The Muscle Ankyrin Repeat Proteins CARP, Ankrd2, and DARP Are Not Essential for Normal Cardiac Development and Function at Basal Conditions and in Response to Pressure Overload

Marie-Louise Bang1*, Yusu Gu2, Nancy D. Dalton2, Kirk L. Peterson2, Kenneth R. Chien3,4, Ju Chen2*

1 Institute of Genetic and Biomedical Research, UOS Milan, National Research Council and Humanitas Clinical and Research Center, Rozzano (Milan), Italy, 2 Department of Medicine, University of California San Diego, La Jolla, California, United States of America, 3 Department of Cell and Molecular Biology and Medicine, Karolinska Institutet, Stockholm, Sweden, 4 Harvard University, Department of Stem Cell and Regenerative Biology, Cambridge, Massachusetts, United States of America

Introduction

In cardiac and skeletal muscle, a family of muscle specific ankyrin repeat proteins (MARPs) includes cardiac ankyrin repeat protein (CARP/Ankrd1) [1,2,3,4], Ankyrin repeat domain protein 2 (Ankrd2/Arpp) [5,6], and diabetes related ankyrin repeat protein (DARP/Ankrd23) [7]. The three proteins share ~50% sequence identity and all contain a N-terminal nuclear localization signal and four ankyrin repeats [8]. CARP is mainly expressed in cardiac muscle, Ankrd2 in skeletal muscle, and DARP at similar amounts in both cardiac and skeletal muscle; however all three proteins can be induced both in heart and skeletal muscle in response to various forms of stress. All three MARPs are localized in the nucleus as well as the sarcomeric I-band where they are thought to be involved in mechanосensing. Together with their strong induction during cardiac disease and the identification of causative Ankrd1 gene mutations in cardiomyopathy patients, this suggests their important roles in cardiac development, function, and disease. To determine the functional role of MARPs in vivo, we studied knockout (KO) mice of each of the three family members. Single KO mice were viable and had no apparent cardiac phenotype. We therefore hypothesized that the three highly homologous MARP proteins may have redundant functions in the heart and studied double and triple MARP KO mice. Unexpectedly, MARP triple KO mice were viable and had normal cardiac function both at basal levels and in response to mechanical pressure overload induced by transverse aortic constriction as assessed by echocardiography and hemodynamic studies. Thus, CARP, Ankrd2, and DARP are not essential for normal cardiac development and function at basal conditions and in response to mechanical pressure overload.

Abstract

Ankrd1/CARP, Ankrd2/Arpp, and Ankrd23/DARP belong to a family of stress inducible ankyrin repeat proteins expressed in striated muscle (MARPs). The MARPs are homologous in structure and localized in the nucleus where they negatively regulate gene expression as well as in the sarcomeric I-band, where they are thought to be involved in mechanosensing. Together with their strong induction during cardiac disease and the identification of causative Ankrd1 gene mutations in cardiomyopathy patients, this suggests their important roles in cardiac development, function, and disease. To determine the functional role of MARPs in vivo, we studied knockout (KO) mice of each of the three family members. Single KO mice were viable and had no apparent cardiac phenotype. We therefore hypothesized that the three highly homologous MARP proteins may have redundant functions in the heart and studied double and triple MARP KO mice. Unexpectedly, MARP triple KO mice were viable and had normal cardiac function both at basal levels and in response to mechanical pressure overload induced by transverse aortic constriction as assessed by echocardiography and hemodynamic studies. Thus, CARP, Ankrd2, and DARP are not essential for normal cardiac development and function at basal conditions and in response to mechanical pressure overload.
knockdown of CARP in rat cardiomyocytes resulted in sarcomere disarray and inhibition of myofilament gene transcription [23]. CARP can bind to both the titin N2A region and myopalladin in the I-band, suggesting its role in mechanosensing and regulation of gene expression in response to muscle stress [8,24]. In addition, CARP has been shown to be able to dimerize and bind to desmin, the muscle specific RING finger proteins MuRF1 and MuRF2, cardiac caldescin 2 [25,26,27], and the tumor suppressor protein p53 [20], suggesting a versatile role of CARP in the heart.

Ankyrin repeat protein 2 (stretch responsive-muscle) (Ankrd2)/Ankyrin Repeat protein with PEST and Proline-rich region (Arpp) was first described by Kemp as a striated muscle specific gene induced by mechanical stretch and involved in mechanically-induced skeletal muscle hypertrophy [5]. Further studies revealed that Ankrd2 is selectively expressed in type I fibers of skeletal muscle [29,30] and present only at low levels in the left and right ventricles, the interventricular septum, and the apex of the heart [6]. During fetal development Ankrd2 is diffusely expressed in skeletal muscles and is barely detectable in the heart [29]. Like CARP, Ankrd2 is highly responsive to acute stress and has been found to be upregulated during myoblast differentiation [29] and in response to mechanical muscle stretch [5,31], exercise [32], eccentric contractions [33,34], and denervation [35]. Furthermore, Ankrd2 has been shown to translate to the nuclei in myofibers in the vicinity of injured fibers [36]. In human, Ankrd2 is upregulated in congenital myopathies [37] and downregulated in muscular dystrophy [30,37,38] and likely due to changes in fiber type distribution [39]. Furthermore, Ankrd2 was found to be upregulated in human dilated cardiomyopathy [16], suggesting that Ankrd2 may also be involved in cardiac pathologies. In addition to the titin N2A domain, Ankrd2 has been shown to interact with T-cap/telethonin as well the transcription factors YB-1, promyelocytic leukemia protein (PML), and p53, suggesting that Ankrd2 may also be involved in muscle gene regulation [40]. Furthermore, our recent results have demonstrated that Akt2-phosphorylated Ankrd2 can bind directly to the NF-kB p50 subunit and negatively regulate inflammatory responses during muscle differentiation in response to oxidative stress [41].

Diabetes-related ankyrin repeat protein (DARP) is expressed in heart, skeletal muscle, and brown adipose tissue [7] and is the least studied of the three proteins. It was found to be upregulated in the hearts of type 2 diabetic and insulin resistant mice and to show altered expression in response to mechanical stretch [5] and denervation [35]. Further, in response to muscle stress [8,24], DARP can bind directly to the NF-kB p50 subunit and negatively regulate inflammatory responses during muscle differentiation in response to oxidative stress [41].

Taken together, several lines of evidence suggest that the MARPs belong to a family of stress responsive proteins, which plays critical roles in the heart. However, although the analysis of the skeletal muscle phenotype of MARP triple KO mice has been published [42], the generation of the KO mice has not previously been described in detail. Genomic DNA clones were isolated from a mouse 129/SvJ genomic DNA library (Stratagene, La Jolla, CA), using full-length cDNA of CARP, Ankrd2, and DARP, respectively. The first 2–3 exons of each gene were replaced by cDNA encoding lacZ and a pGK neo cassette. In this manner, the β-galactosidase cDNA was brought under the control of the endogenous promoters, while also ablating the endogenous CARP, Ankrd2, and DARP genes, respectively. The generation of Ankrd2 KO mice has been described previously [41]. The targeting constructs were verified by sequencing and linearized before electroporation into 129/SvJ derived ES cells at the Transgenic Core Facility at the University of California, San Diego. ES clones were screened for homologous recombination by Southern blot analysis with probes as shown in Fig. 1A, E, and Bean et al [41]. Cells from two independent targeted clones from each construct were microinjected into C57BL/B6 blastocysts and transferred into pseudopregnant mice. Male chimeras resulting from the microinjections were bred with female Black Swiss mice to generate germ line transmitted heterozygous mice. These were subsequently intercrossed to generate homozygous mice. Offspring from intercrosses were genotyped by PCR analysis using mouse tail DNA and wildtype (WT) and mutant allele specific primers. The following primers were used: CARP WT (sense: ATAGACTCTACGGCTGCTCAAAAG; reverse: CTCCCATTTTCGAACTCCCGAGG), mutant (sense: TGGGGATGACTCGCATTGCTGAG; reverse: AGATGAAAACGGGGAGTTAAGGCC); Ankrd2/Arpp WT (sense: AACCTTCAGATGCCGGTCCTGG; reverse: CATCAACTGTCGACG), mutant (sense: CAACCTGGAACGCGCTTTTTCC; reverse: AGATGAAAACGGGGAGTTAAGGCC); and DARP WT (sense: GCAGTTTGATGAGTGGTGG; reverse: TGGCAATCGGAGGAAACCTTACAA), mutant (sense: CTTGCGCCACAGGCTTTTGCTCCT; reverse: AGATGAAAACGGGGAGTTAAGGCC). Double and triple MARP KO mice were generated by interbreeding of single KO mice. To ensure that WT control mice had the same genetic background as double and triple MARP KO mice, also WT mice were generated from the crosses.

### Protein Isolation and Western Blot Analysis

Animals were anesthetized by intraperitoneal injection of a mixture of ketamine (100 mg/kg) and xylazine (5 mg/kg) and sacrificed by cervical dislocation before removal of tissues. Total protein extracts were prepared from heart and skeletal muscle [43] and subjected to Western blot analysis using polyclonal antibodies against CARP and DARP (5 μg/ml, described in Miller et al. [8]).

### The MARPs Are Not Essential for Cardiac Function

**Materials and Methods**

**Ethics Statement**

All animal procedures were approved by the University of California San Diego Animal Care and Use Committee (Approval reference number: S01049) and performed in full compliance with the guidelines of the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Special attention was paid to animal welfare and to minimize the number of animals used and their suffering.

**Generation of Single, Double, and Triple MARP KO Mice**

Although the analysis of the skeletal muscle phenotype of MARP triple KO mice has been published [42], the generation of the KO mice has not previously been described in detail. Genomic DNA clones were isolated from a mouse 129/SvJ genomic DNA library (Stratagene, La Jolla, CA), using full-length cDNA of CARP, Ankrd2, and DARP, respectively. The first 2–3 exons of each gene were replaced by cDNA encoding lacZ and a pGK neo cassette. In this manner, the β-galactosidase cDNA was brought under the control of the endogenous promoters, while also ablating the endogenous CARP, Ankrd2, and DARP genes, respectively. The generation of Ankrd2 KO mice has been described previously [41]. The targeting constructs were verified by sequencing and linearized before electroporation into 129/SvJ derived ES cells at the Transgenic Core Facility at the University of California, San Diego. ES clones were screened for homologous recombination by Southern blot analysis with probes as shown in Fig. 1A, E, and Bean et al [41]. Cells from two independent targeted clones from each construct were microinjected into C57BL/B6 blastocysts and transferred into pseudopregnant mice. Male chimeras resulting from the microinjections were bred with female Black Swiss mice to generate germ line transmitted heterozygous mice. These were subsequently intercrossed to generate homozygous mice. Offspring from intercrosses were genotyped by PCR analysis using mouse tail DNA and wildtype (WT) and mutant allele specific primers. The following primers were used: CARP WT (sense: ATAGACTCTACGGCTGCTCAAAAG; reverse: CTCCCATTTTCGAACTCCCGAGG), mutant (sense: TGGGGATGACTCGCATTGCTGAG; reverse: AGATGAAAACGGGGAGTTAAGGCC); Ankrd2/Arpp WT (sense: AACCTTCAGATGCCGGTCCTGG; reverse: CATCAACTGTCGACG), mutant (sense: CAACCTGGAACGCGCTTTTTCC; reverse: AGATGAAAACGGGGAGTTAAGGCC); and DARP WT (sense: GCAGTTTGATGAGTGGTGG; reverse: TGGCAATCGGAGGAAACCTTACAA), mutant (sense: CTTGCGCCACAGGCTTTTGCTCCT; reverse: AGATGAAAACGGGGAGTTAAGGCC). Double and triple MARP KO mice were generated by interbreeding of single KO mice. To ensure that WT control mice had the same genetic background as double and triple MARP KO mice, also WT mice were generated from the crosses.
Gluceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody was used for normalization (1:1000; Sigma-Aldrich).

**Histology**

Dissected mouse hearts were fixed with 4% paraformaldehyde followed by dehydration and paraffin embedding. 10 μm sections were stained with hematoxylin and eosin or by Masson’s trichrome method.

**Cardiac Functional Studies**

For transthoracic echocardiography, mice were anesthetized with 1% isoflurane and imaged as previously described [44]. For surgical procedures, mice were anesthetized by intraperitoneal injection of a mixture of ketamine (100 mg/kg) and xylazine (5 mg/kg) and anesthesia depth was monitored by toe pinching. Cardiac hemodynamic parameters were evaluated in 8-week-old female and 17-month-old male mice by insertion of a 1.4 French Millar catheter-tip micromanometer catheter through the right carotid artery into the left ventricle [10]. Pressure was recorded both and baseline and following stimulation with graded doses of the β-adrenergic agonist dobutamine (0.75, 2, and 4 μg/kg/min) as previously described [10]. Transverse aortic constriction (TAC) was performed with a 27-gauge needle on 17 to 18-week-old male mice as described elsewhere [44]. 14 days after TAC, cardiac morphology and function was evaluated by echocardiography and

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**Figure 1. Generation of CARP and DARP KO mice.**

(A, E) Targeting strategies. Restriction maps of relevant genomic regions of *Ankrd1* (A) and *Ankrd23* (E) are shown on top, targeting constructs are shown in the center, and mutated loci after homologous recombination are shown at the bottom. B, BamHI; E, EcoRI; H, HindIII; K, KpnI; P, PstI; S1, Sall, neo, neomycin resistance gene; TK, thymidine kinase, E, Exon, P, Primer. (B, F) Detection of WT and targeted alleles by Southern blot analysis. *Ankrd1* (B) and *Ankrd23* (F) DNAs from electroporated ES cells were digested with BamHI and HindIII, respectively and analyzed by Southern blot analysis with probes as shown in A. (C, G) Detection of *Ankrd1* (C) or *Ankrd23* (G) mRNA by Northern blot analysis. Aliquots of 10 μg of total RNA isolated from adult ventricles of WT and KO mice were analyzed by using a cDNA probe spanning the entire coding regions of *Ankrd1* and *Ankrd23*, respectively. A GAPDH probe was used as a control for equal loading. (D, H) Detection of CARP (D) and DARP (H) protein by Western blot analysis. Protein prepared from adult ventricles of WT and KO mice were analyzed with polyclonal antibodies against CARP and DARP. Monoclonal antibodies against GAPDH were used as loading control.

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the gradient for the arterial blood pressure between the
contraction was measured by accumulation as described [44]. Only
mice showing an adequate pressure gradient (>50 mmHg) were
inclusion in the analysis. SHAM operated mice were used as
controls.

Statistical Analysis
Data are indicated as average ± standard deviation. Statistical
comparisons between WT and KO mice were done using the
unpaired Students t-test. A P value of <0.05 is considered
significant.

Results

Generation of CARP, Ankrd2, and DARP KO Mice
To study the functional role of the muscle ankyrin repeat family
in vivo, mice deficient for each of the three family members,
CARP, Ankrd2, and DARP KO mice were generated. The
generation of Ankrd2 KO mice has been described elsewhere [41].
For each of the three genes, the targeting vector was generated by
replacement of the first 1–3 exons by lacZ and the neomycin
resistance gene (neo), thereby disrupting gene expression [Fig. 1A,
E, and Bean et al [41]]. Screening for homologous recombination
in ES cells was performed by Southern blot analysis with primers
as indicated in Fig. 1A, B, E, F, and Bean et al [41]. Correctly
targeted clones were injected into C57/B6 blastocysts and
implanted into the uterus of pseudopregnant mice. The resulting
chimeras were mated to Black Swiss mice and gave rise to
germline transmitted heterozygous mice, which were subsequently
interbred to generate homozygous mice as determined by PCR.
Successful ablation of each of the three genes was confirmed by
Northern (Fig. 1C, G, and Bean et al [41]) and Western blot
analyses (Fig. 1D, H, and Bean et al [41]) using polyclonal
antibodies against CARP, Ankrd2, and DARP. In agreement with
previously published data, Western blot analysis of cardiactargeted
clones revealed highest expression of CARP in heart, Ankrd2 in skeletal muscle, and DARP in both heart and
skeletal muscle (Fig. 1D, H, and Bean et al [41]).

Generation of MARP Triple KO Mice
CARP, Ankrd2, and DARP KO mice were born at expected
Mendelian ratios, were fertile, and survived till adulthood. KO
mice were indistinguishable from wildtype (WT) littermate control
cells and detailed analyses revealed normal cardiac morphology
and physiology and no significant differences in heart/body weight
ratios (see below). Since the three proteins are highly homologous
in structure and are all expressed in the heart, we hypothesized
that they may have overlapping functions and generated double
and triple MARP KO mice by intercrossing single KO mice. WT
mice generated from these crosses were used as controls to ensure
that KO and WT control mice had the same genetic background.
The successful ablation of all three proteins was confirmed by
PCR as well as Northern and Western blot analyses (Fig. 1C, D,
G, H, and Bean et al [41]).

MARP Triple KO Mice have Normal Cardiac Morphology
MARP triple KO mice were born at expected Mendelian ratios
and were indistinguishable from WT control mice. No statistical
difference in heart/body weight ratios between MARP triple KO
and WT mice was observed and cardiac histological analysis by
hematoxylin and eosin and Masson’s trichrome staining showed
no signs of hypertrophy, infarction, fibrosis, necrosis, calcification,
or fat infiltration in MARP triple KO mice up to 17 months of age
(data not shown).

MARP Triple KO Mice have Normal Cardiac Function
Cardiac function was evaluated noninvasively by echocardiogra-
phy at 4 and 17 months of age. As shown in Table 1 and 2, left
ventricular chamber dimensions, fractional shortening, and heart
rate were normal in MARP triple KO mice compared to WT
control mice. Furthermore, hemodynamic function was evaluated
in 8-week- and 17-month-old mice by cardiac catheterization in
the absence of pharmacological stimuli. In the absence of graded dosages of isoproterenol, no significant difference in contractile function was found between
MARP triple KO and WT mice either at baseline or in the
presence of the beta-adrenergic agonist isoproterenol (Table 3).

MARP Triple KO Mice Show a Normal Hypertrophic
Response to Transverse Aortic Constriction
It is well known that CARP, Ankrd2, and DARP are
upregulated in response to stress such as cardiac hypertrophy
[5,9,10,12,15]. Therefore, to study the effect of MARP triple KO
on mechanical pressure overload-induced hypertrophy, we per-
formed transverse aortic constriction (TAC) in 4-month-old
MARP triple KO mice and WT control mice. Cardiac function
before and 14 days after TAC was evaluated by echocardiography
and pressure gradients generated by the aortic constriction were
measured. As shown in Table 2, similar pressure gradients were
produced in both groups. Following TAC, left ventricular wall
thickness and mass were increased by similar amounts in both
groups (Table 2), indicating that the response to hypertrophic
stimuli is unaffected in MARP triple KO mice.

Discussion
To study the functional role of the MARP family members
in vivo, we generated CARP, Ankrd2, and DARP KO mice. Since
no phenotype was found in any of the individual KO mice, we
hypothesized that the three highly homologous proteins have
overlapping functions in the heart and generated MARP triple
KO mice. However, in our detailed analyses, we were unable to
detect any significant abnormalities in cardiac development and
basal cardiac function up to 17 months of age and the
hypertrophic response in response to acute pressure overload
induced by TAC was similar between WT and triple KO mice.
On the other hand, transgenic mice with cardiac specific
overexpression of CARP were recently found to show attenuated
cardiac hypertrophy and fibrosis in response to pressure overload
and isoproterenol infusion, although the they were not protected
against heart failure [22]. One explanation for this apparent
discrepancy could be that the constitutive upregulation of CARP
non-physiological levels in the heart results in non-specific
effects. Alternatively, since the MARP KO mouse models are
constitutive KO mice, it is possible that compensatory mechanisms
counteract the effect of the absence of the MARPs. To clarify this,
it would be necessary to study the effect of conditional KO or
overexpression of the MARP members in adult mice in vivo.
Nevertheless, in light of the strong induction of MARPs in the
heart in response to various forms of stress, including cardiac
hypertrophy and cardiac disease, as well as an accumulating body
of evidence suggesting important roles of the MARP protein
family in cardiogenesis, gene regulation, sarcomeric stress/stretch
sensing, and disease, it is very surprising that the absence of all
three MARP family members does not appear to have any effect
on cardiac function in vivo, in particular since an increasing
number of mutations in the Ankrd1 gene have been identified in
patients with dilated and hypertrophic cardiomyopathy
[19,20,21]. The most likely explanation for this is that the
identified Ankrd1 mutations have dominant negative effects by

The MARPs Are Not Essential for Cardiac Function
interfering with the binding of CARP to its many interaction partners, while complete absence of the MARPs has a less damaging effect and may be compensated for by other mechanisms. This is for example the case for knockout mice of the Z-line protein myotilin, which show no phenotype although mutations in myotilin are causative for limb girdle muscular dystrophy 1A [45].

The lack of a cardiac phenotype in MARP triple KO mice following 14 days of TAC does not exclude that the MARP KO mice may show a phenotype after long-term TAC or in response to other types of stress conditions, such as cardiac ischemia, myocardiitis, and chronic β-adrenergic stimulation. Also, it is possible that MARPs might play a role in other organs, such as in skeletal muscle and the vasculature. We previously studied skeletal muscle function in MARP triple KO mice and found a significant increase in resting sarcomere length associated with the expression of longer titin isoforms [42]. Furthermore, MKO mice showed greater muscle injury following eccentric contraction exercise as indicated by a greater torque loss, and although they recovered normally, this was associated with increased expression of the regulatory genes MyoD and Muscle LIM protein (MLP), suggesting a role of the MARP family members in regulating muscle gene expression. In regard to the vasculature, CARP has

Table 1. Echocardiographic analysis on 17-month-old male MARP triple KO mice compared to WT at basal conditions.

<table>
<thead>
<tr>
<th></th>
<th>WT (n = 6)</th>
<th>tKO (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>54±2</td>
<td>44±7*</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>540±37</td>
<td>558±44</td>
</tr>
<tr>
<td>LVIDd (mm)</td>
<td>3.97±0.18</td>
<td>3.83±0.41</td>
</tr>
<tr>
<td>LVIDd (mm)</td>
<td>2.26±0.27</td>
<td>2.09±0.38</td>
</tr>
<tr>
<td>IVSd (mm)</td>
<td>0.78±0.03</td>
<td>0.76±0.04</td>
</tr>
<tr>
<td>IVSd (mm)</td>
<td>1.29±0.08</td>
<td>1.30±0.13</td>
</tr>
<tr>
<td>LVPWd (mm)</td>
<td>0.78±0.03</td>
<td>0.78±0.04</td>
</tr>
<tr>
<td>LVPWs (mm)</td>
<td>1.41±0.14</td>
<td>1.33±0.11</td>
</tr>
<tr>
<td>LV FS, %</td>
<td>43.3±5.0</td>
<td>45.6±6.2</td>
</tr>
<tr>
<td>LVM (mg)</td>
<td>111.6±6.4</td>
<td>103.8±14.2</td>
</tr>
<tr>
<td>LVM/BW (mg/g)</td>
<td>2.08±0.08</td>
<td>2.38±0.40</td>
</tr>
<tr>
<td>VCF (circ/s)</td>
<td>9.37±1.66</td>
<td>9.98±1.60</td>
</tr>
</tbody>
</table>

All data are presented as mean ± standard deviation. WT, wildtype; tKO, MARP triple knockout; BW, body weight; LVID, left ventricular inner diameter; LVS, interventricular septum; LVPW, left ventricular posterior wall thickness; LV FS, left ventricular fractional shortening; LVM, left ventricular mass; VCF, velocity of circumferential fiber shortening; bpm, beats per minute; circ, circumference; d, diastole; s, systole. *P<0.01.

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Table 2. Echocardiographic analysis of 4-month-old male MARP triple KO mice compared to WT before and after induction of cardiac hypertrophy by TAC.

<table>
<thead>
<tr>
<th></th>
<th>Before TAC</th>
<th>14 days after TAC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT (n = 6)</td>
<td>tKO (n = 6)</td>
</tr>
<tr>
<td></td>
<td>WT (n = 6)</td>
<td>tKO (n = 6)</td>
</tr>
<tr>
<td>Age (weeks)</td>
<td>18.3±0</td>
<td>17.3±0.3</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>36.0±4.9</td>
<td>37.0±3.5</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>484±81</td>
<td>537±55</td>
</tr>
<tr>
<td>LVIDd (mm)</td>
<td>3.79±0.19</td>
<td>3.50±0.30</td>
</tr>
<tr>
<td>LVIDd (mm)</td>
<td>2.16±0.35</td>
<td>1.93±0.24</td>
</tr>
<tr>
<td>IVSd (mm)</td>
<td>0.70±0.07</td>
<td>0.68±0.13</td>
</tr>
<tr>
<td>IVSd (mm)</td>
<td>1.26±0.09</td>
<td>1.19±0.12</td>
</tr>
<tr>
<td>LVPWd (mm)</td>
<td>0.73±0.09</td>
<td>0.71±0.08</td>
</tr>
<tr>
<td>LVPWs (mm)</td>
<td>1.21±0.08</td>
<td>1.16±0.08</td>
</tr>
<tr>
<td>LV FS, %</td>
<td>43.0±7.6</td>
<td>44.8±5.9</td>
</tr>
<tr>
<td>VCF (circ/s)</td>
<td>8.2±1.5</td>
<td>9.7±2.0</td>
</tr>
<tr>
<td>LVMD (mg)</td>
<td>92.4±8.0</td>
<td>79.0±18.4</td>
</tr>
<tr>
<td>LV/BW (mg/g)</td>
<td>2.57±0.42</td>
<td>2.13±0.58</td>
</tr>
<tr>
<td>PG (mm Hg)</td>
<td>83.8±13.2</td>
<td>76.9±13.2</td>
</tr>
</tbody>
</table>

See the legend to Table 1 for details and description of abbreviations. TAC, transverse aortic constriction; PG, Pressure gradient. Data comparison was carried out before and after TAC. *P<0.05 and **P<0.01 for WT TAC vs. WT baseline; *P<0.05 and **P<0.01 for KO TAC vs. KO baseline.

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be demonstrated to be a downstream target of TGF-β/Smad signaling in vascular smooth muscle cells and vascular endothelial cells [46,47], and to be upregulated during arteriogenesis and angiogenesis, suggesting its involvement in these processes [48,49]. In addition, a role of CARP in the induction of angiogenesis and neovascularization during wound healing has been proposed [46,50]. Future studies of the MARP KO mice will reveal whether the MARPs may play important roles in the vasculature in vivo.

Table 3. Hemodynamic properties following dobutamine stimulation in MARP triple KO mice compared to WT.

<table>
<thead>
<tr>
<th></th>
<th>8-week-old females</th>
<th>17-month-old males</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DOB (n = 9)</td>
<td>tKO (n = 8)</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>basal</td>
<td>356 ± 34</td>
<td>373 ± 34</td>
</tr>
<tr>
<td>0.75 u</td>
<td>370 ± 39</td>
<td>378 ± 34</td>
</tr>
<tr>
<td>2 u</td>
<td>386 ± 48</td>
<td>403 ± 38</td>
</tr>
<tr>
<td>4 u</td>
<td>430 ± 69</td>
<td>475 ± 26</td>
</tr>
<tr>
<td>LVP_{max} (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>basal</td>
<td>96 ± 11</td>
<td>91 ± 11</td>
</tr>
<tr>
<td>0.75 u</td>
<td>95 ± 17</td>
<td>93 ± 14</td>
</tr>
<tr>
<td>2 u</td>
<td>100 ± 14</td>
<td>101 ± 16</td>
</tr>
<tr>
<td>4 u</td>
<td>107 ± 13</td>
<td>107 ± 9</td>
</tr>
<tr>
<td>dP/dt_{max} (mmHg/s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>basal</td>
<td>7941 ± 966</td>
<td>7400 ± 1035</td>
</tr>
<tr>
<td>0.75 u</td>
<td>8027 ± 931</td>
<td>7746 ± 1652</td>
</tr>
<tr>
<td>2 u</td>
<td>9591 ± 1016</td>
<td>9882 ± 2198</td>
</tr>
<tr>
<td>4 u</td>
<td>12163 ± 1502</td>
<td>12759 ± 1162</td>
</tr>
<tr>
<td>dP/dt_{min} (mmHg/s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>basal</td>
<td>−5163 ± 617</td>
<td>−5237 ± 495</td>
</tr>
<tr>
<td>0.75 u</td>
<td>−5386 ± 441</td>
<td>−5413 ± 765</td>
</tr>
<tr>
<td>2 u</td>
<td>−5927 ± 653</td>
<td>−6279 ± 1509</td>
</tr>
<tr>
<td>4 u</td>
<td>−7069 ± 573</td>
<td>−7371 ± 1333</td>
</tr>
<tr>
<td>EDP (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>basal</td>
<td>5.4 ± 0.0</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td>0.75 u</td>
<td>5.1 ± 0.3</td>
<td>3.7 ± 3.4</td>
</tr>
<tr>
<td>2 u</td>
<td>4.9 ± 5.9</td>
<td>3.6 ± 3.5</td>
</tr>
<tr>
<td>4 u</td>
<td>4.7 ± 5.8</td>
<td>3.4 ± 3.8</td>
</tr>
<tr>
<td>Exp. Tau (ms)</td>
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<td></td>
</tr>
<tr>
<td>basal</td>
<td>13.1 ± 1.4</td>
<td>12.4 ± 1.4</td>
</tr>
<tr>
<td>0.75 u</td>
<td>12.0 ± 1.3</td>
<td>12.4 ± 1.5</td>
</tr>
<tr>
<td>2 u</td>
<td>12.0 ± 1.0</td>
<td>12.0 ± 2.0</td>
</tr>
<tr>
<td>4 u</td>
<td>10.5 ± 0.7</td>
<td>11.0 ± 0.6</td>
</tr>
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</table>

Values are mean ± standard deviation. WT, wildtype; tKO, MARP triple knockout; LVP_{max}, maximum end-systolic left ventricular pressure; dP/dt_{max}, maximum positive first derivative of LVP (contractility); dP/dt_{min}, maximum negative first derivative of LVP (relaxation); EDP, end-diastolic pressure; Exp. Tau, experimental Tau; DOB: dobutamine; u: units.
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Author Contributions
Conceived and designed the experiments: MLB KLP KRC JC. Performed the experiments: MLB YG ND. Analyzed the data: MLB YG ND. Wrote the paper: MLB JC.

References


