients, the post-LVAD situation may be associated with a return of gene expression to control values. This may lead to a reduction of fibrosis as is shown in various studies. So, pro- and anti-fibrotic gene expression is in agreement with previously described reduction of fibrosis after LVAD support (Goldsmith and Borg, 2002; Gabrielsen et al., 2007), although the mechanisms responsible differed between the two entities.

In view of the changes in mRNA expression that did not seem to be paralleled by corresponding protein expression, special emphasis was given to miR expression during LVAD support. These miRs are important in the post-transcriptional regulation of mRNAs, also in the heart (Chen, 2007; Couzin, 2008). The miRs tested (miR-1, miR-133a and miR133b) had relatively low expression in the myocardium of heart failure patients compared to controls. In IHD patients the level of miR expression tended to return to control levels upon LVAD support. In DCM, however, the miR expression levels tended to decrease even further, which suggests that genes under the control of these miRs could be expressed even stronger. Chen et al. (2006) have described that miR-1 and miR-133 promote skeletal muscle myogenesis and myoblast proliferation, respectively (Townley-Tilson et al, 2010). Similar data have been produced by Liu et al. (2007) and Ikeda et al. (2008) for the failing myocardium. The relatively low expression of miRs in the failing heart, compared to control, may be related to the presence of myocardial hypertrophy (De Jonge et al., 2002), as overexpression of both miR-1 and miR-133 leads to cardiac hypertrophy (Care et al., 2007).

This difference in miR expression between DCM and IHD patients after LVAD support may be explained by the lack of need for cell proliferation in DCM unlike in IHD where there is a need for cell proliferation. Remodeling of DCM involves mainly a reduction of hypertrophy of cardiomyocytes, whereas IHD involves tissue repair including cell proliferation. This may indicate that the studied miRs are primarily involved in regulation of proliferative processes rather than in reduction of hypertrophy. As already mentioned, the reduction in miRNA expression in IHD patients is not restored completely to control levels during LVAD support, not in patients supported for a short period of time nor in patients supported for more than over one year.

The miR data do show that myocardial expression of miRs changes upon heart failure (Busk and Cirera, 2010) and upon LVAD support. In that respect it is interesting to note that there are initial indications that miR released in the serum (Cheng et al. 2010) may act as biomarkers to screen for cardiac diseases (Adachi et al., 2010) and be targets for therapy (Seok and Wang, 2010).

In conclusion, the set of genes coding for proteins involved in mechanotransduction, selected for the analysis of changes in mRNA expression pre- and post-LVAD, resulted in an identification of IHD and DCM as separate entities. The morphologic and structural changes observed in the failing human heart upon LVAD support are only partly reflected in changes of mRNA expression of genes encoding proteins involved in mechanotransduction. This suggests that most changes in ECM and intracellular filaments are not regulated at the mRNA level. However, expression of genes encoding membrane-bound proteins such as cadherin and integrins, and anchoring proteins such as collagen type VI and proteoglycans, is clearly affected by LVAD support and contributes to adaptation to improved loading conditions. Also the genes involved in fibrosis showed adaptation to LVAD support, and their expression runs parallel to the observed morphological changes. These genes may

prove to be important biomarkers in the development of protocols which decide whether LVAD supported patients should undergo heart transplantation, can be weaned from the device, or could rather continue their LVAD therapy for a longer period of time. The role of miR as biomarkers in this decision making, but also as therapeutic targets, is promising but still needs further investigation (Montgomery and van Rooij, 2010).

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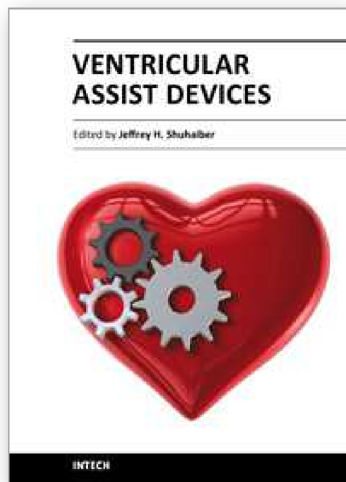

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The assist devices will continue adding a large number of years of life to humans globally and empower the medical society to optimize heart failure therapy. While expensive and cumbersome task, the foundation provided in this book reflects a contemporary product of original research from a multitude of different experts in the field. We hope this cumulative international effort provides the necessary tools for both the novice as well as the active practitioner aiming to change the outcome of these complex patients.

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