Genetics of hypoplastic left heart syndrome: New insights

Paul Grossfeld, M.D.

Professor of Pediatrics

UCSD School of Medicine/RCHSD

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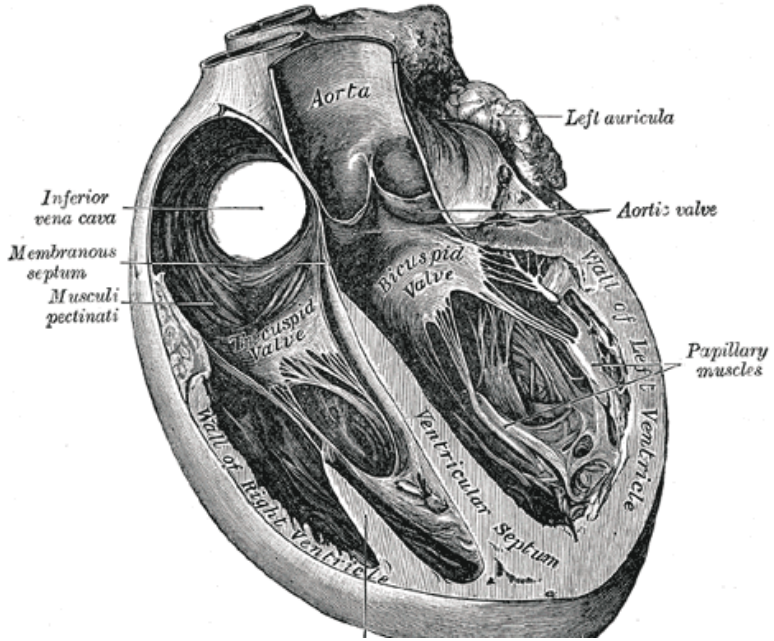
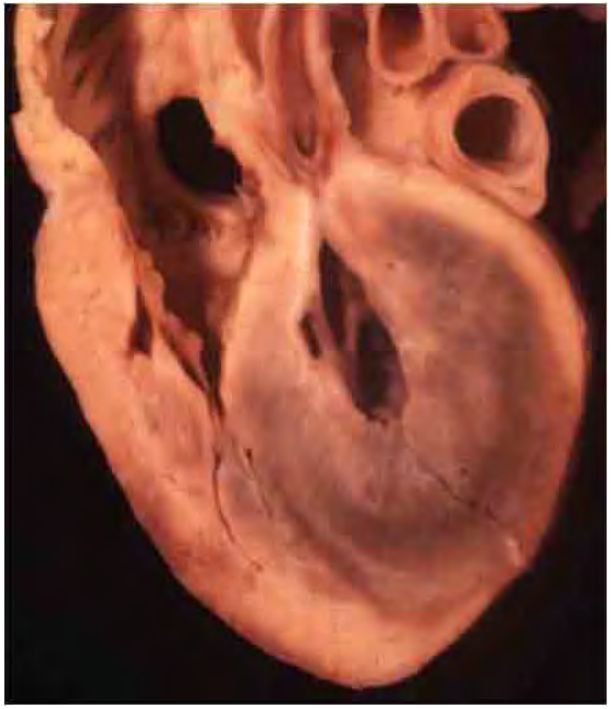
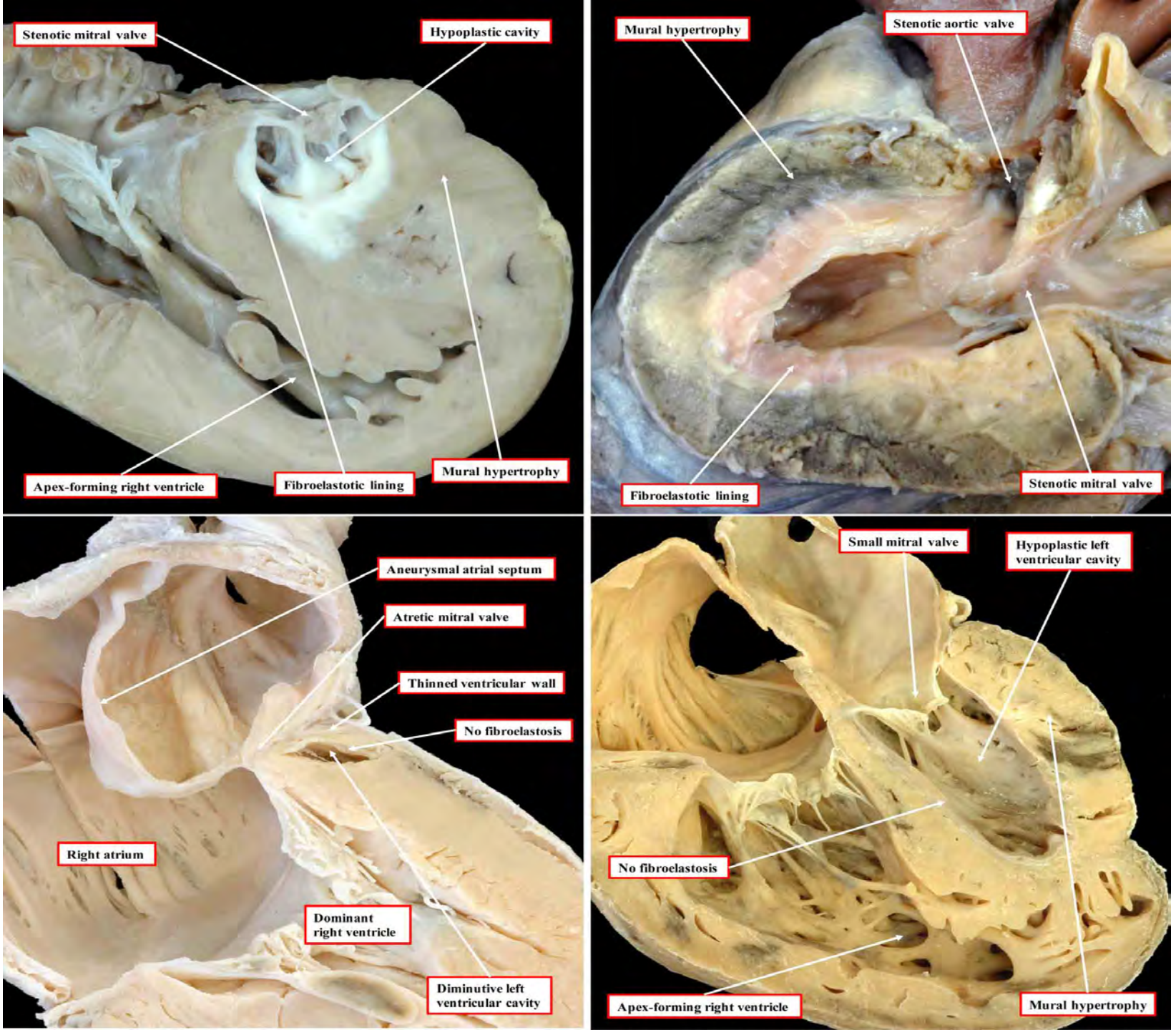
Overview

I). Evidence for a genetic etiology

II). New insights into the mechanisms underlying HLHS


Aorta
Left auricula
Inferior vena cava
Aortic valve
Membranous septum
Musculi pectinati
Bicuspid Valve
Tricuspid Valve
Wall of Left Ventricle
Papillary muscles
Ventricular Septum
Wall of Right Ventricle




Stenotic mitral valve
Hypoplastic cavity
Apex-forming right ventricle
Fibroelastotic lining
Mural hypertrophy
Mural hypertrophy
Stenotic aortic valve
Fibroelastotic lining
Stenotic mitral valve
Aneurysmal atrial septum
Atretic mitral valve
Thinned ventricular wall
No fibroelastosis
Right atrium
Dominant right ventricle
Diminutive left ventricular cavity
Small mitral valve
Hypoplastic left ventricular cavity
No fibroelastosis
Apex-forming right ventricle
Mural hypertrophy

Evidence for a genetic etiology

HLHS in Twins

Concordance for hypoplastic left heart syndrome in a monochorionic twin pregnancy.

Andrews RE, Cook AC, Yates RW. Heart. 2003 Apr;89(4):e13.


ND IN H

Syndromes Associated with Hypoplastic Left Heart

SYNDROME/LOCUS	GENE
Trisomy 13	?
Trisomy 18	?
XO (Turner)	?
Smith-Lemli-Opitz(11q21)	Delta7 Sterol Reductase
Holt-Oram (12q12)	TBX-5
6q-	Connexin 43(?)
8q-	?
DiGeorge (22q11)	TBX-1, CRKL
Fryn's	?
Palister-Hall	Gli-3
Holsgreve	?
Apert	?
11q-	**ETS-1**

Evidence for a genetic etiology of hypoplastic left heart syndrome

Prevalence of congenital cardiovascular malformations among relatives of infants with hypoplastic left heart, coarctation of the aorta, and d-transposition of the great arteries.

Loffredo CA, Chokkalingam A, Sill AM, Boughman JA, Clark EB, Scheel J, Brenner JI.

Am J Med Genet. 2004 Jan 30;124A(3):225-30.

1). **19%** of all first degree relatives, and **30%** of siblings of HLHS patients have heart defects. Inheritance can be autosomal recessive or dominant. Recurrence rate can be as high as **50%.**

2). When a parent had a heart defect, it was **THREE** times more likely to be the father.

Heart Defects in First Degree Relatives of Patients with HLHS

BAV/AS	54%
HLHS*	12%
Coarctation	12%
TOF, VSD, ASD, PDA	23%

*3.5% recurrence rate

Ascending aorta dilation in first degree relatives

- Mayo group has identified a high frequency of dilation of the ascending aorta in parents of patients with HLHS (suggestive of a neural crest cell defect)

Key Points:

- 1). There is strong evidence for a genetic etiology for HLHS
- 2). A mutation in a single gene may give rise to the full spectrum of left-sided heart defects, suggesting there are modifying factors (genetic, epigenetic)
- *3). HLHS is a “polygenic” disease

Human HLHS Genes: Only account for about 10% of total cases

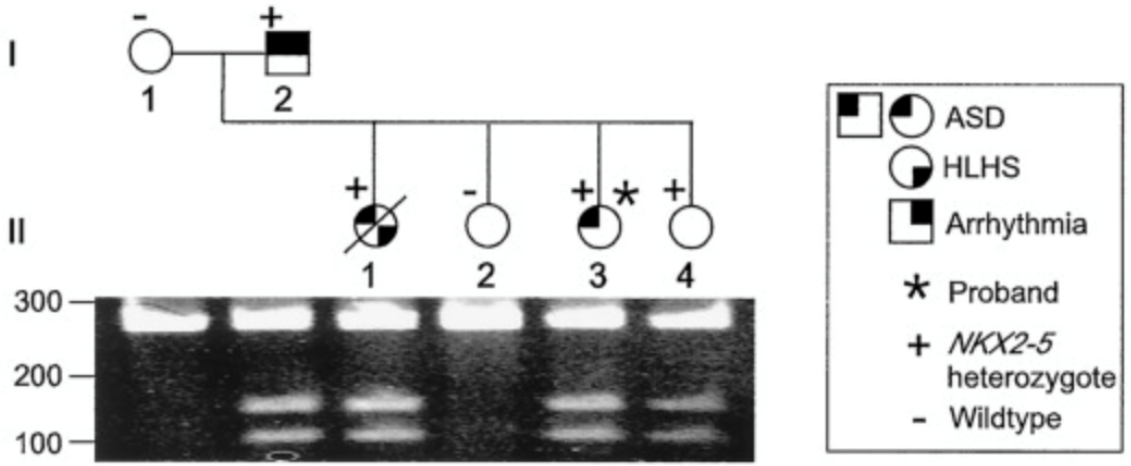
- NKX2-5
- NOTCH1
- ETS1
- rbFOX2

Mayo: WGS on ~120 HLHS families

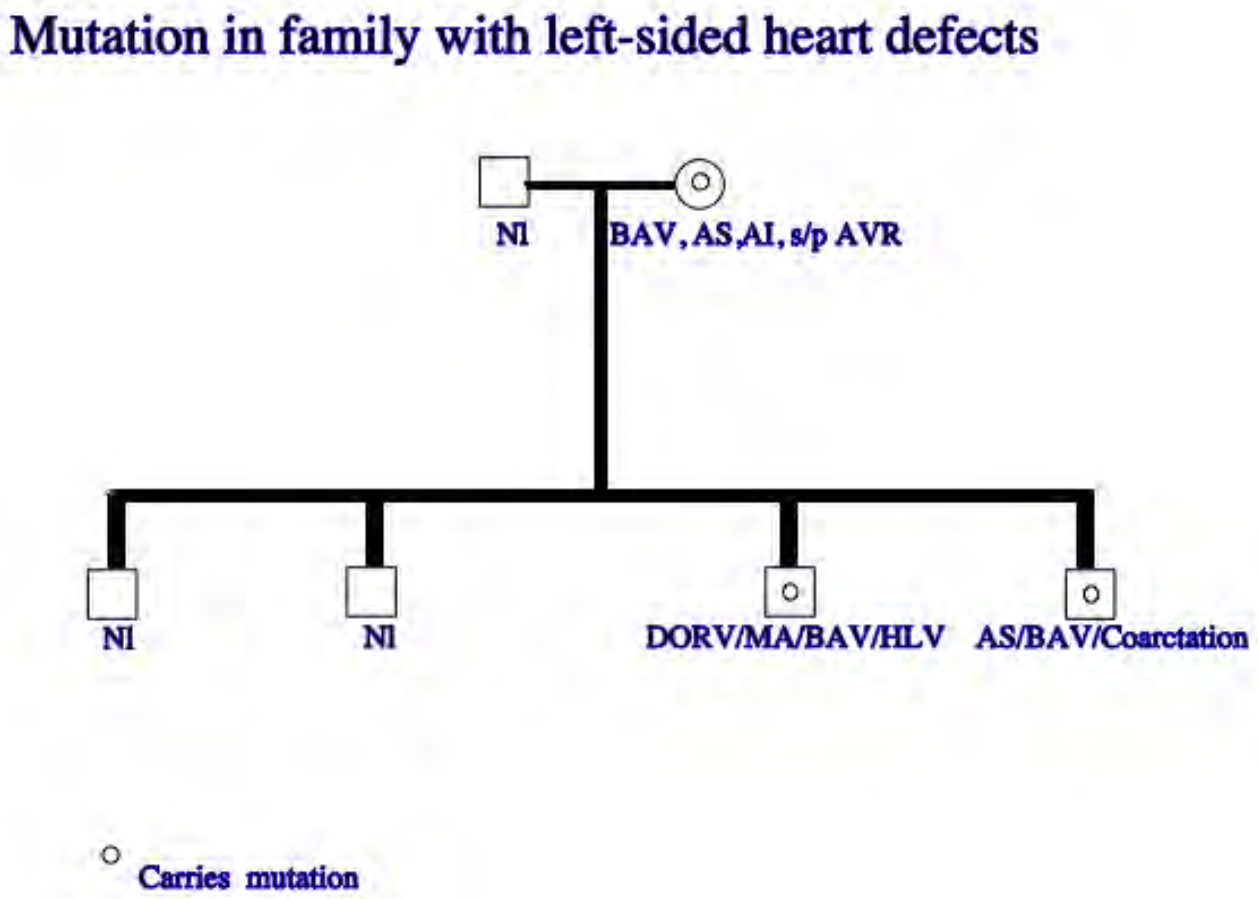
- Very few obvious single gene causes, consistent with a “multi-hit” model
- Notch signaling defects in multiple families

Cardiac homeobox gene NKX2-5 mutations and congenital heart disease: associations with atrial septal defect and hypoplastic left heart syndrome.

Elliott DA, Kirk EP, Yeoh T, Chandar S, McKenzie F, Taylor P, Grossfeld P, Fatkin D, Jones O, Hayes P, Feneley M, Harvey RP.
J Am Coll Cardiol. 2003 Jun 4;41(11):2072-6



Notch-1 mutation in a family with left-sided heart defects



The maternal-age-associated risk of congenital heart disease is modifiable

- Schulkey CE[1], Regmi SD[1], Magnan RA[1], Danzo MT[1], Luther H[1], Hutchinson AK[1], Panzer AA[1], Grady MM[1], Wilson DB[2], Jay PY[3]

- Nature. 2015 Apr 9;520(7546):230-3.

Age of the mother, not of the oocyte, affects frequency of a VSD in NKX2-5 mutant mice. In addition, the effect could be offset by exercise

Summary

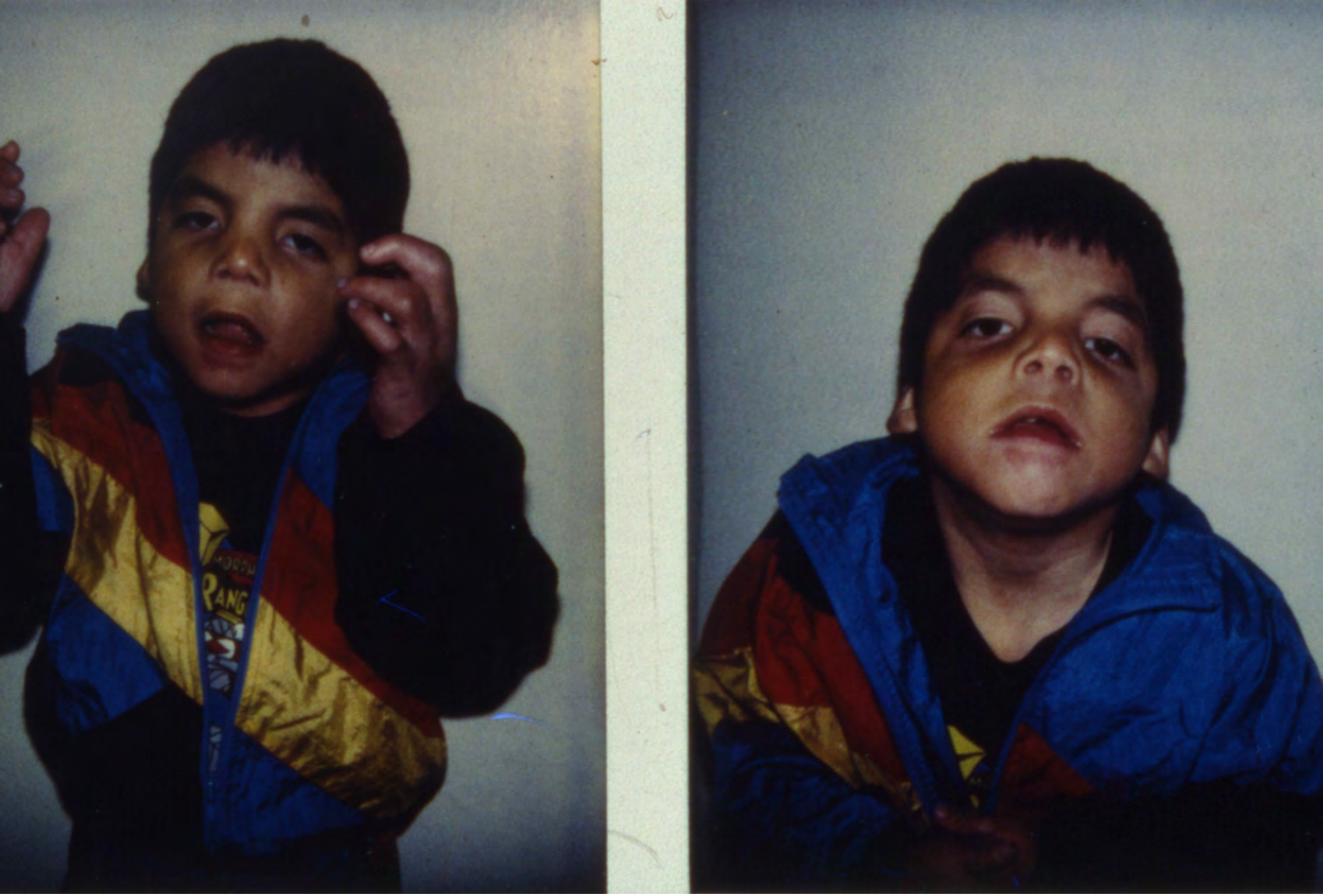
- In most cases, a specific genetic cause is NOT known
- There are likely to be MANY genes that can cause HLHS, through one or more mechanisms affecting early heart development

Environmental factors

- BWAS study demonstrated a seven-fold higher frequency of exposure to organic solvents in parents of HLHS patients

Mechanism of HLHS

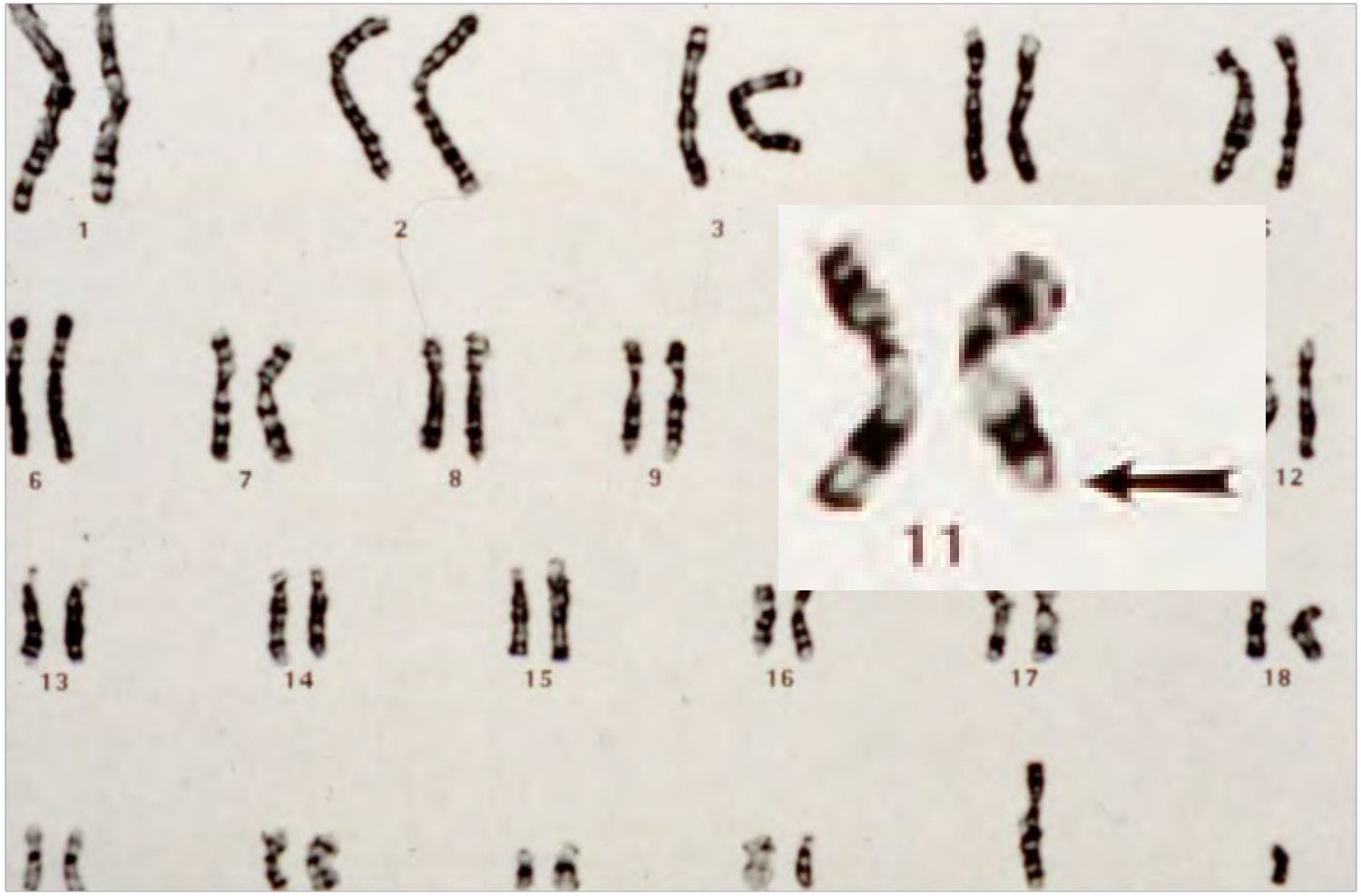
- Flow theory: "no flow, no grow" (either from outflow obstruction, i.e., aortic valve, or inflow obstruction, i.e., mitral valve)
- Ventricle theory: LV hypoplasia is the primary event
- **Multi-hit model: Genetic and/or hemodynamic alterations (from aortic/mitral defects) lead to ENDOCARDIAL injury and impaired ventricular development**



Congenital heart defects in 11q- (summary)

- About half have major heart defects
- Extremely high frequency of Hypoplastic Left Heart Syndrome (HLHS): ~2000-fold higher than general population), and about 1-2% of all cases of HLHS are caused by 11q-
- Many of the major congenital heart defects observed in the general population occur in 11q-

11q terminal deletion disorder

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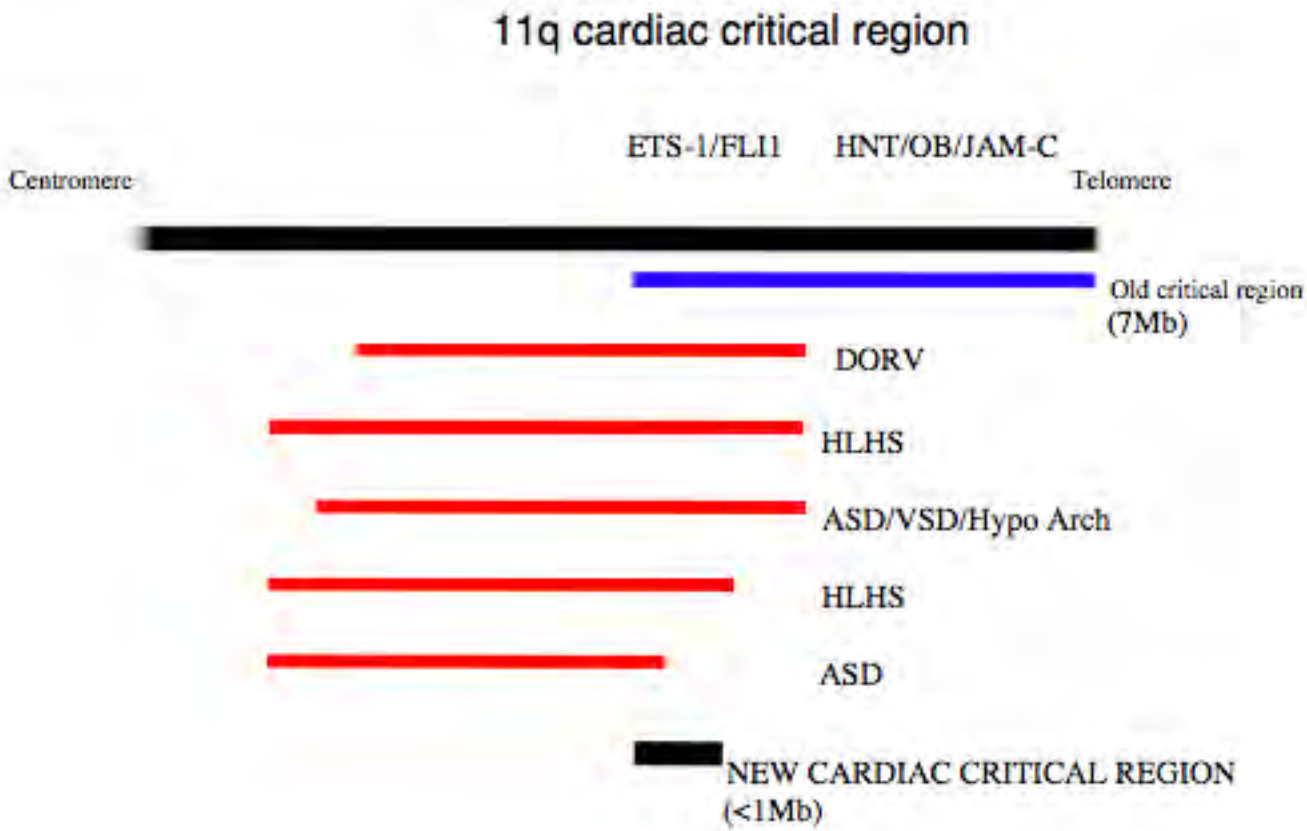
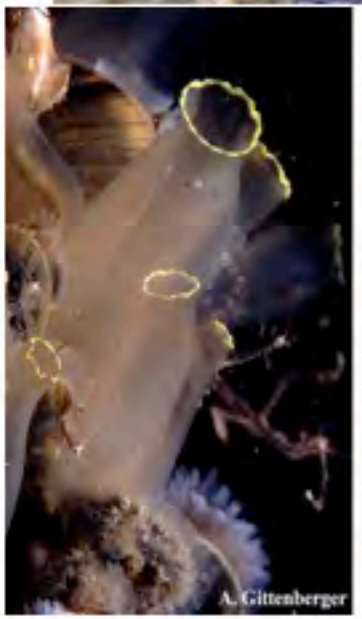
For Research Use Only
LIBERTY
IN GOD WE TRUST
QUARTER DOLLAR

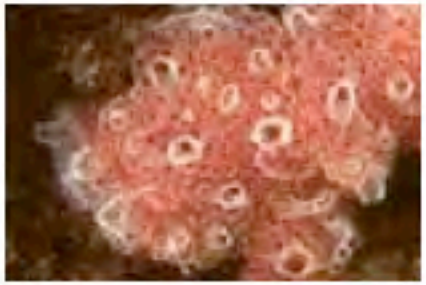

11q cardiac critical region
ETS-1/FLI1
HNT/OB/JAM-C
Centromere
Telomere
Old critical region
(7Mb)
DORV
HLHS
ASD/VSD/Hypo Arch
HLHS
ASD
NEW CARDIAC CRITICAL REGION
(<1Mb)

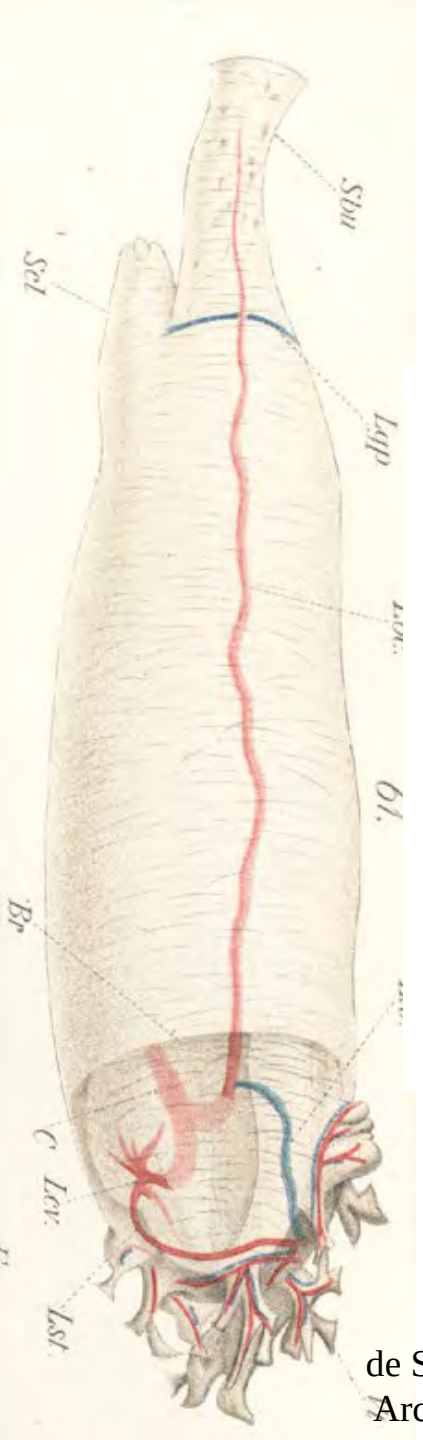
Genes in 1.2Mb region

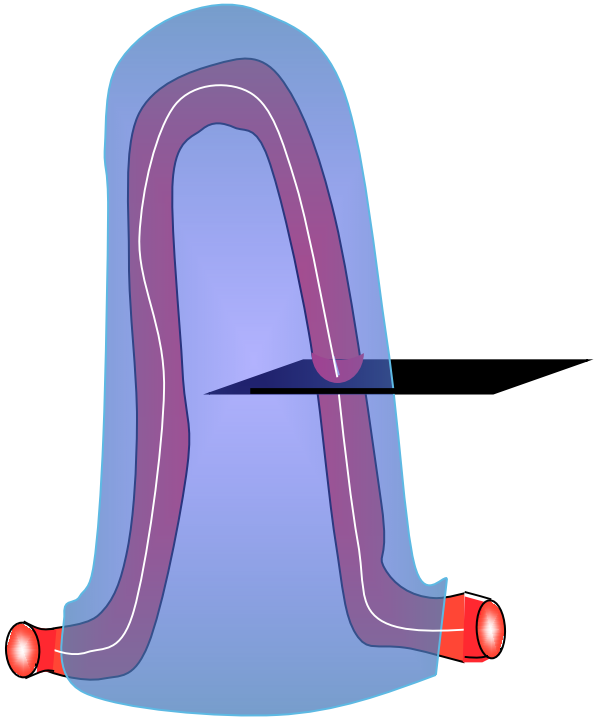
- **ETS-1**: Endothelial transcription factor/leukemia, heart development (sea squirt)
- FLI-1: Endothelial transcription factor/leukemia
- KCNJ1: renal potassium channel/Bartter syndrome
- KCNJ5: cardiac potassium channel/sinus arrhythmias
- p53AIP-1: mediates mitochondrial apoptosis, Induced by p53 (only in primates)
- RICS-1: axonal guidance

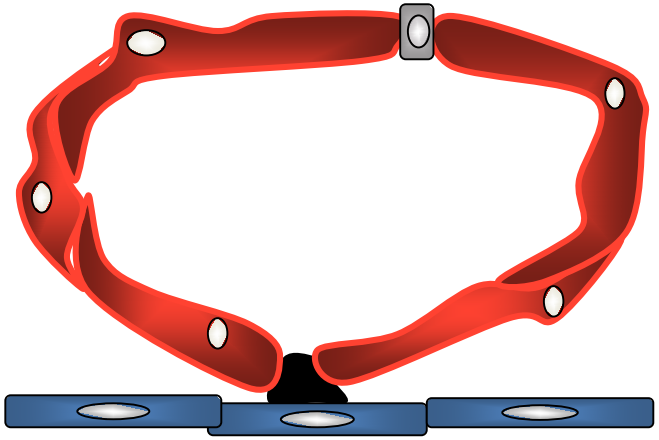

A. Gittenberger


A. Gittenberger



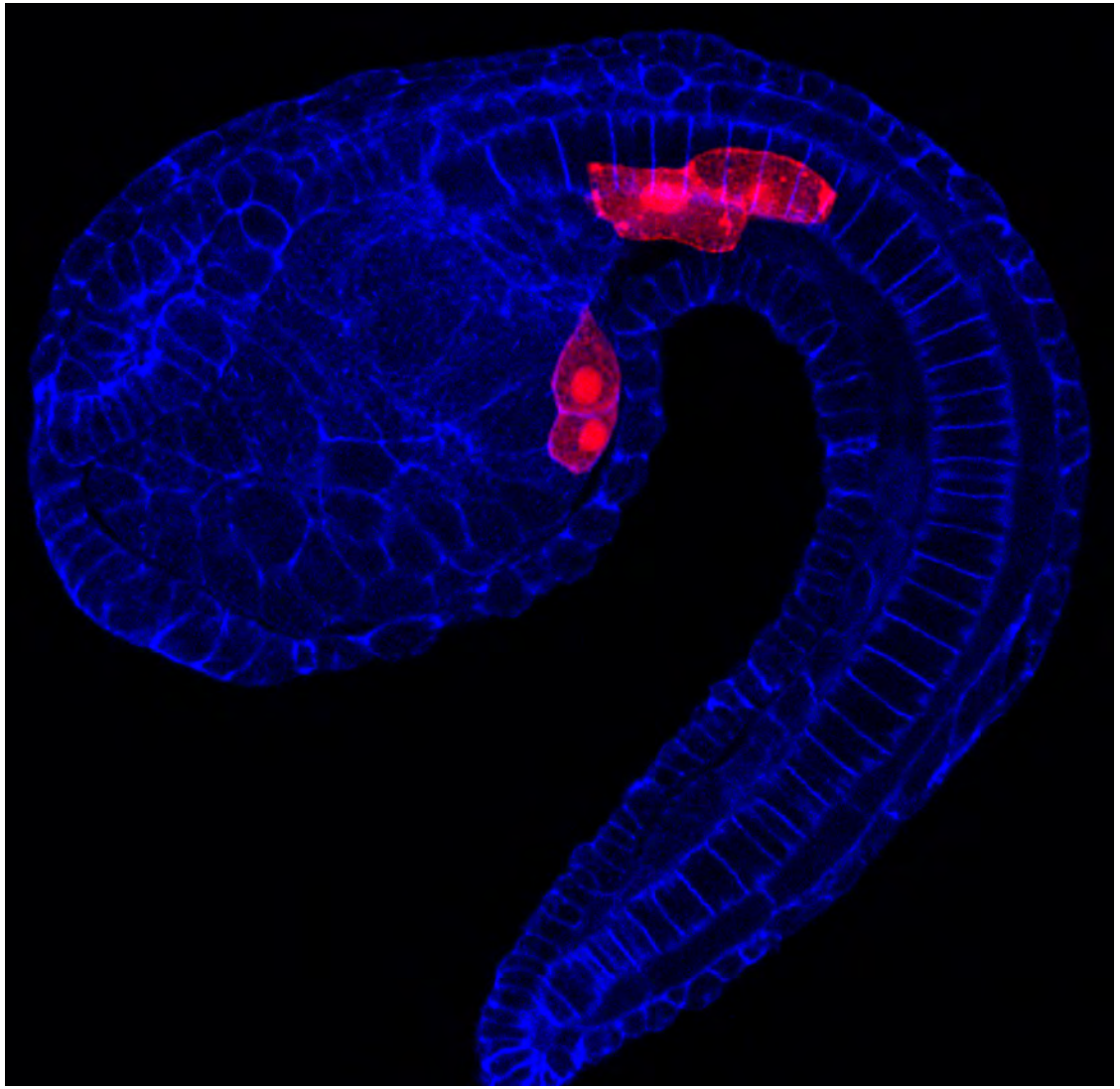




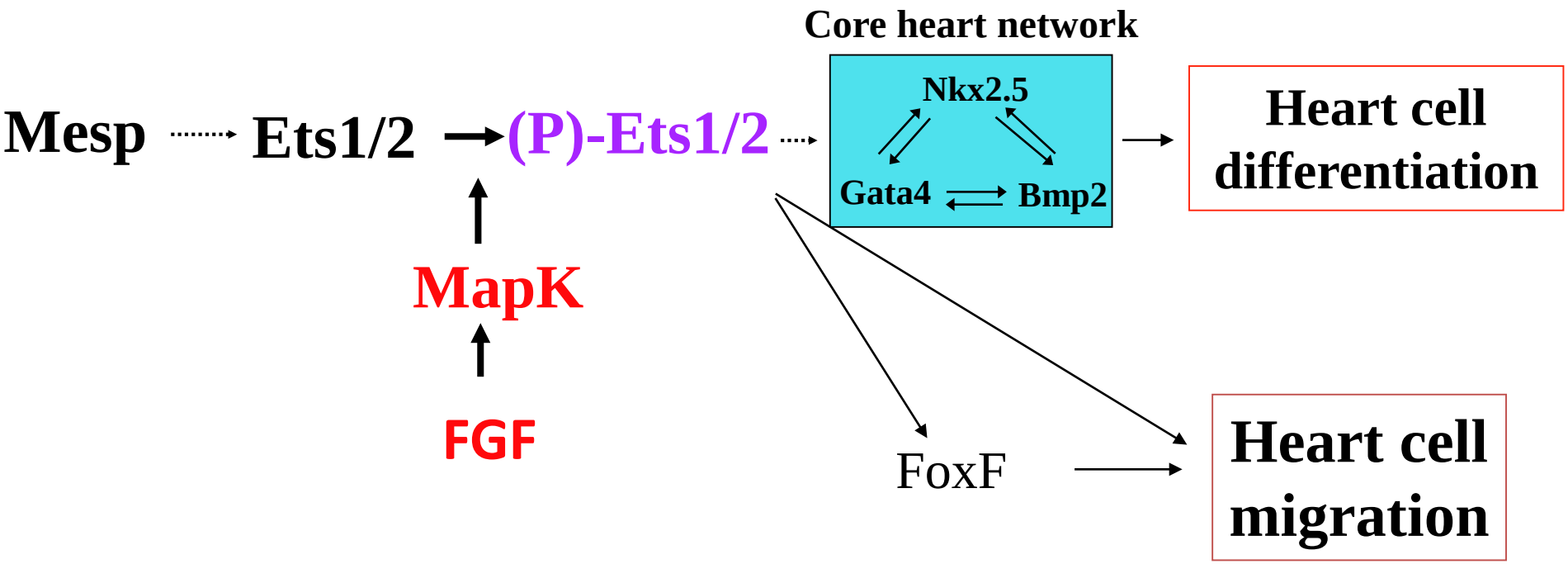


de Selys-Longchamps M. Dévelopment du coeur, du péricarde, et des épicardes chez Ciona intestinalis. Arch Biologie. 1900;17:499–542.

Comprehensive analysis of the heart gene network in the basal chordate, *Ciona intestinalis*



Ciona heart specification network



Development 134, 3297-3305 (2007) doi:10.1242/dev.010140

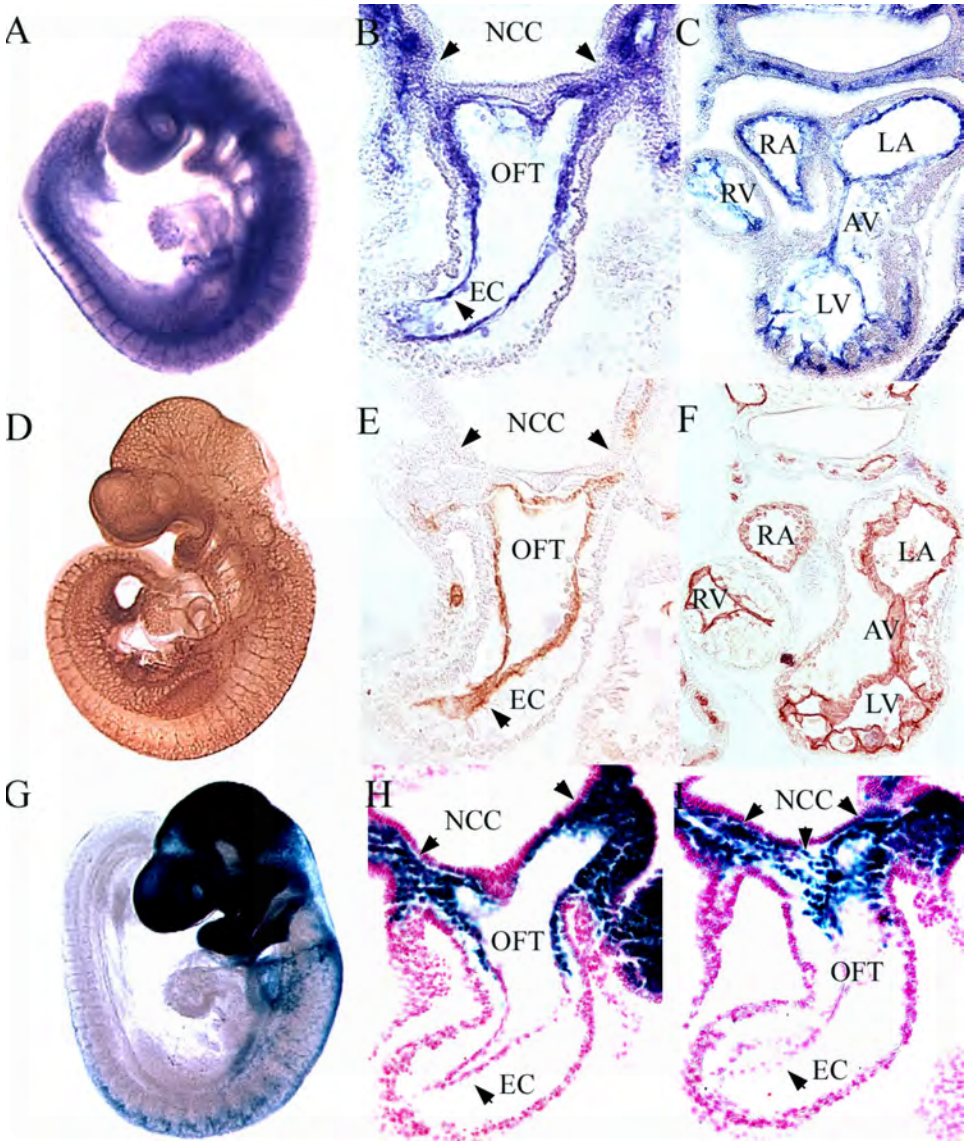
FoxF is essential for FGF-induced migration of heart progenitor cells in the ascidian *Ciona intestinalis*

Jeni Beh, Weiyang Shi, Mike Levine, Brad Davidson*,† and Lionel Christiaen†

Increased Frequency of De Novo Copy Number Variations in Congenital Heart Disease by Integrative Analysis of SNP Array and Exome Sequence Data. Joseph Glessner et al. and the Pediatric Cardiac Genomics Consortium. Circulation Research, Sept. 9, 2014.

- Identified **a *de novo ETS-1* frame shift** mutation in a CHD patient **without** 11q- with Jacobsen syndrome: hypoplastic left heart and mitral valve atresia; pt also has mild developmental delays, autistic-like features, mild dysmorphic features

ETS-1is expressed in two cell lineages critical for heart development: Neural Crest and Endocardium



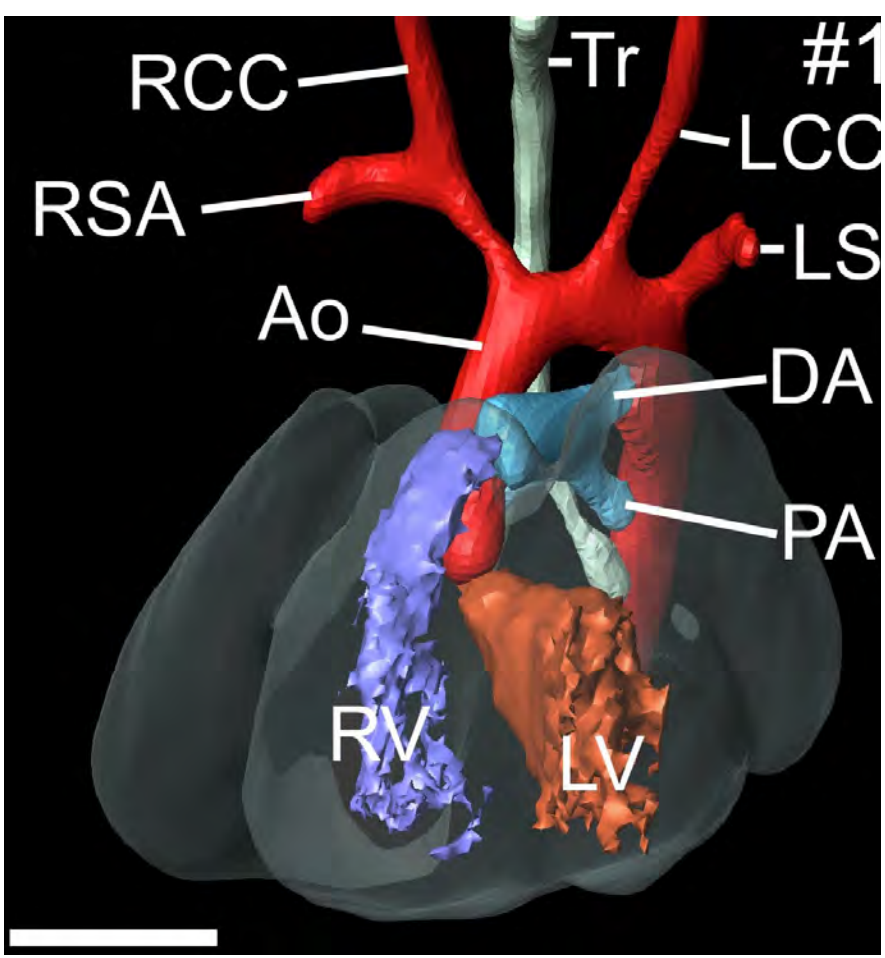
Question

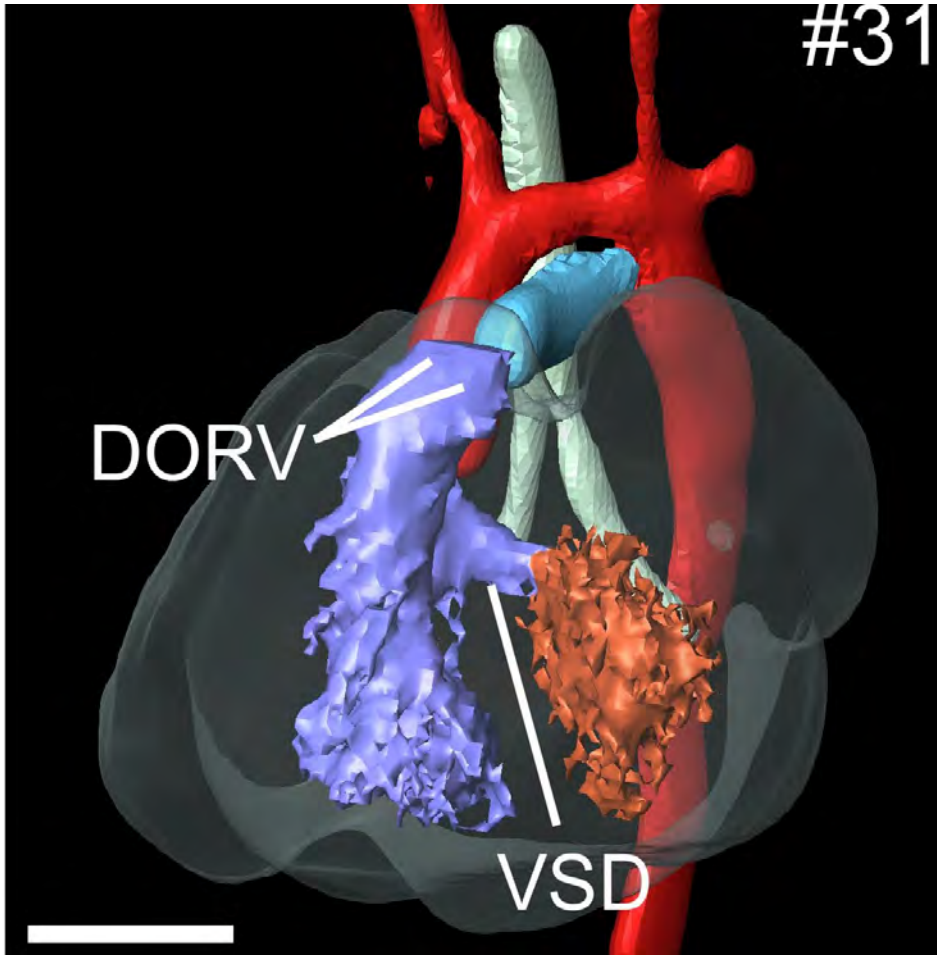
- Can a defect affecting cardiac neural crest and/or endocardial function cause congenital heart defects, including HLHS?

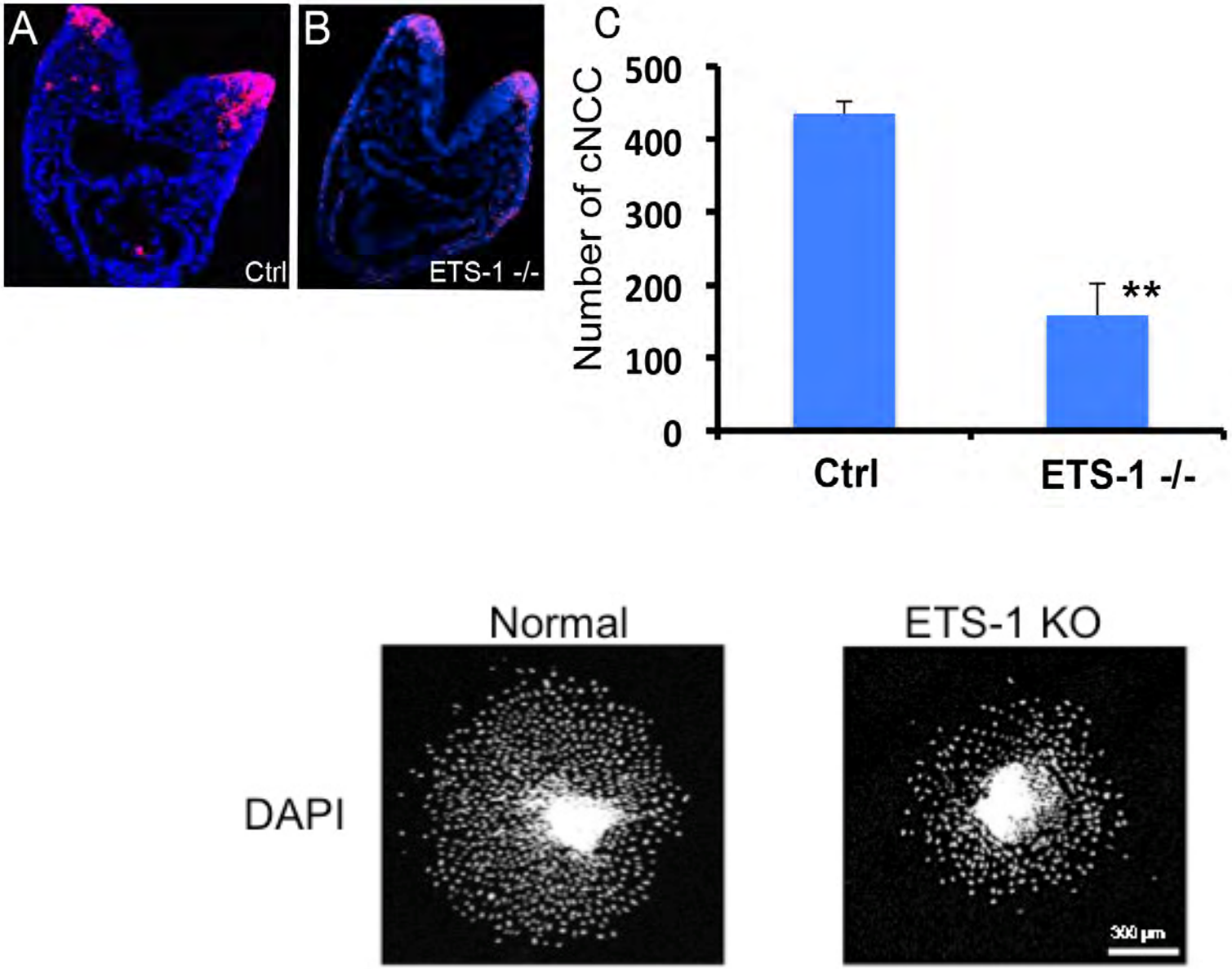
- If so, HOW???

- Based on this knowledge, could it be possible to prevent these heart defects?

Global and conditional deletion of ETS-1 in the neural crest causes DORV



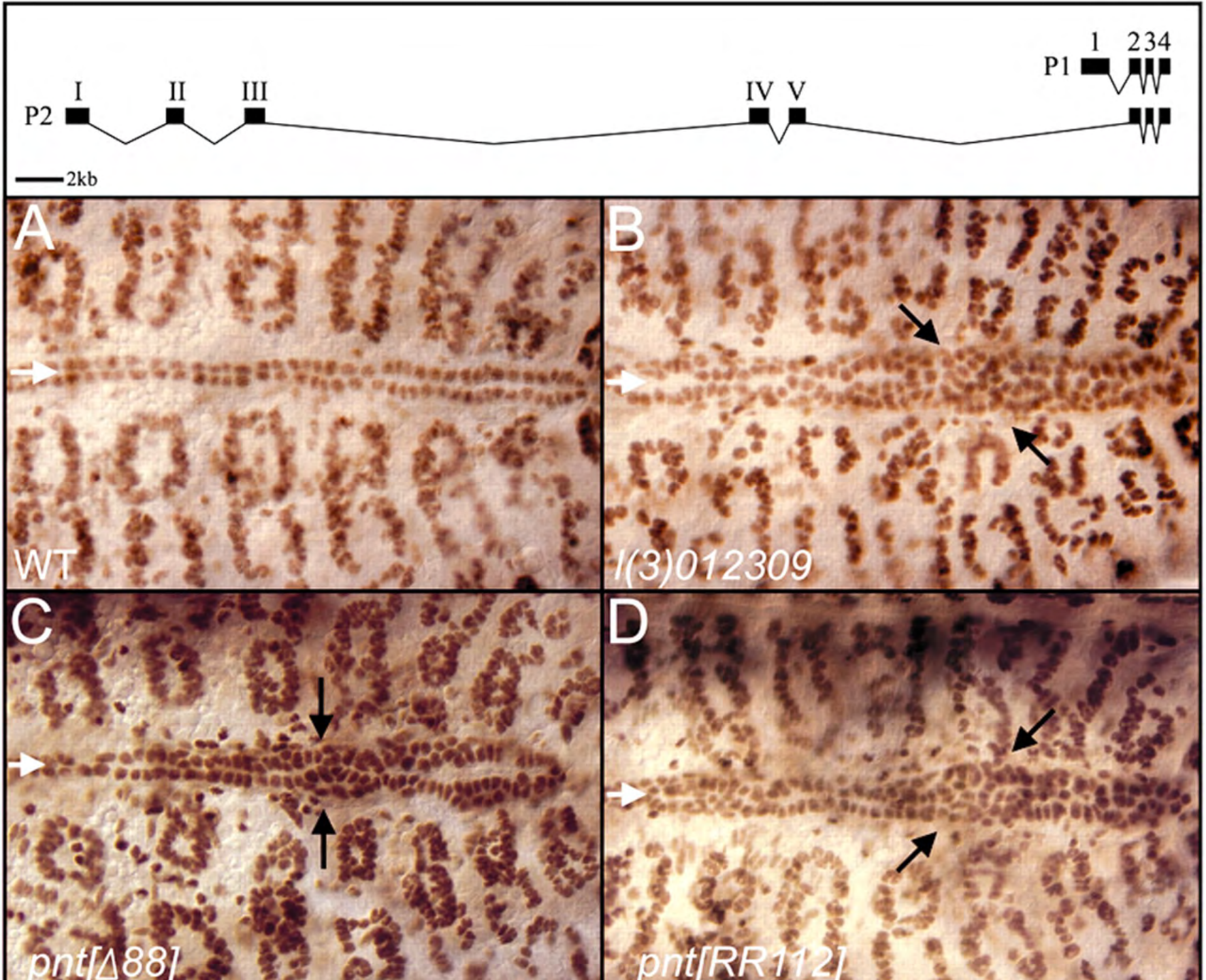



A
Ctrl
B
ETS-1 -/-
C
Number of cNCC
500
400
300
200
100
0
**
Ctrl
ETS-1 -/-
Normal
ETS-1 KO
DAPI
300 μm

Can loss of ETS-1 cause an HLHS phenotype in an animal model?

- Drosophila
- Xenopus

Which lineages are necessary to cause HLHS?


P2
I
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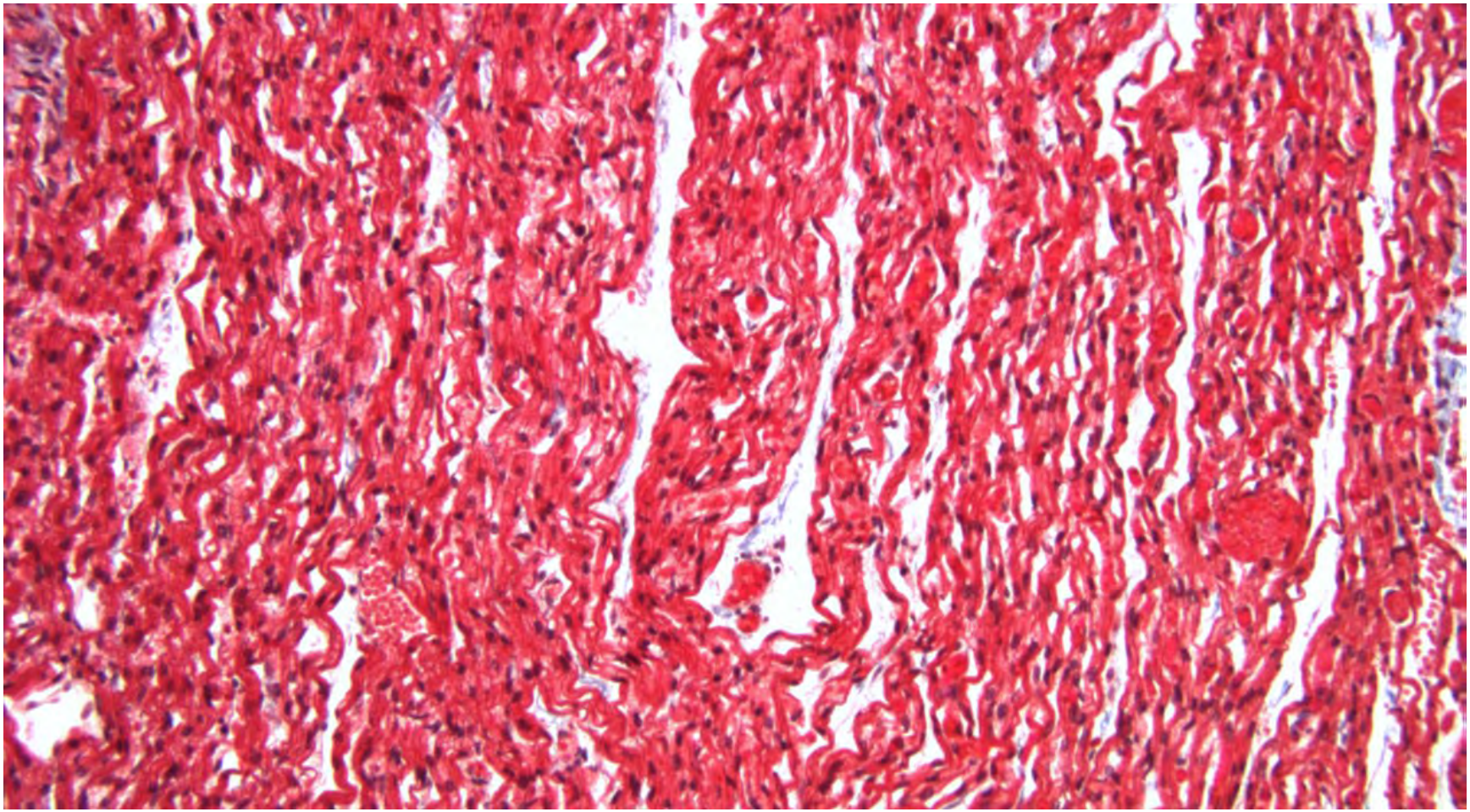
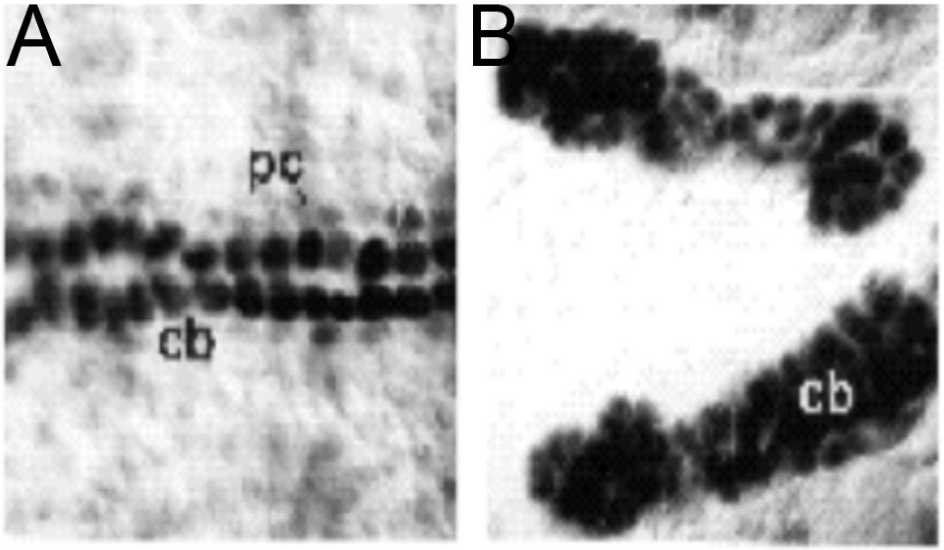
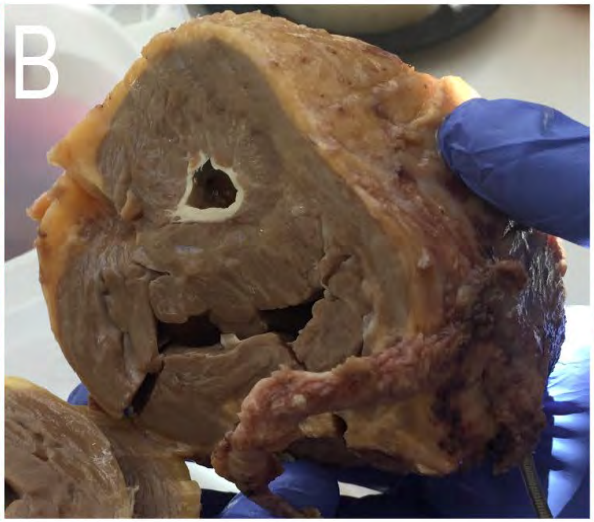
LV from a patient with JS and HLHS

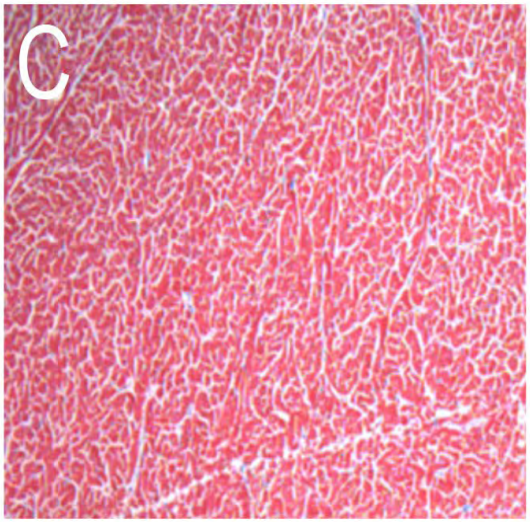


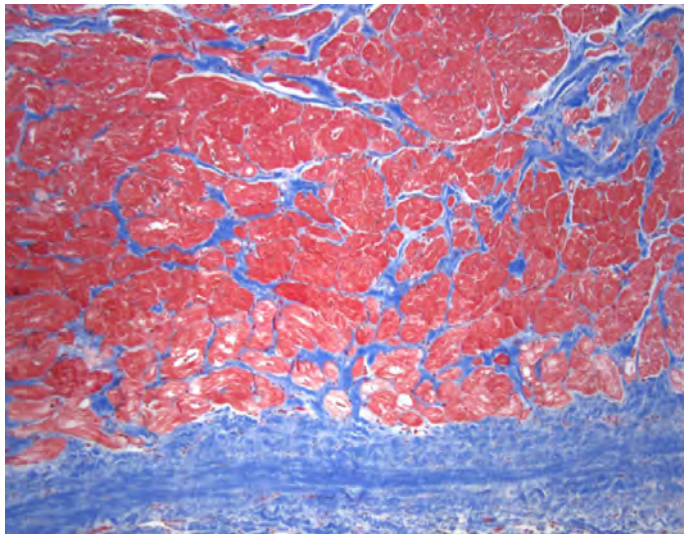
Loss of NOTCH in Drosophila causes increased cardiac myocytes (Hartenstein, et al., 1992)



Idiopathic HLHS





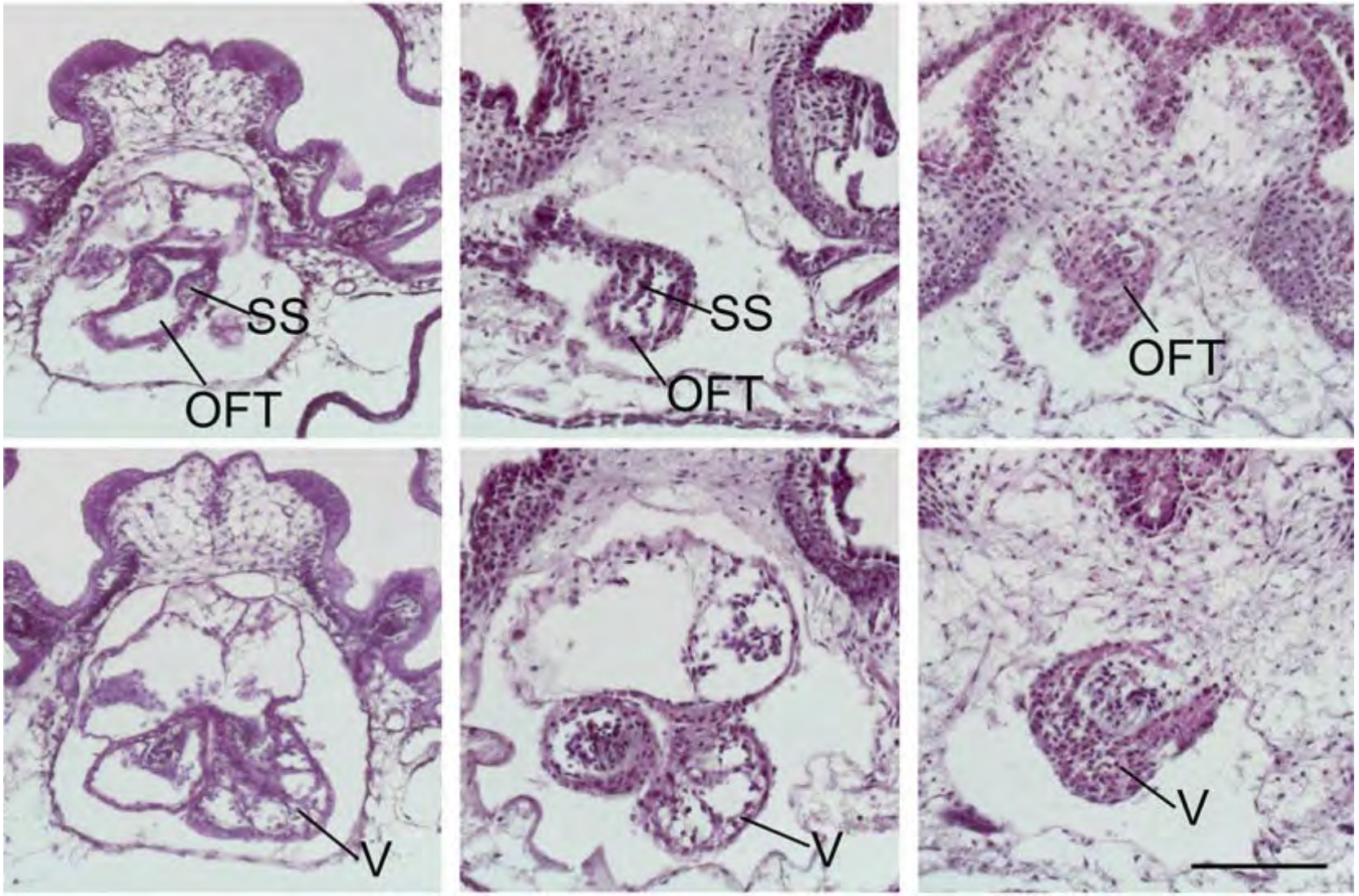


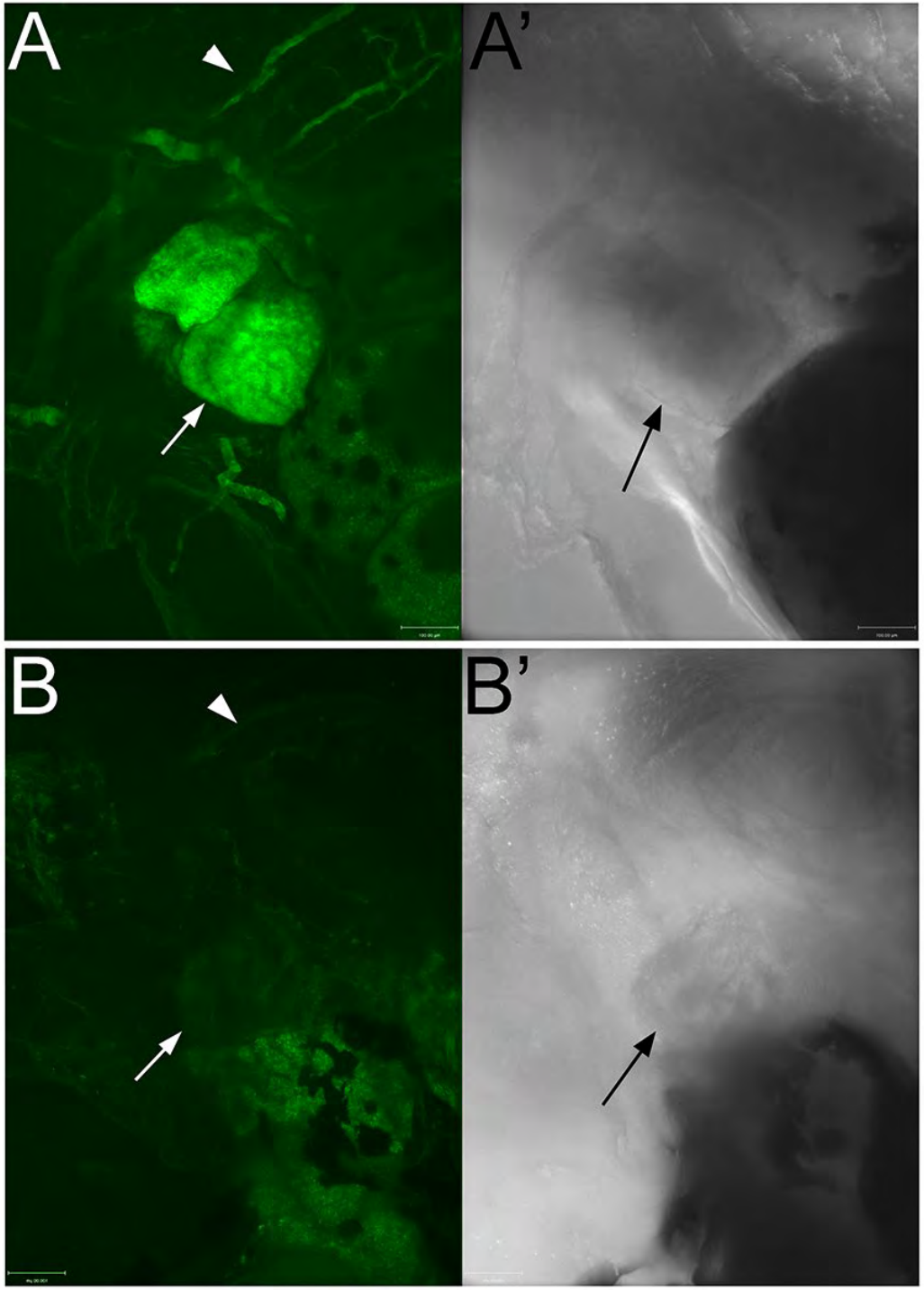
Explanted HLHS heart

LV

RV

KD of ETS-1 in the cardiac mesoderm in *Xenopus* causes an HLHS-like phenotype (Stage 45), with loss of endocardial cells (Nie and Bronner, 2015)

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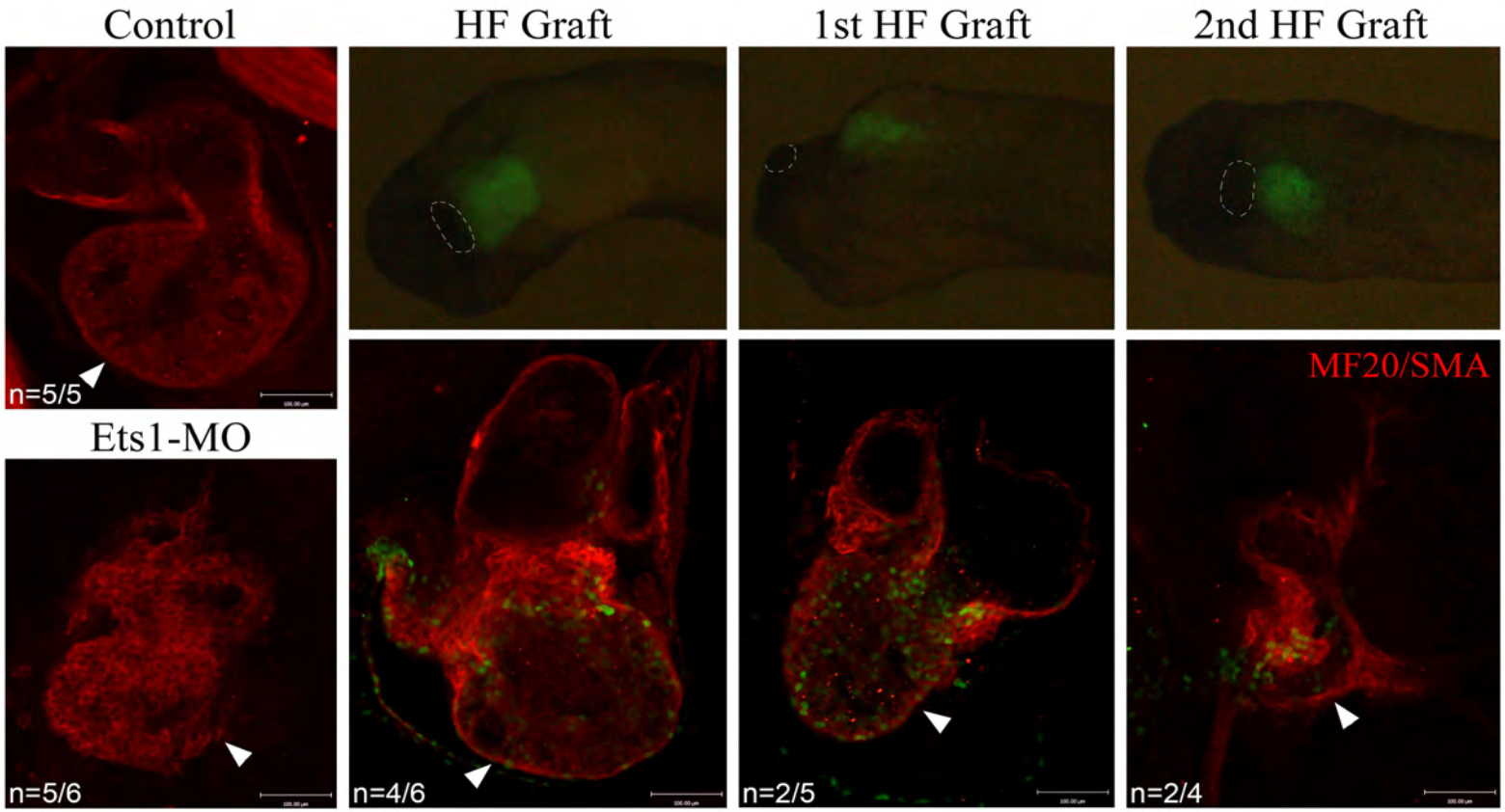
Grafting normal cardiac mesoderm tissue can prevent the HLHS ventricle

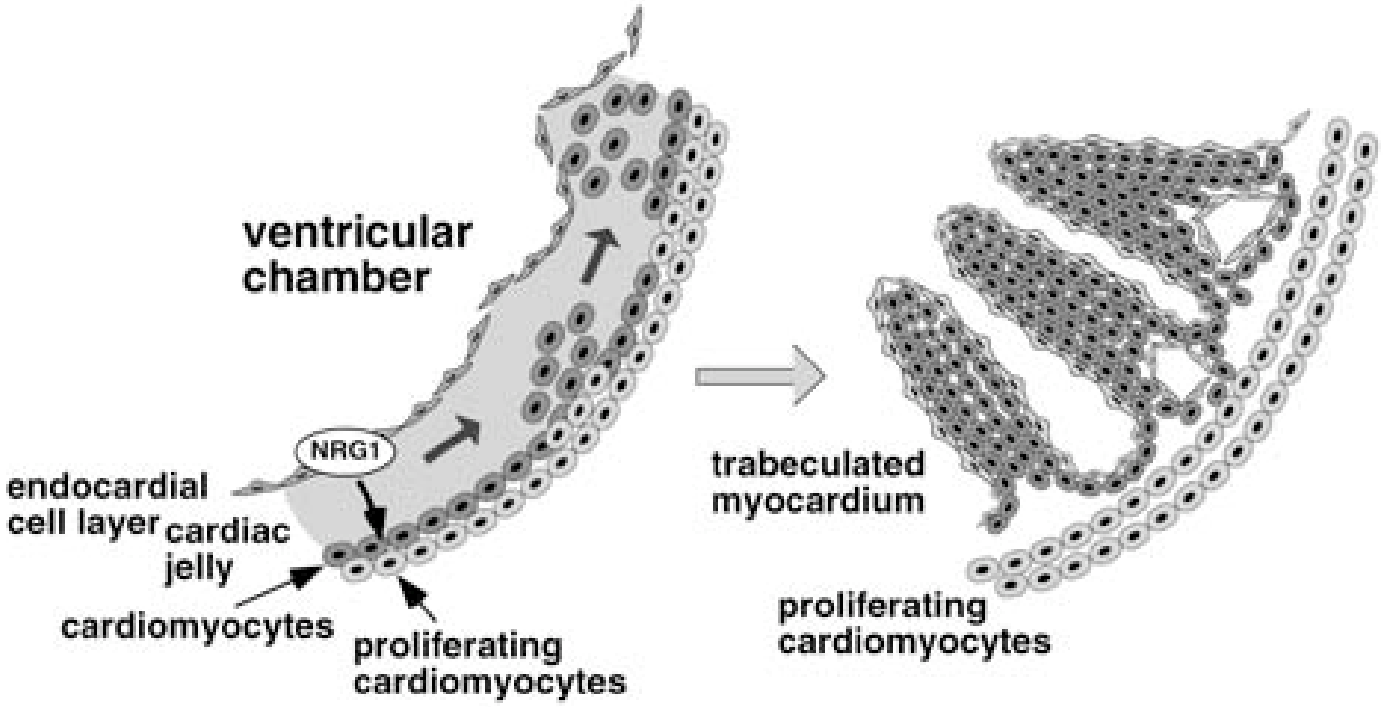


Transplantation of heart field tissue into Ets1-MO embryos partially rescued the heart development. Heart field (or 1st /2nd HF) tissue was dissected from H2bEGFP donor embryo and grafted into Ets1-MO expressing host embryos. The transplanted embryos were raised to stage 45-46. MF20 and SMA antibodies were used to detect outflow tract and ventricle, while anti-EGFP antibody was used to detect donor tissue. Then the embryos were cleared and imaged.

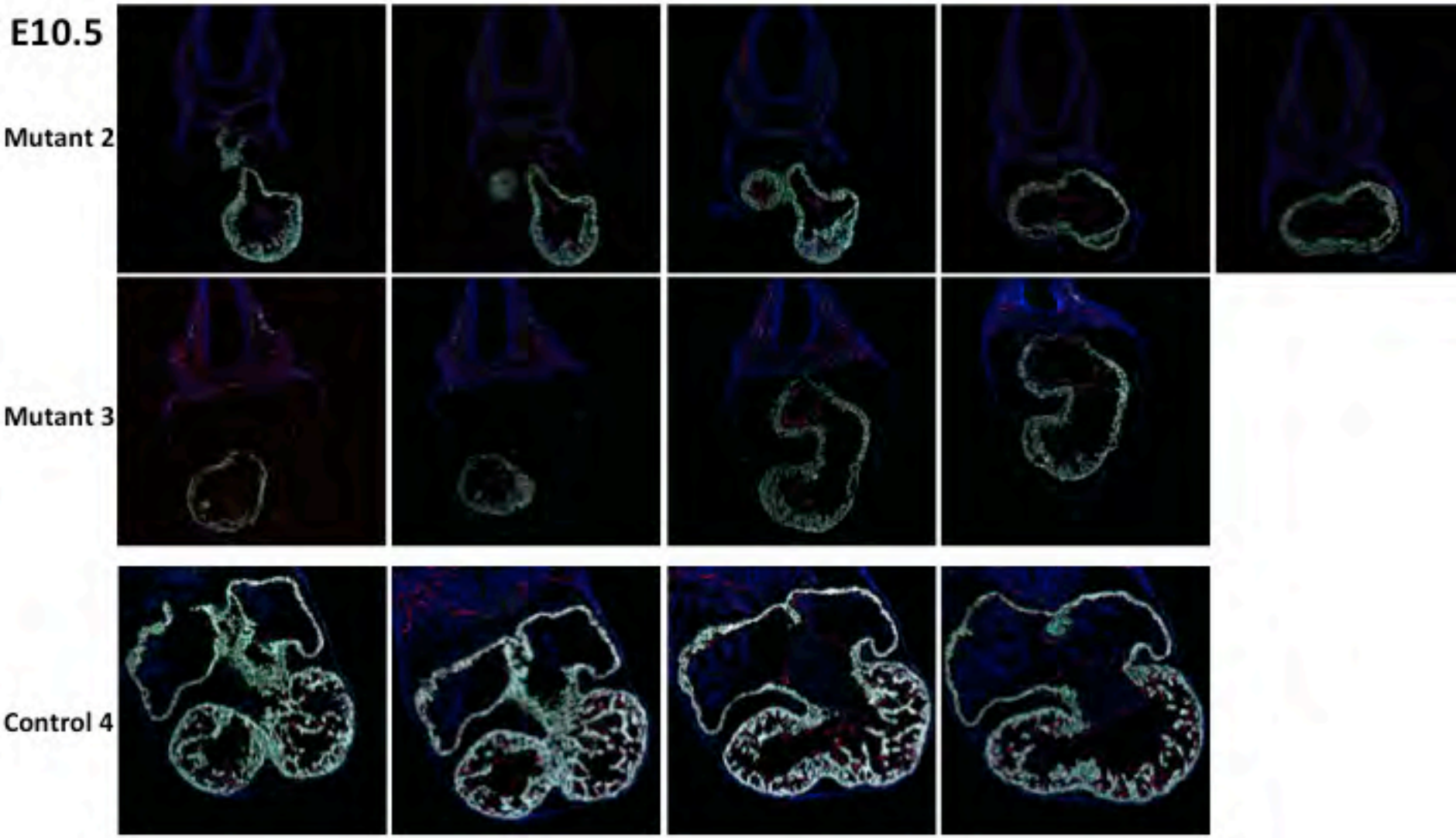
Summary of Xenopus studies

- Knockdown of ETS-1 in the neural crest causes outflow tract defects, but does NOT affect ventricular development
- Knockdown of ETS-1 in the cardiac mesoderm causes an HLHS-like ventricle, with a concomitant loss of endocardial cells
- The HLHS-like ventricular phenotype can be rescued by grafting normal cardiac mesodermal tissue
- Together, this suggests that the **endocardium** regulates ventricular development, specifically cardiac myocyte proliferation, such that loss of the endocardium may be the cause of the HLHS ventricle

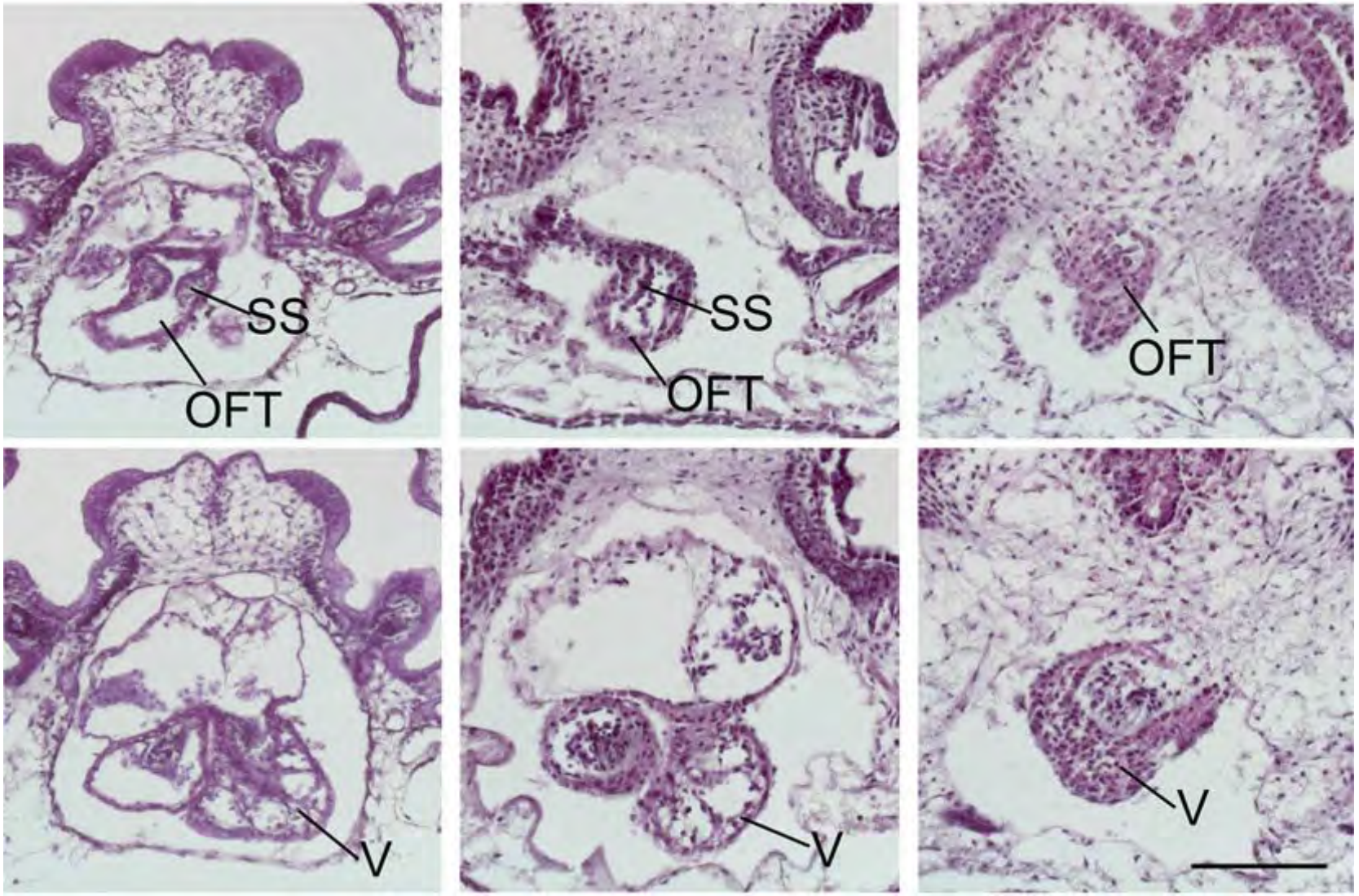
Signaling from the endocardium regulates myocardial growth and development



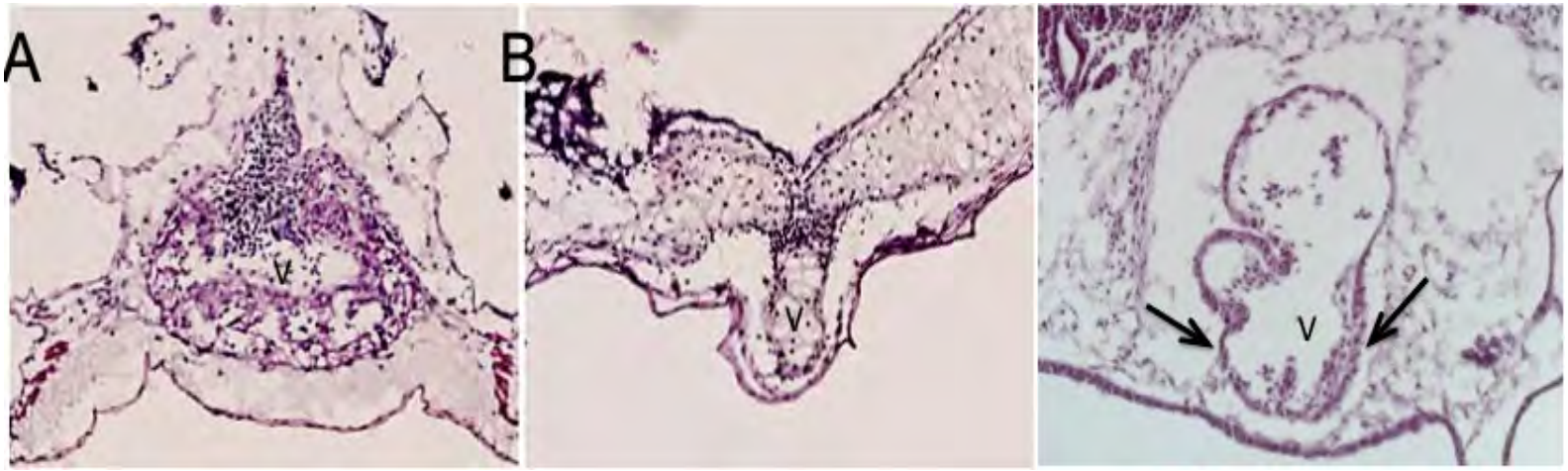
Can loss of the endocardial cells cause a hypoplastic LV?



KD of ETS-1 in the cardiac mesoderm in *Xenopus* causes an HLHS-like phenotype (Stage 45), with loss of endocardial cells (Nie and Bronner, 2015)



KD of ETS1 in the cardiac mesoderm causes growth arrest in a subset of the hearts, which may be the first step in the development of the HLHS ventricle



- Is the HLHS ventricle due to just too many cardiac myocytes, or is it also due to intrinsically abnormal cardiac myocytes?

- The answer could have profound clinical implications

novoheart™ hvCOC

human ventricular Cardiac Organoid Chamber

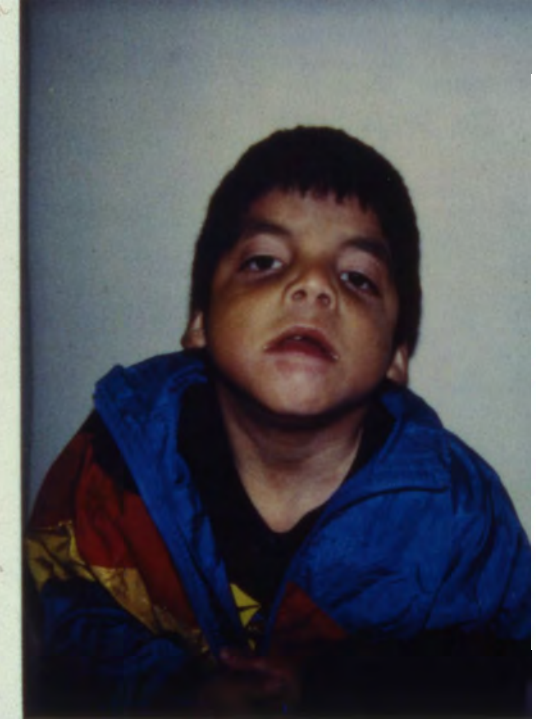
Human "heart-in-a-jar" for disease modeling and drug screening

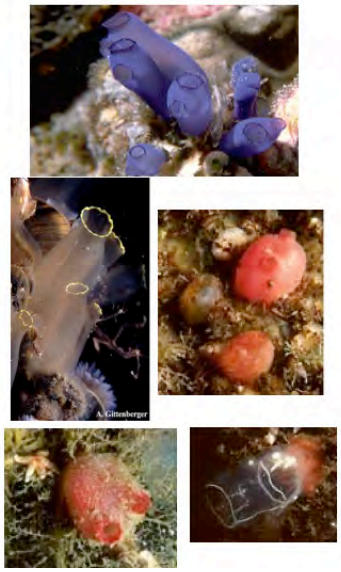
"Heart-in-a-jar" provides the most sophisticated model of cardiac function

- ✓ **3D Cardiac Organoid**
  - Miniature, functional human ventricular chamber
  - Capabilities for characterization of clinically relevant readouts
- ✓ **Comprehensive Analysis**
  - Contractility: pressure-volume loop, ejection fraction, cardiac output
  - Electrophysiology: volumetric electrical propagation
  - Integrated cardio-mimetic 3D platform

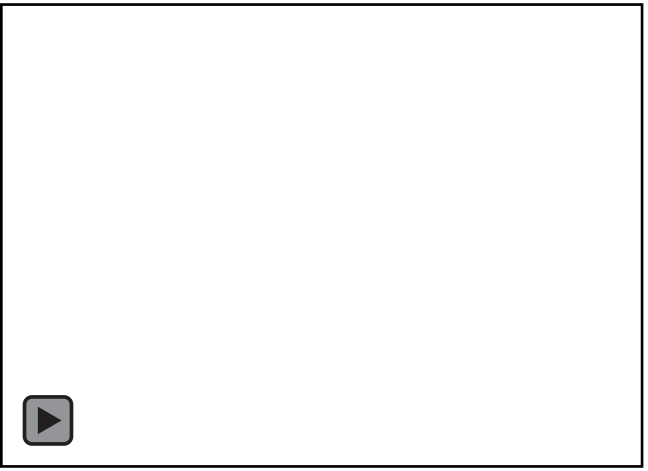
Summary

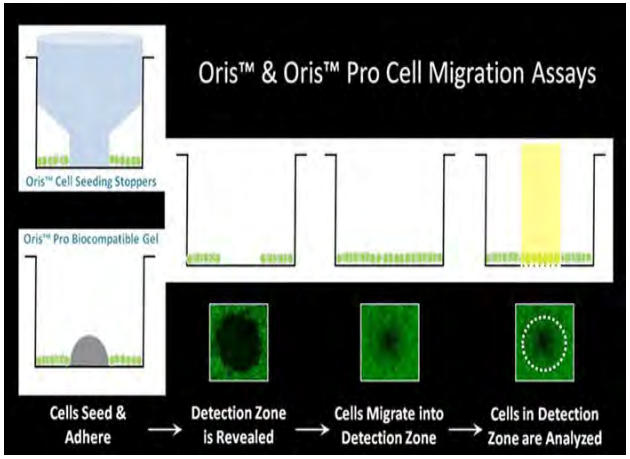
- There is a strong genetic component to HLHS, but it is clearly a multifactorial disease
- The endocardium may have a critical role in causing the “HLHS” LV such that loss of endocardial function leads to ventricular growth arrest, followed by cardiac myocyte proliferation
- Consequently, fetal balloon angioplasty at mid-gestation may be too late to be beneficial
- Prevention through EARLY intervention might be possible






A. Gittenberger




Oris™ & Oris™ Pro Cell Migration Assays
Oris™ Cell Seeding Stoppers
Oris™ Pro Biocompatible Gel
Cells Seed & Adhere
Detection Zone is Revealed
Cells Migrate into Detection Zone
Cells in Detection Zone are Analyzed

HLHS pt, holding his explanted heart

Acknowledgements

- Grossfeld lab
  - Maoqing Ye
  - Yin Yan
  - Kazumi Fukatsu
  - Lizhu Lin
  - Lu Wang

Sylvia Evans Lab (UCSD)

Rolf Bodmer (SBP)

Karen Ocorr (SBP)

Alex Colas(SBP)

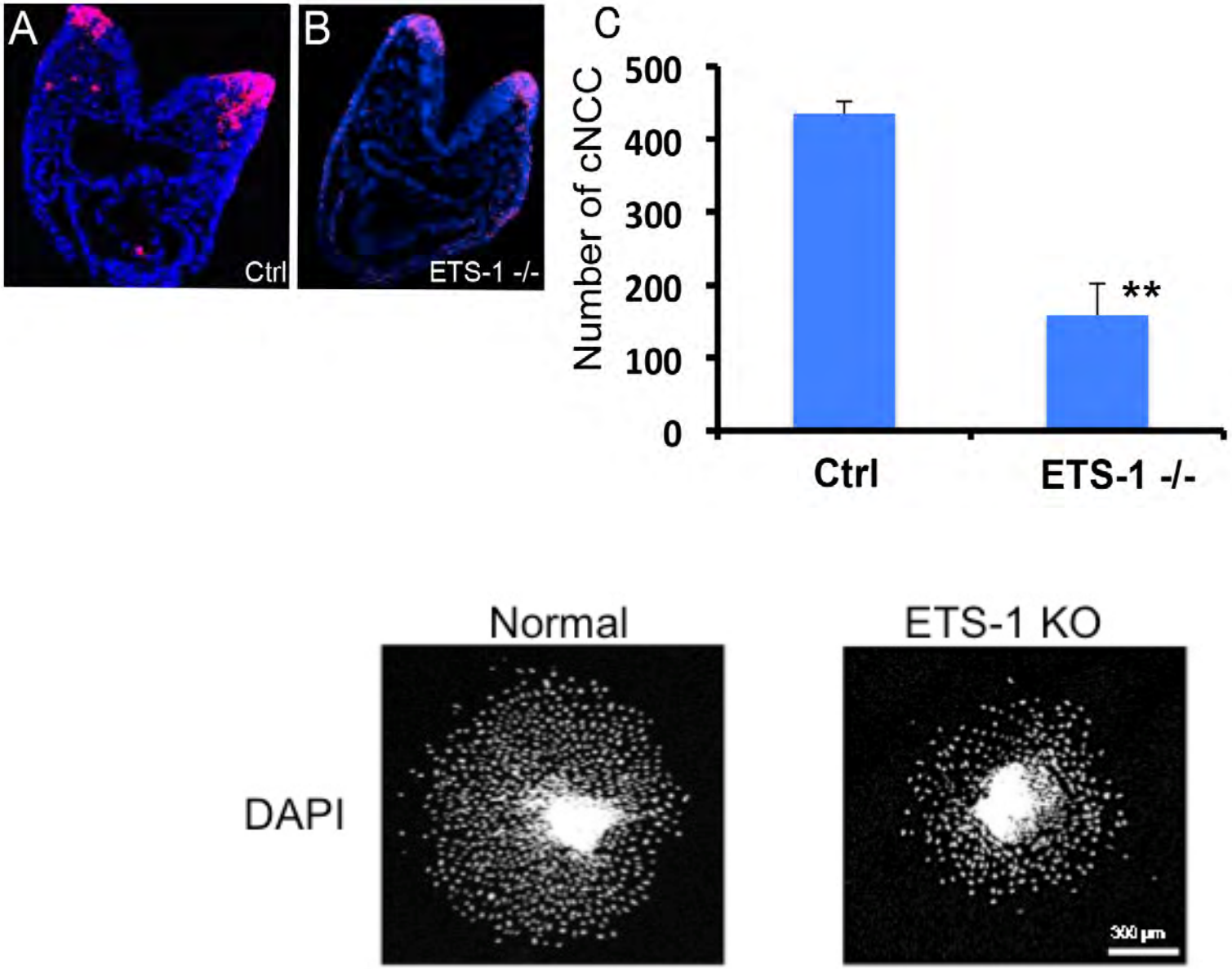
Alexey Terskikh (SBP)

Marianne Bronner (Cal Tech)

Shuyi Nie (Georgia Tech)

Mayo Clinic

Robert Anderson(UK)


A
Ctrl
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ETS-1 -/-
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Number of cNCC
500
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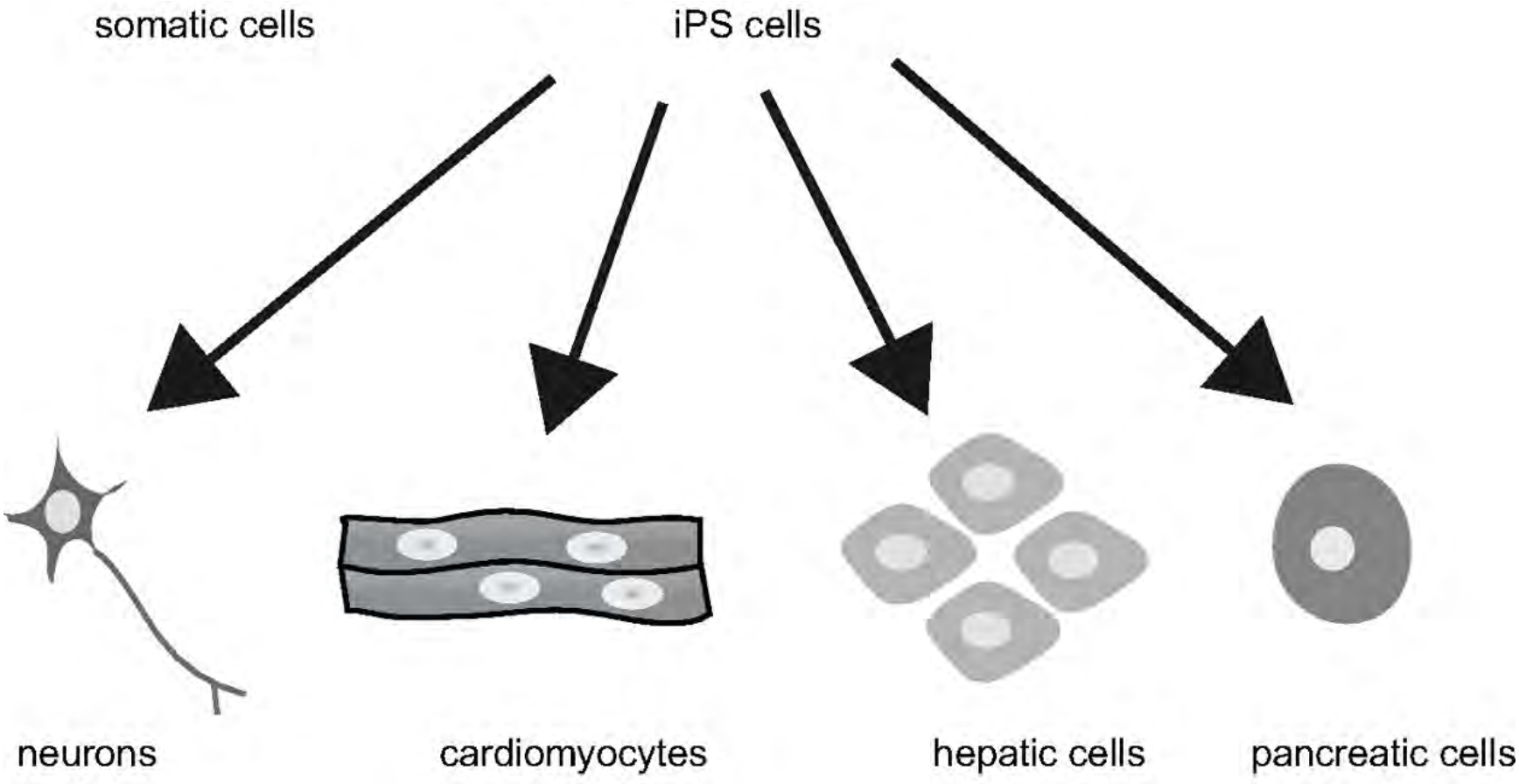
Induced Pleuripotent Stem Cells to Study “Disease in a Dish”



Neural crest cell migration defect in patients with HLHS