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Myocardial Gene Expression in Heart Failure Patients Treated With Cardiac Resynchronization Therapy

Responders Versus Nonresponders

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Objectives We studied whether functional improvement after cardiac resynchronization therapy (CRT) is associated with reversal of the heart failure (HF) gene program.

Background Cardiac resynchronization therapy improves exercise tolerance and survival in patients with advanced congestive HF and dyssynchrony.

Methods Twenty-four patients referred for CRT underwent left ventricular (LV) endomyocardial biopsies immediately before CRT implantation (baseline). In addition, 17 of them underwent LV endomyocardial biopsy procurement 4 months later (follow-up). In 6 control patients with normal LV function, LV biopsies were obtained at the time of coronary artery bypass grafting. The LV messenger ribonucleic acid (mRNA) levels of contractile and calcium regulatory genes were measured by quantitative real time polymerase chain reaction and normalized for glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The HF patients showing an improvement in New York Heart Association (NYHA) functional class by >1 score and a relative increase in LV ejection fraction ≥25% at 4 months after CRT were considered as responders.

Results The HF patients were characterized by lower LV mRNA levels of α-myosin heavy chain (α-MHC), β-myosin heavy chain (β-MHC), sarcoplasmic reticulum calcium ATPase 2α (SERCA), phospholamban (PLN), and higher brain natriuretic peptide (BNP) mRNA levels as compared with control subjects. Responders to CRT ($n = 11$) showed an increase in LVEF ($p < 0.001$), a decrease in left ventricular end-diastolic diameter ($p = 0.003$), and NYHA functional class ($p = 0.002$), and a reduction in N-terminal proBNP levels ($p = 0.032$) as compared with baseline. This was associated with an increase in mRNA levels of α-MHC ($p = 0.035$), SERCA ($p = 0.032$), a decrease in BNP mRNA levels ($p = 0.002$), and an increase in the ratio of α-/β-MHC ($p = 0.018$) and SERCA/PLN ($p = 0.012$). No significant changes in molecular profile were observed in nonresponders.

Conclusions In HF patients with electromechanical cardiac dyssynchrony, functional improvement related to CRT is associated with favorable changes in established molecular markers of HF, including genes that regulate contractile function and pathologic hypertrophy. (J Am Coll Cardiol 2008;51:129–36) © 2008 by the American College of Cardiology Foundation

The adverse left ventricular (LV) remodeling and the reduced contractile function observed in heart failure (HF) is associated with altered gene expression profile. One of the hallmarks of the altered molecular response is the activation of the "fetal" gene program including isoform switch in myosin heavy chain (MHC) gene expression with down-regulation of the fast α-myosin isoform (1–3) and up-regulation of natriuretic peptides. Other molecular changes include alterations in expression of genes

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encoding excitation-contraction coupling such as sarcoplasmic reticulum calcium ATPase 2α (SERCA) and phospholamban (PLN). End-stage HF is associated with decreased levels of SERCA relative to PLN as well as reduced activity, resulting in impaired calcium cycling thereby ac-

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Abbreviations and Acronyms

BNP = brain natriuretic peptide

CRT = cardiac resynchronization therapy

HF = heart failure

LV = left ventricle/ventricular

MHC = myosin heavy chain

mRNA = messenger ribonucleic acid

Nt-proBNP = N-terminal part of the pro-brain natriuretic peptide

PLN = phospholamban

RT-PCR = real-time polymerase chain reaction

SERCA = sarcoplasmic reticulum calcium ATPase 2α

counting for the contractile deficit of the failing heart (4). These changes seem to represent basic molecular mechanisms underlying LV dysfunction and HF. Accordingly, it was postulated that clinical strategies should be designed to target these adverse molecular changes in order to effectively improve contractile performance of the failing myocardium (5).

Few clinical studies have addressed the reversibility of adverse molecular profile in human HF. In terminal HF, unloading with LV assist devices resulted in a decrease in cellular hypertrophy and fibrosis in parallel with deactivation of the "fetal" gene program and improvements in myocyte contractile properties and beta-adrenergic responsiveness (6–9). In addition, in idiopathic dilated cardiomyopathy, functional improvement related to beta-blocker therapy was associated with an increase in SERCA and α-MHC messenger ribonucleic acid (mRNA) together with a decrease in β-MHC mRNA (10).

Cardiac resynchronization therapy (CRT) acutely improves cardiac performance by restoring the coordination between left and right ventricles, leading to improved mechanical efficiency in parallel with improved contraction and relaxation (11). These effects seem to be persistent over time and translate into a reversal of LV remodeling and improved clinical prognosis (12–14). However, alterations in the molecular fingerprint associated with this reversed remodeling have not been elucidated. Accordingly, we investigated whether functional improvement after CRT was associated with favorable changes in expression of established molecular structural and calcium regulatory markers of HF.

Methods

Study population. The study population consisted of 24 consecutive New York Heart Association (NYHA) functional class III to IV patients undergoing CRT. Patients were studied the day before and 4 months after CRT implantation. All patients had to be on optimal stable medical therapy for at least 3 months and all cardiovascular medications, comprising angiotensin-converting enzyme inhibitors (n = 24), beta-blockers (n = 23), aldosterone antagonists (n = 20), and diuretics (n = 24), were continued during the study period. Only the dose of the loop diuretics was changed during follow-up. All patients were in sinus rhythm, had an LV ejection fraction (EF) <35%, left bundle branch block with QRS duration >140 ms, and significant mechanical dyssynchrony assessed by tissue Doppler echocardiography (15). All patients underwent LV endomyocardial biopsy sampling at baseline before CRT implantation. In 17 patients, LV endomyocardial biopsies were obtained at baseline and 4 months after CRT. The control group consisted of 6 patients with normal LV systolic and diastolic function undergoing elective coronary artery bypass grafting, owing to stable coronary artery disease. All patients gave informed consent, and the study was approved by the institutional ethics committee.

Biventricular pacemaker implantation. Biventricular pacing devices were implanted as previously described (16). The LV pacing electrode was positioned with a transvenous approach through the coronary sinus into the lateral or posterolateral cardiac vein. The device was programmed in biventricular-DDD mode with a fixed atrioventricular delay (115 ± 24 ms) optimized by echocardiography (17).

Doppler echocardiography. Two-dimensional Doppler echocardiography was performed (Acuson Sequoia C512, Siemens, Malvern, Pennsylvania). Images were acquired in semi-supine position at rest by 2 experienced echocardiographers blinded to the moment of study examination. The following morphological and functional analyses were performed off-line from digitally-stored images: mitral regurgitation, assessed semi-quantitatively on a scale of 1 to 4 (18); LV end-diastolic and -systolic volumes; diameters; and LVEF, with the Simpson's formula (19).

In addition, pulsed-wave tissue Doppler imaging was used to assess interventricular and LV intraventricular dyssynchrony from regional time intervals between the onset of QRS complex and the onset of systolic myocardial velocity in basal segments of the left and right ventricle. Left ventricular dyssynchrony was defined as the maximum delay between basal LV segments. Interventricular dyssynchrony was assessed by comparison of the most delayed basal segment of the left ventricle with the right ventricle free wall delay (15,17).

Endomyocardial biopsies. Left ventricular endomyocardial biopsies were obtained with a long guiding sheath and a disposable transfemoral bioptome (Cordis Corp., Miami, Florida) at the level of the distal interventricular septum. Seventeen patients consented at the time of enrollment to undergo repeat biopsies after 4 months. Control LV endomyocardial biopsies (n = 6) were obtained from the free LV wall at the time of coronary artery bypass grafting in patients with normal LV function before initiation of extracorporeal circulation. In all patients, biopsies were snap frozen in liquid nitrogen and stored at −80°C for subsequent RNA analyses.

Brain natriuretic peptide. Venous levels of N-terminal pro-brain natriuretic peptide (BNP) (Elecsys 2010, Roche Diagnostics, Mannheim, Germany) were determined from blood samples collected the day before CRT implantation and 4 months later.

Data analysis. Four months after CRT, patients were divided into 2 groups according to their response to CRT.

Responders were identified by a relative increase in EF of ≥25% together with an improvement in NYHA functional class score >1 (15,20). LV mRNA levels of α-MHC, β-MHC and BNP, SERCA, and PLN all established molecular markers of heart failure were analyzed.

Quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR). Highly sensitive RT-PCR was used for RNA quantifications as previously described (21). Briefly, total RNA was isolated from LV endomyocardial biopsies with the RNeasy Fibrous Tissue Mini Kit (Qiagen, Valencia, California) and deoxyribonuclease digested. The RNA was reverse transcribed with random primers with the high-capacity complementary deoxyribonucleic acid (cDNA) Archive Kit (Applied Biosystems, Foster City, California). The RT-PCR was performed in 96-well plates on the ABI Prism 7000 Sequence Detection System (Applied Biosystems) with TaqMan Universal PCR Master Mix and Assays-On-Demand (Applied Biosystems), with a final reaction volume of 25 μl. The PCR primers and 6-carboxyfluorescein (FAM) probes for all of the target genes were purchased as Assays-On-Demand. The assay numbers for the target genes were Hs01564008_m1, Hs00160179_m1, Hs00411899_m1, Hs01110632_m1, and Hs00173590_m1 for SERCA, PLN, α-MHC, β-MHC, and BNP, respectively. Human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as endogenous control (Applied Biosystems). All samples were performed in triplicate. The relative expression of the target genes was normalized to the level of GAPDH in the same cDNA.

Statistical analysis. Data are expressed as mean ± SEM or as median. An exact Wilcoxon signed-rank test, a Mann-Whitney test, and a Spearman correlation coefficient were used for appropriate comparisons. A p value of <0.05 was considered significant for comparisons and correlations.

Results

Baseline characteristics. Table 1 shows baseline clinical characteristics. All patients had severe LV dysfunction, significant intraventricular and interventricular dyssynchrony, and severe LV dilatation. All were in NYHA functional class ≥3 HF. Heart failure was due to ischemic heart disease in 13 patients and idiopathic dilated cardiomyopathy in 11 patients. All patients were receiving optimal medical therapy that remained unchanged at follow-up. All control patients had normal LV function with stable coronary artery disease; none of them had a history of acute coronary syndrome.

Baseline LV endomyocardial gene expression: HF patients versus control population. Table 2 shows LV gene expression of the entire study population. No infiltrative or inflammatory processes were identified at diagnostic histopathological analysis. As expected, HF patients showed lower levels of mRNA for SERCA, PLN, α-myosin, and β-MHC and higher levels of BNP mRNA compared with control subjects. The ratio of SERCA to PLN was significantly lower in the HF patients compared with the control group. No differences in myocardial gene expression were noted between patients with ischemic and nonischemic dilated cardiomyopathy (data not shown). In the entire study population, LVEF was inversely related to BNP mRNA ($r = -0.615$; $p = 0.003$) and directly related to SERCA mRNA ($r = 0.660$; $p = 0.001$).

Table 1 Baseline Characteristics of the Control and Heart Failure Patients

Variable	Control (n = 6)	CRT (n = 24)	p Value
Clinical characteristics			
Age (yrs)	61 ± 5	68 ± 2	NS
Gender (% male)	6 (100)	21 (87)	NS
Etiology (% ischemic cardiomyopathy)	6 (100)	13 (55)	
NYHA functional class (n)			
III	—	8	<0.001
IV	—	16	<0.001
ICD implantation (%)	—	6 (25)	
Hemodynamic parameters			
LVEDD (mm)	51 ± 3	66 ± 3	0.004
LVEDV (ml)	158 ± 27	315 ± 29	0.018
EF (%)	72 ± 4	25 ± 2	<0.001
LV dyssynchrony (ms)	—	55 ± 7	
InterV dyssynchrony (ms)	—	57 ± 7	
Medical therapy (%)			
Beta-blockers	3 (50)	23 (95)	<0.001
ACE-I	0 (0)	24 (100)	<0.001
Loop diuretics	0 (0)	24 (100)	<0.001
Spironolactone	0 (0)	20 (85)	<0.001

ACE-I = angiotensin-converting enzyme inhibitors; CRT = cardiac resynchronization therapy; EF = ejection fraction; ICD = implantable cardioverter-defibrillator; InterV = interventricular; LV = left ventricular; LVEDD = left ventricular end-diastolic diameter; LVEDV = left ventricular end-diastolic volume; NYHA = New York Heart Association.

Effects of CRT on ventricular function and myocardial gene expression. Table 3 shows serial changes in echocardiographic indices and myocardial gene expression in 17 HF patients undergoing serial LV biopsies. Cardiac resynchronization therapy overall resulted in a significant increase in LVEF together with a decrease in LV dimensions and volumes. This was associated with a reduction in mitral regurgitation, cardiac dyssynchrony, and serum N-terminal part of the pro-brain natriuretic peptide (Nt-proBNP) levels. Consequently, NYHA functional class decreased in all but 4 patients. In the entire patient population, LV BNP mRNA levels significantly decreased 4 months after CRT, whereas no significant changes were observed in the expression of contractile or Ca^{2+}-regulating genes.

Myocardial gene expression in responders versus nonresponders to CRT. Table 4 and Figures 1 and 2 summarize the effects of CRT upon LV function and gene expression in responders versus nonresponders. By definition responders were identified by a relative increase in EF of ≥25% together with an improvement in NYHA functional class score >1 (15,20). At baseline, responders and nonresponders had similar LVEF and volumes. Nonresponders tended to have lower LV dyssynchrony and mitral regurgi-

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Table 2 Baseline mRNA Expression of the Control and Heart Failure Patients

Gene Expression (Relative Units)	Control Subjects (n = 6)	Heart Failure (n = 24)	p Value
SERCA (median; 25%–75%)	4.08 ± 1.34 (2.76; 1.83–7.63)	1.47 ± 0.15 (1.27; 0.97–1.57)	0.002
PLN (median; 25%–75%)	11.65 ± 2.02 (10.87; 7.29–16.99)	6.47 ± 0.53 (6.06; 4.69–7.94)	0.002
α-MHC (median; 25%–75%)	2.22 ± 0.51 (2.17; 0.99–3.48)	1.12 ± 0.21 (0.73; 0.40–1.50)	0.037
β-MHC (median; 25%–75%)	66.02 ± 16.69 (57.90; 37.66–108.50)	32.58 ± 3.46 (29.24; 22.78–40.93)	0.005
BNP (median; 25%–75%)	0.13 ± 0.06 (0.08; 0.01–0.30)	13.18 ± 2.60 (8.75; 2.39–22.16)	0.014

Data are normalized for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and expressed as relative units.
α-MHC = α-myosin heavy chain; β-MHC = β-myosin heavy chain; BNP = brain natriuretic peptide; mRNA = messenger ribonucleic acid; PLN = phospholamban; SERCA = sarcoplasmic reticulum calcium ATPase 2α.

tation but more significant interventricular dyssynchrony and larger LV volumes compared with responders. At follow-up, responders showed a greater increase in LVEF and a greater reduction in LV end-diastolic dimensions and volume, whereas LV and interventricular dyssynchrony were reduced to a similar extent in both groups. Serum Nt-proBNP levels decreased significantly in responders and remained unchanged in nonresponders.

At baseline, LV mRNA levels PLN and β-MHC were similar between responders and nonresponders. There was a non significant trend towards lower SERCA and α-MHC gene expression and higher BNP gene expression in responders versus nonresponders.

At follow-up a significant increase in mRNA of α-myosin and in the ratio of α-/β-MHC was noted in responders in parallel to a reduction in mRNA levels of BNP. The α-/β-MHC ratio was significantly higher in responders versus nonresponders ($p < 0.05$). In responders, LV SERCA message levels increased together with the SERCA/PLN ratio. In contrast, no significant changes in gene expression were noted in nonresponders.

Table 3 Serial LV Endomyocardial mRNA Expression, Echocardiographic, and Functional Characteristics in the CRT Population

	BL (n = 17)	FU (n = 17)	p Value BL vs. FU
Hemodynamics			
EF	24 ± 1	39 ± 2	<0.001
LVEDD (mm)	68 ± 3	63 ± 4	0.014
LVEDV (ml)	291 ± 24	183 ± 17	<0.001
LV dyssynchrony (ms)	53 ± 6	27 ± 3	0.001
InterV dyssynchrony (ms)	64 ± 9	17 ± 3	<0.001
MR grade >2 (%)	75	25	<0.001
Functional			
NYHA functional class	3.4 ± 0.1	2.6 ± 0.1	0.001
Nt-proBNP (pg/ml)	2,897 ± 495	1,480 ± 303	0.003
Gene expression			
SERCA (U)	1.392 ± 0.16	1.54 ± 0.12	NS
PLN (U)	6.56 ± 0.38	6.75 ± 0.40	NS
SERCA/PLN	0.21 ± 0.02	0.23 ± 0.02	NS
α-MHC (U)	1.01 ± 0.21	1.28 ± 0.4	NS
β-MHC (U)	33.60 ± 2.33	31.72 ± 2.99	NS
α-/β-MHC	0.03 ± 0.01	0.04 ± 0.01	NS
BNP (U)	16.77 ± 3.07	6.09 ± 1.27	0.005

Responders and nonresponders. Gene expression data are normalized for GAPDH and expressed as relative units.
BL = baseline; FU = 4-month follow-up; LV = left ventricular; MR = mitral regurgitation; Nt-proBNP = N-terminal part of the pro brain natriuretic peptide; other abbreviations as in Tables 1 and 2.

Variability of gene expression. In 3 patients biopsy samples from different sites of the LV were used. In these patients the variability of SERCA, PLN, BNP, β-MHC, and α-MHC mRNA concentrations were 10 ± 4%, 12 ± 3%, 33 ± 6%, 12 ± 5%, and 18 ± 5%, respectively.

Discussion

The present study is the first to investigate the effects of CRT on established molecular structural and calcium regulatory markers of HF in human subjects. Our findings demonstrate that the beneficial effects of CRT on LV function and remodeling are associated with "reversed molecular remodeling" characterized by an increase in expression of genes regulating excitation-contraction coupling and a reversal of the isoform switching of the contractile genes. These data suggest that gene expression profile in human HF patients—receiving optimal medical therapy with angiotensin-converting enzyme inhibitors, beta-blockers, and spironolactone—is at least partially reversible and that molecular changes in structural and functional proteins might contribute to favorable effects of CRT on myocardial performance.

HF and myocardial gene expression. Activation of so-called "fetal gene program" is the best-described myocardial molecular alteration relevant to the pathophysiology of HF. It is characterized by isoform switching in the expression of genes regulating contractile proteins and the down-regulation of genes regulating excitation-contraction coupling (22,23). Although an altered molecular profile is part of an adaptive response to persistent mechanical overload, changes in contractile proteins are responsible for depressed performance and contribute to further negative LV remodeling and failure. This is corroborated by single cardiac myocytes or isolated heart experiments showing negative inotropic effects secondary to increased expression of the slow β-MHC isoform (24,25). Accordingly, it was postulated that only those therapeutic strategies that interfere with or reverse these molecular changes will result in an effective functional improvement and survival benefit in congestive HF patients (5,26). This postulate is supported by experimental studies demonstrating improved contractil-

Table 4 Serial LV Endomyocardial mRNA Expression, Echocardiographic, and Functional Characteristics in Responders and Nonresponders to CRT

	Responders (n = 11)		p Value	Nonresponders (n = 6)		p Value
	BL	FU	BL vs. FU	BL	FU	BL vs. FU
Hemodynamics						
EF (%)	23 ± 1	35 ± 3	<0.001	20 ± 3	23 ± 1*	0.004
LVEDD (mm)	68 ± 4	61 ± 4	0.003	71 ± 4	69 ± 4	0.056
LVEDV (ml)	290 ± 26	191 ± 21	<0.001	343 ± 69	304 ± 56*	0.070
LV dyssynchrony (ms)	69 ± 9	28 ± 3	0.002	40 ± 4	27 ± 6	0.154
InterV dyssynchrony (ms)	61 ± 10	18 ± 5	0.003	79 ± 17	14 ± 4	0.013
MR grade >2 (%)	36	0	0.010	14	1	0.034
Functional						
NYHA functional class	3.4 ± 0.5	2.3 ± 0.5	0.00210	3.4 ± 0.5	3.1 ± 0.4†	NS
Nt-proBNP (pg/ml)	3,334 ± 692	1,634 ± 322	0.032	2,230 ± 703	1,153 ± 1012	NS
Gene expression						
SERCA (U)	1.19 ± 0.11	1.50 ± 0.13	0.032	1.64 ± 0.35	1.60 ± 0.27	NS
PLN (U)	6.28 ± 0.46	6.4 ± 0.56	NS	6.70 ± 0.68	7.41 ± 0.46*	NS
SERCA/PLN	0.19 ± 0.01	0.24 ± 0.02	0.012	0.23 ± 0.03	0.22 ± 0.04	NS
α-MHC (U)	0.69 ± 0.10	1.14 ± 0.26	0.035	1.40 ± 0.48	1.52 ± 1.13	NS
β-MHC (U)	32.29 ± 2.99	28.67 ± 3.80	NS	32.55 ± 4.73	37.32 ± 4.26	NS
α-/β-MHC	0.021 ± 0.005	0.044 ± 0.008	0.018	0.041 ± 0.010‡	0.040 ± 0.030*	NS
BNP (U)	19.76 ± 3.33	6.79 ± 1.27	0.002	10.65 ± 5.08	4.79 ± 2.86	NS

Gene expression data are normalized for GAPDH and expressed as relative units. *$p < 0.05$ follow-up nonresponders versus responders; †$p < 0.01$ follow-up nonresponders versus responders; ‡$p < 0.05$ baseline nonresponders versus responders.

Abbreviations as in Tables 1, 2, and 3.

ity after adenoviral gene transfer of SERCA in animal models of HF or failing human cardiac myocytes (27,28). Likewise, increased SERCA levels relative to PLN mRNA levels were associated with improved SERCA mediated Ca^{2+} sequestration and enhanced systolic function (4,29).

CRT and myocardial gene expression. Cardiac resynchronization therapy is associated with improved performance and survival in patients with advanced HF and electromechanical dyssynchrony (11,13,30). Nevertheless, approximately one-third of these patients do not exhibit any

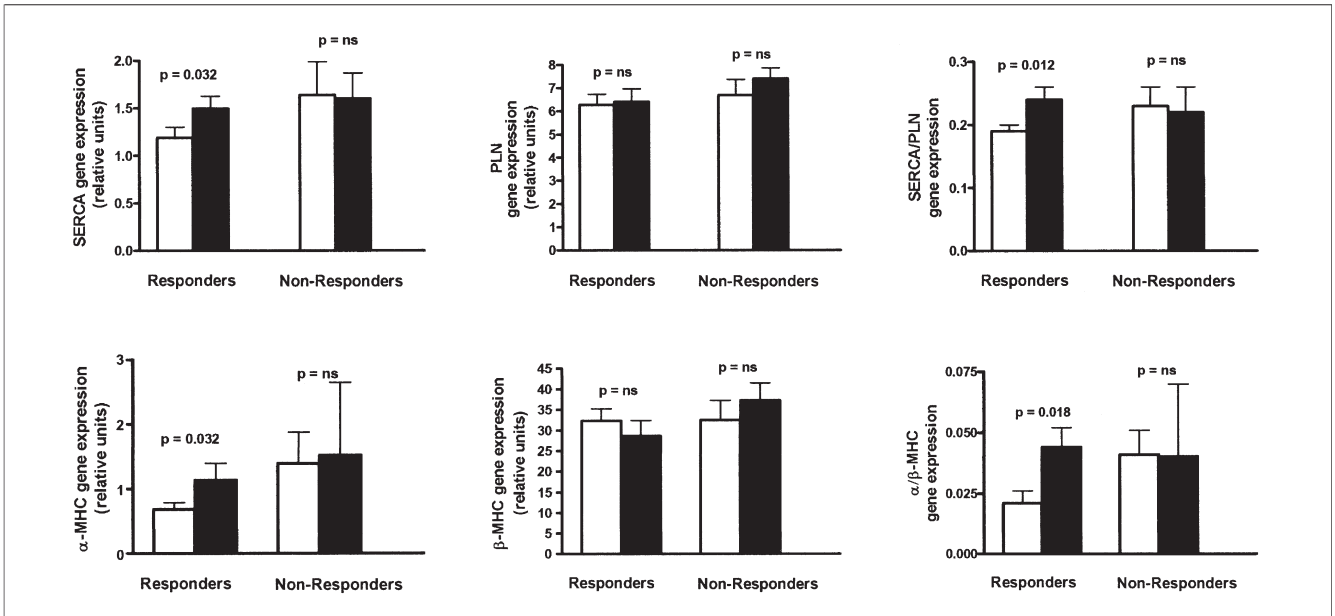

Figure 1 LV Endomyocardial Expression of Functional and Contractile Genes

Left ventricular (LV) endomyocardial expression of functional and contractile genes at baseline **(open bars)** and at 4-month follow-up **(solid bars)** in responders and nonresponders. Data are normalized for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and expressed as relative units. α-MHC = α-myosin heavy chain; β-MHC = β-myosin heavy chain; BNP = brain natriuretic peptide; PLN = phospholamban; SERCA = sarcoplasmic reticulum calcium ATPase 2α.

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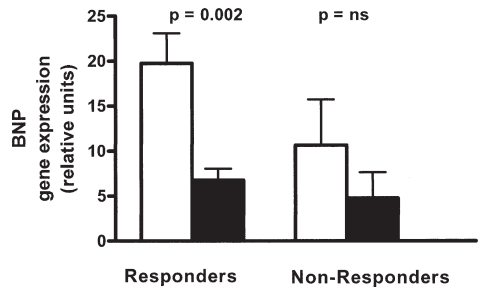

Figure 2 **LV Endomyocardial Gene Expression of BNP Gene**

The LV endomyocardial gene expression of BNP gene at baseline **(open bars)** and at 4-month follow-up **(solid bars)** in responders and nonresponders. Data are normalized for GAPDH and expressed as relative units. Abbreviations as in Figure 1.

benefit from this therapy. We hypothesized that reversed LV remodeling and improved myocardial performance in patients undergoing resynchronization therapy was associated with changes in established molecular structural and calcium regulatory markers of HF. As expected, before CRT, HF patients showed altered expression of genes that regulate Ca^{2+} handling and contractile proteins as compared with control subjects. Of note, in the entire population, CRT was not associated with changes in gene expression of these proteins. Only the subgroup of patients with improved LV function, reversed LV remodeling, and reduction in NYHA functional class, categorized as responders to CRT, demonstrated an increase in expression of the fast α-MHC and an increase of the α-myosin/β-MHC mRNA ratio. Moreover, in these responders, improved myocardial performance was associated with a significant increase in LV SERCA mRNA levels and in the SERCA/PLN ratio as compared with baseline. In contrast, the absence of LV remodeling and functional beneficial effects of CRT in nonresponders was associated with no changes in the gene profile of the structural or calcium regulatory proteins. Furthermore, the significant reduction of serum Nt-proBNP levels in all patients was paralleled by a significant down-regulation of myocardial BNP gene expression only in the responders. Our observations are consistent with experimental studies demonstrating beneficial effects of molecular intervention on myocardial performance (27,28). In addition, they corroborate previous observations demonstrating increased expression of fast α-myosin isoform or calcium handling proteins after beta-blocker therapy or LV unloading with assist devices (6,10). Taken together, these observations support the postulate that interventional therapies in HF could lead to sustained functional and clinical improvement only if they will efficiently alter adverse molecular remodeling.

Interestingly, we noticed a trend toward higher mRNA expression of α-MHC and SERCA in the nonresponders at baseline as compared with responders, indicating that despite a similar degree of LV dysfunction, dyssynchrony, or clinical HF class, nonresponders had paradoxically a better, "more favorable," molecular profile of established structural and calcium regulatory markers at baseline as compared with responders. This seems to indicate that these patients were less sick in terms of "the molecular remodeling," and therefore less prone to improve after CRT. In addition, this indirectly suggests that the current selection criteria based on clinical, electrocardiographic, and echocardiographic-derived parameters are not sufficient enough to predict the individual response to CRT. We speculate that baseline gene expression profiling might be a new and more accurate tool in predicting the response to CRT therapy. Further studies using large scale microarray profiling are needed to address this hypothesis and identify novel molecular markers predictive of reversed remodeling after CRT.

Our study, similar to previous clinical reports on the effects of beta-blocker therapy (10), does not address causative or mechanistic relationship between LV remodeling after CRT and molecular changes. Nevertheless, LV dyssynchrony and increased neurohormonal activation are associated with down-regulation of contractile regulating genes (31–34). A more synchronous contraction together with the reduction of muscle sympathetic nerve activity coinciding with reduced levels of catecholamines in responders to CRT (35,36) might thus account for the observed molecular changes. Alternatively, the reduced wall stress induced by LV remodeling after chronic CRT reduces the stretch-induced up-regulation of BNP, which beneficially affects LV remodeling. This hypothesis is supported by our observation of inverse relationship between BNP and EF and positive correlation between SERCA and EF, and a previous report on BNP-mediated reduction in SERCA gene expression indirectly supports this hypothesis (31). The extent to which the restoration of synchrony, LV remodeling, and reduced neurohormonal activation contribute to the observed molecular remodeling requires further investigation. Experimental studies with serial endomyocardial biopsies could unravel the time- and spatial-myocardial relationship of relevant molecular changes and further elucidate causative mechanisms underlying CRT-induced molecular changes.

Study limitations. First, LV dyssynchrony generates alterations in transmural and transchamber protein expression, which are most prominent in the late-activated, high-stress lateral endocardium (32) and might cause differences in regional gene expression (33). To limit the impact of regional differences, LV biopsies were obtained from the apicoseptal regions in all patients at baseline and at follow-up. Furthermore, multiple biopsies were procured from various LV sites in 3 patients. In these patients, the variability in SERCA, PLN, α-MHC, β-MHC, and BNP mRNA levels in the different biopsies were minimal. Thus, in the current study, gene expression data seem to reflect global rather than local changes related to LV remodeling. Second, the underlying etiology of HF might affect the

molecular response to CRT. Although our study might be underpowered to compare smaller subgroups, no difference in gene expression was noted between HF patients with ischemic versus idiopathic dilated cardiomyopathy. In addition, the variability was limited by performing paired comparisons in samples from the same individuals. Third, as expected, the dose of diuretics had to be adapted following CRT. In contrast, the dose of beta-blocker and angiotensin-converting enzyme inhibitor therapy remained unchanged, and there were no differences in HF medication between responders and nonresponders excluding potential drug effects on remodeling. Fourth, only RNA analysis and no protein analyses or functional activity studies of calcium handling genes were possible, owing to the small size of the LV biopsies. In this regard, it should be noted that the relationship between transcriptional and translational changes in SERCA expression is controversial. Nevertheless, an increase in SERCA gene expression was concomitant with the functional improvement in responders, suggesting that the increase in SERCA message was functionally meaningful. Fifth, although GAPDH is generally accepted as a very reliable housekeeping gene, we do admit that doing the whole procedure again with another housekeeping gene could add some incremental value to the study and compensate for variability in the gene expression of the housekeeping gene itself (37). However, lack of tissue samples made this impossible. Finally, although we clearly demonstrated improved expression of the most established molecular markers of HF, it is likely that many other relevant genes are participating in the complex tissue remodeling and need to be identified in studies using microarray analysis.

Conclusions

In congestive HF patients responding to CRT, an improvement in LV function and reduction in NYHA class are associated with "reversed molecular remodeling," characterized by increased expression of contractile and calcium regulatory proteins. This suggests that reversed "molecular remodeling" might be the key mechanism contributing to sustained improvement in LV function and survival after CRT.

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