Neonatal-lethal dilated cardiomyopathy due to a homozygous *LMOD2* donor splice-site variant.

Dr. Michaela Yuen PhD[1,2*], Dr. Lisa Worgan MD[3*], Miss. Jessika Iwanski MS[4], Dr. Christopher T. Pappas PhD[4,5], Mr. Himanshu Joshi[1], Dr. Jared M. Churko PhD[4,5], Dr. Susan Arbuckle MD[6], Prof. Edwin Kirk MD[7,8,9], Dr. Tony Roscioli FRACP, PhD[7,9], Dr. Carol C. Gregorio PhD[4,5,#], Prof. Sandra T. Cooper PhD[1, 2,10,#]

**,# these authors contributed equally*

[1]Kids Neuroscience Centre, The Children's Hospital at Westmead, Australia; [2]Discipline of Child and Adolescent Health, Sydney Medical School, University of Sydney, Australia; [3]Department of Medical Genomics, Royal Prince Alfred Hospital, Australia; [4]Department of Cellular and Molecular Medicine, and Sarver Molecular Cardiovascular Research Program, The University of Arizona, AZ, United States; [5]Department of Histopathology, The Children's Hospital at Westmead, Australia; [6]New South Wales Health Pathology Randwick Genomics Laboratory, Australia; [7]School of Women's and Children's Health, University of New South Wales, Australia; [8]Centre for Clinical Genetics, Sydney Children's Hospital, Australia; [9]The Children's Medical Research Institute, Australia

Introduction

Inherited dilated cardiomyopathy is characterised by enlargement of the heart, ventricular dilation and normal or reduced thickness of the left ventricular wall (**Figure 1**). Patients display impaired ventricular contractility and commonly progressive heart disease which may result in heart failure if left untreated.[1] Inherited dilated cardiomyopathy has been associated with mutations in >50 genes, many genes encode for structural proteins of the cardiac sarcomere (e.g. *TTN*, *MYH7*, *TNNT2*, *MYBPC3*).[2,3]

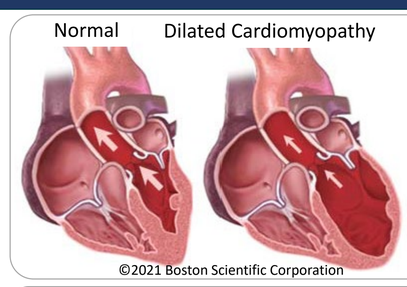

Figure 1:
Structural changes to the heart in dilated Cardiomyopathy:
- enlargement,
- ventricular dilation,
- reduced left ventricular wall thickness

LMOD2 encodes leiomodin-2 (LMOD2), an actin filament associated protein. LMOD2 binds the pointed-end of the sarcomeric thin filament and regulates thin filament length in chicken, mouse and human cardiomyocytes.[4-7]

Recently, a single patient with homozygous nonsense variant in *LMOD2* (c.1193G>A, p.Trp398*) has been identified, suggesting *LMOD2* may be a novel dilated cardiomyopathy disease gene.[4]

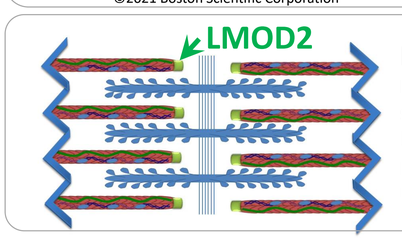

Figure 2:
LMOD2 is a protein of the cardiac sarcomere
LMOD2 localises to the thin filament pointed ends where it is involved in regulating thin filament length.

An *LMOD2* splice-site variant associated with neonatal heart failure

Here we report two siblings (III:3 and 4) which died shortly after birth due to heart failure (Pedigree in **Figure 3**).

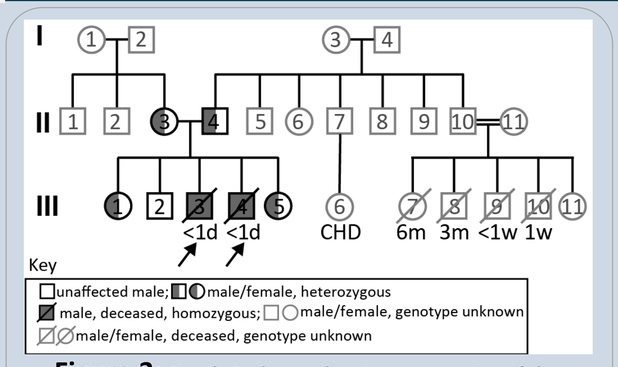

Figure 3: **Family Pedigree showing segregation of the LMOD2 splice-site variant**

Cardiac ultrasound and autopsy (proband III:4) were consistent with dilated cardiomyopathy (showing bi-ventricular dilation and cardiac enlargement, **Figure 4**).

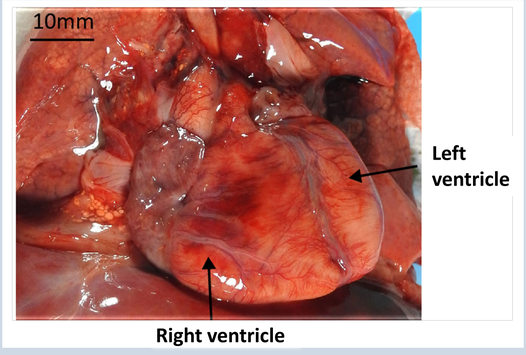

Figure 4: **Anterior view of the heart of III:4 in situ demonstrates enlargement of the heart**

Exome sequencing identified a segregating homozygous LMOD2 variant ablating the donor 5' splice-site of intron 1

((GRCh37)chr7:g.123296291G>A; NM_207163.2:c.273+1G>A).

In silico splicing analysis (Alamut Visual® and SpliceAI) suggests this variant abolishes the 5' splice-site of intron 1 (**Figure 5**).

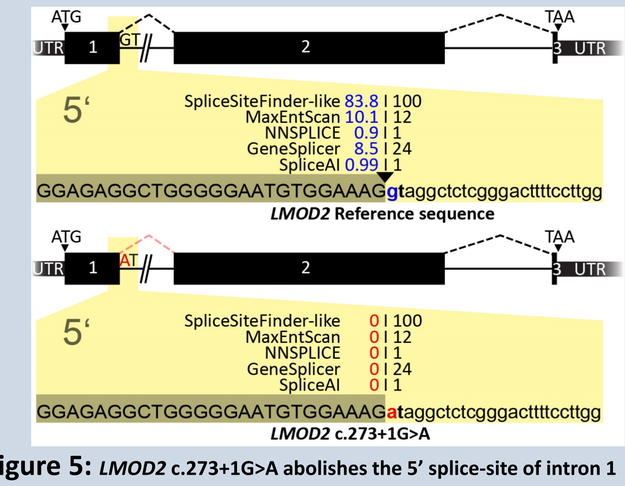

Figure 5: ***LMOD2* c.273+1G>A abolishes the 5' splice-site of intron 1**
A splice-site is located before the affected nucleotide (reference sequence splicing scores in blue, maximum score in black). The *LMOD2* c.273+1G>A variant ablates this 5' splice-site (variant shown in red at bottom, along with prediction scores of 0 in red).

Consistent with previous reports of LMOD2s role as a regulator of thin filament length, thin filaments were significantly shortened in cardiac muscle of proband III:4 (**Figure 6**).

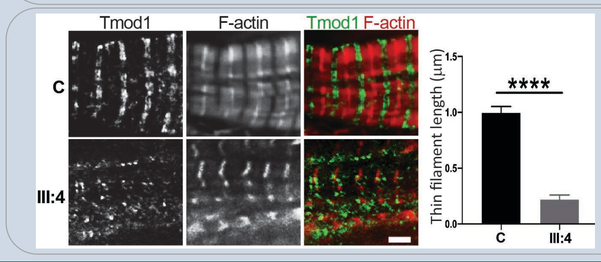

Figure 6:
Thin filaments are shorter in the heart muscle of proband III:4

LMOD2 c.273+1G>A - loss of correctly spliced *LMOD2* transcript and LMOD2 protein

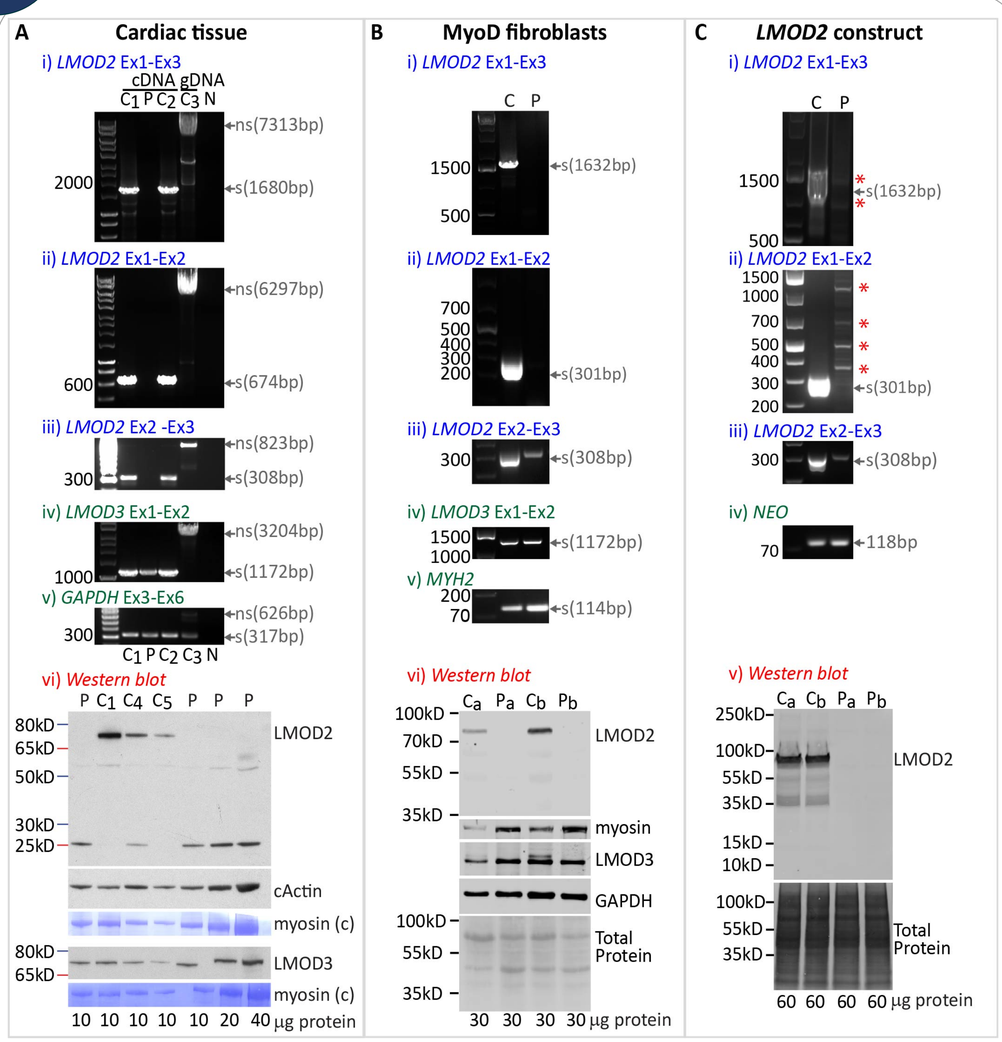

Figure 7: **Reverse-transcription PCR and Western blot show *LMOD2* c.273+1G>A a lack of transcripts or mis-spliced transcripts in proband III:4 cardiac tissue, proband III:4 MyoD-transdifferentiated skin fibroblasts and *LMOD2* gene-construct transfected HEK293 cells.**

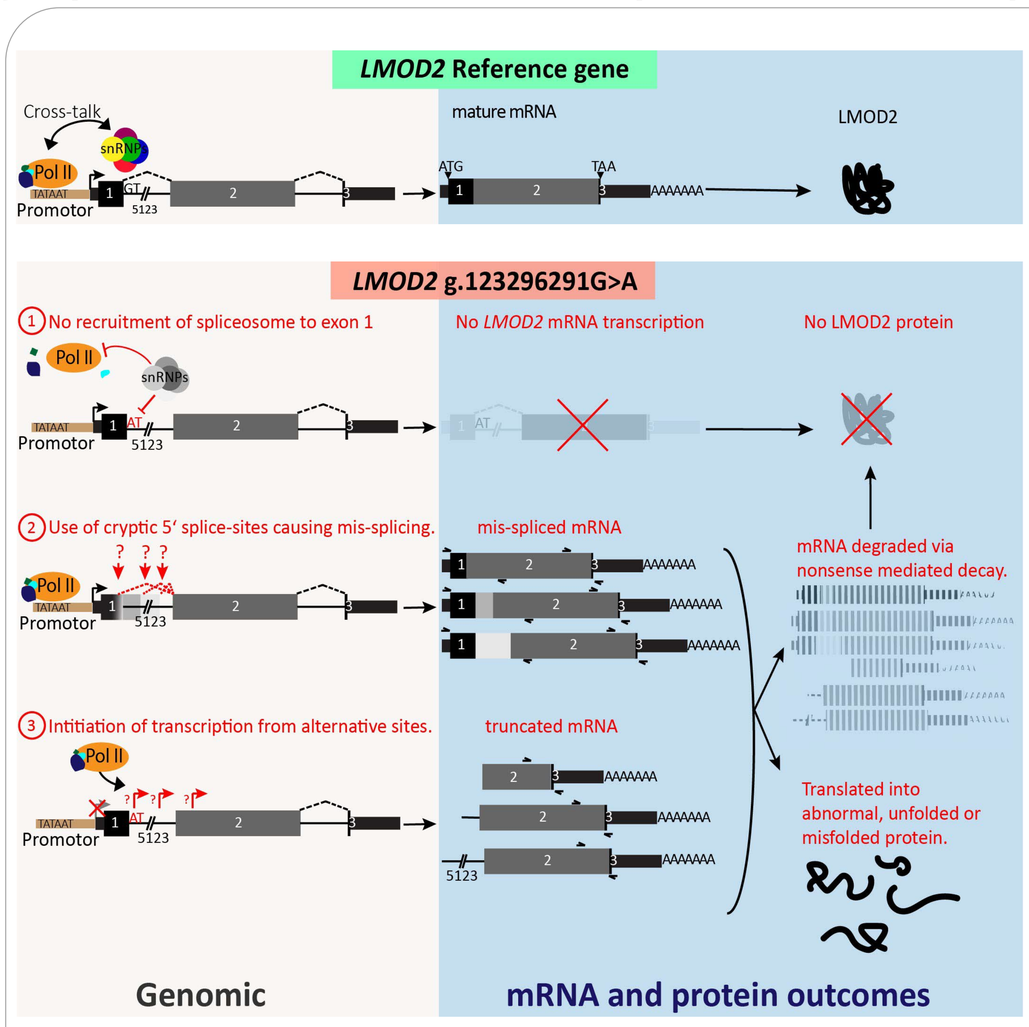

Figure 8: **Proposed pathogenic mechanism resulting in loss of transcripts and protein from LMOD2 g.123296291 G>A**

Potential effects of Intron 1 5' splice-site mutagenesis are:

(1) The splicosome does not recognise and assemble on the mutated splice-site resulting in loss of recruitment of the transcription machinery and no *LMOD2* transcripts.

(2) Transcription machinery transcribes LMOD2 pre-mRNA and transcripts are spliced at alternative (cryptic) splice-sites (indicated by ?). Mis-spliced mRNA transcripts are either removed via nonsense-mediated decay or result in frame-shift/truncated or otherwise abnormal protein.

(3) The transcription machinery is recruited to an alternative transcription start site. This results in start-loss transcripts which are either targeted by nonsense-mediated decay or result in aberrant protein similar to mechanism (2).

Conclusion: *LMOD2* c.273+1G>A splice-site variant causes dilated cardiomyopathy by abolishing LMOD2 protein expression resulting in thin filament shortening and contractile dysfunction.

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Michaela Yuen I Postdoctoral Research Fellow
Kids Neuroscience Center I Kids Research Institute
email: michaela.kreissl@sydney.edu.au
http://www.kidsneuroscience.org.au/