ORIGINAL ARTICLE

Multiple short-chain dehydrogenases/reductases are regulated in pathological cardiac hypertrophy.

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Cardiac hypertrophy (CH) is an important and independent predictor of morbidity and mortality. Through expression profiling, we recently identified a subset of genes (*Dhrs7c*, *Decr*, *Dhrs11*, *Dhrs4*, *Hsd11b1*, *Hsd17b10*, *Hsd17b8*, *Blvrb*, *Pecr*), all of which are members of the short-chain dehydrogenase/reductase (SDR) superfamily and are highly expressed in the heart, which were significantly dysregulated in a rat model of CH caused by severe aortic valve regurgitation (AR). Here, we studied their expression in various models of CH, as well as factors influencing their regulation. Among the nine SDR genes studied, all but *Hsd11b1* were downregulated in CH models (AR rats or mice infused with either isoproterenol or angiotensin II). This regulation showed a clear sex dimorphism, being more evident in males than in females irrespective of CH levels. In neonatal rat cardiomyocytes, we observed that treatment with the alpha-1 adrenergic receptor agonist, phenylephrine, mostly reproduced the observations made in CH animals models. Retinoic acid, on the other hand, stimulated the expression of most of the SDR genes studied, suggesting that their expression may be

Abbreviations: SDR: short-chain dehydrogenase/reductase; CH: cardiac hypertrophy; HF: heart failure; AR: aortic valve regurgitation; LV: left ventricle; ACE: angiotensin I converting enzyme; LVH: left ventricle hypertrophy; RA: retinoic acid; ANGII: angiotensin II.

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related to cardiomyocyte differentiation. Indeed, levels of expression were found to be higher in the hearts of adult animals than in neonatal cardiomyocytes. In conclusion, we identified a group of genes modulated in animal models of CH and mostly in males. This could be related to the activation of the fetal gene expression program in pathological CH situations, in which these highly expressed genes are down-regulated in the adult heart.

KEYWORDS

short-chain dehydrogenase/reductase , *Dhrs7c*, heart hypertrophy, aortic regurgitation, volume overload, pressure overload, angiotensin II

1 | INTRODUCTION

Abnormal hemodynamic overloads induce cardiac hypertrophy (CH); a compensatory adaptive response aimed at maintaining cardiac output. If this adaptive response is sustained, it can lead to decompensation and heart failure (HF), an important cause of morbidity and mortality[1].

CH is characterized by an important remodelling of the myocardial structure, a consequence of cardiomyocytes size increase and extracellular matrix rearrangement. Neurohormonal factors as well as mechanic stress cause alterations in myocardial gene expression including the reactivation of the fetal gene program[2]. This feature is common to a variety of pathological conditions including ischemia, atrophy, hypoxia, diabetes in addition of hypertrophy. This return to the fetal gene program has long been considered detrimental whereas others have suggested that it protects the heart against irreversible impairment and cell death.

Genes often associated with the fetal gene program include atrial and brain natriuretic peptide (*Anp* and *Bnp*), contractile protein beta-myosin heavy chain (beta-MHC or *Myh7*) and early response genes such as *c-myc* and *c-fos* among many others. This reactivation of the fetal gene program in the stressed heart is accompanied with the down-regulation of the adult gene program[3].

We study a rat model of chronic volume overload caused by severe aortic valve regurgitation (AR), which is associated with a long asymptomatic period during which the left ventricle (LV) progressively dilates (eccentric remodelling) and hypertrophies[4]. This process is accompanied by a decrease in LV function, occurrence of symptoms and eventually HF[5]. Chronic AR often secondary to rheumatic fever is a condition still frequent in developing countries and in populations having less than adequate access to health care[6, 7].

Gene expression profiles have been established in several animal models of LV eccentric hypertrophy; including by us in a rat model after two weeks of severe AR, a period characterized by intense LV remodelling[8, 9, 10, 11]. We recently reported a LV gene expression profiling late in the disease (9 months)[12]. We observed the expected activation of many genes associated with the fetal gene program both early and late in the disease. At nine months, a general down-regulation of genes involved in fatty acid oxidation and bioenergetics was observed. Among the cardiac genes that were modulated in our model, a significant number (26) were tagged with the molecular function mention, oxidoreductase activity[12].

Here, we report that many members of the short-chain dehydrogenase/reductase (SDR) superfamily are strongly expressed in the adult heart and that their expression is reduced in the hypertrophied myocardium.

2 | METHODS

Animal experiments:

Rats: Wistar rats, male (350g) and female (225-250g) were obtained from Charles River (St. Constant, QC, Canada). Pregnant rats (day 15 of gestation) were also obtained from the same provider. Severe AR was induced by retrograde puncture of the aortic valve leaflets under echocardiographic guidance as previously described[13, 14, 15]. Only animals with >65% regurgitation were included in the study. A complete echo exam was performed before AR induction and at the end of the protocol as previously described[16, 17]. Captopril treatment (1g/l in drinking water) was initiated 14 days after AR induction[17]. Pregnant female rats were ordered also from Charles River and delivered on the 14-15 days of gestation. Mice: C57Bl6J mice (20-25g) were also purchased from Charles River. Micro-osmotic pumps (Model 1004; Alzet, Cupertino, Palo Alto, CA), gradually releasing isoproterenol (Iso: 30 mg/kg/day), angiotensin II (AngII; 4 mg/kg/d) or vehicle (saline), were implanted subcutaneously for 14 days. The protocols were approved by the Universite Laval's animal protection committee and followed the recommendations of the Canadian Council on laboratory animal care.

Cell culture:

Neonatal rat cardiomyocytes (NRCMs) were prepared following an adapted protocol[18]. Briefly, ventricular tissue from 1- to 2-day old Wistar rats was subjected to enzymatic digestion in collagenase/dispase solution. Cells were then collected by low-speed centrifugation. Enrichment of cardiomyocytes was accomplished by plating cells on culture dishes for 2 hours. NRCMs were cultured in DMEM/M199 (4:1) supplemented with 4% horse serum, antibiotics and 1-b-D-arabinofuranoside in order to prevent the growth of contaminating non-myocytes. H9C2 cells (ATCC CRL-1446) were cultured in DMEM supplemented with 10 % fetal bovine serum.

Micro-array analysis:

The micro-array analysis was conducted on 9-month AR male rats as reported previously [12]. Complete data (complying with MIAME guidelines) are available at the GEO database (NCBI) under the accession number GSE17050. Genes displaying low levels of expression were discarded from analysis. In order to do so, we calculated the mean + 5 times the standard deviation of the signals obtained from genes encoding for olfactory receptors as a threshold of minimal expression to be considered as meaningful[11].

Analysis of mRNA accumulation by quantitative RT-PCR:

The analysis of LV mRNA levels by quantitative RT-PCR has been described in details elsewhere[11]. QuantiTect® and IDT (Coralville, Iowa) Primer Assays (pre-optimized specific primer pairs; and QuantiFast® SYBR Green PCR kits (Qiagen) were used (Table S1). We also used one pair of non-pre-optimized primers for (5'-TGCAGAAAGCTGACCTATGG-3' and 5'-GGGAAGAAGGTGCGGATAAA-3'; 95bp transcript) for the rat *Dhrs7c* gene. Cyclophilin A (*Ppia*) was used as the control "housekeeping" gene.

Western blot analysis:

Crude LV homogenates were separated by SDS-PAGE. Immunoblotting was performed as described elsewhere[13]. All primary antibodies were used at a 1:1000 dilution and were purchased from Cell Signaling Technology (Beverly, MA) or from Santa Cruz Biotechnology (Santa Cruz,CA).

Tubulin immunostaining:

H9C2 cells were culture in Labtek chamber slides. After culture medium removal, cells were fixed in cold 4%

paraformaldehyde in PBS and permeabilized with 0.1% Triton X-100. Primary (anti-tubulin; Sigma) and secondary (Alexa Fluor 488 anti-mouse IgG) antibodies were both used at 1:1000 dilution. After several washes with PBS, nuclei were stained with Hoechst 33342 (1:10000 in PBS). Cells were then mounted with Fluoromount G and observed under a Zeiss LSM 800 confocal microscope.

Statistical analysis :

Results are presented as the mean and SEM: standard error of the mean. Statistical significance was set at p below 0.05. Student's T-test were used when two groups were compared. One-way ANOVA: analysis of variance was used followed by Tukey's post-test when more than two groups were compared. Data and statistical analyses were performed using Graph Pad Prism version 7.04 for Windows, Graph Pad Software (San Diego, CA).

3 | RESULTS

SDRs gene expression in eccentric LVH.

After 9 months, eight of fifteen (8/15) AR animals were still alive, whereas all sham-operated animals were alive as previously reported[12]. From published micro-array data, we had identified an enriched category of genes down-regulated in the myocardium of AR male rats tagged "oxidoreductase"[12]. Among them, we noticed the presence of many SDR genes. In all, we identified 36 SDR genes in the rat LV displaying a higher expression signal than the fixed threshold and divided them in three groups (high, mid and low-expression) as illustrated in Figure 1A. Of these genes, fourteen had significantly different levels of LV expression between AR and sham-operated male rats. We then chose to focus on nine of them (the most expressed) namely *Dhrs7c*, *Decr1*, *Dhrs11*, *Dhrs4*, *Hsd11b1*, *Hsd17b10*, *Hsd17b8*, *Blvrd* and *Pecr*. These nine genes will be referred as SRD genes from now on. With the exception of *Hsd11b1*, all of them were down-regulated in the LV of AR rats. This was confirmed when assayed by real-time RT-PCR (Figure 1B). We also observed that Dhrs7c and Decr protein contents were also strongly reduced in AR LV crude homogenates compared to shams. Only a small trend for 11beta-hsd1 increase was observed (Figure 1C). We studied acute regulation of these SDR genes in the AR rat model after 48 hours. Only *Dhrs7c* and *Hsd17b8* mRNA levels were down-regulated in the hearts of AR rats 48 hours post-AR induction (Figure S1).

Evidence for sex dimorphism in the control of SDR genes in AR LVH.

Baseline levels of expression of the studied SDR genes were similar between male and female rats (Figure 2A). After 6 months of severe AR, the hypertrophic response to similar levels of aortic valve regurgitation was more important in female rats (Figure 2B). On the other hand, SDR genes remained mostly unregulated in AR females compared to males (Figure 2C).

SDR genes are less regulated in physiological LV remodeling

We tested two rat models of physiological heart remodeling. The first was moderate endurance training on a treadmill for a period of 6 months in both male and female Wistar rats[16]. Most of the SDR genes studied were not regulated in trained rats (Figure S2). We then tested the effects of gestation. Hearts of the dams were collected at three time points namely late pregnancy (LP; 19 days of gestation), one day post-partum (1PP) and 4 days post-partum (4PP). As illustrated in Figure S3, Heart hypertrophy was present at LT and 1PP while heart weight had returned to normal 4 days after delivery. Only mild changes in expression were observed for 3 of the 9 genes studied namely down-regulation of *Dhrs7c*, *Dhrs11* and *Dhrs4*.

Blocking LVH development with an ACE inhibitor tends to normalize SDR gene expression.

Male AR rats were treated with captopril (an angiotensin I converting enzyme inhibitor; ACEi) for 6 months. This treatment mostly blocked CH development as illustrated in Figure 3A. Six of the nine studied SDR genes had their

TABLE 1 Echocardiographic data after two weeks of continuous isoproterenol (Iso) infusion in male C57Bl6J mice.

Parameters	Saline ($n = 8$)	Iso ($n = 8$)	P value
End-diastolic LV diameter, mm	4.5 +/- 0.07	4.5 +/- 0.10	0.98
End-systolic LV diameter, mm	3.0 +/- 0.11	2.8 +/- 0.12	0.30
LV fractional shortening, %	34 +/- 2.8	38 +/- 2.0	0.24
Septal wall thickness, %	0.68 +/- 0.023	0.78 +/- 0.029	0.017
Posterior wall thickness, mm	0.68 +/- 0.031	0.87 +/- 0.026	0.0003
Relative wall thickness, mm	0.3 +/- 0.011	0.37 +/- 0.016	0.0046

Echocardiography measurements obtained under inhaled 1.5% isoflurane anesthesia. LV: left ventricle. Relative wall thickness was calculated using the following equation: (Septal wall thickness + Posterior wall thickness) / end-diastolic LV diameter. Values are expressed as mean +/- SEM of the indicated number of animals Statistical significance between groups was determined using the Student's T-test.

expression normalized by the ACEi treatment (Figure 3B).

SDR genes are also regulated in two mouse models of concentric LVH.

In order to confirm that this SDR genes regulation was not limited to eccentric CH, male mice were infused continuously for 14 days with either one of the two following pro-hypertrophic agents namely isoproterenol (Iso; beta1-adrenergic agonist) or angiotensin II (AngII). Both agents induced moderate levels of CH (Figure 4A). Heart rate was significantly increased in mice receiving Iso (Figure 4A). Echocardiography imaging confirmed the development of concentric LV hypertrophy with a significant increase in wall thickness (Tables 1 and 2). Systolic function remained unchanged for both models. All SDR genes saw their expression decreased in mice infused with either Iso or AngII. This effect was stronger in mice treated with AngII (Figure 4B). As illustrated in Figures S4 and S5, female mice receiving either Iso or Ang II, had a similar hypertrophic response compared to males. On the other hand, SDR genes regulation was less marked compared to males.

Adrenergic agonists and retinoic acid regulates SDR genes in opposite ways in vitro.

Isolated neonatal rat cardiomyocytes were treated with either 1 micro M Iso, 1 micro M phenylephrine (Phe; alpha1-adrenergic agonist) or 10 nM all-trans retinoic acid (RA) for 24 hours. With the exception of the *Dhrs7c* gene, which was strongly down-regulated by Iso and Phe, response of other SDR genes was milder in cells treated with Iso than by Phe (Figure 5A). RA-treated cells showed a pattern of SDRs gene expression that was a mirror image of the one observed in Phe-treated cardiomyocytes. We also examined the expression of hypertrophy markers in these cells namely *Anp*, *Bnp*, *Myh6* and *Myh7*. Iso treatment only induced minor changes in the expression of these genes. *Anp* and *Bnp* mRNA levels were markedly increased by Phe treatment and decreased by RA. The opposite was observed for *Myh6* (Figure 5B).

SDR gene expression during cardiac muscle cell differentiation.

As illustrated in Figure 6A, Dhrs7c, Decr and 11-HSD protein levels are higher in LV crude homogenates (rat or mouse) than in NRCMs (same amounts of total protein) . Expression of *Dhrs7c* gene increased with time in NRCMs. The same was true for *Decr1* (not shown). Expression of troponin T (*Tnnt*), a marker of differentiation for cardiomyocytes also goes up (Figure 6A). We then studied H9C2 cells. After 24 hours of RA treatment (10 nM) , mRNA levels of 7 out of 9 SDR genes studied were increased compared to untreated H9C2 cells (Figure 6B). This cardiac myoblast cell line can be made to differentiate into cardiomyocytes upon treatment with all-trans-retinoic acid (RA) and reduction of serum

TABLE 2 Echocardiographic data after two weeks of continuous angiotensin II (AngII) infusion in male C57Bl6J mice.

Parameters	Saline ($n = 6$)	AngII ($n = 7$)	P value
End-diastolic LV diameter, mm	4.4 +/- 0.13	3.9 +/- 0.07	0.013
End-systolic LV diameter, mm	2.9 +/- 0.18	2.6 +/- 0.24	0.38
LV fractional shortening, %	34 +/- 2.7	34 +/- 5.3	0.99
Septal wall thickness, %	0.62 +/- 0.024	0.74 +/- 0.024	0.0039
Posterior wall thickness, mm	0.73 +/- 0.046	0.83 +/- 0.035	0.099
Relative wall thickness, mm	0.31 +/- 0.012	0.40 +/- 0.016	0.001

Echocardiography measurements obtained under inhaled 1.5% isoflurane anesthesia. LV: left ventricle. Relative wall thickness was calculated using the following equation: (Septal wall thickness + Posterior wall thickness) / end-diastolic LV diameter. Values are expressed as mean +/- SEM of the indicated number of animals Statistical significance between groups was determined using the Student's T-test.

concentration in culture medium. After 7 days, the differentiation culture medium (1% serum + 10 nM RA) induced formation of myotubes as illustrated in Figure 6C. This was accompanied with a spike of expression of Tnnt. After 7 days of this treatment with RA, *Tnnt* expression was up by more than 12 times which was not the case for cells cultured without RA. (Fig. 6D). This differentiation process lead to increase in expression of several SDR genes namely *Dhrs7c*, *Decr1*, *Dhrs4* and *Hsd17b8*.

4 | DISCUSSION

The results presented herein indicate that expression of highly expressed cardiac SDR genes is regulated in the stressed heart. Furthermore, this regulation is mostly present in males and less so in females. SSDR genes are modulated strongly in pathological heart hypertrophy. In physiological situations leading to myocardial remodelling such as exercise and gestation, this modulation was only present for a few genes. Cardiac SDR gene expression seems to be increased during heart maturation and/or cardiac myoblasts differentiation into cardiac myotubes suggesting that these genes are part of the adult gene program.

The SDR super-family represents one of the largest and oldest protein families [19, 20, 21]. They share low sequence identity but are relatively the same size and usually catalyze NAD(P)(H)-dependent reduction/oxidation. Their large spectrum of substrates includes retinoids, steroids, polyols, fatty acid derivatives, prostaglandins and xenobiotics[19]. Over 75 human SDR proteins have been identified. If enzymes of this super-family involved in the biosynthesis of retinoids, sex hormones and glucocorticoids have received considerable attention, 30% of all SDRs are not characterized and their cellular functions remain undetermined.

Dhrs7c is mainly expressed in cardiac and skeletal muscles and is localized in the endoplasmic/sarcoplasmic reticulum[22, 23]. It is predicted to have NAD/NADH activity but its function is still debated. It was first proposed to be a retinol dehydrogenase but this then has been put into question in a recent study[23, 24]. A role for DHRS7C in maintaining intracellular Ca2+ homeostasis in the myoblastic C2C12 cell line has been proposed. Functional loss of DHRS7C function leads to myotube enlargement. Dhrs7C deficiency, on the other hand, was associated with higher

resting Ca2+ cytosolic concentrations and general Ca2+ overload in C2C12 skeletal muscle cells, a feature also relevant to cardiac hypertrophy[24]. The NAD/NADH-dependant dehydrogenase activity was also showed to be essential for Dhrs7C action on calcium control. More recently, in transgenic mice over-expressing Dhrs7C in skeletal muscle, a role for this SDR was identified as an enhancer of glucose metabolism and muscle performance. Dhrs7C was shown to increase glucose uptake into muscles. The role of Dhrs7c would be mediated via its retinol dehydrogenase activity and increased synthesis of retinoic acid (RA). RA is an enhancer of insulin signalling, which results in increased plasma membrane localization of glucose transporter 4[25]. Here, we demonstrate that Dhrs7c was down-regulated in various models of CH. This had been also reported in HF in humans as well as in HF animal models[22, 26]. Interestingly, we showed that female AR rats did not modulate their Dhrs7C levels as well as most of the SDRs studied here. Cardiac myocyte calcium levels handling is different between the sexes and is usually better maintained in the failing heart of females[27]. The reasons and the consequences of the disappearance of Dhrs7C in CH and HF at least in males have to be better explored in the future.

A metabolic link to gene expression in the stressed heart has been proposed[2]. The fetal heart relies mostly on carbohydrates for energy production. After birth, this state rapidly switches to the oxidation of fatty acids. The opposite happens in various heart pathological conditions with the return to the fetal gene program and an increased reliance again on carbohydrates for energy production[28]. *Dhrs7c* and *Decr1* mRNA levels increased in the adult heart compared to neonatal rat cardiomyocytes. Retinoic acid, a pro-differentiation factor, stimulates the expression of almost all SDR genes studied with the exception of *Hsd11b1*, the only gene initially observed to be up-regulated in CH. *Decr1*, *Pecr* and *Hsd17b10* genes all encode for enzymes implicated in fatty acid metabolism. Decr1 and Pecr are both 2,4-dienoyl-CoA reductases[29, 30]. Decr1 is expressed in the mitochondria and is an auxiliary enzyme implicated in the metabolism of poly-unsaturated fatty acids. Pecr fulfils the same function as Decr1 in the peroxisomal compartment. Unlike Decr1, Pecr is able to metabolize very long-chain fatty acid (C>20). *Hsd17b10* encodes for a mitochondrial form of 17beta-hydroxysteroid dehydrogenase[31]. Also named short chain L-3-hydroxyacil-CoA dehydrogenase (SCHSD), it is essential for the metabolism of branched-chain and straight chain fatty acids as well as isoleucine. Other roles for this enzyme include inactivation of 17beta-estradiol as well as conversion of other steroids via a 3alpha-hydroxysteroid dehydrogenase activity. We recently showed that expression of virtually all genes implicated in fatty acid -oxidation was down-regulated in male rats with severe AR but not females including *Decr1*[32]. Here, we show that *Pecr* and *Hsd17b10* gene also follow this trend.

Hsd11b1 gene encodes for the 11beta-hydroxysteroid dehydrogenase type 1 (11betaHSD1). This enzyme catalyses the regeneration of active glucocorticoids such as cortisone or corticosterone from their 11-keto inactive forms. Its inhibition was shown to be able to reverse established hypertrophy in a mouse model of perfusion deficit-induced cardiac remodelling[33]. Progression towards HF is slowed in mice lacking 11betaHSD1 in a myocardial infarct model[34, 35]. Of the highly expressed SDR genes studied, *Hsd11b1* was the only one to see its expression increased in the AR rat. This was also the case in Iso-treated neonatal rat cardiomyocytes. However, in mice where cardiac hypertrophy was induced by infusion of either Iso or AngII, *Hsd11b1* expression was reduced. In the Iso- or AngII-induced hypertrophy mice, *Hsd11b1* expression was reduced, however. It is not clear which myocardial cell type (endothelial cells, cardiomyocytes or fibroblasts), is responsible for 11betaHSD1 action in the heart[36].

Two other Dhrs (4 and 11) were found down-regulated in CH. The putative roles of these enzymes in the heart remain obscure. *Dhrs4* encodes for a carbonyl reductase with a dual function in the metabolism of endogenous signalling molecules and the detoxification of exogenous carbonyl compounds. Although, highly expressed in the heart, its physiologic role remains mostly unexplored[37]. On the other hand, *Dhrs11* gene encodes for a recently characterized 17beta-hydroxysteroid dehydrogenase with unusual secondary 3-ketosteroid reductase activity[38]. *HSD17b8* gene encodes for the 17beta-hydroxysteroid dehydrogenase type 8, which has been shown to catalyse steroid oxida-

tion/reduction in vitro[39]. Interestingly, the 17betaHSD8 can form a heterotetramer with the carbonyl reductase type 4 to generate an mitochondrial enzyme implicated in fatty acid synthesis[40]. Again, the role of 17betaHSD8 has not been studied.

Blvrb or biliverdin reductase has been identified to be a multifunctional enzyme. One of its function is the reduction of biliverdin to bilirubin. The other functions of Blvrb include a dual-specificity kinase activity, it being a transcription factor and molecular scaffold and cellular transporter of kinases and regulatory factors[41]. Biliverdin reductase in addition of heme oxidases is part of the defence against cytotoxicity in cardiac myocytes[42].

Short chain dehydrogenases/reductases catalyses oxidation/reduction reactions using NAD(H)/NADP(H) as co-factors. Control of NAD homeostasis is central for the heart energy metabolism. A broad range of activities related to cell signalling, Ca2+ handling, reactive oxygen species detoxification and others also relies on the NAD+ family of co-factors. NAD+ levels go down in the ageing heart and in HF[43, 44]. The decrease described here in the expression of various SDR genes encoding for enzymes using NAD(H)/NADP(H) may be a strategy used by the myocardium to focus the use of these co-factors on their main task, producing enough energy to maintain contraction. The return the fetal gene program has been proposed in the past as a way to protect the heart against stresses such as hypoxia, ischemia, hypertrophy or atrophy[3]. In the AR dilated LV, we already observed a decrease in the capillary density suggesting that access to oxygen and nutrients may be impaired[12, 45]. The myocardium thus has to rely less on aerobic metabolism and more on glycolysis. This study relied mainly on evaluation of gene expression levels and more thorough analyses at the level of protein content, activity and localization are needed.

5 | CONCLUSION

In this study, we identified a group of genes part of the SDR super-family highly expressed in the heart muscle and regulated in response to pathological hypertrophic stresses. Many of these SDR genes seem to be part of the adult gene program which becomes inhibited when the fetal cardiac gene program is reactivated during cardiac hypertrophy and HF.

6 | SUPPLEMENTARY DATA

Supporting information is included with the published article. Table S1. Primer Assays used in qPCR analysis of gene expression. Figure S1: Evaluation by real-time quantitative RT-PCR of the mRNA levels of SDRs genes in the LV of rats with acute and severe AR (2 days). Figure S2: Impact of moderate intensity training for a period of 3 months on SDR gene expression in male and female rat cardiac tissue. Figure S3: Impact of gestation on SDR gene expression in female mouse cardiac tissue. Figure S4: Impact of pro-hypertrophic factors on SDR gene expression in female mouse cardiac tissue. Figure S5: Echocardiographic data from female mice treated with either continuous infusion of isoproterenol (Iso) or angiotensin II (AngII) for 14 days.

AUTHOR CONTRIBUTIONS

ER performed most in vitro studies, compiled and analysed data. MCD performed animal studies, compiled and analysed data. AML performed studies in Figure 6. MA helped conceiving the study and writhing the manuscript. JC conceived the study and wrote the manuscript.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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Figure 1 Roussel et al. 2017

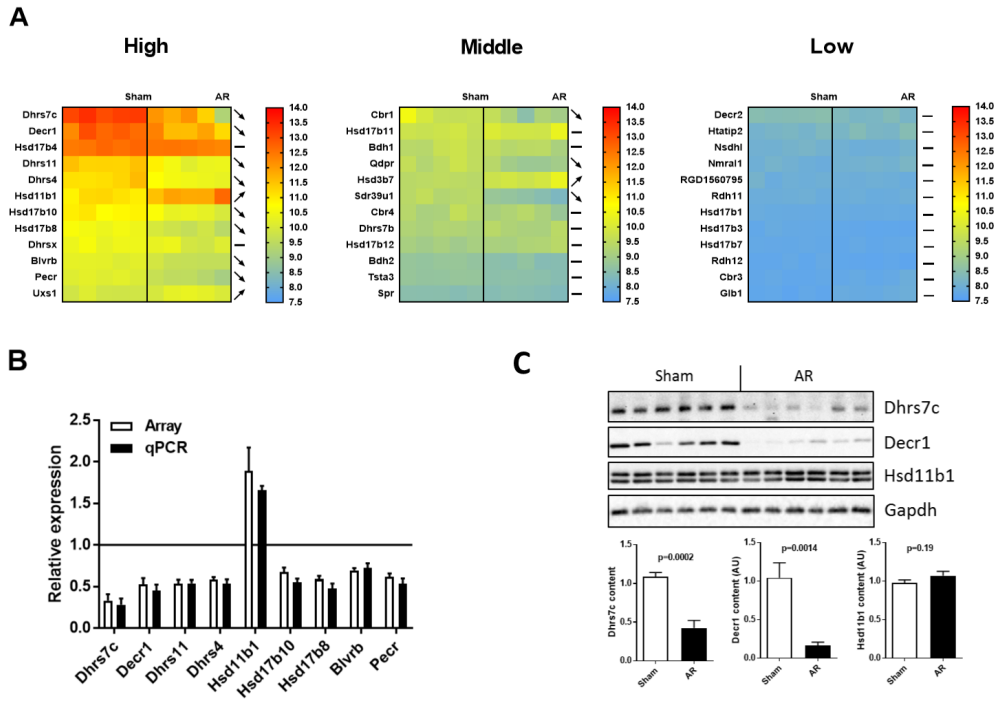

FIGURE 1 Many SDR genes highly expressed in the left ventricle of rats are down-regulated after chronic aortic valve regurgitation (AR). A. Heat maps of expression of 35 SDR genes in the LV of sham-operated or AR male rats for 9 months. Genes were separated into 3 heat maps depending of their level of expression in sham rats. B. Comparison of gene expression measured by micro-array (array) technology vs. qPCR: quantitative RT-PCR for nine highly expressed SDR genes. The solid bar set at one represents expression of the gene in sham animals. Results are expressed as the mean +/- SEM (n=5) C. Protein contents of Dhrs7c, Decr1 and 11beta-HSD1 in the myocardium of sham-operated and AR rats. Gapdh protein was used as control. P values were determined using the Student's T-test.

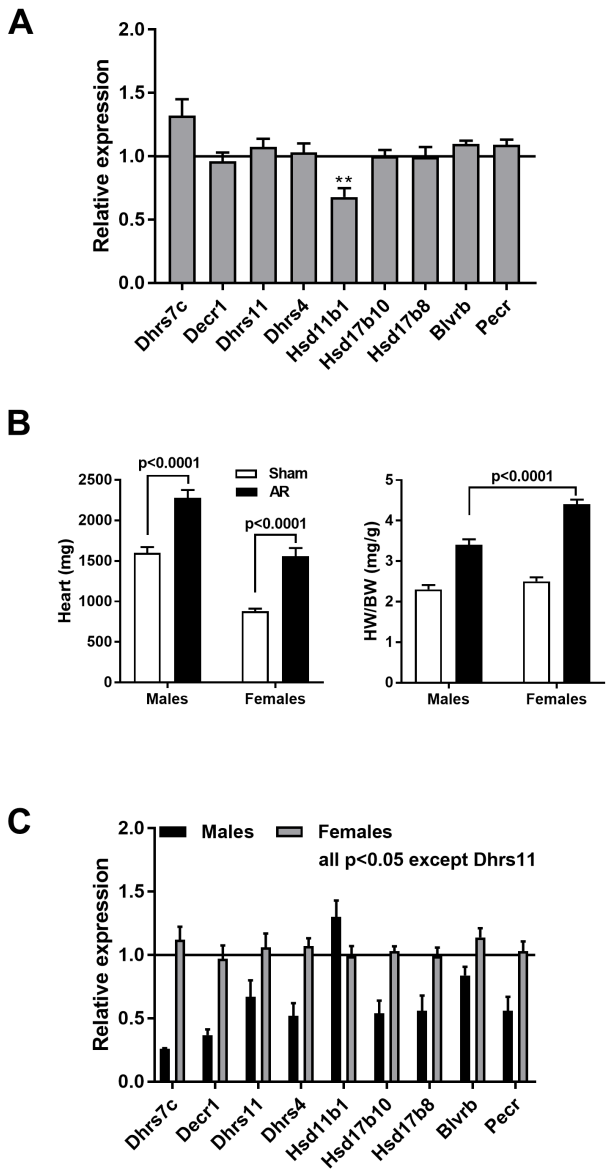

FIGURE 2 Sexual dimorphism in the regulation of SDR genes in AR rats. A. Relative gene expression of the SDR genes in sham females compared to males. The solid bar set at one represents expression of the gene in male animals. B. Cardiac hypertrophy in male and female rats after 6 months of volume overload from AR. HW/BW: heart weight/body weight. C. After 6 months of AR, no SDR genes are regulated in the LV of AR female rats, whereas a similar regulation of SDR genes as observed in 9-month AR rats is observed in 6-month AR males. Results are expressed as the mean +/- SEM (n=8-10/gr.) Statistical significance between groups was determined using the Student's T-test.

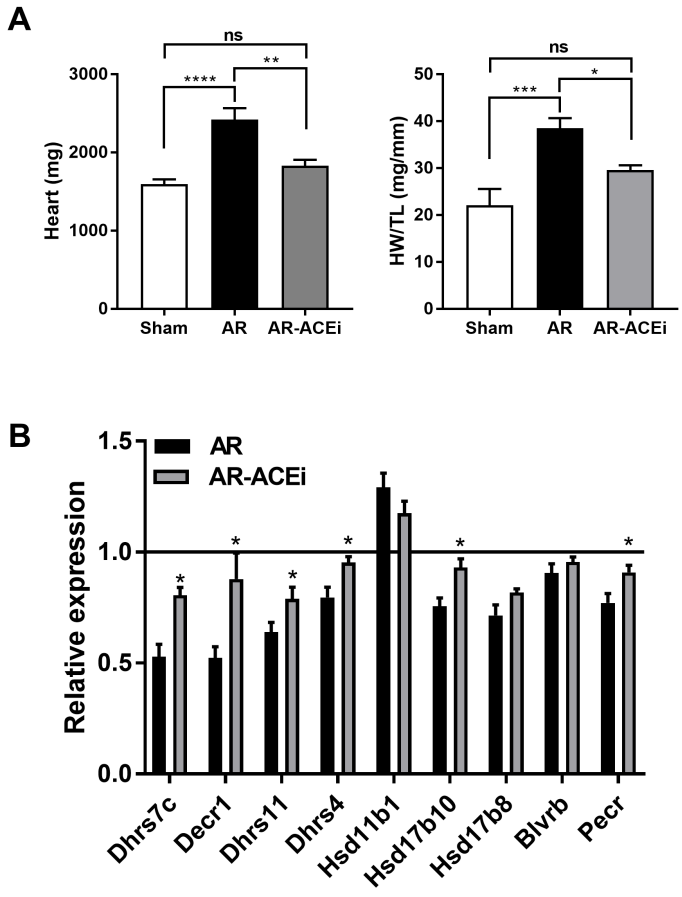

FIGURE 3 The angiotensin I converting enzyme inhibitor (ACEi), captopril, reverses the hypertrophic response (A) to experimental volume overload in male AR rats and helps normalize SDR gene expression (B). Rats were treated with captopril for 6 months starting 2 weeks post-AR induction. The solid bar (B) set at one represents expression of the gene in untreated sham animals. Results are expressed as the mean +/- SEM (n=6/gr.) A. *: $p < 0.05$, **: $p < 0.01$ and ***: $p < 0.001$ between groups. ns: non significant as determined using ANOVA followed by the Tukey's post-test. B. *: $p < 0.05$ vs. AR as determined using the Student's T-test.

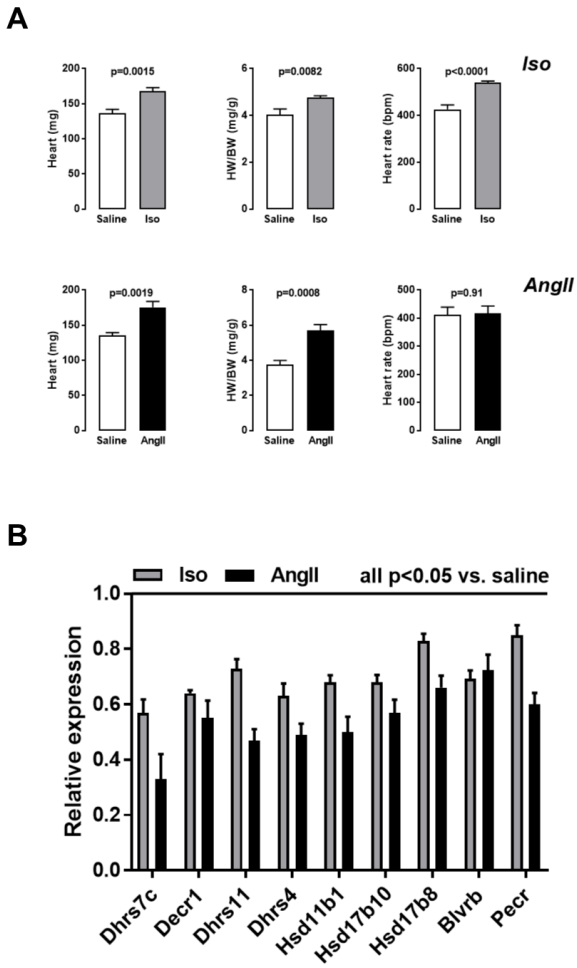

FIGURE 4 A 14-day continuous infusion of either isoproterenol (Iso) or angiotensin II (AngII) in male mice induces mild cardiac hypertrophy (A) and general down-regulation of SDR genes (B). Results are expressed as the mean +/- SEM (n=8-10/gr.) Statistical significance between groups was determined using the Student's T-test.

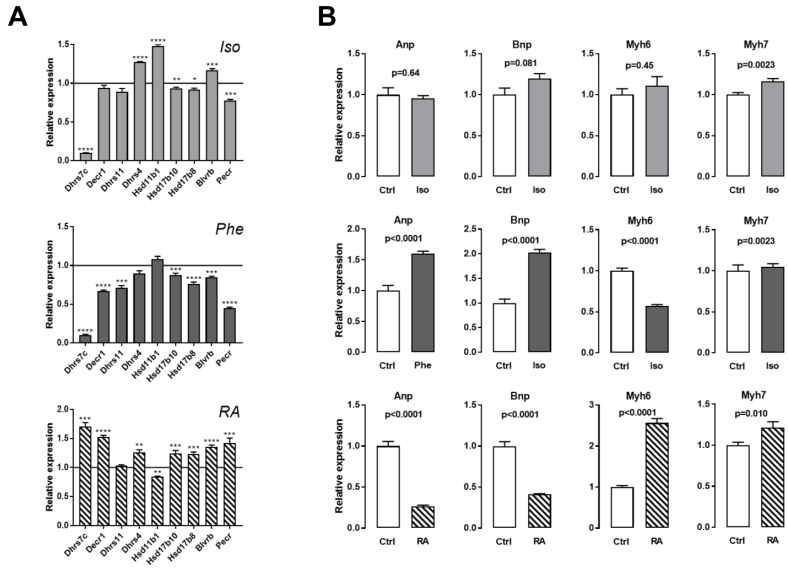

FIGURE 5 Treatment of neonatal rat cardiac myocytes with Iso, Phe or all-trans retinoic acid (RA) for 24 hours regulate SDRs gene expression (A). B. Regulation of hypertrophy markers in cardiac myocytes by the aforementioned treatments. Results are expressed as the mean and SEM (n=6/gr.) *: p below 0.05, **: p below 0.01 and ***: p below 0.001 between groups (A) or vs. untreated cardiac myocytes (Bar set to 1). Statistical significance between groups was determined using the Student's T-test.

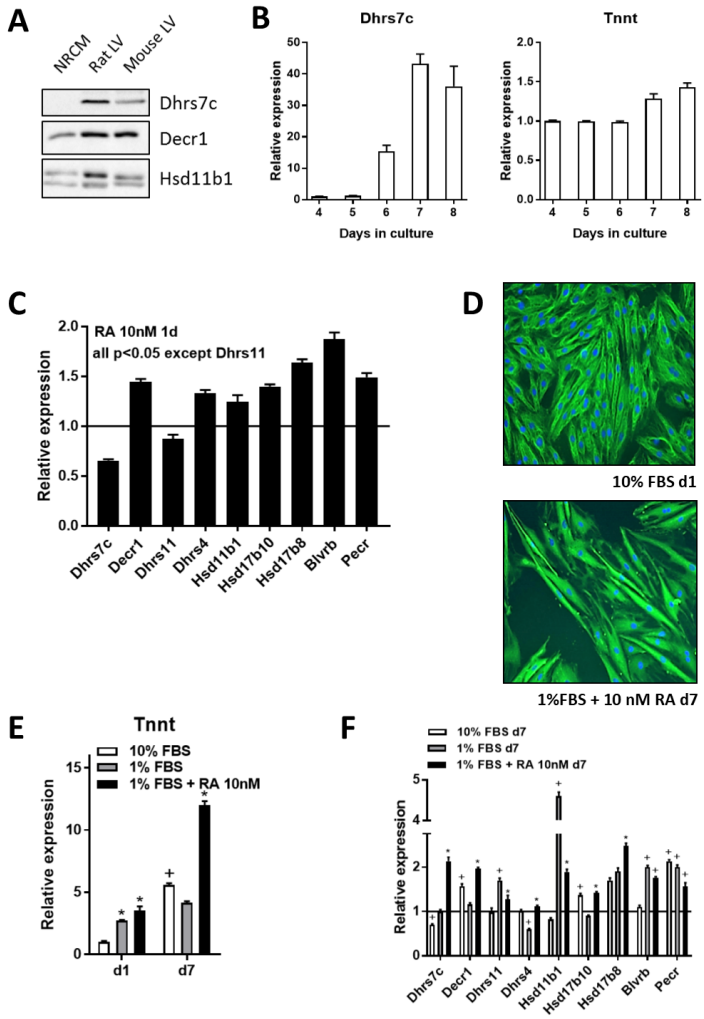

FIGURE 6 SDR gene expression and protein content are up-regulated upon cardiac myocytes differentiation. A. Dhrs7c, Decr1 or Hsd11b1 protein content is more abundant in adult rat or mouse LV tissue than in neonatal rat cardiac myocytes (NRCMs). B.*Dhrs7c* and *Decr1* mRNA levels are up-regulated with time in culture in NRCMs. C. Treatment of H9C2 cardiac myoblasts with RA for 24 hours up-regulates SDRs gene expression. D. Treatment with differentiation culture medium (1% serum (FBS) + RA 10 nM) results in H9C2 morphological changes and strong induction of troponin t (Tnnt) gene expression (E). F. Most SDR genes are up-regulated upon H9C2 cardiac myoblasts differentiation (7 days). +: p below 0.05 vs. H9C2 cells after 24 hours of culture in normal medium (10% FBS) and *: $p < 0.05$ vs. cells after 7 days of culture in 1%FBS. Results are expressed as the mean +/- SEM. (n=6/gr.) Statistical significance between groups was determined using the Student's T-test (C) or ANOVA and Tukey's post-test (E and F).