Creation of Nonischemic Functional Mitral Regurgitation by Annular Dilatation and Nonplanar Modification in a Chronic In Vivo Swine Model

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Background—Mechanisms and treatments of nonischemic functional mitral regurgitation (NIMR) are not fully established, in part, because of a lack of proper large animal models. We developed a novel technique of NIMR creation in a swine model by making multiple small incisions in the mitral annulus.

Methods and Results—Ex vivo experiments using isolated swine hearts (n=10) showed a 15% increase in annular area (6.8–7.8 cm²) after 16 incisions were made along the posterior mitral annulus of a pressurized left ventricle. In an in vivo swine model (n=7; 46.4±2.2 kg), NIMR was created by making fourteen to twenty-six 2-mm incisions in the atrial aspect of the mitral annulus using a cardioport video-assisted imaging system in the beating heart. Animals were euthanized at 4 weeks (n=4) and 6 weeks (n=3). Three-dimensional (3D) echocardiography was obtained before and immediately after NIMR creation and at euthanasia; vena contracta area, mitral annular dimension, left ventricular volume, and inter-papillary muscle distance were measured. The mitral annular incisions resulted in mild to moderate mitral regurgitation and an increased vena contracta area. NIMR creation altered mitral valve geometry by decreasing mitral annular nonplanarity and increasing annular area, primarily in the anteroposterior dimension. NIMR creation did not significantly change left ventricular volume or inter-papillary muscle distance. Longer follow-up period did not significantly affect these outcomes.

Conclusions—NIMR can successfully be created in a beating heart swine model and results in dilatation and 3D changes in mitral annular geometry. This model can enhance the experimental validation of new valve repair devices and techniques. (Circulation. 2013;128[suppl 1]:S263-S270.)

Key Words: endoscopic surgical procedure ■ echocardiography, three-dimensional ■ mitral valve insufficiency ■ models, animal ■ ventricular remodeling

Functional mitral regurgitation (MR) is a major prognostic factor for patients with ischemic or dilated cardiomyopathy1 and closely associated with mitral annular dilatation and leaflet tethering.2–6 The 3-dimensional (3D) geometry of the mitral annulus (saddle shape) has been recognized as an important factor to minimize annular and leaflet stresses7,8 and enhance leaflet coaptation by coordinated annular hinge motion from planar shape in diastole to saddle shape in systole.9 Although functional MR is associated with a less saddle shape and more planar annulus,10,11 the mechanisms and treatment strategy for functional MR have yet to be fully established, in part, because of a lack of optimal large animal models.

To date, ischemic12 and nonischemic13,14 large animal models for functional MR have been developed to mimic the characteristics of ischemic and dilated cardiomyopathy, respectively. Unfortunately, ischemic models, which involve creation of a posterolateral left ventricular (LV) wall infarction, are not reproducible for inducing ischemic functional MR because of significant LV dysfunction and low survival rate.12 Tachycardia-induced cardiomyopathy by rapid ventricular pacing has been established in a sheep model for understanding mechanisms of nonischemic functional mitral regurgitation (NIMR). This method is not optimal for a chronic model to evaluate mitral valve (MV) repair techniques because the dilated annulus and ventricle can recover by themselves after rapid pacing is ceased.13 Phenol application to the mitral annulus has also been attempted for NIMR creation, but this could only dilate the annulus by 13% and induce no MR.14 Hence, a more reproducible and less invasive method of NIMR creation with sufficient 3D annular remodeling would be beneficial.

The principle aim of this study was to test a novel method of creating NIMR, involving multiple small incisions along the posterior mitral annulus in a beating heart.

From the Department of Cardiac Surgery, Boston Children’s Hospital, Harvard Medical School, Boston, MA. Presented at the 2012 American Heart Association meeting in Los Angeles, CA, November 3–7, 2012. The online-only Data Supplement is available with this article at http://circ.ahajournals.org/lookup/suppl/doi:10.1161/CIRCULATIONAHA.112.000396/-/DC1. Correspondence to Pedro J. del Nido, MD, Department of Cardiac Surgery, Boston Children’s Hospital, Harvard Medical School, 300 Longwood Ave, Boston, MA 02115. E-mail Pedro.delnido@childrens.harvard.edu © 2013 American Heart Association, Inc. Circulation is available at http://circ.ahajournals.org DOI: 10.1161/CIRCULATIONAHA.112.000396
using a cardiport video-assisted system. The cardiport was designed for 2 purposes: (1) to visualize structures inside the beating heart by housing an endoscope and (2) to provide a working channel for instrument access inside the beating heart.\textsuperscript{14} The cardiport is an all-in-one system that can easily be introduced into the heart, in contrast to some other existing cardioscopy concepts.\textsuperscript{17} Using our cardiport system, we have successfully developed a swine model of functional tricuspid regurgitation by making tricuspid annular incisions to induce annular dilatation.\textsuperscript{18} Therefore, the objectives of this study were to examine the feasibility of creating NIMR by making mitral annular incisions with the cardiport and to analyze the effects of the resultant NIMR on annular and LV geometry.

### Materials and Methods

Expanded methods are available in the online-only Data Supplement.

#### Ex Vivo Swine Heart Model

Isolated fresh swine hearts were mounted in a saline-filled tank as described previously\textsuperscript{11} (Figure I in the online-only Data Supplement), and the LV chamber was pressurized with 120 mmHg. Sixteen 2-mm incisions were made along the posterior mitral annulus. The anteroposterior and intercommissural diameters of the mitral annulus were measured, and the estimated annular area was calculated \[ \text{area} = \pi \times (\text{anteroposterior diameter}/2 \times \text{intercommissural diameter}/2). \]

#### Surgical Technique to Create Mitral Regurgitation (In Vivo Swine Model)

The study protocol was approved by the Boston Children’s Hospital/Harvard Medical School Animal Care and Use Committee. Yorkshire pigs (n=7; 46.4±2.2 kg) were prepared for anesthesia and surgery, as previously described.\textsuperscript{18} With the animal placed in the right decubitus position, the left chest cavity was entered through the fourth intercostal space. The pericardium was open. After heparinization (150 U/kg), the cardiport was inserted through the purse-string suture into the left atrium. The cardiport comprises a plastic shaft with 2 channels, one for a 5-mm rigid 30° endoscope and the other for a cutting rod, and the head of the cardioport is covered by a transparent cap filled with saline solution to examine the intracardiac structures using a video-assisted imaging system (Figure 1A and 1B).\textsuperscript{15,16,18} Fourteen to twenty-six 2-mm annular incisions were made along the atrial aspect of the posterior mitral annulus between the fibrous trigones by pivot turning the cardioport around the annulus from the purse string suture (Figure 1C) under endoscopic guidance through the cardioport (Figure 1D). When the cardiport head was apart from the mitral annulus, the annulus was not visible because of the surrounding blood (Figure 1E-a); however, when the cardiport head was pushed against the annulus, the surrounding blood was displaced and the annulus was stabilized and visible through the endoscope (Figure 1E-b). The incisions were made using a cutting metal rod with a sharpened blade at its tip; this rod was passed through the instrument channel of the cardiport (Figure 1E-c). The metal rod was passed no more than 3 to 4 mm beyond the cardiport head so as not to damage perianular structures (eg, coronary artery and vein) and LV wall. Because the instrument channel was not centrally located (Figure 1B), the endoscopic portion of the cardiport head was kept facing to the side of left atrial wall to stabilize the moving annulus firmly. After the annular incisions were made, the cardiport was withdrawn and the purse string was secured on the left atrium. The chest wound was closed in multiple layers. Prophylactic cephalixin (500 mg twice daily) was administered orally for 7 days postoperatively. At 4 weeks (n=4; #299, #336, #360, and #361) or 6 weeks (n=3; #314, #373, and #374) after NIMR creation, the pigs were anesthetized as described.\textsuperscript{17} With the animal supine the chest was opened twice daily was administered orally for 7 days postoperatively. At 4 weeks (n=4; #299, #336, #360, and #361) or 6 weeks (n=3; #314, #373, and #374) after NIMR creation, the pigs were anesthetized as described.\textsuperscript{17} With the animal supine the chest was opened by median sternotomy. After echocardiographic examination, the animals were euthanized by intravenous injection of overdosed pentobarbital sodium (Fatal-Plus solution, 86 mg/kg; Vortech Pharmaceuticals, Dearborn, MI).

#### Echocardiographic Analysis

Two-dimensional and 3D echocardiography were performed before and immediately after NIMR creation and at euthanasia. The 2D color Doppler images were used to grade MR, and the 3D echocardiographic images were analyzed offline using the multiplanar 3D quantification mode of the Qlab software (Phillips, Healthcare, Andover, MA) to measure the annular area and dimensions (Figure 2A). The maximal vena contracta area (VCA) of the MR jet in systole was measured, as described elsewhere.\textsuperscript{20} The inter-papillary muscle (PM) distance at mid-systole was also measured in the plane cutting through both PM heads. The LV end-diastolic volume, LV end-systolic volume (LVESV), and LV ejection fraction (LVEF) were measured using Qlab’s algorithms.

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**Figure 1.** Cardiport-guided creation of mitral regurgitation. Panel A shows a cardiport inserted into the left atrium (LA). Panel B is a schematic drawing of the cardiport head covered by a transparent cap. Panel C shows an endoscopic view of the cardiport. Panel D is a schematic illustration showing a posterior mitral valve. Panel E shows a sequence of endoscopic views showing (a) how to make annular incisions; (b) a cardiport approaches to mitral annulus; (c) a cutting rod makes annular incisions; (d) LV indicates left ventricle; and (e) MV, mitral valve.
In addition, 3D data from the latter 4 animals (#360, #361, #373, and #374) were analyzed in the MV quantification mode of Qlab to visualize the MV’s end-systolic 3D geometry and to measure the annular height and the nonplanarity angle of mitral leaflets (Figure 2B and 2C).

Histology
The posterior mitral annulus was excised and treated for hematoxylin and eosin staining and Masson trichrome staining.

Statistical Analysis
Measurements are presented as means±SE. Hemodynamic data were evaluated using 1-way ANOVA with repeated measures to compare blood pressures, heart rate, and central venous pressure among the 3 time points (before MR creation [MRc], after MRc, and 4–6 weeks after MRc) with the F test used to determine significance with a Bonferroni adjustment of the P value. Two-way repeated measures ANOVA with Bonferroni correction was applied to evaluate changes in echocardiographic parameters accounting for the paired data (within-animal measurements), as well as the between-effects of 4-week versus 6-week euthanasia. Interobserver and intraobserver reliability of the 2 blinded observers (H.Y. and S.S.) were determined using the intraclass correlation coefficient based on a 2-way random effects model. Simple linear regression analysis was performed to ascertain the relationship between the number of annular incisions on annular diameter and area for anteroposterior and intercommissural dimensions using the method described elsewhere. The criterion for statistical significance was a 2-tailed P<0.05 with Bonferroni correction to protect against committing type I errors because of multiple comparisons. Statistical analysis was conducted with the SPSS software package (version 20.0, SPSS Inc/IBM, Chicago, IL).

Results
Mitral Annular Dimension (Ex Vivo)
The saline-tank experiments showed that the anteroposterior and intercommissural annular diameters of the MV increased by 2.0 mm (6%) and 2.1 mm (8%) after the annular incision procedure, respectively (P<0.001; Figure IIA and IIB in the online-only Data Supplement). The mitral annular area increased from 6.8 to 7.8 cm² (15%) after 16 annular incisions (P<0.001; Figure IIC and IID in the online-only Data Supplement).

Mitral Regurgitation
All animals survived without complication. Hemodynamic variables before, immediately after, and 4 to 6 weeks after NIMR creation are shown in the Table. Central MR was trivial in 4 animals and trivial to mild in the other 3 animals before NIMR creation. Central MR was enhanced to trivial to mild in 2 animals, mild to moderate in 1, and moderate to severe in 2 after 4 to 6 weeks (Figure 3A). MR was holosystolic except for 1 animal with early systolic mild MR (#299). In 5 animals, 1 to 3 leaflet punctures occurred near the hinge point during the annular incision procedure, but any resultant regurgitant jets had entirely vanished in 3 of the 5 animals by 4 to 6 weeks postoperatively. The VCA of the central MR jet had significantly increased to by 4 to 6 weeks after NIMR creation, whereas the total VCAs of the MR via leaflet punctures were almost negligible as compared with the central MR after 4 to 6 weeks (0.02 versus 0.19 cm²; P<0.001; Figure 3B). The VCA was smaller at 6 weeks after NIMR creation compared with 4 weeks postoperatively (P=0.012; Figure 3C).

Mitral Annular Dimension (In Vivo)
Geometrically, at baseline the mitral annulus had a saddle shape with concave mitral leaflets at end systole. By 4 weeks after NIMR creation, the annulus had become dilated and more planar, and the mitral leaflets had flattened (Figure 4A). The mitral annular area at mid-systole had dilated by 46%
The anteroposterior annular diameter was 25% longer ($P<0.001$; Figure 4C); however, the intercommissural diameter was only 7% longer, and this increase was not statistically significant (Figure 4D). No significant difference was observed between the 4-week and 6-week follow-up groups for any of the parameters. The mitral annular height at end systole was 35% lower after 4 to 6 weeks ($P=0.012$; Figure 4E). The nonplanarity angle of mitral leaflets was increased after 4 to 6 weeks ($P<0.001$; Figure 4F). The mitral annular dimensions throughout the different phases of the cardiac cycle (mid-systole, end systole, mid-diastole, and end diastole) were also examined (Figure 5). Mitral annular area and anteroposterior annular diameter measurements increased significantly at 4 to 6 weeks after MRC compared with pre-MRC during mid-systole, end systole, mid-diastole, and end diastole (Figures 5A and 5B; all $P<0.001$). For intercommissural annular diameter, there was a significant increase during mid-systole ($P<0.001$), mid-diastole ($P=0.013$), and end diastole ($P<0.001$) but no difference during end systole ($P=0.18$; Figure 5C).

**LV Dimension**

LV geometry was evaluated after 4 to 6 weeks, and 2-way repeated measures ANOVA detected no significant changes over time for 4- or 6-week animals (Figure 6). Compared with pre-MRC, there were no significant differences detected at 4 to 6 weeks after MRC in LV end-diastolic volume (Figure 6A; 4 weeks, $P=0.14$; 6 weeks, $P=0.28$), LVESV (4 weeks, $P=0.27$; 6 weeks, $P=0.37$), LVEF (4 weeks, $P=0.14$; 6 weeks, $P=0.53$), or inter-PM distance (4 weeks, $P=0.42$; 6 weeks, $P=0.46$). In pooling the 7 animals together, the only parameter that showed a significant change was LVEF with an increase noted.

### Table. Hemodynamic Parameters

<table>
<thead>
<tr>
<th></th>
<th>Pre-MRC</th>
<th>Post-MRC</th>
<th>4–6 Wk Post-MRC</th>
<th>Overall P Value</th>
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<tbody>
<tr>
<td>Systolic BP, mm Hg</td>
<td>93±4</td>
<td>86±3</td>
<td>80±4</td>
<td>&lt;0.001*</td>
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<tr>
<td>Diastolic BP, mm Hg</td>
<td>57±2</td>
<td>50±2</td>
<td>46±2</td>
<td>0.007*</td>
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<tr>
<td>Heart rate, bpm</td>
<td>81±4</td>
<td>77±4</td>
<td>75±4</td>
<td>0.59</td>
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<tr>
<td>CVP, mm Hg</td>
<td>2.6±0.5</td>
<td>2.7±0.5</td>
<td>3.6±0.5</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Data are mean±SE. BP indicates blood pressure; bpm, beats per minute; CVP, central venous pressure; and MRC, mitral regurgitation creation.

*Statistically significant (repeated measures ANOVA). Compared with pre-MRC, systolic BP was significantly lower at post-MRC ($P=0.034$) and at 4 to 6 wk post-MRC ($P<0.001$). Compared with pre-MRC, diastolic BP was significantly lower at post-MRC ($P=0.045$) and at 4 to 6 wk post-MRC ($P=0.004$).
at 4 to 6 weeks after MRc ($P = 0.039$). Overall, these results confirm that LV measurements were essentially unaffected after creation of the MR model, with the caveat that LVEF may actually be improved at 4 to 6 weeks after MRc.

**Assessment of Observer Reliability**

Interobserver and intraobserver reliability were acceptable and showed moderate to high level of concordance between and within observers for each echocardiographic parameter, as assessed by the intraclass correlation coefficient statistic. Interobserver reliability was 0.75 for VCA, 0.81 for mitral annular area, 0.66 for anteroposterior diameter, 0.76 for intercommissural diameter, 0.75 for LV end-diastolic volume, and 0.67 for LVESV. Intraobserver reliability was 0.85 for VCA, 0.82 for mitral annular area, 0.84 for anteroposterior diameter, 0.68 for intercommissural diameter, 0.65 for LV end-diastolic volume, and 0.75 for LVESV. All intraclass correlation coefficient values were found to be statistically significant ($P < 0.001$).

**Histological Analysis**

The annular tissue healed well with all incised regions of the annulus covered by regenerated tissue by 4 to 6 weeks after NIMR creation (Figure 7A). By light microscopy, the myocardium surrounding the annular incisions was destroyed and replaced by fibroblasts and collagen (Figure 7B through 7D).

**Discussion**

In this study, cardiopoint-assisted MV annular incisions successfully produced functional MR with 46% annular dilatation predominantly in the anteroposterior direction and 3D annular changes, which is comparable with clinically observed annular dilatation (36%–52%) in functional MR and with tachycardia-induced cardiomyopathy (46%). Given that not significant annular dimensional changes but minor MR were observed immediately after NIMR creation, the repeated forces imposed on the annulus and leaflets during the cardiac cycle are likely important in gradual annular and leaflet remodeling.
Nguyen et al reported that pure MR without annular injuries induced 31% annular dilatation with maintained annular saddle shape after 12 weeks. Thus, we speculate that direct annular injuries may attenuate annular contraction, and in the setting of intracardiac forces the annular geometry remains closer to its diastolic conformation: larger and more planar.

Notably, LV dilatation and impaired contraction, as well as PM dyssynchrony, has also been regarded as determinants of functional MR severity because they lead to MV tethering. In the present study, we did not observe substantial changes of LV dimensions and inter-PM distances after NIMR creation (Figure 6). Although not significant, LVESV tended to decrease and LVEF increased after NIMR creation. Given that hemodynamic data were not correlated with increased LV function (Table), it may be apparently affected by MR volume; a part of LV volume regurgitates into left atrium, resulting in smaller LVESV. The discrepancy between our NIMR model and the previously reported ischemic model showing ventricular remodeling and dysfunction could be explained as follows: (1) our technique does not directly compromise...
LV myocardium; (2) the 4- to 6-week follow-up period may be insufficient to demonstrate LV remodeling. Although our NIMR swine model is not ideal for examining LV remodeling/dysfunction, it does demonstrate Carpentier type I MR by annular dilatation. Actually, MR in tachycardia-induced cardiomyopathy was mainly by Carpentier type I mechanism because MV tethering was modest even with some LV dilatation. Otsuji et al reported that isolated annular dilatation observed in patients with lone atrial fibrillation did not cause moderate or greater functional MR. The MR grade observed in our model was mild to moderate, but not severe (Figure 3A), and it is comparable with that observed in the animal model of tachycardia-induced cardiomyopathy and this clinical report.

For potential future application, this NIMR model will enhance the ability to evaluate new annuloplasty rings (eg, a saddle-shaped rings and other novel valve interventions involving 3D MV repair) that are currently being tested in an in vitro setting or in vivo nondiseased MV model, and which would be more challenging to test in animals with significantly depressed LV function. Our method of mitral annular incisions could be improved by full opening of pericardium at the time of NIMR creation because we opened only the upper part of pericardium. It was reported that full opening of pericardium helped to dilate LV in an acute model. In addition, our method can also be combined with others (eg, coronary ligation and rapid ventricular pacing) to induce Carpentier type III MR because of restricted leaflet motion, depending on what type of large animal model would be required for the future study purposes. Combining techniques of MRc will require further investigation to evaluate the feasibility and durability of combined methods.

Study Limitations

An in vivo swine model was used in this study because human and swine hearts are similar in size and anatomy. The pathophysiology of our NIMR model is different from that in a clinical setting with functional MR because our model does not compromise LV function, and the positions of subvalvular apparatus (eg, chorda tendinae and PMs) are not exactly coincident with those in a clinical setting. Nevertheless, this technique is beneficial because it is well tolerated, reproducible, and persistent as compared with the previously reported models.

Conclusions

NIMR can be successfully created by multiple small annular incisions in a beating heart swine model. Mitral annular incisions result in dilatation and 3D geometric changes in the mitral annulus and leaflets ≥4 weeks after NIMR creation. This annular dilatation and 3D geometric changes effectively mimic what is observed with annulus clinically in NIMR. The annulus dilatation and 3D change are likely secondary to a synergistic relationship between annular tissue injury/healing and the intracardiac forces. Longer follow-up might be...
necessary to determine whether LV remodeling and MV leaflet tethering occur over a longer time period. Given the importance of annular and leaflet geometry for MV mechanics and function, this model can serve as a high-fidelity platform for better understanding MV mechanics and for developing new devices and techniques for MV repair.

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Disclosures

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