An Image Guided Transapical Mitral Valve Leaflet Puncture Model of Controlled Volume Overload from Mitral Regurgitation in the Rat

Daniella Corporan, Emory University
Takamori Kono, Emory University
Daisuke Onohara, Emory University
Muralidhar Padala, Emory University

Journal Title: JOVE-JOURNAL OF VISUALIZED EXPERIMENTS
Volume: Volume 2020, Number 159
Publisher: JOURNAL OF VISUALIZED EXPERIMENTS | 2020-05-01
Type of Work: Article | Post-print: After Peer Review
Publisher DOI: 10.3791/61029
Permanent URL: https://pid.emory.edu/ark:/25593/vwq7s

Final published version: http://dx.doi.org/10.3791/61029
Accessed December 30, 2023 6:02 AM EST
An image guided transapical mitral valve leaflet puncture model of controlled volume overload from mitral regurgitation in the rat

Daniella Corporan¹, Takanori Kono¹, Daisuke Onohara¹, Muralidhar Padala¹,²
¹Structural Heart Research and Innovation Laboratory, Carlyle Fraser Heart Center, Emory University Hospital Midtown, Atlanta, GA, USA
²Division of Cardiothoracic Surgery, Joseph P. Whitehead Department of Surgery, Emory University School of Medicine, Atlanta, GA, USA

Abstract
Mitral regurgitation (MR) is a widely prevalent heart valve lesion which initiates cardiac remodeling over a period of time till failure. While uncorrected MR is associated with greater risks of adverse cardiac events, the current knowledge of the underlying mechanisms in the disease progression remain incomplete. To understand the molecular mechanisms which contribute to let ventricular adverse remodeling, animal models have been described. Small animal models of volume overload induced cardiac remodeling have been reported and widely used, with the aorto-caval fistula model being the most commonly used model to study volume overload. However, this model does not accurately represent the low-pressure volume overload which is seen clinically in patients with primary MR. Here, we describe a rodent model of severe volume overload induced MR in which the mitral leaflet is perforated with a 23G needle in the beating heart with echocardiographic image guidance. Severity of MR is assessed with echocardiography and is reproducible. This feasible model can be used to study longitudinal changes to the left ventricle.

SUMMARY:
A rodent model of cardiac volume overload from chronic mitral regurgitation is reported. Mitral regurgitation is induced by puncturing the mitral valve leaflet using an ultrasound guided needle inserted into the beating heart and advanced into the valve leaflet.

Keywords
Mitral regurgitation (MR); volume overload; heart valve; echocardiography; cardiac remodeling; rodents

Corresponding author: Muralidhar Padala (spadala@emory.edu).

DISCLOSURES:
M P is an advisor to Heart Repair Technologies (HRT), for which he has received consulting fees. HRT did not have any role in this study, nor did it provide any funding to support this work.
INTRODUCTION:

Mitrval regurgitation (MR) is a common heart valve lesion, diagnosed in 1.7% of the general US population and in 9% of the elderly population greater than 65 years of age. In this heart valve lesion, improper closure of the mitral valve leaflets in systole, causes regurgitation of blood from the left ventricle into the left atrium. MR can occur due to various etiologies, however, primary lesions of the mitral valve (primary MR) are diagnosed and treated more frequently compared to secondary MR. Isolated primary MR is often a result of myxomatous degeneration of the mitral valve, resulting in elongation of the leaflets or chordae tendineae, or rupture of some chordae, all of which contribute to the loss of systolic coaptation of the valve.

MR resulting from such valve lesions elevates the blood volume filling the left ventricle in each heart beat, increasing the end diastolic wall stress and providing a hemodynamic stressor that incites cardiac adaptation and remodeling. Cardiac remodeling in this lesion is often characterized by significant chamber enlargement, mild wall hypertrophy, with preserved contractile function for prolonged periods of time. Since ejection fraction is often preserved, correction of MR using surgical or transcatheter means is often delayed, until the onset of symptoms such as dyspnea, heart failure, and arrhythmias. However, uncorrected MR is associated with high risks of cardiac adverse events, though currently our knowledge of the ultrastructural changes underlying these events are unknown.

Animal models of MR provide a valuable model to investigate such ultrastructural changes in the heart, and study longitudinal progression of the disease. Previously, researchers have induced MR in large animals including pigs, dogs, and sheep, by creating an external ventriculo-atrial shunt, intracardiac chordal rupture, or leaflet perforation. While surgical techniques are easier in large animals, these studies have been limited to sub-chronic follow-up in a small sample size, owed to the high costs of performing such studies in large animals. Furthermore, molecular analysis of tissue from these models is often challenging due to limited species-specific antibodies and annotated genome libraries for alignment.

Small animal models of MR can provide a suitable alternative to study this valve lesion and its impact on cardiac remodeling. Historically, the rat model of aorto-caval fistula (ACF) of cardiac volume overload has been used. First described in 1973 by Stumpe et al., in this model a arterio-venous fistula is surgically created to bypass high pressure arterial blood from the descending aorta into the low pressure inferior vena cava. The high flow rate in the fistula induces a drastic volume overload on both sides of the heart, causing significant right and left ventricular hypertrophy and dysfunction occurring within days of creating the ACF. Despite its success, ACF does not mimic the hemodynamics of MR, a low-pressure volume overload, which elevates preload but also reduces afterload. Due to such limitations of the ACF model, we sought to develop and characterize a model of MR that better mimics the low-pressure volume overload.

Herein, we describe the protocol for a model of mitral valve leaflet puncture to create severe MR in rats. A hypodermic needle was introduced into the beating rat heart, and advanced into the anterior mitral valve leaflet under real-time echocardiographic guidance.
The technique is highly reproducible and a relatively good model that mimics MR as seen in patients. MR severity is controlled by the size of the needle used to perforate the mitral leaflet and severity of MR can be assessed using transesophageal echocardiography (TEE).

**PROTOCOL:**

Procedures were approved by the Animal Care and Use Program at Emory University under the protocol number EM63Rr, approval date 06/06/2017.

1. **Pre-surgical preparation**
   1.1. Steam sterilize surgical instruments prior to the procedure.
   1.2. On the procedure day, transfer rats from housing to surgery, and weigh them.
   1.3. Draw pre-operative and post-operative drugs according to the weight.
      1.3.1. Two doses of Carprofen (2.5 mg/kg each), one dose of Gentamycin (6 mg/kg), and one dose of Buprenorphine (0.02 mg/kg) are drawn.
   1.4. Ensure adequate volume of isoflurane in the gas mixer, and oxygen in the tanks are available for the surgery. One full tank of oxygen (24 ft³) is often adequate.

2. **Animal preparation**
   2.1. Adult Sprague-Dawley male rats weighing 350–400 g were used in this study.
      NOTE: The surgical techniques are amenable to slightly smaller or larger animals, if desired.
   2.2. Sedate the rat in an induction chamber with 5% isoflurane mixed in 1 LPM of 100% oxygen. Adequate level of sedation is determined from slower respiratory rate under visual observation, and loss of twitch upon pinching the rat’s toe.
   2.3. Intubate the rat with a 16G angiocath, fitted for use as an endotracheal tube.
      2.3.1. Visualize the trachea and vocal chords using an otoscope, and use a cotton tip applicator to clear pharyngeal secretions.
      2.3.2. Introduce the endotracheal tube on a 0.034-inch guidewire, into the vocal chords. Once the tube is appropriately placed in the trachea, push the tube inwards and withdraw the wire.
   2.4. Place the rat on the heated surgical pad maintained at 37°C and connect the endotracheal tube to a mechanical ventilator. Input weight of the rat into the ventilator control software, which calculates the ventilation rate and tidal volume.
      2.4.1. 66 breaths per minute with a tidal volume of 1 ml/100 g body weight were used in this study.
      2.4.2. 100% oxygen (1 LPM) mixed with 2–2.5% isoflurane is used as inhalant anesthetic, and level of anesthesia is confirmed with loss of jaw tone, and loss of response to toe pinch (Figure 1).
2.4.3. If properly intubated, chest motion should synchronize with the ventilator.

2.4.4. If improperly intubated, chest motion will not synchronize with the ventilator. To test for improper intubation, compress the abdomen of rat, which creates backpressure on the ventilator, generating an over-pressure alarm. In this scenario, retract the angiocath gently, and return the rat to the induction chamber with 5% isoflurane for few minutes to ensure the rat is sufficiently anesthetized, and re-intubate the rat.

2.4.5. Once properly intubated, secure the endotracheal tube by suturing the proximal end of the tube to the cheek of the rat with a 4–0 silk suture to avoid extubation during the procedure.

2.5. Insert a rectal temperature probe to monitor body temperature, and a four-terminal electrocardiogram to monitor ECG during the entire procedure.

2.5.1. An overhead heating lamp is used if heat from the surgical platform is insufficient. Turn off lamp if body temperature rises above 37 °C.

2.5.2. Visually assess the electrocardiogram for any arrhythmias or signs of myocardial ischemia. If none are present, record the baseline electrocardiogram.

2.6. Perform transthoracic echocardiography (TTE) for baseline cardiac function (Figure 2A).

2.6.1. Turn the rat to supine position and shave the left side of the thorax. To obtain clear echo views, hair can be removed using a depilatory cream.

2.6.2. Any ultrasound system with adequate frequency for high heart rate imaging can be used. In this study we used the Visualsonics 2100 system with a 21 MHz probe, which is appropriate for cardiac imaging in rats.

2.6.3. Obtain B-mode images in the parasternal long-axis plane, to calculate left ventricular volumes. In the same plane, obtain M-mode images to measure wall dimensions.

2.6.4. Turn the probe by 90 degrees, and obtain B-mode and M-mode parasternal short-axis views at the mid-papillary level to measure cross-sectional wall dimensions.

2.7. Perform transesophageal echocardiography (TEE) for baseline imaging (Figure 2B).

2.7.1. Place the rat in the right decubitus position and insert an 8Fr intracardiac ultrasound probe (8 MHz) into the esophagus of the rat with a small amount of gel applied to the tip. A GE Vivid I or Siemens SC2000 prime system were used for ICE (intracardiac
echocardiography) imaging. The frequency of the ICE probe is sufficient to obtain 4–6 frames per heartbeat, which are adequate to visualize the valve motion.

2.7.2. Obtain a high esophageal view to obtain a two-chamber view of the left side of the heart. This view is ideal to visualize the left atrium, mitral valve, and left ventricle. The probe should be positioned such that anterior and posterior leaflets are visualized and coaptation is central. This angle also allows Doppler measurements across the mitral valve, without angle correction.

2.7.3. Left atrial area and mitral valve annulus dimensions can be measured in this view.

2.7.4. Color Doppler imaging is performed to confirm valve competence and lack of MR at baseline. Pulsed wave and continuous wave Doppler imaging are performed, to quantify mitral inflow and confirm lack of regurgitant flow.

2.7.5. B-mode and pulsed wave Doppler imaging of the aorta is also performed to measure the aortic root diameter and calculate aortic flow.

2.7.6. Pulsed wave Doppler imaging of the pulmonary vein is performed to measure pulmonary venous flow.

2.8. Inject a single dose of Carprofen (2.5 mg/kg, SQ, non-steroidal anti-inflammatory), Gentamycin (6 mg/kg, SQ, antibiotic), and sterile saline (1 ml, SQ) to pre-emptively compensate for blood loss during the procedure.

2.9. Shave the left side of the thorax as needed to remove any remaining hair from the surgical field. Shaving from the lower neck region to the xiphoid, and from the left arm down to the mid-sternum should be sufficient to ensure a field that is devoid of hair and reduce the risk of surgical site contamination.

2.10. Scrub the surgical area with a gauze soaked in Betadine, followed by a gauze soaked in 70% ethanol. Scrub the area in circular motions on the skin, such that the gauze does not contact a previously scrubbed area.

2.11. Repeat this step three times to achieve an adequately sterile field for surgery.

2.12. Drape the animal with sterile covers, opening a window to access the sterile surgical area.

3. Left thoracotomy

3.1. The entire surgical procedure is performed using aseptic techniques, with isoflurane maintained at 2–2.5% in 1 LPM of oxygen. All the instruments are placed in a sterile tray, and placed back in the tray after each use.
3.2. Sterile gloves, a mask and a surgical cap should be worn by the surgeon for the entire procedure. A sterile surgical gown may be worn as well, but it is optional, unless you expect any contamination.

3.3. Use a surgical scalpel with a No. 15 blade to make a skin incision on the left side of the thorax, approximately 1 cm proximal to the xyphoid. Use a blunted dissecting tip scissors to separate the skin layer from the muscle layer and make a longitudinal incision.

3.4. Dissect the muscle layers in the same manner until the ribs are exposed.

3.5. Carefully make a 2–3 cm longitudinal incision in the fifth intercostal space, adequate to insert retractors and expose the heart.

3.6. Use fine tipped forceps to lift the pericardium, and micro scissors to excise it in the region surrounding the apex of the heart. This step helps to avoid postsurgical adhesions of the heart to the chest walls and diaphragm.

NOTE: Avoid surgical incisions close to the sternum to minimize bleeding. Transecting the internal mammary arteries that run along the sternum, may cause excessive bleeding. If encountered with such bleeding, identify the bleeder and cauterize it.

4. **Echo guided MR procedure (Figure 3 & 4)**

4.1. Use a 6–0 prolene suture and a micro needle holder, to place a purse string suture on the apex of the left ventricle. If required, use micro forceps to stabilize the heart.

4.2. Gently tether the apical suture to stabilize the apex and insert a 23G needle (flushed with saline, and with a stopcock at its distal end) in the center of the purse string suture, into the left ventricular cavity.

4.3. Use one hand to stably hold and guide the needle, and the other hand to simultaneously manipulate the transesophageal echo probe to achieve an optimal echo view to visualize the needle, as described above.

4.4. With real time ultrasound guidance, advance the needle toward the ventricular side of the anterior mitral leaflet. Once the needle position is confirmed on ultrasound, advance the needle in one fine motion through the valve leaflet. If a resistance is felt, twist the needle as it is advanced into the leaflet to perforate it.

NOTE: Advancing the needle too far into the left atrium could result in left atrial perforation, causing excessive bleeding and animal death. The needle should be visualized on ultrasound at all times.

4.5. Retract the needle into the left ventricular chamber, away from the mitral valve, and confirm MR by turning on color Doppler imaging.

4.6. If MR is not seen on color Doppler imaging, repeat step 4.4 and 4.5. Adjust the echo probe if required to obtain a better view. After practice in few rats, it is possible to induce a leaflet puncture in one motion of the needle, inducing a hole.
that is the size of the outer diameter of the needle. This was confirmed after necropsy of the rat hearts.

4.7. Once MR is confirmed, retract the needle out of the left ventricular cavity and gently tie the purse string suture.

4.8. Use a sterile gauze to soak any blood on the apex and in the thoracic cavity.

NOTE: Touching the echo probe with the surgical gloves may result in contamination of the sterile environment. Spray your gloves with 70% ethanol or replace the gloves with new ones, appropriately.

5. Animal recovery and post-operative care

5.1. After 5–10 minutes of stable cardiac function (normal ECG and heart rate), the thoracotomy is closed in layers with 4–0 vicryl, while reducing isoflurane in steps.

5.2. Use an interrupted suture to approximate the ribs, with isoflurane maintained at 2%. Insert a chest tube into the sixth intercostal space and secure it to the sterile drapes to avoid inadvertent advancement of the tube into the thoracic cavity.

5.3. Use a continuous suture to close the muscle layer with isoflurane maintained at 1.5%.

5.4. Use a continuous suture to close the skin layer with isoflurane maintained at 1%.

5.5. Connect a 10 mL Luer-lock valve tipped syringe to the chest tube and drain 10–12 mL of air from the chest cavity and then remove the chest tube. Close the final incision completely.

5.6. Administer a final dose of Carprofen (2.5 mg/kg, SQ) and turn off isoflurane.

5.7. Continue mechanical ventilation while rat weans from anesthesia, monitoring vital signs (SpO2 and heart rate). At the onset of spontaneous breathing, turn off ventilation to test the ability of the rat to maintain such breathing and good SpO2.

5.8. If SpO2 levels start to fall below 90%, turn on the ventilator. Once the rat is able to maintain SpO2 levels without ventilation, the anchoring suture to the endotracheal tube is cut, and the animal is prepared for extubation.

5.9. Once the rat shows signs of alertness including whisker or eye movements, the animal is extubated.

5.10. Place a nose cone with 100% oxygen until the rat is ambulatory.

5.11. Transfer rat to a clean cage with minimal bedding and continue to monitor vital signs using a handheld SpO2 monitor, placed on the rat’s foot or tail, until rat is ambulatory.

5.12. To reduce the risk of injury to the surgical site and avoid the risk of infection, single house rats after surgery.
5.13. Administer Buprenorphine within three hours after the rat is awake and sufficiently ambulatory.

5.14. Buprenorphine may cause respiratory distress when administered early in the perioperative recovery period, thus delay it until the rat is breathing without difficulty.

6. Validation of MR severity with echocardiography (Figure 5)

6.1. Repeat TEE at two weeks after surgery, using the same steps specified in section 2.7. Two weeks post-surgery is adequate time for the hemodynamics to stabilize.

6.2. Obtain color Doppler imaging on a 2-chamber view using transesophageal ultrasound imaging, visualizing the left ventricle and left atrium. Measure the area of the left atrium and MR jet. Calculate the MR jet area fraction using

\[
MR \text{ jet area} \% = \frac{MR \text{ jet area} (mm^2)}{Left \text{ atrial area} (mm^2)}
\]  

Severe MR is defined as MR jet area ≥30%.

6.3. The area of the regurgitant orifice is approximated by calculating the area of 23G needle, using the outer diameter of the needle. This equation assumes that the area of the regurgitant orifice is equal to the area of the 23G needle.

\[
Area (cm^2) = \pi \times 0.03207^2 (cm^2)
\]  

6.4. Obtain continuous-wave Doppler imaging with the Doppler gate at the orifice of the regurgitant jet. Trace the waveform to compute VTI of the regurgitant jet. MR volume can be estimated using

\[
MR \text{ volume}(uL) = 1000 \times [MR \text{ VTI} (cm) \times Area of MR \text{ orifice} (cm^2)]
\]  

Severe MR is defined as MR volume ≥95 μL.

6.5. Obtain pulse wave Doppler imaging of the pulmonary vein by rotating the echo probe laterally, clockwise. Measure the systolic and diastolic wave velocities and use the following equation to calculate the ratio.

\[
Pulmonary \text{ flow ratio} = \frac{S \text{ wave}(m/s)}{D \text{ wave}(m/s)}
\]  

A negative pulmonary flow ratio indicates severe MR.

7. Sham Surgery

7.1. Steps 1–3 were performed as described.

7.2. Step 4 was modified such that the 23G needle was inserted into the left ventricular chamber, through a purse string suture on the left ventricular apex, but it was not advanced into the mitral valve to create MR. The needle was
inserted into the left ventricular chamber and retracted immediately, followed by tightening and closure of the ventricular apex.

7.3. Step 5 was performed as described.

7.4. Mitral valve assessment was performed as described in step 6. However, MR was not present in any of the animals, thus quantification as described was not necessary.

REPRESENTATIVE RESULTS

Feasibility and reproducibility:

The proposed MR model is highly reproducible, with a well defined hole in the mitral leaflet achieved in 100% of the rats used in this study. Figure 6A depicts the direction of the needle as it is inserted into the mitral valve. Figure 6B depicts a hole in the mitral valve leaflet from a representative rat explanted at 2 weeks after the procedure.

Survival & Adverse Events:

Sixteen rats were induced with MR using the described methods. Severe MR was created in all the rats. One rat died within an hour of creating MR from acute respiratory failure. Therefore, overall survival at 2 weeks after creating MR was 93.75%. Mortality or major cardiac adverse events, such as bleeding, arrhythmias, or stroke were not observed in any animals in the two weeks of observation.

Severity of mitral regurgitation:

Table 1 summarizes the hemodynamic profile of the left heart at baseline and at 2 weeks after inducing MR. A paired t-test was used to determine statistical significance between baseline and MR severity at 2 week, with a statistical significance defined as p<0.05. An MR jet was vivid at two weeks after the surgery, with an average area of 21.15±8.11 mm² (p<0.0001 compared to baseline) and a mean velocity time integral of 39.72±7.52 cm. Normalized MR fraction at 2 weeks was 41.91±8.3%, which is considered severe according to the guidelines of the American Society of Echocardiography. The severity of MR was adequate to induce pulmonary flow reversal, with a decrease in S/D ratio from 0.91±0.17 at baseline to −0.69±0.65 at 2 weeks (p<0.0001).

Cardiac chamber remodeling:

Figure 7 shows morphological changes in a representative heart after severe MR for 2 weeks, compared to a heart from a rat that underwent sham surgery. After two weeks post-surgery, the heart from the rat with MR was spherical and severely dilated, with a 29.65% increase in end diastolic volume (baseline EDV: 462.49±39.62 μl; and post-2week MR EDV: 599.79±58.59 μl, p<0.0001). End systolic volume increased by 10.06%, from 153.90±18.78 μl at baseline, to 169.36±24.64 μl (p=0.01) at 2 weeks after MR induction. Hypercontractility of the heart was observed in the first two weeks as expected, due to afterload reduction, as evident from an elevated ejection fraction (66.77±2.02% at baseline to 71.82±2.31% at 2 weeks (p<0.0001)). Exposure to MR for two weeks, increased the left atrial area by 99.59% (p<0.0001).
**DISCUSSION:**

A reproducible rodent model of severe MR, with good survival (93.75% survival after surgery) and without significant post-operative complications is reported. Real-time imaging with transesophageal echocardiography, and introduction of a needle into the beating heart to puncture the mitral leaflet are feasible and can be taught. Severe MR was produced with the 23G needle size in this study, which can be varied as desired using a smaller or larger needle. MR induced in this model creates a low-pressure volume overload on the left ventricle, which is a better representation of clinically observed mitral valve lesions. Severe left atrial and left ventricular dilatation are observed within two weeks after MR onset in this model, but without contractile dysfunction measured by ejection fraction. Analogous to such a situation are patients with primary MR, who remain asymptomatic without heart failure for prolonged periods, despite progressive dilatation of their left sided cardiac chambers.

This MR model of volume overload differs in several ways from the widely used aorto-caval fistula model of volume overload. Procedural ease of ACF, which requires a simple laparotomy without the need for intubation and mechanical ventilation, has encouraged its adoption by the scientific community. Despite its clear procedural advantages, arterio-venous fistulae shunt a large volume of blood into the vena cavae which overloads the venous reservoir, and also the right ventricle. Elevated central venous pressure from venous congestion may induce hepatic congestion and suboptimal renal filtration, which may cause hepatic fibrosis or activation of the renin-angiotensin-aldosterone (RAAS) system. The confounding effect of the RAAS system on ventricular-arterial coupling is known, and thus the ACF model fails to present a true volume overload on the left ventricle as seen in the setting of mitral regurgitation. When compared to the mitral valve defect model, lack of afterload reduction further diverges this model from the clinical situation of MR. Altogether, a significant different hemodynamic stress on the LV in the ACF model, introduces rapid changes with pronounced hypertrophy, dilation, and dysfunction that were not observed in our model.

Beyond the novelty of introducing MR with a needle stick, our model has multiple applications in answering clinically important questions. Patients with primary MR that emerges from a mitral valve lesion, often are asymptomatic for long periods, and receive correction of their MR only at the onset of pulmonary or cardiac failure symptoms. Recent clinical data indicates that such delayed correction of MR does not enable functional recovery of the left ventricle, despite relief of fatigue and symptoms. In a recent study using this rodent model, we demonstrated that MR introduces rapid and early remodeling of the cardiac extracellular matrix, which is a precursor of structural changes in the left ventricle. Such mechanistic insights that provide a physiological basis for mitral valve intervention can be developed using this model. Combined with cardiac imaging, it is possible to develop biomarkers that represent these early left ventricular changes to guide the timing of intervention. Additionally, this model of MR can be combined with ventricular cardiomyopathies such as ischemic, non-ischemic and other etiologies, to understand the effect of MR on remodeling of diseased left ventricles. For example, secondary MR, a frequent occurrence in myopathic ventricles after an infarction or with chronic ischemia, is a lesion that is clinically challenging to manage. Whether MR is a bystander in this disease...
state and a product of LV dysfunction, or if it actively contributes to cardiac remodeling are controversial. We recently extended this model of MR to investigate if post-infarction hearts with MR differ in their cardiac remodeling potential compared to those without MR\textsuperscript{11}, elucidating potential mechanisms involved in worsening heart failure in patients with MR. This model provides the flexibility to investigating the impact of early onset versus late onset of MR on cardiac remodeling to failure, which could have a significant clinical impact in guiding interventions.

As with any experimental model, there are some advantages and limitations that should be considered when applying results from animals to humans. The clear advantage of this model is the reproducible severity of MR, which aids in understanding cardiac chamber remodeling in clinically diagnosed conditions such as primary MR from chordal rupture. The increase in cardiac chamber volumes observed in this model and extracellular matrix remodeling observed in the myocardium, represent the changes observed previously in larger animals and humans with primary MR\textsuperscript{14, 15}. The limitation of this leaflet perforation model is that MR develops acutely, representing only a subset of patients with primary MR from acute chordal rupture. Notwithstanding the limitations, acute onset of MR accounts for a significantly large patient population that undergo mitral valve interventions, and this model is very relevant to such a situation. Another limitation of this model is that MR is not reversible or repairable, which does not enable studies on the effect or timing of intervention on cardiac remodeling.

**ACKNOWLEDGMENTS:**

This work was funded by grant 19PRE34380625 and 14SDG20380081 from the American Heart Association to D.Corporan and M.Padala respectively, grants HL135145, HL133667, and HL140325 from the National Institutes of Health to M.Padala, and infrastructure funding from the Carlyle Fraser Heart Center at Emory University Hospital Midtown to M.Padala.

**REFERENCES:**


Figure 1. Intubation technique -
(A) 16G angiocath with a guidewire used for endotracheal intubation in this rat model; (B) Image of the pharyngeal view using an otoscope, and the target region to insert the endotracheal tube; (C) Final configuration of the endotracheal tube; (D) Attachment of the endotracheal tube to the mechanical ventilator.
Figure 2. Transthoracic imaging:
(A1) Setup for transthoracic imaging of the rat, depicting the angle of the imaging probe; (A2) Parasternal long axis view of the heart; (A3) Short axis view of the heart; Transesophageal imaging: (B1) 8Fr intracardiac echo probe with probe inserted into the esophagus while the animal is intubated; (B2) High esophageal views of the left heart, depicting the left atrium, mitral valve and left ventricle.
Figure 3.

(A) Surgical layout showing left thoracotomy at 5th intercostal space, and ICE catheter into the esophagus of the rat for image guidance, and a 23G needle inserted into the LV apex where the purse-string suture is placed. (B) Surgical view during transesophageal echo guided leaflet perforation. (C) Echocardiographic image of the needle insertion into the left ventricle in diastole. (D) Echocardiographic image of the needle insertion into the left ventricle in systole. (E) Echocardiographic image of the needle pierced through the anterior leaflet.
Figure 4.
(A) Baseline echo 2 chamber view prior to creating MR; (B) 23G needle, visualized on echo during beating heart, advanced into the left atrium through the anterior mitral valve leaflet; (C) Color Doppler imaging showing MR jet seen in systole.
Figure 5.
Representative echo images to validate MR severity at 2wks post-surgery. (A) Left atrial area traced in white and MR jet area traced in red; (B) MR VTI trace in red; (C) Pulmonary flow showing systolic reversal.
Figure 6.
(A) Orientation of needle puncture on an ex-vivo heart. Needle punctured through apex of the LV at an angle, a longitudinal section of the LV with the needle directed towards the mitral valve leaflet, and the needle punctured through the mitral valve leaflet into atrial space. (B) Representative explant photograph depicting a hole in the anterior mitral leaflet.
Figure 7.
Gross morphology of whole hearts of a sham operated control rat (A) and a rat which underwent MR surgery (B) 2 weeks post-surgery. The rat with severe MR has significant left ventricular dilation and chamber enlargement compared to the sham operated control.
Table 1:

Mitrval regurgitation characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n=15)</th>
<th>2wk MR (n=15)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left atrial area (mm²)</td>
<td>25.03 ± 8.70</td>
<td>49.95 ± 14.78</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>MR jet area (mm²)</td>
<td>0</td>
<td>21.15 ± 8.11</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>MR fraction (%)</td>
<td>0</td>
<td>41.91 ± 8.30</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>MR VTI (cm)</td>
<td>0</td>
<td>39.72 ± 7.52</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>S wave (m/s)</td>
<td>0.39 ± 0.07</td>
<td>-0.51 ± 0.41</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>D wave (m/s)</td>
<td>0.44 ± 0.04</td>
<td>0.70 ± 0.17</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>S/D wave ratio</td>
<td>0.91 ± 0.17</td>
<td>-0.69 ± 0.65</td>
<td>p&lt;0.0001</td>
</tr>
</tbody>
</table>
## Table of Materials

<table>
<thead>
<tr>
<th>Name of Material/ Equipment</th>
<th>Company</th>
<th>Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACUSON AcuNav Ultrasound probe</td>
<td>Biosense Webster</td>
<td>10135936</td>
</tr>
<tr>
<td>ACUSON PRIME Ultrasound System</td>
<td>Siemens</td>
<td>SC2000</td>
</tr>
<tr>
<td>ACE Light Source</td>
<td>Schott</td>
<td>A20500</td>
</tr>
<tr>
<td>Betadine</td>
<td>McKesson</td>
<td>1073829</td>
</tr>
<tr>
<td>Blunted microdissecting scissors</td>
<td>Roboz</td>
<td>RS5990</td>
</tr>
<tr>
<td>Buprenorphone</td>
<td>Patterson Veterinary</td>
<td>99628</td>
</tr>
<tr>
<td>Carprofen</td>
<td>Patterson Veterinary</td>
<td>7847425</td>
</tr>
<tr>
<td>Chest tube (16G angiocath)</td>
<td>Terumo</td>
<td>SR-OX1651CA</td>
</tr>
<tr>
<td>Disposable Surgical drapes</td>
<td>Med-Vet</td>
<td>SMS40</td>
</tr>
<tr>
<td>Electric Razor</td>
<td>Oster</td>
<td>78400-XXX</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>Patterson Veterinary</td>
<td>78057791</td>
</tr>
<tr>
<td>Heat lamp with table clamp</td>
<td>Braintree Scientific</td>
<td>HL-1 120V</td>
</tr>
<tr>
<td>Hemostatic forceps, straight</td>
<td>Roboz</td>
<td>RS7110</td>
</tr>
<tr>
<td>Hemostatic forceps, curved</td>
<td>Roboz</td>
<td>RS7341</td>
</tr>
<tr>
<td>Induction chamber</td>
<td>Braintree Scientific</td>
<td>EZ-1785</td>
</tr>
<tr>
<td>Injection Plug, Cap, Luer Lock</td>
<td>Exel</td>
<td>26539</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>Patterson Veterinary</td>
<td>6679401725</td>
</tr>
<tr>
<td>Mechanical ventilator</td>
<td>Harvard Apparatus</td>
<td>Inspira ASV</td>
</tr>
<tr>
<td>Microdissecting forceps</td>
<td>Roboz</td>
<td>RS5135</td>
</tr>
<tr>
<td>Microdissecting spring scissors</td>
<td>Roboz</td>
<td>RS5603</td>
</tr>
<tr>
<td>Needle holder</td>
<td>Roboz</td>
<td>RS6417</td>
</tr>
<tr>
<td>No. 15 surgical blade</td>
<td>McKesson</td>
<td>1642</td>
</tr>
<tr>
<td>Non-woven sponges</td>
<td>McKesson</td>
<td>446036</td>
</tr>
<tr>
<td>Otoscope</td>
<td>Welch Allyn</td>
<td>23862</td>
</tr>
<tr>
<td>Oxygen</td>
<td>Airgas Healthcare</td>
<td>UN1072</td>
</tr>
<tr>
<td>Pulse Oximeter</td>
<td>Nonin Medical</td>
<td>2500A VET</td>
</tr>
<tr>
<td>Retractor, Blunt 4x4</td>
<td>Roboz</td>
<td>RS6524</td>
</tr>
<tr>
<td>Rodent Surgical Monitor</td>
<td>Indus Instruments</td>
<td>113970</td>
</tr>
<tr>
<td>Scale</td>
<td>Salter Brecknell</td>
<td>LPS 150</td>
</tr>
<tr>
<td>Scalpel Handle</td>
<td>Roboz</td>
<td>RS9843</td>
</tr>
<tr>
<td>Silk suture 3–0</td>
<td>McKesson</td>
<td>220263</td>
</tr>
<tr>
<td>Small Animal Anesthesia System</td>
<td>Ohio Medical</td>
<td>AKDL03882</td>
</tr>
<tr>
<td>Sterile saline (0.9%)</td>
<td>Baxter</td>
<td>281322</td>
</tr>
<tr>
<td>Surgical cap</td>
<td>McKesson</td>
<td>852952</td>
</tr>
<tr>
<td>Surgical gloves</td>
<td>McKesson</td>
<td>854486</td>
</tr>
<tr>
<td>Surgical Mask</td>
<td>McKesson</td>
<td>188696</td>
</tr>
<tr>
<td>Syringe 1mL</td>
<td>McKesson</td>
<td>1031817</td>
</tr>
<tr>
<td>Syringe 10mL</td>
<td>McKesson</td>
<td>1031801</td>
</tr>
<tr>
<td>Ultra-high frequency probe</td>
<td>Fujifilm Visualsonics</td>
<td>MS250</td>
</tr>
<tr>
<td>Ultrasound gel</td>
<td>McKesson</td>
<td>150690</td>
</tr>
</tbody>
</table>

*J Vis Exp. Author manuscript; available in PMC 2021 May 19.*
<table>
<thead>
<tr>
<th>Name of Material/ Equipment</th>
<th>Company</th>
<th>Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEVO Ultrasound System</td>
<td>Fujifilm Visualsonics</td>
<td>VEVO 2100</td>
</tr>
<tr>
<td>4–0 vicryl</td>
<td>Ethicon</td>
<td>J496H</td>
</tr>
<tr>
<td>6–0 prolene</td>
<td>Ethicon</td>
<td>8307H</td>
</tr>
<tr>
<td>23G needle</td>
<td>McKesson</td>
<td>16-N231</td>
</tr>
<tr>
<td>25G needle, 5/8 inch</td>
<td>McKesson</td>
<td>1031797</td>
</tr>
<tr>
<td>70% ethanol</td>
<td>McKesson</td>
<td>350600</td>
</tr>
</tbody>
</table>