Gene profiling of left ventricle eccentric hypertrophy in aortic regurgitation in rats: rationale for targeting the β-adrenergic and renin-angiotensin systems.

Serge Champetier, Azadeh Bojmehrani, Jonathan Beaudoin, Dominic Lachance, Éric Plante, Élise Roussel, Jacques Couet* and Marie Arsenault.

Groupe de Recherche en Valvulopathies, Centre de Recherche Hôpital Laval, Institut de cardiologie de Québec, Université Laval, Québec, Canada

Running title: Gene profiling in volume-overload LV hypertrophy

*Corresponding author: Jacques Couet PhD

Groupe de Recherche en Valvulopathies, Centre de Recherche Hôpital Laval,

2725, Chemin Sainte-Foy, Québec, (Québec), Canada, G1V 4G5

Phone: 1-418-656-4760; Fax: 1-418-656-4509

Email: jacques.couet@med.ulaval.ca

This work was supported by an operating grant from the Canadian Institutes for Health Research (MOP 68989), Canadian Heart and Stroke Foundation and Quebec Heart Institute Corporation to Doctors Arsenault and Couet. Drs Arsenault and Couet are both senior scholars from the Fonds de la recherche en santé du Québec. Mr Lachance is a recipient of a PhD scholarship and Mr Plante of a postdoctoral fellowship from the Canadian Institutes for Health Research.

No disclosures to declare.

Keywords: Aortic regurgitation, heart hypertrophy, volume overload, gene expression, renin-angiotensin system, adrenergic system.

Abstract

Aortic valve regurgitation (AR) imposes a severe volume overload to the left ventricle (LV) which results in dilation, eccentric hypertrophy and eventually loss of function. Little is known about the impact of AR on LV gene expression. We therefore conducted a gene expression profiling study in the LV of rats with acute and severe AR. We identified 64 genes that were specifically up-regulated and 29 that were down-regulated out of 21910 genes after 2 weeks. Of the up-regulated genes, a good proportion was related to the extracellular matrix. We subsequently studied a subset of 19 genes by quantitative RT-PCR (qPCR) to see if the modulation seen in the LV after two weeks persisted in the chronic phase (after 6 and 12 months) and found that it did persist. Knowing that the adrenergic and renin-angiotensin systems are over-activated in our animal model, we were interested to see if blocking those systems using metoprolol (25 mg/kg/d) and captopril (100 mg/kg/d) would alter the expression of some up-regulated LV genes in AR rats after 6 months. By qRT-PCR we observed that up-regulations of LV mRNA levels encoding for pro-collagens type I and III, fibronectin, atrial natriuretic peptide (ANP), transforming growth factor β2 (TGFβ2) and connective tissue growth factor (CTGF) were totally or partially reversed by this treatment. These observations provide a molecular rationale for a medical strategy aiming these systems in the medical treatment of AR and expand the paradigm in the study of this form of LV volume overload.

Introduction

Severe aortic valve regurgitation (AR) is associated with a long asymptomatic period during which the left ventricle (LV) progressively dilates and hypertrophies in response to a chronic volume overload. This process is accompanied by a decrease in LV function, occurrence of symptoms and eventually heart failure (4; 7). No drug has yet been clearly shown in humans to be effective to slow LV dilation, hypertrophy, and loss of systolic function or to have any impact on morbidity or mortality in chronic AR(5; 15).

At the microscopic level, AR is associated with cardiomyocytes elongation and also believed by excessive but mostly non-collagen myocardial fibrosis (fibronectin) (6; 23). Although gene expression profiling in LV eccentric hypertrophy resulting from volume overload has been studied recently in an aortocaval fistula rat model (25), very little is known about the mechanisms of LV hypertrophy and extracellular matrix remodeling associated with a more clinically common form of LV volume overload namely aortic valve regurgitation. Aorto-caval fistula models relate to a clinically rather rare complication of aortic aneurysm or secondary to abdominal trauma in humans (21). Moreover, aorto-caval fistulae result in a global form of volume overload affecting all right and left heart chambers resulting in rapid and massive congestive heart failure. On the other hand, chronic aortic valve regurgitation often secondary to rheumatic fever is a condition still very frequent in developing countries and in populations having non-adequate access to health care (3; 13; 20; 36). Aorto-caval fistulae as well as mitral valve regurgitation are both characterized by a low pressure LV overload while aortic valve regurgitation represents a mixed form of volume overload with a component of pressure overload (10). Contrarily to the natural evolution of aorto-caval fistula models,

we have shown that our model of chronic AR is associated with severe LV hypertrophy but moderate (and late) loss of function with a low rate of congestive heart failure (29; 31). Significant LV fibrosis is present later in the evolution of this disease and is associated with increased mortality (31). We hypothesize that in AR, activation of genes encoding collagens and non-collagens components of the extracellular matrix is a feature of this type of LV eccentric hypertrophy.

We present here the first study of LV gene expression profiling in an eccentric hypertrophy model caused by severe aortic valve regurgitation during the acute phase of LV remodeling two weeks post AR-inducing surgery in male Wistar rats. We have also investigated the LV expression of a subset of genes acutely modulated at later times (chronic period: 6 and 12 months).

Considering that we have previously shown that both the adrenergic and renin-angiotensin systems are over-activated in our model of AR, we tested the effects on LV gene expression of a drug therapy consisting of a combination of a β_1-adrenergic blocker (metoprolol) and an angiotensin II converting enzyme inhibitor (ACEi) (captopril), two drugs we have previously demonstrated to effectively slow LV hypertrophy and loss of systolic function in our model (14; 30; 32).

Methods

Animals: Male adult Wistar rats (300-350g body weight) were purchased from Charles River (Saint-Constant QC, Canada).

Acute study: Twelve animals were equally divided in two groups: Sham-operated (Sh2) and aortic regurgitation (AR2). Sacrifice was done two weeks after surgery.

Six-month study: Thirty-six animals were equally divided in three groups: Sham-operated (6 months (Sh26) AR (AR26) and treated AR (ART) animals receiving in their drinking water both captopril (100 mg/kg/day) and metoprolol (25 mg/kg/day) for six months starting two weeks after AR induction.

Twelve-month study: Thirty animals were divided in two groups: Sham-operated (Sh52; n=10), AR (AR52; n=20).

This protocol was approved by the Université Laval's animal protection committee and was consistent with the recommendations of the Canadian Council on animal care.

Aortic regurgitation: AR was induced by retrograde puncture of the aortic valve leaflets under anesthesia as previously described (2; 29; 32). Sham-operated animals had their right carotid artery cannulated under anesthesia without puncturing the aortic valve. AR was considered severe by echocardiography by the following criteria at the time of surgery: color-Doppler ratio of regurgitant jet width to LVOT diameter >50%, retrograde holo-diastolic flow in proximal descending aorta with end-diastolic velocity >18 cm/s, ratio of time-velocity integral of reversed diastolic flow to forward systolic flow in descending thoracic aorta >60% and acute increase in LV diastolic dimension during the surgical procedure. Echocardiographic criteria of AR severity had to be accompanied by

an acute drop of aortic diastolic pressure of at least 30% to qualify. Animals not meeting the echographic and hemodynamic criteria were not included in the study. Animals were clinically evaluated daily by experienced animal laboratory technicians for the presence of signs of heart failure (increased respiratory rate/distress and/or peripheral edema) and were weighed weekly. At the end of the protocol, surviving animals were sacrificed, hearts were quickly dissected and all cardiac chambers were weighed. Pieces of LV were snap-frozen in liquid nitrogen and kept at -80^{o} Celsius for further analysis while a mid-ventricular slice (~2-3 mm thick) was kept in RNAlater solution (Ambion, Austin TX) for RNA studies.

Echocardiography

A complete M-Mode, 2D and Doppler echocardiogram was performed on the animals under 1.5% inhaled isoflurane anesthesia using a 12 MHz probe with a Sonos 5500 echograph (Philips Medical Imaging, Andover, MA) immediately before and during surgery and at the end of the protocol. Left ventricular dimensions, wall thickness, ejection fraction, diastolic function, cardiac output (ejection volume in the left ventricular outflow tract X heart rate) were evaluated as previously reported (14; 33). AR was semi-quantified at each time-point as described in the previous section. Animals had to meet all the criteria of severe AR by semi-quantization at each time-point to remain included in the protocol.

Array experiments

Total RNA was extracted from stored tissues using Trizol (Invitrogen, Carlsbad, CA), and further purified on column with RNeasy MinElute (Qiagen, Valencia, CA). RNA

concentration and integrity were assessed by UV spectrophotometry and microfluidic electrophoresis (Bioanalyzer 2100, Agilent Technologies, Santa Clara, CA), respectively. Total RNA extracts were treated for double-stranded cDNA synthesis and labeling of cRNA with biotin according to the Eberwine method (38), using the Illumina TotalPrep RNA Amplification kit reagents and protocol (Ambion cat no IL1791; Austin, TX). The lyophilized biotin-labeled cRNA preparations were hybridized to BeadChip RatRef-12 microarrays (Illumina; San Diego, CA) according to supplier's protocol (11286340 rev. A), using 750 ng cRNA per array. After hybridization and washes, arrays were incubated in streptavidin-Cy3 solution, washed, and fluorescence data were collected on a BeadArray reader (Illumina). Treatment of data was performed with the FlexArray software package (Blazejczyk et al, 2007). Raw fluorescence data were processed and normalized with the lumi Bioconductor package (http://bioconductor.org/) version 1.1.0., and differential expression was determined according to the random variance model of Wright and Simon (SAM analysis) (43). Complete data are available at the ArrayExpress database (EMBL-EBI) under the accession number E-TABM-490.

Analysis of mRNA accumulation by quantitative RT-PCR

As previously described (31), LV RNA samples were diluted to 500ng/µl. One µl RNA (500 ng) was converted to cDNA using the QuantiTect® Reverse Transcription kit (Qiagen), a procedure which included a genomic DNA elimination step. The cDNA obtained was further diluted 11-fold with water prior to amplification (final concentration corresponding to 4.54 ng/µl of initial RNA). Five µl diluted cDNA were amplified in duplicate by Q-PCR in a Rotor-Gene™ thermal cycler (Corbett Life Science. Sydney, Australia), using QuantiTect® Primer Assays (pre-optimized specific primer pairs from

Qiagen; Table 1) and either QuantiTect® or QuantiFast® SYBR Green PCR kits (Qiagen). Each run included one tube with water only (no template control) and a series of three 10-fold dilutions of a representative cDNA sample to check efficiency of the amplification reactions. In the case of intronless genes, minus-RT control reactions were made on ten randomly chosen samples to ensure that genomic DNA did not yield significant amplification. The quantification of gene expression was based on the $-2^{\Delta\Delta Ct}$ method (22). Briefly, mean Ct values of duplicates for each gene of interest were subtracted from the mean Ct value (hence ΔCt) of the control "housekeeping" gene cyclophilin A. The difference in the mean ΔCts between groups of rats ($\Delta\Delta$Ct) allows the calculation of relative levels of induction/repression of genes of interest.

Cardiomyocyte cross-sectional area (CSA) and evaluation of fibrosis:

Sections from paraffin-embedded mid-LV portions were stained using the Trichrome-Masson staining. Three sections per slide from 10 animals per group were studied for the evaluation of cross sectional area (CSA) of the cardiomyocytes as previously described (19). Total myocardial and peri-vascular fibrosis was also evaluated from the Trichrome-Masson stained LV midwall section as previously described (19).

Other statistical analysis

Results are presented as mean ± SEM unless specified otherwise. Inter-group comparisons were done using one or two-way ANOVA and Tukey's post-test and intra-group comparisons with paired t tests. Statistical significance was set at a $p<0.05$. Data

and statistical analysis were performed using Graph Pad Prism version 4.02 for Windows, Graph Pad Software (San Diego CA).

Results

All animals survived in the acute two-week study. Table 2 shows the findings at the time of sacrifice. As expected, severe aortic valve regurgitation induced a marked increase in heart weight which was almost totally due to left ventricle hypertrophy. Using echocardiography, this LV hypertrophy was characterized by clear chamber dilation (increased LV end-diastolic diameters and stroke volume and decreased relative wall thickness (RWT). During this acute 2-week phase, neither systolic function (Table 2) nor diastolic function (LV filling parameters) parameters were significantly changed (not shown). By histology using Trichrome-Masson staining, no increase in myocardial fibrosis was observed but a tendency for increased peri-vascular fibrosis was observed in AR left ventricle midwall sections. (45% vs. 57%; p=0.09).

Figure 1 and Tables 3 and 4 show results from the array study. As illustrated in the upper panel of figure 1, general levels of gene expression tended to be increased in AR animals compared to sham-operated controls. For further analysis, we chose to discard genes displaying low levels of expression. In order to do so, we calculated the mean + 5 times the standard deviation of the signals obtained from the DNA chips of genes encoding for olfactory receptors as a threshold of minimal expression to be considered as meaningful. As illustrated in the middle panel of Figure 1, around 16000 genes were excluded this way. We chose to arbitrarily fix the fold change levels between AR LV samples and sham controls to 1.5 times with a P value below 0.001 and a SAM d-score <-3 of >3 in order to consider a gene as regulated. The volcano plot in Figure 1 (upper panel) illustrates the number of genes exceeding these cut offs. We then performed an analysis comparing SAM d-scores between our two groups (AR vs. Sham animals) vs. the d-scores obtained if animals were divided with three animals of each condition in

each group). As expected, higher d-scores were present when animals were divided according to their condition (Figure 1; bottom right panel).

As listed in Table 3 and 4, respectively, we found 64 up-regulated genes (>1.5 fold change) in AR left ventricles compared to controls and 29 down regulated genes (<0.67 fold change).

As illustrated in Figure 2, we found a good correlation (r^2=0.79 and p<0.0001) between these results and those obtained from quantitative RT-PCR (qPCR) in terms of fold change for a subset of 20 genes (3 down-regulated, 4 non-regulated and 13 up-regulated) studied using both techniques. However, the correlation between fold changes obtained for each gene by qPCR compared for the intensities of the signal on the DNA chip was also significant but less solid (r^2=0.38 and p=0.003; not shown).

In this first step of our study, we performed gene profiling experiments on the LV from AR rats during a still very active and acute period of adaptation to a severe volume overload and observed the modulation of several genes. In order to see if this modulation was still present at later times in more chronic phases of the disease we chose to look at two later time points: 6 and 12 months. At 6 months, survival rate was 83% (10/12) but only 40% at 12 months (8/20; p<0.0001 vs. S52 group) in AR animals while all sham animals were still alive at both 6 and 12 months. Most of the animals died from sudden death and only one by heart failure as previously observed (31). As shown in Table 5, dilated LV hypertrophy was present in AR animals and systolic function as depicted be the ejection fraction parameter were still in the normal range (>50%) although significantly reduced compared to sham controls.

A subset of 19 genes was studied for their mRNA levels in the surviving AR animals and fold change comparison to their sham-operated counterparts was made at those three

time points (2, 26 and 52 weeks). As illustrated in Figure 3, most of the genes studied were modulated similarly at the different time points.

Pharmaceutical strategies (β-blockade, ACE inhibition) aiming at the adrenergic or the renin-angiotensin systems have been shown in this rat model to improve either survival or LV function or hypertrophy (30-32). We therefore tested if we could observe benefits by combining these drugs and hopefully normalize the LV expression of various genes especially ones implicated in the control of fibrosis and markers of LV dysfunction such as ANP and BNP. As illustrated in the right column of Table 5, the combination of a β_1-adrenergic receptor blocker (metoprolol) and an angiotensin II converting enzyme inhibitor (captopril) did help slow LV hypertrophy and tended to improve systolic function. Moreover, we observed normalization of LV gene expression for Collagen Type I and III as well as for fibronectin and CTGF (Figure 4). In addition, levels of ANP, BNP and TGFβ2 were also reduced in AR animals by this drug combination.

Discussion

We present in this study the first gene profiling of the left ventricles of rats with volume overload due to severe aortic valve regurgitation. We report that many genes (related to the extra-cellular matrix components) are up-regulated in the LV of AR rats and that this up-regulation persists from the acute to the chronic phase of the disease. A drug combination targeting both the β-adrenergic and renin-angiotensin systems was able to prevent the over-expression of some genes such as those of natriuretic peptides (ANP and BNP), collagens I and 3, fibronectin, CTGF and TGFβ2.

Our AR rats develop a severe LV hypertrophy that can be sustained for a long period of time before heart failure occurs (31). Our model is not a model of rapid heart failure such as the aorto-caval fistula model (9) but a one of well tolerated severe volume overload. It may not be surprising that only a rather modest amount of genes were shown to be regulated in our array experiment compared to what has previously been published in more drastic heart failure models (24). Moreover, our array results leave the impression that most gene expression changes originated more likely from fibroblasts than from cardiac myocytes. It is however difficult to infer completely physiological consequences to these changes. In addition, profiling gene expression may not be the method of choice to assess myocyte hypertrophy since very little information can be obtained of the levels of activation of signaling pathways implicated in myocyte hypertrophy such as MAP kinases for example.

Unlike other LV hypertrophy gene expression studies involving pressure overload (25; 40-42; 44) as well as in the only one so far interested in volume overload (25), we found only 22 genes (18 up- and 4 down-regulated) having 2-fold change factor between AR

and control rats and this with a similar extent of dilation compared to the latter study. Moreover, several genes such as ANP, BNP, Collagen 1, skeletal muscle α-actin, LOX-like protein among others which were shown previously to be modulated in the aortocaval shunting model were also modulated in our study (25).

The animals included in our study protocols all had more than 65% of regurgitation which is considered clearly severe in humans. They all developed important LV dilation and hypertrophy but remained with a normal or close to normal ejection fraction. Although the array used may be one factor explaining this rather modest effect of AR on LV gene expression compared to previous studies, the nature of the volume overload is probably a more important factor. In addition, levels of changes in mRNA expression as evaluated by qRT-PCR were not different than those estimated with the array.

Compared to the aortocaval fistula model which affects directly both right and left heart chambers and is associated with early heart failure, our model of volume overload is centered principally on the LV and affects the right chambers of the heart and the lungs rather modestly. Our model relates to a more frequent clinical condition of volume overload which is known to evolve over decades before the apparition of clinical symptoms requiring valve replacement. Unlike gene expression profiling in cultured neonatal cardiomyocytes stretching (an *in vitro* model of isolated volume overload (16)) results obtained from a whole organ in a live animal trying to preserve its homeostasis and tending to reach a compensated state should lead to less strongly modulated genes.

An interesting feature highlighted by our study is the chronic activation of the extracellular matrix remodeling in the LV. Although AR is usually not associated with

severe myocardial collagen deposition, it is to be noted that at least 8 collagen genes were up-regulated. We observed here as in the past (19), that peri-vascular fibrosis tended to be increased while elsewhere in the myocardium, there was no marked accumulation of collagen. In addition, genes encoding for fibronectin, fibrillin, elastin and TIMP1 amongst others were all activated in the LV of AR rats. The implication for this observation points towards a constant state of LV ECM remodeling that is still active at later times even when LV hypertrophy evolves at a slower rate. One can propose that the myocardium is either always in a constant state of remodeling or that once some "compensated" form of LV hypertrophy settles then the process of fibrosis accumulation (other than in the vicinity of blood vessels) takes place, a phenomenon we observed in older AR rats (31). One limitation of this study in this regard is that the gene expression analysis could only be performed on tissues from surviving animals and this is especially important in the 12-month protocol where survival rate of AR animals was down to 40%. This can thus bias the results towards a probably less severe situation since only animals which coped well with the important volume overload were investigated. Among the genes shown to be up-regulated in the AR animals, it is interesting to note the rather important stimulation of the CTGF and TGFβ2. In the heart, CTGF can be produced both by cardiomyocytes and fibroblasts (12; 24). This multifunctional peptide which synthesis is often under TGFβ control is believed to be associated with myocardial fibrosis (11) through its ability to increase expression of fibronectin and collagens I and III, a situation we observed here. CTGF also has been shown to induce hypertrophy in rat neonatal cardiomyocytes via the Akt pathway making it an interesting target to study in the future (17).

This study also highlights that aiming at both the β_1-adrenegic and the renin-angiotensin system is an interesting avenue to explore in the treatment of chronic aortic regurgitation and that this approach is also based on molecular observations. We showed that gene expression of several ECM proteins as well as that of growth factors associated with myocardial fibrosis can be normalized using a combination of metoprolol and captopril. We observed in previous studies that these drugs independently were effective in slowing loss of cardiac function, development of hypertrophy and fibrosis and in the case of metoprolol, improving survival (30-32).

The identification in the present study of new molecular players that are regulated during LV eccentric hypertrophy caused by severe AR opens new avenues of research in the field and new ways to assess the effectiveness of a therapeutic intervention in our model.

Among the new molecular players identified in this study, it is worth mentioning Dscr1 (MCIP; modulatory calcineurin-interacting protein) and S100a1. Dscr1/MCIP which was first believed to be a negative regulator of calcineurin pro-hypertrophic signaling (thus protective for the heart) was more recently shown to be a calcineurin signaling facilitator(34; 35; 39). The Dscr1/MCIP up-regulation during the first 6 months observed here hints towards a calcineurin implication in the development of the eccentric LV hypertrophy caused by a chronic volume overload. Calcineurin signaling has been more linked in the past to pressure-overload hypertrophy(37) and was observed in mitral valve regurgitation in humans to be unregulated (8).

S100a1 gene expression was down-regulated in AR LV of our animals at every time point observed. The S100a1 protein has been shown to help calcium cycling in cardiac

myocytes thus helping contraction (1; 26; 28). Gene expression of S100a1 has been shown to be decrease in heart failure (18; 28) and to help restore contractility by gene therapy in a heart failure model (1; 27). It is interesting here to note that in our AR rat model, the evolution towards heart failure has seldom happened and most of the deaths observed were sudden thus probably caused by fatal arrhythmias. The significance of the down-regulation of this inotropic molecule, S100a1, in our model is thus intriguing and deserves more attention.

In conclusion, we have performed a gene expression profiling in the LV of rats with severe AR and showed for an important subset of modulated genes at two weeks that this regulation persisted in time and could be normalize by β-blockade and ACEi combination therapy

References

1. **Ackermann GE, Marenholz I, Wolfer DP, Chan WY, Schafer B, Erne P and Heizmann CW**. S100A1-deficient male mice exhibit increased exploratory activity and reduced anxiety-related responses. *Biochim Biophys Acta* 1763: 1307-1319, 2006.

2. **Arsenault M, Plante E, Drolet MC and Couet J**. Experimental aortic regurgitation in rats under echocardiographic guidance. *J Heart Valve Dis* 11: 128-134, 2002.

3. **Bitar FF, Hayek P, Obeid M, Gharzeddine W, Mikati M and Dbaibo GS**. Rheumatic fever in children: a 15-year experience in a developing country. *Pediatr Cardiol* 21: 119-122, 2000.

4. **Bonow RO**. Aortic Regurgitation. *Curr Treat Options Cardiovasc Med* 2: 125-132, 2000.

5. **Bonow RO, Carabello BA, Kanu C, de LA, Jr., Faxon DP, Freed MD, Gaasch WH, Lytle BW, Nishimura RA, O'Gara PT, O'Rourke RA, Otto CM, Shah PM, Shanewise JS, Smith SC, Jr., Jacobs AK, Adams CD, Anderson JL, Antman EM, Faxon DP, Fuster V, Halperin JL, Hiratzka LF, Hunt SA, Lytle BW, Nishimura R, Page RL and Riegel B**. ACC/AHA 2006 guidelines for the management of patients with valvular heart disease: a report of the American

College of Cardiology/American Heart Association Task Force on Practice Guidelines (writing committee to revise the 1998 Guidelines for the Management of Patients With Valvular Heart Disease): developed in collaboration with the Society of Cardiovascular Anesthesiologists: endorsed by the Society for Cardiovascular Angiography and Interventions and the Society of Thoracic Surgeons. *Circulation* 114: e84-231, 2006.

6. **Borer JS, Herrold EM, Hochreiter CA, Supino PG, Yin A, Krieger KH and Isom OW**. Aortic regurgitation: selection of asymptomatic patients for valve surgery. *Adv Cardiol* 39: 74-85, 2002.

7. **Borer JS, Truter SL, Gupta A, Herrold EM, Carter JN, Lee E and Pitlor L**. Heart failure in aortic regurgitation: the role of primary fibrosis and its cellular and molecular pathophysiology. *Adv Cardiol* 41: 16-24, 2004.

8. **Braun MU, LaRosee P, Simonis G, Borst MM and Strasser RH**. Regulation of protein kinase C isozymes in volume overload cardiac hypertrophy. *Mol Cell Biochem* 262: 135-143, 2004.

9. **Brower GL and Janicki JS**. Pharmacologic inhibition of mast cell degranulation prevents left ventricular remodeling induced by chronic volume overload in rats. *J Card Fail* 11: 548-556, 2005.

10. **Chandrashekhar Y**. Embracing diversity in remodeling: a step in therapeutic decision making in heart failure? *J Am Coll Cardiol* 49: 822-825, 2007.

11. **Chen MM, Lam A, Abraham JA, Schreiner GF and Joly AH**. CTGF expression is induced by TGF- beta in cardiac fibroblasts and cardiac myocytes: a potential role in heart fibrosis. *J Mol Cell Cardiol* 32: 1805-1819, 2000.

12. **Chen MM, Lam A, Abraham JA, Schreiner GF and Joly AH**. CTGF expression is induced by TGF- beta in cardiac fibroblasts and cardiac myocytes: a potential role in heart fibrosis. *J Mol Cell Cardiol* 32: 1805-1819, 2000.

13. **Chockalingam A, Gnanavelu G, Elangovan S and Chockalingam V**. Current profile of acute rheumatic fever and valvulitis in southern India. *J Heart Valve Dis* 12: 573-576, 2003.

14. **Couet J, Gaudreau M, Lachance D, Plante E, Roussel E, Drolet MC and Arsenault M**. Treatment of combined aortic regurgitation and systemic hypertension: insights from an animal model study. *Am J Hypertens* 19: 843-850, 2006.

15. **Evangelista A, Tornos P, Sambola A, Permanyer-Miralda G and Soler-Soler J**. Long-term vasodilator therapy in patients with severe aortic regurgitation. *N Engl J Med* 353: 1342-1349, 2005.

16. **Frank D, Kuhn C, Brors B, Hanselmann C, Ludde M, Katus HA and Frey N**. Gene expression pattern in biomechanically stretched cardiomyocytes: evidence for a stretch-specific gene program. *Hypertension* 51: 309-318, 2008.

17. **Hayata N, Fujio Y, Yamamoto Y, Iwakura T, Obana M, Takai M, Mohri T, Nonen S, Maeda M and Azuma J**. Connective tissue growth factor induces cardiac hypertrophy through Akt signaling. *Biochem Biophys Res Commun* 370: 274-278, 2008.

18. **Kiewitz R, Acklin C, Minder E, Huber PR, Schafer BW and Heizmann CW**. S100A1, a new marker for acute myocardial ischemia. *Biochem Biophys Res Commun* 274: 865-871, 2000.

19. **Lachance D, Plante E, Roussel E, Drolet MC, Couet J and Arsenault M**. Early left ventricular remodeling in acute severe aortic regurgitation: insights from an animal model. *J Heart Valve Dis* 17: 300-308, 2008.

20. **Lebowitz NE, Bella JN, Roman MJ, Liu JE, Fishman DP, Paranicas M, Lee ET, Fabsitz RR, Welty TK, Howard BV and Devereux RB**. Prevalence and correlates of aortic regurgitation in American Indians: the Strong Heart Study. *J Am Coll Cardiol* 36: 461-467, 2000.

21. **Leon LR, Jr., Arslan B, Ley E and Labropoulos N**. Endovascular therapy of spontaneous aortocaval fistulae associated with abdominal aortic aneurysms. *Vascular* 15: 35-40, 2007.

22. **Livak KJ and Schmittgen TD**. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25: 402-408, 2001.

23. **Maron BJ, Ferrans VJ and Roberts WC**. Myocardial ultrastructure in patients with chronic aortic valve disease. *Am J Cardiol* 35: 725-739, 1975.

24. **Matsui Y and Sadoshima J**. Rapid upregulation of CTGF in cardiac myocytes by hypertrophic stimuli: implication for cardiac fibrosis and hypertrophy. *J Mol Cell Cardiol* 37: 477-481, 2004.

25. **Miyazaki H, Oka N, Koga A, Ohmura H, Ueda T and Imaizumi T**. Comparison of gene expression profiling in pressure and volume overload-induced myocardial hypertrophies in rats. *Hypertens Res* 29: 1029-1045, 2006.

26. **Most P, Bernotat J, Ehlermann P, Pleger ST, Reppel M, Borries M, Niroomand F, Pieske B, Janssen PM, Eschenhagen T, Karczewski P, Smith GL, Koch WJ, Katus HA and Remppis A**. S100A1: a regulator of myocardial contractility. *Proc Natl Acad Sci U S A* 98: 13889-13894, 2001.

27. **Most P, Pleger ST, Volkers M, Heidt B, Boerries M, Weichenhan D, Loffler E, Janssen PM, Eckhart AD, Martini J, Williams ML, Katus HA, Remppis A and Koch WJ**. Cardiac adenoviral S100A1 gene delivery rescues failing myocardium. *J Clin Invest* 114: 1550-1563, 2004.

28. **Most P, Remppis A, Pleger ST, Katus HA and Koch WJ**. S100A1: a novel inotropic regulator of cardiac performance. Transition from molecular physiology to pathophysiological relevance. *Am J Physiol Regul Integr Comp Physiol* 293: R568-R577, 2007.

29. **Plante E, Couet J, Gaudreau M, Dumas MP, Drolet MC and Arsenault M**. Left ventricular response to sustained volume overload from chronic aortic valve regurgitation in rats. *J Card Fail* 9: 128-140, 2003.

30. **Plante E, Gaudreau M, Lachance D, Drolet MC, Roussel E, Gauthier C, Lapointe E, Arsenault M and Couet J**. Angiotensin-converting enzyme inhibitor captopril prevents volume overload cardiomyopathy in experimental chronic aortic valve regurgitation. *Can J Physiol Pharmacol* 82: 191-199, 2004.

31. **Plante E, Lachance D, Champetier S, Drolet MC, Roussel E, Arsenault M and Couet J**. Benefits of long-term {beta}-blockade in experimental chronic aortic regurgitation. *Am J Physiol Heart Circ Physiol* 294: H1888-H1895, 2008.

32. **Plante E, Lachance D, Gaudreau M, Drolet MC, Roussel E, Arsenault M and Couet J**. Effectiveness of beta-blockade in experimental chronic aortic regurgitation. *Circulation* 110: 1477-1483, 2004.

33. **Plante E, Lachance D, Roussel E, Drolet MC, Arsenault M and Couet J**. Impact of anesthesia on echocardiographic evaluation of systolic and diastolic function in rats. *J Am Soc Echocardiogr* 19: 1520-1525, 2006.

34. **Rothermel BA, McKinsey TA, Vega RB, Nicol RL, Mammen P, Yang J, Antos CL, Shelton JM, Bassel-Duby R, Olson EN and Williams RS**. Myocyte-enriched calcineurin-interacting protein, MCIP1, inhibits cardiac hypertrophy in vivo. *Proc Natl Acad Sci U S A* 98: 3328-3333, 2001.

35. **Sanna B, Brandt EB, Kaiser RA, Pfluger P, Witt SA, Kimball TR, van Rooij E, De Windt LJ, Rothenberg ME, Tschop MH, Benoit SC and Molkentin JD**. Modulatory calcineurin-interacting proteins 1 and 2 function as calcineurin facilitators in vivo. *Proc Natl Acad Sci U S A* 103: 7327-7332, 2006.

36. **Schaffer WL, Galloway JM, Roman MJ, Palmieri V, Liu JE, Lee ET, Best LG, Fabsitz RR, Howard BV and Devereux RB**. Prevalence and correlates of rheumatic heart disease in American Indians (the Strong Heart Study). *Am J Cardiol* 91: 1379-1382, 2003.

37. **Shimoyama M, Hayashi D, Takimoto E, Zou Y, Oka T, Uozumi H, Kudoh S, Shibasaki F, Yazaki Y, Nagai R and Komuro I**. Calcineurin plays a critical role in pressure overload-induced cardiac hypertrophy. *Circulation* 100: 2449-2454, 1999.

38. **Van Gelder RN, von Zastrow ME, Yool A, Dement WC, Barchas JD and Eberwine JH**. Amplified RNA synthesized from limited quantities of heterogeneous cDNA. *Proc Natl Acad Sci U S A* 87: 1663-1667, 1990.

39. **van Rooij E, Doevendans PA, Crijns HJ, Heeneman S, Lips DJ, van Bilsen M, Williams RS, Olson EN, Bassel-Duby R, Rothermel BA and De Windt LJ**. MCIP1 overexpression suppresses left ventricular remodeling and sustains cardiac function after myocardial infarction. *Circ Res* 94: e18-e26, 2004.

40. **Wagner RA, Tabibiazar R, Powers J, Bernstein D and Quertermous T**. Genome-wide expression profiling of a cardiac pressure overload model identifies major metabolic and signaling pathway responses. *J Mol Cell Cardiol* 37: 1159-1170, 2004.

41. **Wang D, Oparil S, Feng JA, Li P, Perry G, Chen LB, Dai M, John SW and Chen YF**. Effects of pressure overload on extracellular matrix expression in the heart of the atrial natriuretic peptide-null mouse. *Hypertension* 42: 88-95, 2003.

42. **Weinberg EO, Mirotsou M, Gannon J, Dzau VJ, Lee RT and Pratt RE**. Sex dependence and temporal dependence of the left ventricular genomic response to pressure overload. *Physiol Genomics* 12: 113-127, 2003.

43. **Wright GW and Simon RM**. A random variance model for detection of differential gene expression in small microarray experiments. *Bioinformatics* 19: 2448-2455, 2003.

44. **Yang DK, Choi BY, Lee YH, Kim YG, Cho MC, Hong SE, Kim dH, Hajjar RJ and Park WJ**. Gene profiling during regression of pressure overload-induced cardiac hypertrophy. *Physiol Genomics* 30: 1-7, 2007.

Figure legends:

Figure 1: Gene expression microarray observations. Upper panel: Volcano plot showing in the hatched region genes that were identified as unregulated. The line indicates the $\log_{10}$ of the P value (0.001) that was set as a threshold of significance for genes falling outside the hatched region and thus considered as regulated in the LV of AR rats. Middle panel: Array intensity vs. the number of expressed sequence tags (EST). The line indicates the lower threshold under which the expression was considered too low for further analysis. Bottom panel: Plot of Sam analysis d-score comparison between AR vs. sham animals in the Y axis and mixed individuals (3 out of 6 RNA samples from each group randomly assigned) in the X axis. The line indicates the expected relation if d-score obtained were only by chance with a ±3 variation in d-score indicated by the dotted lines on each side.

Figure 2: Correlation between fold change results obtained from the array experiment and by quantitative RT-PCR for a subset of genes. Results are expressed as mean ± SEM (n=6 animals per group) as fold change compared to sham-operated control rats and were calculated as described in the Material and methods section.

Figure 3: Changes over time in LV mRNA levels of selected genes in rats with severe AR. Results are illustrated as mean of fold change relative to sham-operated animals of the same age ± SEM (n= 6 per group at 2 weeks; 10 at 26 weeks and 7 at 52 weeks). ns: Fold change not significantly different from age-matched sham controls. All other values have a p value less than 0.05 compared to sham controls.

Figure 4: Treatment with metoprolol and captopril reversed the up-regulation of the mRNA expression of several genes in the LV of AR animals as assessed by real-time quantitative RT-PCR. Results are expressed in arbitrary units (AU) as mean ± SEM (n=7-9/gr.). Sham (sham-operated animals) group mRNA levels were normalized to 1. AR: untreated and ART: group treated with metoprolol and captopril. ¶: $p<0.05$ vs. AR, ¶¶: $p<0.01$ vs. AR, ¶¶¶: $p<0.001$ vs. AR. *: $p<0.05$ vs. sham, **: $p<0.01$ vs. sham, ***: $p<0.001$ vs. sham.

Table 1: QuantiTect® Primer Assays used in Q-PCR analysis of gene expression.

mRNA	Symbol	Acc. No.	Cat. No.	Amplicon (bp)	Exon junction
Connective tissue growth factor	Ctgf	NM_022266	QT00182021	102	yes
Down syndrome critical region homolog 1	Dscr1	NM_153724	QT00181293	76	yes
Elastin	Eln	NM_012722	QT01575924	74	yes
Enolase 3, beta	Eno3	NM_012949	QT00180138	106	yes
Extracellular matrix protein 1	Ecm1	NM_053882	QT01619310	90	yes
Fibrillin 1	Fbn1	NM_031825	QT00187215	105	yes
Fibronectin type III domain-positive	Fndc1	NM_001038615	QT01627472	91	yes
Fibronectin 1	Fn1	NM_019143	QT00179333	92	yes
Lysyl oxidase	Lox	NM_017061	QT00185591	148	yes
Monoamine oxidase A	Maoa	XM_001058993	QT01625071	81	yes
Natriuretic peptide precursor A	Nppa, ANP	NM_012612	QT00366170	107	no
Natriuretic peptide precursor B	Nppb, BNP	NM_031545	QT00183225	94	yes
Neuropeptide Y	Npy	NM_012614	QT00180355	150	yes
Procollagen-1 alpha-1	Col1a1	NM_053304	QT00370622	92	no
Procollagen-3 alpha-1	Col3a1	NM_032085	QT01083537	111	yes
S100 calcium binding protein A1	S100a1	NM_001007636	QT00377034	79	no
Tissue inhibitor of metallopeptidase 1	Timp1	NM_053819	QT00185304	113	yes
Titin	Ttn	XM_001065955	QT00504672	86	no
Transforming growth factor beta 1	Tgfb1	NM_021578	QT00187796	145	yes
Transforming growth factor beta 2	Tgfb2	NM_031131	QT00187320	139	yes
Transforming growth factor beta 3	Tgfb3	NM_013174	QT00177065	138	yes

Table 2. Sacrifice and echocardiography data of two-week AR study.

Parameters	S2 (n=6)	AR2 (n=6)	P value
Body weight, g	449 ± 8.2	427 ± 10.9	0.18
Heart weight, g	1.18± 0.05	1.42 ± 0.04	0.002
LV weight, mg	812 ± 39.0	1025 ± 23.7	0.0008
LV/Body weight	1.8± 0.05	2.4 ± 0.06	0.00014
EDD, mm	8.2 ± 0.09	9.8 ± 0.22	<0.0001
ESD, mm	4.0 ± 0.02	4.9 ± 0.01	0.0004
RWT (unitless)	0.31 ± 0.003	0.28 ± 0.007	0.0006
Heart rate	395 ± 5.0	376 ± 5.4	0.061
Stroke volume, µl	183 ± 3.0	266 ± 12.5	<0.0001
EF, %	77 ± 0.74	75 ± 0.78	0.17

Values are expressed as mean ± SEM for the indicated number of animals. LV: left ventricle. EDD: end-diastolic diameter ESD: end-systolic diameter, RWT: relative wall thickness ((Septal wall + posterior wall)/EDD) and EF: ejection fraction.

Table 3: Genes upregulated in the LV of rats with severe aortic valve regurgitation.

Target ID	SYMBOL	DEFINITION	Fold change	SAM d-score	P-value
ILMN_61583	Nppa	Natriuretic peptide precursor type A	14.40	10.15	<0.00001
ILMN_48417	Acta1	Actin, alpha 1, skeletal muscle	2.89	6.22	0.00011
ILMN_48683	Ctgf	Connective tissue growth factor	2.56	8.30	0.00001
ILMN_66092	Col3a1	Collagen, type III, alpha 1	2.52	3.23	0.00904
ILMN_64489	LOC499410	PREDICTED: LOC499410	2.50	13.31	<0.00001
ILMN_70153	Lox	PREDICTED: Lysyl oxidase	2.45	9.58	<0.00001
ILMN_70226	Col8a1_predicted	PREDICTED: Procollagen, type VIII, alpha 1	2.42	5.29	0.00037
ILMN_70184	LOC361624	PREDICTED: Similar to C11orf17 protein	2.41	9.96	<0.00001
ILMN_68993	Loxl1_predicted	PREDICTED: Lysyl oxidase-like 1	2.40	13.41	<0.00001
ILMN_55259	Cd164l1_predicted	PREDICTED: CD164 sialomucin-like 1	2.19	11.92	<0.00001
ILMN_67646	Hamp	Hepcidin antimicrobial peptide	2.13	4.55	0.00106
ILMN_50026	LOC363083	PREDICTED: Similar to F-box protein FBL2	2.10	13.52	<0.00001
ILMN_51879	Fbn1	Fibrillin 1	2.09	9.60	<0.00001
ILMN_68990	Col14a1_predicted	PREDICTED: Collagen, type XIV, alpha 1	2.06	9.13	0.00001
ILMN_53766	Mfap4_predicted	PREDICTED: Microfibrillar-associated protein 4	2.03	7.67	0.00002
ILMN_70000	Ptgds	Prostaglandin D2 synthase	2.02	7.13	0.00004
ILMN_59954	Fxyd6	FXYD domain-containing ion transport regulator 6	2.02	9.72	<0.00001
ILMN_53129	Mfap5_predicted	PREDICTED: Microfibrillar associated protein 5	2.01	8.23	0.00001
ILMN_56696	LOC498709	PREDICTED: Similar to Cyclin-dependent kinases regulatory subunit 2	1.97	14.58	<0.00001
ILMN_55682	Loxl2_predicted	PREDICTED: Lysyl oxidase-like 2	1.97	10.83	<0.00001
ILMN_53273	Nppb	Natriuretic peptide precursor type B	1.96	6.17	0.00011
ILMN_48170	Emp1	Epithelial membrane protein 1	1.94	5.75	0.00019
ILMN_68954	Ccnd1	Cyclin D1	1.93	12.89	<0.00001
ILMN_64926	Fstl1	Follistatin-like 1	1.93	12.16	<0.00001
ILMN_49631	Ptgis	Prostaglandin I2 (prostacyclin) synthase	1.88	10.03	<0.00001
ILMN_57003	Dscr1	Down syndrome critical region homolog 1	1.86	12.09	<0.00001
ILMN_49561	Ecm1	Extracellular matrix protein 1 (Ecm1)	1.83	8.11	0.00001
ILMN_64916	LOC498272	PREDICTED: Similar to UDP-N-acteylglucosamine pyrophosphorylase 1 homolog	1.81	7.73	0.00002
ILMN_60211	Anxa1	Annexin A1	1.79	9.09	0.00001
ILMN_61895	LOC360627	PREDICTED: Similar to 65kDa FK506-binding protein	1.76	11.22	<0.00001
ILMN_50637	Col1a1	PREDICTED: Collagen, type 1, alpha 1	1.75	5.19	0.00042
ILMN_57568	Cspg2	PREDICTED: Chondroitin sulfate proteoglycan 2	1.75	8.44	0.00001
ILMN_58183	S100a6	S100 calcium binding protein A6 (calcyclin)	1.73	14.83	<0.00001

ILMN_57623	Maoa	PREDICTED: Monoamine oxidase A	1.73	4.46	0.00121
ILMN_63022	LOC499856	PREDICTED: Similar to RIKEN cDNA 1110018M03	1.73	7.44	0.00003
ILMN_63763	Col4a1_predicted	PREDICTED: Procollagen, type IV, alpha 1	1.72	6.70	0.00006
ILMN_69134	Tgfb2	Transforming growth factor, beta 2	1.71	7.38	0.00003
ILMN_53665	Serpine2	PREDICTED: Serine (or cysteine) proteinase inhibitor, clade E, member 2	1.66	7.89	0.00002
ILMN_59788	S100a11	PREDICTED: S100 calcium binding protein A11 (calizzarin)	1.64	8.52	0.00001
ILMN_50151	Col5a1	Collagen, type V, alpha 1	1.63	6.00	0.00014
ILMN_64570	LOC307351	PREDICTED: Similar to RIKEN cDNA 2310057H16	1.63	17.26	<0.00001
ILMN_53896	Eln	Elastin	1.63	4.40	0.00133
ILMN_51080	Col5a2	PREDICTED: Collagen, type V, alpha 2	1.61	5.81	0.00017
ILMN_59617	Sulf2_predicted	PREDICTED: Sulfatase 2	1.60	9.37	<0.00001
ILMN_65617	LOC498276	PREDICTED: Similar to Fc gamma (IgG) receptor II (low affinity) alpha precursor	1.59	5.34	0.00034
ILMN_64708	RGD1309107_predicted	PREDICTED: Similar to RIKEN cDNA 6530401L14 gene	1.58	9.40	<0.00001
ILMN_57892	G0s2	G0/G1 switch gene 2	1.56	6.63	0.00007
ILMN_54242	Vim	Vimentin (Vim)	1.56	5.03	0.00053
ILMN_48221	Tagln2_predicted	PREDICTED: Transgelin 2	1.56	8.87	0.00001
ILMN_63786	Odc1	Ornithine decarboxylase 1	1.56	8.38	0.00001
ILMN_62559	Timp1	Tissue inhibitor of metalloproteinase 1	1.55	8.82	0.00001
ILMN_52596	Col1a2	Procollagen, type I, alpha 2	1.55	5.67	0.00021
ILMN_50058	Fn1	Fibronectin 1	1.54	4.06	0.00229
ILMN_54050	Pmp22	Peripheral myelin protein 22	1.54	6.52	0.00007
ILMN_51244	Myl9_predicted	PREDICTED: Myosin, light polypeptide 9, regulatory	1.54	6.27	0.00010
ILMN_47707	Aif1	Allograft inflammatory factor 1	1.53	7.42	0.00003
ILMN_57751	Chst1_predicted	PREDICTED: Carbohydrate (keratan sulfate Gal-6) sulfotransferase 1	1.53	7.40	0.00003
ILMN_48308	Kifc1	Kinesin family member C1	1.52	7.07	0.00004
ILMN_57815	Anxa2	Annexin A2	1.52	6.09	0.00012
ILMN_52167	Pdlim7	PDZ and LIM domain 7	1.52	8.42	0.00001
ILMN_66128	Lamb1-1_predicted	PREDICTED: Laminin B1 subunit 1	1.51	8.90	0.00001
ILMN_63287	LOC500040	PREDICTED: Similar to Testis derived transcript	1.51	5.16	0.00044
ILMN_59259	Ctsk	Cathepsin K	1.50	7.85	0.00002
ILMN_59433	LOC286890	PREDICTED: Tropomyosin isoform 6	1.50	6.65	0.00007

Table 4. Genes down-regulated in the LV of rats with severe aortic valve regurgitation.

Target ID	SYMBOL	DEFINITION	Fold change	SAM d-score	P-value
ILMN_66083	Ifit1	Interferon-induced protein with tetratricopeptide repeats 1	0.38	-4.43	0.00126
ILMN_64141	Cyp26b1	Cytochrome P450, family 26, subfamily b, polypeptide 1	0.44	-5.40	0.00031
ILMN_59685	Wfdc1	WAP four-disulfide core domain 1	0.45	-3.45	0.00617
ILMN_53199	Ddit4l	PREDICTED: DNA-damage-inducible transcript 4-like	0.47	-7.27	0.00004
ILMN_59204	LOC500621	PREDICTED: Similar to hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase.	0.53	-8.09	0.00001
ILMN_54246	Atp1a2	ATPase, Na+/K+ transporting, alpha 2 polypeptide	0.54	-4.69	0.00086
ILMN_59459	LOC360546	PREDICTED: Similar to m-ephrin-B3	0.55	-6.01	0.00014
ILMN_64504	Lsamp	Limbic system-associated membrane protein	0.56	-6.44	0.00008
ILMN_67004	Ces3	PREDICTED: Carboxylesterase 3	0.59	-3.56	0.00522
ILMN_61942	Amd1	S-adenosylmethionine decarboxylase 1	0.59	-5.78	0.00018
ILMN_68662	LOC361044	PREDICTED: Similar to HCDI protein	0.59	-6.87	0.00005
ILMN_57629	Snurf	SNRPN upstream reading frame (Snurf)	0.62	-12.60	<0.00001
ILMN_63455	LOC363331	PREDICTED: Similar to S3-12	0.63	-4.50	0.00114
ILMN_63008	Perp_predicted	PREDICTED: PERP, TP53 apoptosis effector	0.63	-7.27	0.00004
ILMN_51872	Gpt1	Glutamic pyruvic transaminase 1, soluble	0.63	-6.79	0.00006
ILMN_58624	Ldhd	Lactate dehydrogenase D	0.63	-5.53	0.00025
ILMN_67626	Eno3	Enolase 3, beta	0.64	-4.38	0.00138
ILMN_56305	LOC314472	PREDICTED: Hypothetical LOC314472	0.64	-3.64	0.00462
ILMN_70398	Lrp16	LRP16 protein	0.64	-8.01	0.00002
ILMN_56897	Npy	Neuropeptide Y	0.64	-3.66	0.00442
ILMN_64510	Bckdha	PREDICTED: Branched chain ketoacid dehydrogenase E1	0.65	-6.34	0.00009
ILMN_59611	S100a1	PREDICTED: S100 calcium binding protein A1	0.65	-6.55	0.00007
ILMN_47906	C7	PREDICTED: Complement component 7	0.65	-3.80	0.00353
ILMN_56121	LOC314467	PREDICTED: Hypothetical LOC314467	0.66	-9.63	<0.00001
ILMN_65867	Boll_predicted	PREDICTED: Bol, boule-like (Drosophila)	0.66	-5.51	0.00026
ILMN_62489	Hdac11_predicted	PREDICTED: Histone deacetylase 11	0.66	-6.03	0.00013
ILMN_62505	Limd1_predicted	PREDICTED: LIM domains containing 1	0.66	-5.66	0.00022
ILMN_52287	Adhfe1_predicted	PREDICTED: Alcohol dehydrogenase, iron containing, 1	0.67	-8.45	0.00001
ILMN_60478	Rev3l_predicted	PREDICTED: REV3-like, catalytic subunit of DNA polymerase zeta RAD54 like (S. cerevisiae)	0.67	-6.58	0.00007

Table 5. Sacrifice and echocardiography data 26 weeks and 52 weeks post AR induction in surviving animals.

Parameters	S26 (n=12)	AR26 (n=10)	ART (n=12)	S52 (n=10)	AR52 (n=8)	p
Body weight, g	666 ± 17.8	684 ± 22.5	605 ± 18.4*	850 ± 25.8	830 ± 18.5	0.56
Heart weight, g	1.4 ± 0.04**	2.1 ± 0.09	1.8 ± 0.08*	1.67 ± 0.05	2.84 ± 0.14	<0.0001
Tibial length, mm	60.5 ± 0.42	60.3 ± 0.35	59.7 ± 0.50	62.2 ± 0.38	61.8 ± 0.39	0.49
LV weight, mg	997 ± 40.9**	1614 ± 65.5	1387 ± 62.8*	1113 ± 77.1	1914 ± 59.9	<0.0001
LV/Body weight	1.5 ± 0.05	2.4 ± 0.13	2.2 ± 0.11	1.3 ± 0.08	2.3 ± 0.08	<0.0001
LV/Tibial length	16.5 ± 0.74**	27.5 ± 0.81	22.9 ± 0.92*	17.9 ± 1.23	30.0 ± 1.10	<0.0001
EDD, mm	8.5 ± 0.22**	11.7 ± 0.30	10.6 ± 0.20*	8.5 ± 0.18	12.6 ± 0.46	<0.0001
ESD, mm	4.3 ± 0.29**	7.9 ± 0.34	6.7 ± 0.21*	4.8 ± 0.23	8.7 ± 0.44	<0.0001
RWT (unitless)	0.45 ± 0.015**	0.36 ± 0.012	0.39 ± 0.009*	0.50 ± 0.015	0.36 ± 0.016	<0.0001
Stroke volume, µl	310 ± 15.0**	488 ± 24.4	433 ± 25.4	310 ± 7.6	504 ± 11.8	<0.0001
Heart rate	334 ± 12.0	340 ± 15.5	355 ± 11.4	334 ± 16.6	293 ± 13.7	0.099
SBP (mm Hg)	125 ± 3.0	134 ± 3.2	129 ± 2.9	122 ± 5.0	119 ± 4.6	0.63
DBP (mm Hg)	81 ± 3.2**	67 ± 3.5	68 ± 3.7	78 ± 4.2	62 ± 4.4	0.002
EF, %	74 ± 2.3**	54 ± 1.9	61 ± 1.9*	68 ± 2.2	53 ± 1.8	0.00013

EDD: end-diastolic diameter; ESD: end-systolic diameter; RWT: relative wall thickness ((Septal wall + posterior wall)/EDD); SBP: systolic blood pressure; DBP: diastolic blood pressure; EF: left ventricular ejection fraction. S26: sham animals 26 weeks; AR26: AR animals 26 weeks; ART: AR animals treated with metoprolol+captopril 26 weeks; S52: sham animals 52 weeks and AR52: AR animals 52 weeks. Values are mean ± SEM of the indicated number animals per group. *: $p<0.05$ vs. AR26 group and **: $p<0.01$ vs. AR26 group. p values: Student T-test between S52 and AR52 groups.