Marfan Foundation Annual Conference 2017
Research Update

“Listening to Nature’s Cues”

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Marfan syndrome

Fibrillin-1

Microfibrils (fibrillin-1)

Elastic fiber

Fibrillin-1 Mutations Lead to Excessive TGFβ Signaling in MFS

Excessive TGFβ Activation

- LAP
- LAP
- TGFβ
- Latent Complex

Microfibrils composed of Fibrillin-1

Excessive TGFβ Signaling

- TGFβ
- Smad2/3
- pSmad2/3
- Smad4
- pSmad2/3

Phenotypic Consequences

- Emphysema
- Aortic Aneurysm
- Mitral Valve Prolapse
- Myopathy

(All rescued by systemic Rx of MFS mice with TGFβ-neutralizing antibody)

- Judge et al. JCI, 2004
- Ng et al. JCI, 2004
The Angiotensin II Type 1 Receptor Blocker (ARB) Losartan

AngI \rightarrow \text{Losartan} \rightarrow \text{AngII}

TGF \rightarrow \text{TGF receptors}

AT1 \rightarrow \text{TGF signaling}

AT2 \rightarrow \text{TSP-1}

Proliferation, Apoptosis, Fibrosis, MMP2, MMP9

Aortic Root Growth

Wild-type n = 11
Placebo n = 10
Propranolol n = 7
Losartan n = 5

C1039G/+ Wild-type

C1039G/+ Placebo

C1039G/+ Propranolol

C1039G/+ Losartan

Habashi...and Dietz, Science, 2006
Lacro et al. NEJM, 2014 (mean z=4.0)

Atenolol
(mean 2.8; up to 4.0mg/kg/day)  +0.069cm/yr  -0.14z/yr

Losartan
(mean 1.2; up to 1.4mg/kg/day)  +0.075cm/yr  -0.11z/yr

Untreated pediatric patients:
Ladoucer et al., 2007  +0.11cm/yr
Rossi-Foulkes et al., 1999  +0.18cm/yr
Tahernia et al., 1993  +0.16cm/yr
Salim et al. 1994  +0.21cm/yr

Treated with typical atenolol dosing:
Brooke et al., 2008 (mean z=3.25)  +0.17cm/yr  +0.24z/yr
Brooke et al., 2008 (mean z=7.21)  +0.35cm/yr  +0.46z/yr
Ao Root Growth (mm / 6 months)

WT

Placebo

n=11

n=16

MFS

<0.001

<0.05

<0.001

<0.00001
Canonical TGFβ Signaling

Noncanonical TGFβ Signaling (MAPK)
Activation of ERK MAPK in Marfan Mice

Pregnant women with MFS or LDS have an elevated risk of aortic tear. While this risk has historically been attributed to hemodynamic stress, the vast majority of dissections occur within the weeks after delivery, and this risk is not altered by Cesarean section or antihypertensive agents.

What initiates toward the end of pregnancy, is maintained after delivery and might synergize with pathogenic events previously defined for aneurysm and dissection? Oxytocin

- needed to initiate uterine contraction and milk letdown
- release peaks at the end of pregnancy and is sustained during breast feeding
- receptor upregulated in the aorta in response to estrogen and pregnancy
- mediates its effects on peripheral tissues through ERK1/2 activation

Habashi et al. unpublished
Profund Protection from Postpartum Aortic Dissection Simply by Removing Pups at Birth (and Prevention of Lactation-Induced Oxytocin Release)

From 4% to 70% survival simply by removing pups at birth (eliminating lactation-induced oxytocin release)

Habashi et al. unpublished
Administration of an Oxytocin-Blocking Drug in the 3rd Trimester and After Birth Provides Full Protection from Aortic Dissection (Despite Retained Ability to Both Deliver Pups and Breast Feed)

desGly-NH2-d(CH2)5[D-Tyr2, Thr4]OVT
Oxytocin antagonist
(150X greater potency for the oxytocin vs. vasopressin receptor)

Habashi et al. unpublished
Predisposition for Pregnancy-Associated Aortic Dissection is Proportional to ERK Activation

Habashi et al. unpublished

Trametinib – FDA approved ERKi

Percent Survival

P<0.0001

Postpartum Day

mgR-/-preg (n=45)
mgR-/-preg, ERKi (n=20)
How does nature modify Marfan syndrome?

Five exceptional families with defined \( FBN1 \) mutation showing discrete intrafamilial variation in phenotypic severity were subjected to linkage analysis to map a protective modifier locus for Marfan syndrome.

32 genes in the critical interval

\( MAP3K4 \) (encodes a MAPK kinase – the kind of protein that activates ERK)

Alex Doyle
(w/ Bart Loeys, Julie DeBacker and Anne De Paepe)
Protection from Vascular Disease in MFS Associates with Reduced Expression of MAP3K4 in Cultured Dermal Fibroblasts
Map3k4 haploinsufficiency abrogates abnormal aortic root growth in a mouse models of MFS.

A comparison of AoR growth (mm/8mo) between WT, M3K4+/−, MFS, and MFS M3K4+/− shows a significant difference in the M3K4+/− and MFS groups compared to WT and MFS M3K4+/− groups. The p-values for MAP3K4, pERK1/2, pp38, and pJNK1/2 are marked as <0.05 and <0.01, indicating statistical significance. β-Actin was used as a loading control.
Definition of an Axis for Vascular Disease in Marfan Syndrome

Angiotensin-II

Losartan → AT1R

AT1R → DAG, IP3 → PKC → pERK1/2 (+/- pJNK, pp38)

NAb → TGFβ → PLC

AT2R → OXTR blocker → AT2KO

OXTR → Oxytocin

Hydralazine

Enzastaurin

RDEA119/ MAP3K4i → pERK1/2 (+/- pJNK, pp38)

Target Genes

Red: Worsens Aneurysm (3)  Blue: Prevents Aneurysm (7)
Modification of Marfan Syndrome in Mice:
The C57BL/6J (BL6) mouse background is protected
The Sv129 mouse background shows accelerated vascular disease

AR Growth: 2-6mo (mm)

% Surviving

Blood Pressure (mmHg)

Pulse Rate (bpm)
The predisposition imposed by the Sv129 background remains responsive to both losartan and ERK1/2 inhibition (RDEA-119).
Smad/PKC/ERK Activation is Increased in the Aortic Root of Marfan Mice on a Sv129 Background

Doyle & Doyle et al. unpublished
Kyphosis Index (mm)

Wild Type

Marfan

WT BL6                WT 129             Marfan BL6

Marfan 129

<0.00001

<0.0001

<0.05

NS

WT BL6 129 BL6 129
Pronounced epistasis – LOD=12.8 when 2 loci are considered in combination
**Modifier Candidate Genes:**
Theme – integration of Ca\(^{+2} /\) TGFβ / MAPK signaling

<table>
<thead>
<tr>
<th>Genes of interest – Mouse Chromosome 5:</th>
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<tbody>
<tr>
<td><strong>Rabgef1</strong></td>
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<tr>
<td><strong>Rasa4</strong></td>
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<td><strong>Mmp17</strong></td>
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<table>
<thead>
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<th>Genes of interest – Mouse Chromosome 11</th>
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<tr>
<td><strong>Bptf</strong></td>
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<tr>
<td><strong>Prkca</strong></td>
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<tr>
<td><strong>Map2k6</strong></td>
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Dosage of knockout alleles

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<th>Total</th>
<th>0</th>
<th>0</th>
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<th>1</th>
<th>2</th>
<th>4</th>
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<tbody>
<tr>
<td>Map2k6</td>
<td>129</td>
<td>+/+</td>
<td>+/-</td>
<td>+/+</td>
<td>+/-</td>
<td>-/</td>
<td>BL6</td>
</tr>
<tr>
<td>Mmp17</td>
<td>129</td>
<td>+/+</td>
<td>+/+</td>
<td>+/-</td>
<td>+/-</td>
<td>-/</td>
<td>BL6</td>
</tr>
</tbody>
</table>

Aortic Root at 2mo (mm)

- Pure 129
- Mixed
- Pure BL6

MFS

Total dosage:
- 0
- 0
- 1
- 1
- 2
- 4
- 4

Means ± SD

Statistical significance:
- NS
- <0.01
Taken together, these data suggest that natural genetic variation that attenuates TGFβ and/or ERK1/2 activation in both people and mice with Marfan syndrome has the ability to protect from vascular disease.

Drugs that mimic nature’s strategies represent novel and potentially potent therapeutic options for the care of people with Marfan syndrome.
Optimistic prospects for disease treatment

Weak tissues $\rightarrow$ Obligate Tissue Failure

TGFβ antagonists
AT1 antagonists (ARBs)
ERK antagonists
Oxytocin antagonists
PLC/IP3/PKC antagonists
MAP3K4 antagonists
MAP2K6 antagonists
p300 inhibitors
EGFR antagonists
Dietz lab:

Hamza Aziz
James Beckett
Ben Brooke
Juan Calderon
Sara Cooke
Alex Doyle
Jef Doyle
Elena Gallo
Russell Gould
Jennifer Habashi
Tammy Holm
Adam Johnson
Dan Judge
Ben Kang

Mark Lindsay
David Loch
Bart Loeys
Javid Moslehi
Sarah Parker
Rosanne Rouf
Joseph Shin
Robert Wardlow
Nicole Wilson

Bart Loeys
Checco Ramirez
Jason Cooke
Dan Rifkin
Anne De Paepe
Julie De Backer
Jennifer van Eyk
Dave Huso

GenTAC Investigators
MIBAVA Investigators

William S. Smilow Center
For Marfan Syndrome Research