Developmental Noncompaction Cardiomyopathy in a Mouse Model of Barth Syndrome

Colin K.L. Phoon, MPhil, MD

Division of Pediatric Cardiology
Mitochondria & heart development

- Mitochondrial disorders
- Mitochondrial disorders as a category suggest a role of mitochondrial functioning in myocardial & heart development.
- Barth syndrome

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Not just the powerhouse of the cell: emerging roles for mitochondria in the heart

Derek J. Hausenloy¹* and Marisol Ruiz-Meana²
Cardiolipin, the center of mitochondrial physiology

Tafazzin (taz) encodes for an acyltransferase involved in the maturation of the phospholipid cardiolipin

Mitochondrial functions:
- Bioenergetics
- Apoptosis
- Calcium homeostasis
- Cellular redox balance
- Biosynthetic pathways
- Transcriptional control, cellular proliferation pathways
- Heme synthesis reactions
- Immune responses

Claypool & Koehler, TiBS 2011
Barth syndrome: cardiolipin deficiency

- X-linked (Xq28): mutations in the taz gene

AN X-LINKED MITOCHONDRIAL DISEASE AFFECTING CARDIAC MUSCLE, SKELETAL MUSCLE AND NEUTROPHIL LEUCOCYTES

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SUMMARY

An X-linked recessive disease is reported in a large pedigree. The disease is characterised by a triad of dilated cardiomyopathy, neutropenia and skeletal...
Myocardial trabeculation & compaction

LV noncompaction in Barth syndrome
Towbin & Bowles, 2001

Trabeculation & compaction in human embryonic hearts
Lamers et al., 1995
Model for Barth syndrome?

- Model organisms: yeast, Drosophila, zebrafish
- Traditional mouse knockout genetics: unsuccessful
- Proprietary shRNA knockdown strategy

Tafazzin Knockdown in Mice Leads to a Developmental Cardiomyopathy With Early Diastolic Dysfunction Preceding Myocardial Noncompaction

Colin K. L. Phoon, MPH; MD; Devrim Acarhan, PhD; Michael Schrumpf, MD; David L. Stokes, PhD; Jiri Edelman-Novensky, PhD; Dawson Yu, PhD; Ying Xu, PhD; Naray Venkatesan, MD; Wenfeng Qin, PhD

**Background**—Barth syndrome is a rare, multisystem disorder caused by mutations in tafazzin that lead to cardiolipin deficiency and mitochondrial abnormalities. Patients most commonly develop an early-onset cardiomyopathy in infancy or fetal life.

**Methods and Results**—Knockdown of tafazzin (TAZKD) in a mouse model was induced from the start of gestation via a doxycycline-inducible shRNA transgenic approach. All liveborn TAZKD mice died within the neonatal period, and in vivo echocardiography revealed prenatal loss of TAZKD embryos at E12.5-14.5. TAZKD E13.5 embryos and newborn mice demonstrated significant tafazzin knockdown, and mass spectrometry analysis of hearts revealed abnormal cardiolipin profiles typical of Barth syndrome. Electron microscopy of TAZKD hearts demonstrated ultrastructural abnormalities in mitochondria at both E13.5 and newborn stages. Newborn TAZKD mice exhibited a significant reduction in total mitochondrial area, smaller size of individual mitochondria, reduced cristae density, and disruption of the normal parallel orientation between mitochondria and sarcomeres. Echocardiography of E13.5 and newborn TAZKD mice showed good systolic function, but early diastolic dysfunction was evident from an abnormal flow pattern in the dorsal aorta. Strikingly, histology of E13.5 and newborn TAZKD hearts showed myocardial thinning, hypertrophy of trabeculae and noncompaction, and defective ventricular septation. Altered cellular proliferation occurring within a narrow developmental window accompanied the myocardial hypertrophy-noncompaction noncompaction.

**Conclusions**—In this murine model, tafazzin deficiency leads to a unique developmental cardiomyopathy characterized by ventricular myocardial hypertrophy-noncompaction and early lethality. A central role of cardiolipin and mitochondrial functioning is strongly implicated in cardiomyocyte differentiation and myocardial patterning required for heart development. (J Am Heart Assoc. 2012;1:e000455 doi: 10.1161/JAHA.111.000455.)

**Key Words**: Barth syndrome • cardiolipin • mitochondrial disease • noncompaction cardiomyopathy • tafazzin

Phoon et al. J Am Heart Assoc 2012
Cardiac dysfunction in TAZKD embryos
Taz knockdown leads to prenatal lethality
Evidence for pre-/perinatal lethality

- Uninduced litters: expected Mendelian ratios at birth
- One litter imaged at E14.5:
  - 8 live+2 resorbed embryos at E14.5
  - 6 live pups born, all WT

<table>
<thead>
<tr>
<th>STAGE</th>
<th>TOTAL</th>
<th>WT Alive</th>
<th>WT Dead</th>
<th>TAZKD Alive</th>
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<td>14</td>
<td>7</td>
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<td>E13.5</td>
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<td>31</td>
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<td>28</td>
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<td>Newborn</td>
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<td>35</td>
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<td>13</td>
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TAZKD mice exhibit noncompaction
### E13.5 Embryos In Vivo

<table>
<thead>
<tr>
<th></th>
<th>End-diastolic Area (biV) (mm²)</th>
<th>Fractional Area Shortening</th>
<th>Dorsal Ao peak velocity (mm/s)</th>
<th>Isovolumic Relaxation Time (msec)</th>
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<tbody>
<tr>
<td><strong>WT</strong></td>
<td>1.969 ± 0.057</td>
<td>42.1% ± 1.7</td>
<td>103 ± 8</td>
<td>53 ± 8</td>
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<tr>
<td><strong>TAZKD</strong></td>
<td>1.832 ± 0.072</td>
<td>45.5% ± 1.3</td>
<td>78* ± 8</td>
<td>42 ± 9</td>
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* *p < 0.05

### Newborn Mice (few hours old)

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<th>End-diastolic Area (LV only) (mm²)</th>
<th>Fractional Area Shortening</th>
<th>LV diastolic wall thickness (mm)</th>
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<tr>
<td><strong>WT</strong></td>
<td>1.413 ± 0.070</td>
<td>50.8% ± 1.4</td>
<td>0.26 ± 0.01</td>
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<td><strong>TAZKD</strong></td>
<td>1.375 ± 0.058</td>
<td>49.4% ± 1.1</td>
<td>0.26 ± 0.01</td>
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</table>

*No significant differences in any indices of heart size or function*
Cardiolipin biochemistry is altered

Claypool & Koehler, *TiBS* 2011
Mitochondria are abnormal: E13.5
Mitochondria-myofibril alignment

Ong, *Cardiovasc Res* 2010

Newborn myocardium
Abnormal mitochondrial morphometrics

A

B

C

D

E

F

G

H

I

NYU School of Medicine
NYU Langone Medical Center
Cardiomyocytes: Less differentiated?

Figure 8. Representative EM’s from 2 control and 2 E13.5 TAZKD embryos suggest TAZKD cardiomyocytes are less well-differentiated: myofibrils are lacking in Z-bands (arrows in control mice) and appear less well-organized.
Developmental window of noncompaction

Induced at E10.5
Abnormal cellular proliferation

Figure 7. Phosphohistone-H3 (PHH3) immunofluorescence staining of representative E13.5 WT and TAZKD left ventricular sections. Left panels: PHH3, single-channel; right panels: PHH3 (green) merged with DAPI (blue) and troponin (red). Differential cardiomyocyte proliferation in trabecular and compact layers is evident.
Microarray data: E12.5 myocardium

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<th>DAVID GO Terms</th>
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<th>Up/Down</th>
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<td>Metal ion binding, zinc finger</td>
<td>1.6-5.6</td>
<td>Up/Down</td>
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<td>Steroid hormone, nuclear hormone receptor</td>
<td>3.5</td>
<td>Down</td>
</tr>
<tr>
<td>Synaptic transmission, neurotransmitter, neuron</td>
<td>2.7-3.5</td>
<td>Down</td>
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<tr>
<td>Protein dimerization, protein binding</td>
<td>2.7</td>
<td>Up</td>
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<tr>
<td>Apoptosis, programmed cell death</td>
<td>2.6</td>
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<td>DNA binding, transcription, regulation of RNA</td>
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<tr>
<td>metabolic process</td>
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<tr>
<td>Membrane glycoprotein</td>
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<td>Down</td>
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<tr>
<td>Cell adhesion, cell-cell adhesion</td>
<td>2.0</td>
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<td>Cell morphogenesis, neuron morphogenesis</td>
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Role of reactive oxygen species (ROS)?
Review

Mitochondria and calcium signaling in embryonic development

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The Permeability Transition Pore Controls Cardiac Mitochondrial Maturation and Myocyte Differentiation

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DOI 10.1016/j.devcel.2011.08.008

SUMMARY

Although mature myocytes rely on mitochondria as the primary source of energy, the role of mitochondria in the developing heart is not well known. Here, we find that closure of the mitochondrial permeability transition pore (mPTP) drives maturation of mitochondrial structure and function and myocyte differ-

that mitochondria are important to the development of the heart, as dysfunction of the mitochondrial electron transport chain (ETC) can cause heart malformation and embryonic death between E8.5 and E10.5, suggesting that mitochondrial function is essential to cardiac function and survival of the embryo (Ingram et al., 2009; Larsson et al., 1998).

Mitochondria in the adult heart are well characterized and occupy over 30% of the cell volume. It is thought that complex
Increased ROS: BTHS, TAZKO cells

Figure 2. MitoSOX™ Red shows increased mitochondrial ROS in fibroblasts from Barth syndrome patients (BTHS, bottom) vs. controls (top). Cells were plated at comparable density.

Figure 3. A, B) Mouse embryoid-derived fibroblast-like cells in which tafazzin was knocked out showed a 2 to 3-fold increase in mitochondrial superoxide in TAZ cells over wildtype (WT). (ESC's courtesy of Dr. Zaza Khuchua; Acehan 2009)
Figure 4. DCF staining shows increased ROS in E12.5 ventricular cardiomyocytes from tafazzin-knockdown (TAZKD, left) vs. wildtype (WT, right). Two representative regions of interest from each plate are shown. Cells were plated at comparable density.
Figure 5. MitoSOX™ Red shows increased mitochondrial ROS in E18.5 taz-knockdown (TAZKD, left panels) ventricular cardiomyocytes vs. wildtype (WT, right panels). Cells were plated at comparable density.
N-acetylcysteine

Berk M. TiPS 2008
Figure 6. N-acetylcysteine (NAC) partially rescues the HT-NC phenotype in TAZKD newborn mice. A) Wildtype; B) TAZKD with HT-NC and ventricular septal defects. C, D) Newborns of pregnant mothers fed NAC: (C) Wildtype and (D) TAZKD. (A & B adapted from Phoon 2012)
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<th>Genes Associated with Human LV Noncompaction</th>
<th>Genes Related to Compact Zone &amp; Trabecular Formation, &amp; Cell Cycle Control, Animal Models</th>
<th>Transcriptional Regulators, Factors</th>
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<td>Sp2, Sp6</td>
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**Table 1**
Figure 1

A

ROS

↓ taz

ΔCL

↑ ROS

mPTP

B

Trabecular layer

Δtranscription

Nucleus

C

Hypertrabeculation

D

Compact zone layer

Δtranscription

Nucleus

Thin compact zone
Conclusions

- The TAZKD mouse is a good model for human BTHS.
  - Ventricular hypertrabeculation-noncompaction
  - Myocardial wall thinning
  - Abnormal mitochondrial morphometrics
  - Abnormal mitochondrial functioning: ROS
- Tafazzin knockdown in embryonic vs. adult hearts indicates entirely different roles for mitochondria.
- Mitochondria & heart development: an emerging field
  - Myocardial patterning: possible role of mito-ROS
  - Not just bioenergetics!
Future directions

- How does cardiolipin contribute to mitochondrial development & normal cardiac myoarchitecture?
  - Cell cycling pathways
  - ROS, Ca$^{2+}$ homeostasis, ECM, cell adhesion, cytoskeleton

![Diagram]

- Altered cardiomyocyte cellular proliferation
- Altered cardiomyocyte differentiation

Myocardial hypertrabeculation-noncompaction
# Acknowledgments

<table>
<thead>
<tr>
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<th>Schlame Lab</th>
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<tr>
<td>Nitya Viswanathan, MD</td>
<td>Michael Schlame, MD</td>
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