A Novel LNK Between Inflammation, Hypertension, & Aortic Dissection

Meena S. Madhur, MD, PhD
Assistant Professor
Department of Medicine
Department of Molecular Physiology
Vanderbilt University
Nashville, TN
What do these people have in common?

Lucille Ball (1911-1989)
Alan Thicke (1947-2016)
What do these people have in common?

They all died from an acute aortic dissection or rupture.

Schematic of the thoracic and abdominal aorta (left) and an aortic dissection (right). Source: https://medlineplus.gov/ency/article/000181.htm
Aortic dissection (AD) **is a tear in the inner layer of the aortic wall**, which allows blood to enter into the wall of the aorta, creating a new passage for blood, known as the “false lumen.”

AD weakens the aortic wall → **Aortic rupture**

**RISK FACTORS**

- Long standing arterial **hypertension**, atherosclerosis
- **Vascular inflammation**
- **Heritable connective tissue disorders** (Marfan, Ehlers-Danlos and Loeys-Dietz syndrome)
Numerous GWAS have identified single nucleotide polymorphisms in LNK that are associated with autoimmune and cardiovascular disorders:
What is LNK (or SH2B3)?

- Member of the SH2B family of intracellular adaptor proteins – proteins without enzymatic function that serve as molecular platforms for the coordination of signaling events.

The PH domain is thought to target LNK to the cell membrane.

The SH2 domain serves to target kinases to their substrates.

How does LNK work?

- Primarily expressed in **hematopoietic cells** and **endothelial cells** where it functions as a **negative** regulator of cytokine signaling and cell proliferation.
A Link Between LNK and Hypertension

Lymphocyte adaptor protein LNK deficiency exacerbates hypertension and end-organ inflammation

Mohamed A. Saleh,1,2 William G. McMaster,1 Jing Wu,1 Allison E. Norlander,1 Samuel A. Funt,3,4 Salim R. Thabet,1,3 Annet Kirabo,1 Liang Xiao,1 Wei Chen,1 Hana A. Itani,1 Danielle Michell,1 Tianxiao Huan,5,6 Yahua Zhang,1 Satoshi Takaki,7 Jens Titze,1 Daniel Levy,5,6 David G. Harrison,1 and Meena S. Madhur1

Saleh et al. JCI 2015
A Link Between LNK and Hypertension

Ang II: 140 ng/kg/min:

Ang II: 490 ng/kg/min:

Saleh et al. *JCI* 2015
What is the impact of LNK deletion on the development of aortic dissection?

Angiotensin II infusion
1200 ng/kg/min

WT and LNK Knockout mice

14 days
No Difference in BP at this dose of Ang II

Ang II: 1200 ng/kg/min:
LNK Deficiency Promotes Aortic Dissection/Rupture

LNK−/− mice

Suprarenal segment of abdominal aorta

![Image of LNK−/− mice with arrow pointing to suprarenal segment of abdominal aorta]

Graph showing percent event-free survival over days elapsed:
- WT (black line)
- LNK−/− (red line)

Significance level: **
LNK Deficiency Promotes Aortic Dissection/Rupture

WT Ang II

LNK−/− Ang II

![Graph showing experimental results](graph.png)

Graph legend:
- WT
- LNK−/−
After 3 days of Ang II infusion, the SAA from LNK<sup>-/-</sup> mice display a predisposition to failure and a decrease in energy storage capacity:

**Collaboration**
Humphrey J.D., Bersi M.R. and Korneva A.
Yale School of Engineering and Applied Sciences

**Causes:**
- Decreased Elastin Integrity
- Increased Collagen

ATA: ascending thoracic aorta, DTA: descending thoracic aorta, SAA suprarenal abdominal aorta, IAA: infra-renal abdominal aorta

#P<0.05 WT Ang II vs LNK<sup>-/-</sup> Ang II
LNK Deficiency Results in Increased Elastin Fragmentation

Verhoeff van Gieson staining

**P<0.01 WT Ang II vs LNK<sup>−/−</sup> Ang II, n=4-6**
LNK Deficiency Results in Reduced Collagen Content

WT Ang II

LNK^{-/-} Ang II

Abdominal aorta

Picrosirius red staining

**P<0.01 WT Ang II vs LNK^{-/-} Ang II, n=4-6
LNK Deficiency Results in Collagen Thinning

Keen A.N. et al. JEB 2017

Thick collagen

PSR staining under polarized light

Thin collagen

WT Sham

LNK-/- Sham

Percentage

n=6

Red
Orange
Yellow
Green

n=6

Red
Orange
Yellow
Green

x40
LNK Deficiency Results in Collagen Thinning

Thick collagen

Thin collagen

PSR staining under polarized light

WT Ang II

LNK⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻{-

Percentage

Keen A.N. et al. JEB 2017

P < 0.001
P < 0.001
P < 0.01
P < 0.05

x40
LNK Deficiency Results in Increased MMP Expression

MMP: Matrix metalloproteinases

*P<0.05, WT Ang II vs LNK<sup>-/-</sup> Ang II, n=7-8
LNK Deficiency Results in Increased Vascular Inflammation

Driven primarily by innate immune cells

#P<0.05, ##P<0.01 WT Ang II vs LNK⁻/⁻ Ang II
LNK Deficiency Results in Increased Vascular Inflammation

WT Ang II

Neutrophils

Macrophages

LNK\(^{-/-}\) Ang II

Neutrophils
Two most enriched categories: hematopoietic cell lineage and natural killer (NK) cell mediated cytotoxicity.

RNA Sequencing Analysis

VANTAGE: Vanderbilt Technologies for Advanced Genomics

Changed by at least 1.5 fold and statistically significant
LNK Deficiency Results in Increased Vascular Inflammation

Natural Killer cell:

Source: Jordan Orange (Childrens Hosp of Philadelphia)

### P<0.01 WT Ang II vs LNK⁻/⁻ Ang II
Conclusions and Future Directions

Loss of LNK

- Aortic Inflammation (Innate Immune Cells)
- Matrix Metalloproteinases 2, 9, and 12
- Elastin Integrity
- Collagen Thickness

Understanding the pathophysiology of aortic dissection and rupture will likely lead to new therapeutic targets for this fatal disease.

Acute Aortic Dissection, Rupture, and Death
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