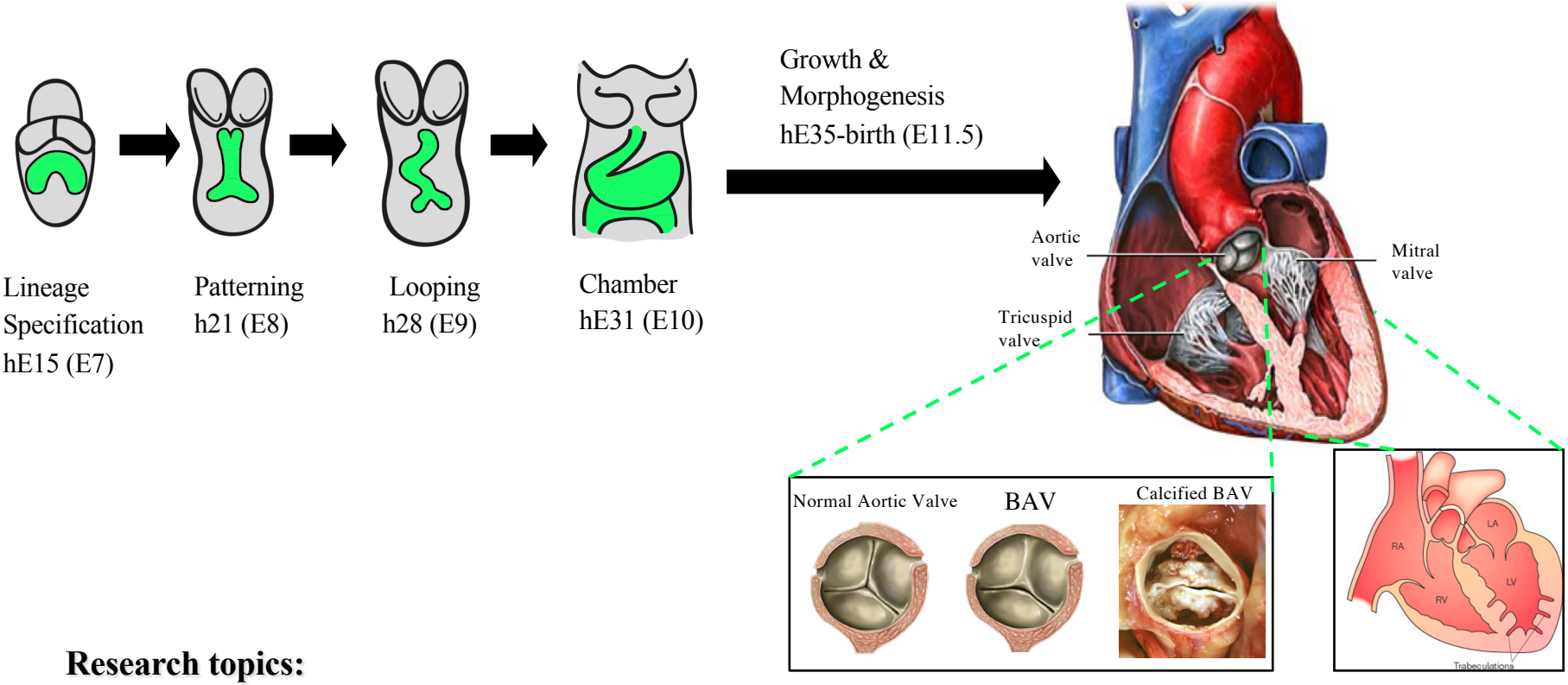
Intercellular signaling in cardiovascular development, homeostasis and disease



Research topics:

-Cardiac Development → Cardiac valves, Chambers & Coronaries

↕

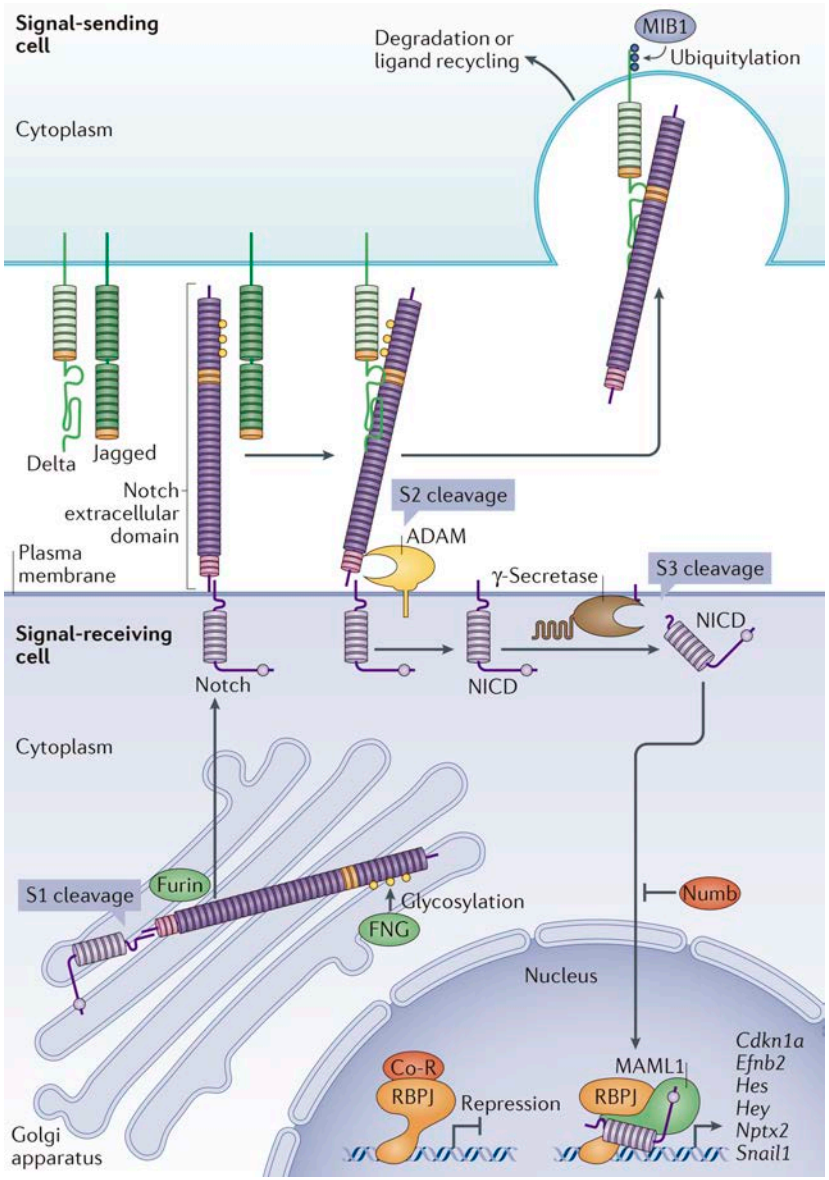
-Cardiac Disease (CHD): Valve Disease & Cardiomyopathy

-Tissue homeostasis/regeneration: Zebrafish Heart regeneration

Signals interplay

GOAL: Identification of novel developmental/pathological mechanisms, disease markers and/or therapeutical targets for CVD

The NOTCH signaling pathway



MacGrogan et al. (2018) *Nat Rev Cardiol.*

FUNCTIONS DESCRIBED

Cell fate specification

Cell proliferation

Cell differentiation

Stem cell maintenance

Tissue patterning

Oncogenesis

PREVIOUS CONTRIBUTIONS

Development 1995: 121, 3291
Development 1997: 124, 1139
Neuron 1998: 20, 469
Curr Biol 1999: 9, 470
Cell 2001: 106, 207
Genes Dev 2003: 17, 1213
Genes Dev 2004: 18, 99
Development 2005: 132, 1117
Dev Cell 2007: 12, 415
Leukemia 2007: 21, 1496
J Exp Med. 2009: 206, 779
PLoS Genet. 2009: 5, e1000662
JCI 2010:120, 3493
Curr Top Dev Biol. 2010: 92, 333
Circ Res 2011: 108, 824
Circ Res 2011: 109, 1429
ATVB 2011: 31, 1580
Cardiovasc Res. 2012: 93, 232
Dev Cell 2012: 22, 244
Development 2013: 140,1402
Genesis 2013: 51, 32.
Breast Cancer Res. 2013:15, R54
Nat Med 2013: 19, 193

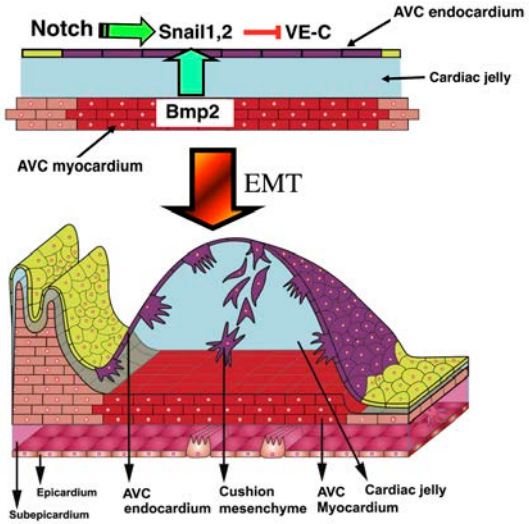
TOOLS GENERATED

Genetically modified mouse models Molecular tools Explant assays Cell lines

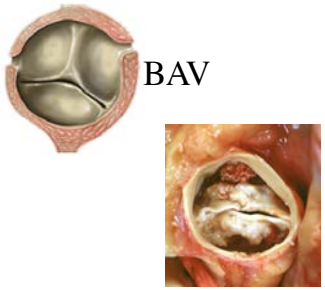
I. Valve development & disease. Previous work: Notch is required for cardiac valve primordium formation

LOF: Notch is required for endocardial EMT [Timmerman et al. *G&D* (2004)]

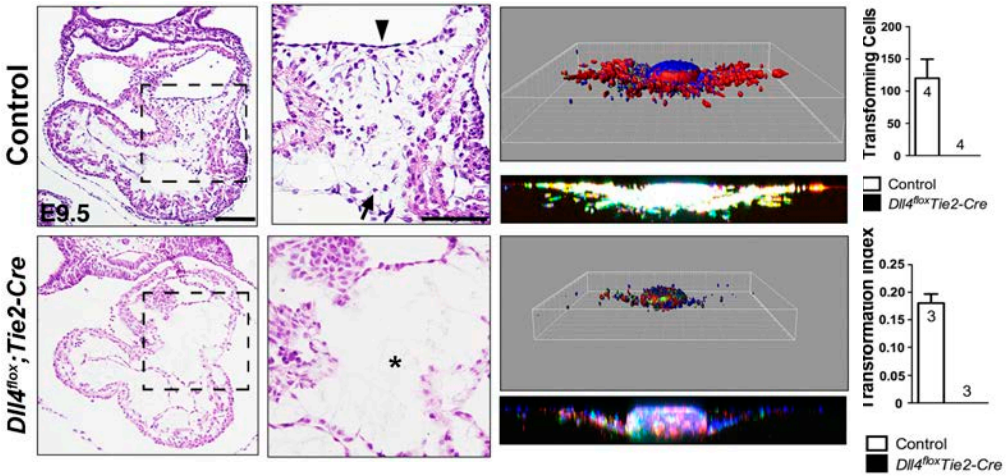
GOF: Notch and Bmp2 co-regulate endocardial EMT [Luna-Zurita et al. *JCI* (2010)]



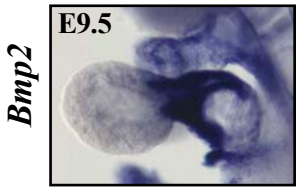
NOTCH1 mutations cause BAV.
Garg *et al. Nature* (2005)



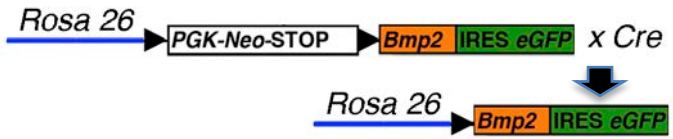
- Recent work: Dll4-Notch1 drives EMT

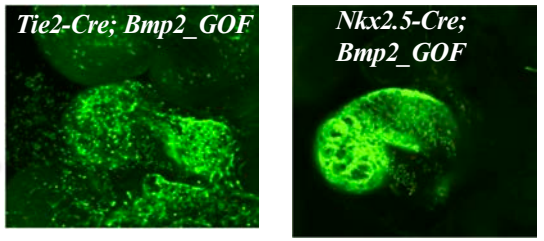


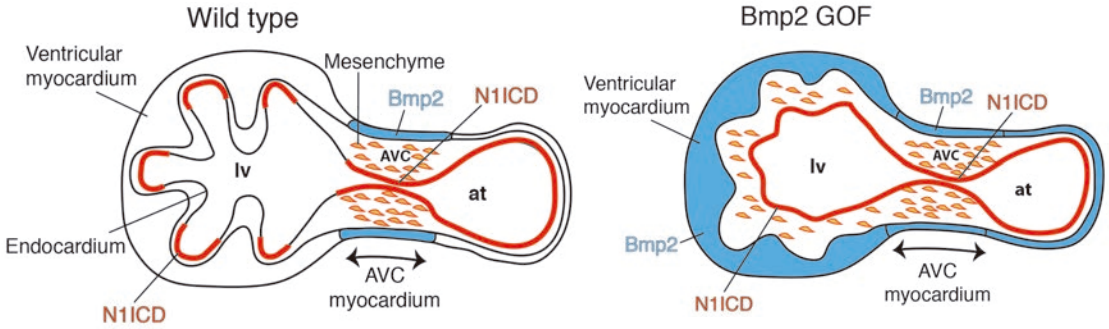
- Recent work: Myocardial Bmp2 patterns the valve-forming region upstream of Notch



New transgenic line







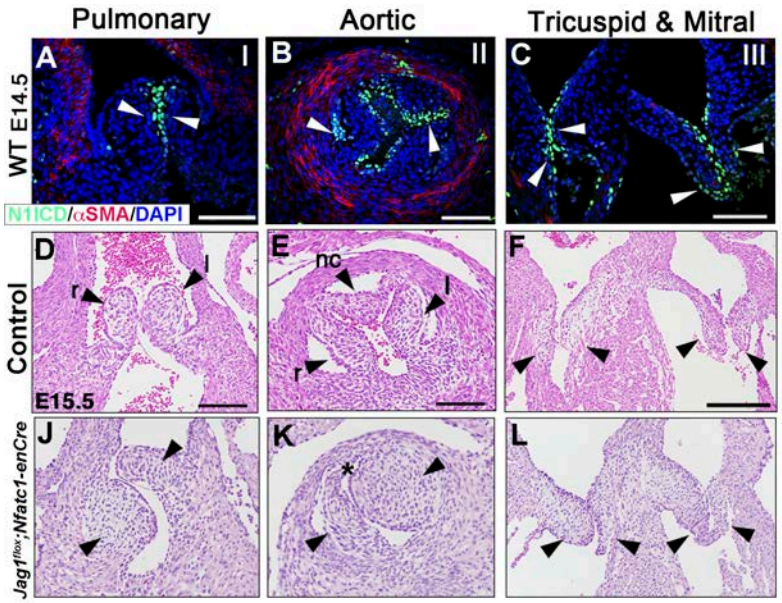
MacGrogan et al. *Circ Res* (2016)

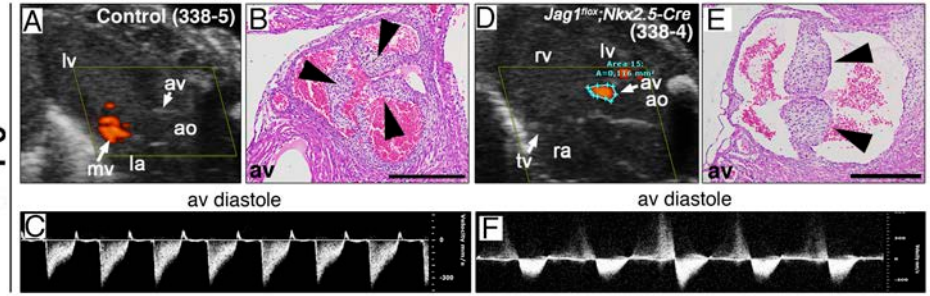
Prados et al. *Cell Death Disease* (2018)

Papoutsi et al. *Development* (2018)

MacGrogan et al. *Nat Rev Cardiol* (2018)

- Recent work: Jag1-Notch signaling regulates mesenchyme proliferation during cardiac valve morphogenesis





- Mouse model of BAV (40%).

MacGrogan et al. *Cold Spring Harb Perspect Med. (*2014)

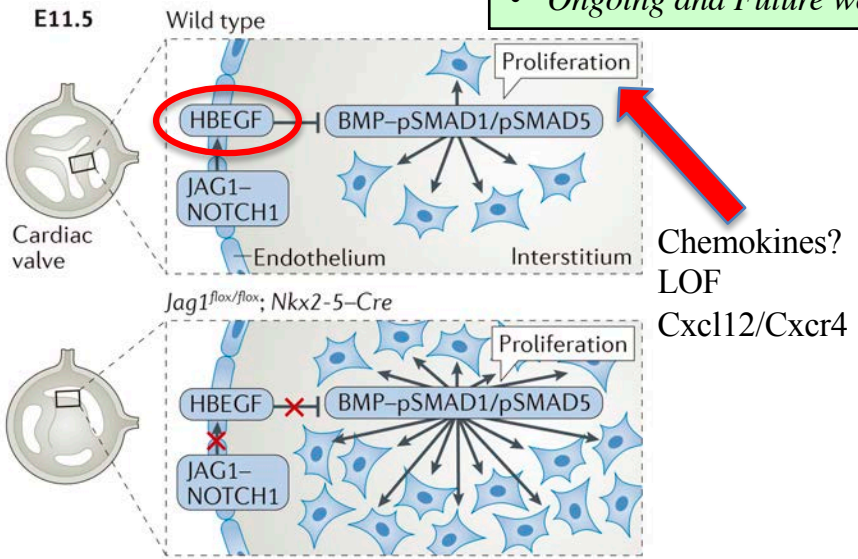
MacGrogan et al. *Circ Res (*2016)

Luxan et al. *Circ Res (*2016)

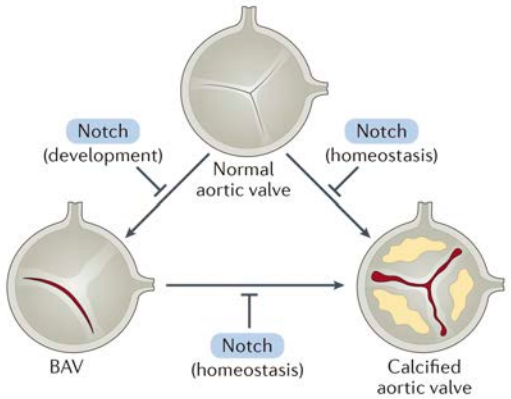
Eley et al. *Elife* (2018)

MacGrogan et al. *Nat Rev Cardiol* (2018)

- *Ongoing and Future work:* Notch in cardiac valve morphogenesis, BAV and valve calcification



Chemokines?
LOF
Cxcl12/Cxcr4



Nus et al. *ATVB* (2011)

Nus et al., *Cardiov Res (*2016)

MacGrogan et al. *Nat Rev Cardiol* (2018)

New mouse models: *NOTCH (MIB1)-GATA 6* and BAV

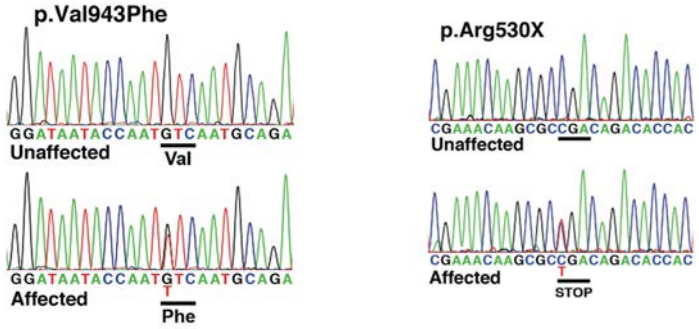
MIB1;TercKO and CAVD

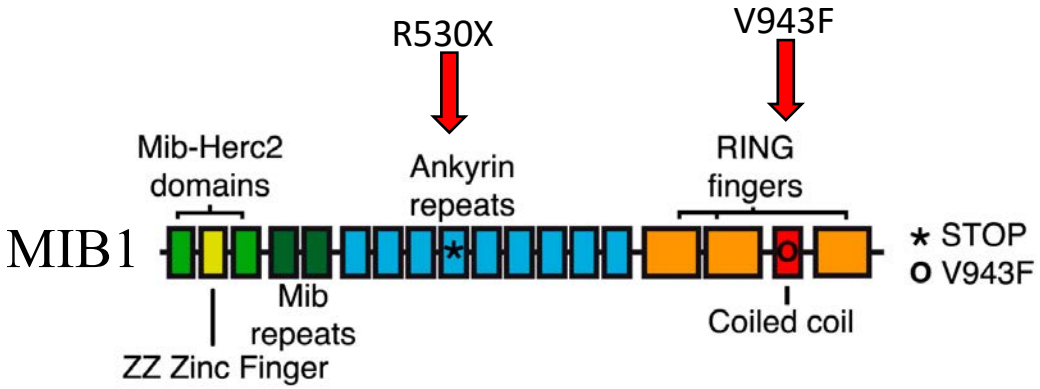
RELEVANCE AND TRANSLATIONAL IMPACT:

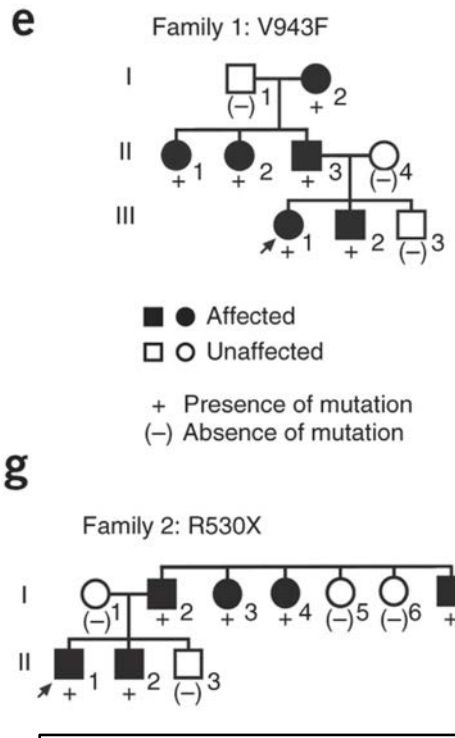
- Understanding the mechanism underlying BAV formation
- Identification of novel valve disease markers

II. Ventricular chamber development & cardiomyopathy. Previous work: Mutations in *MIB1* cause LVNC

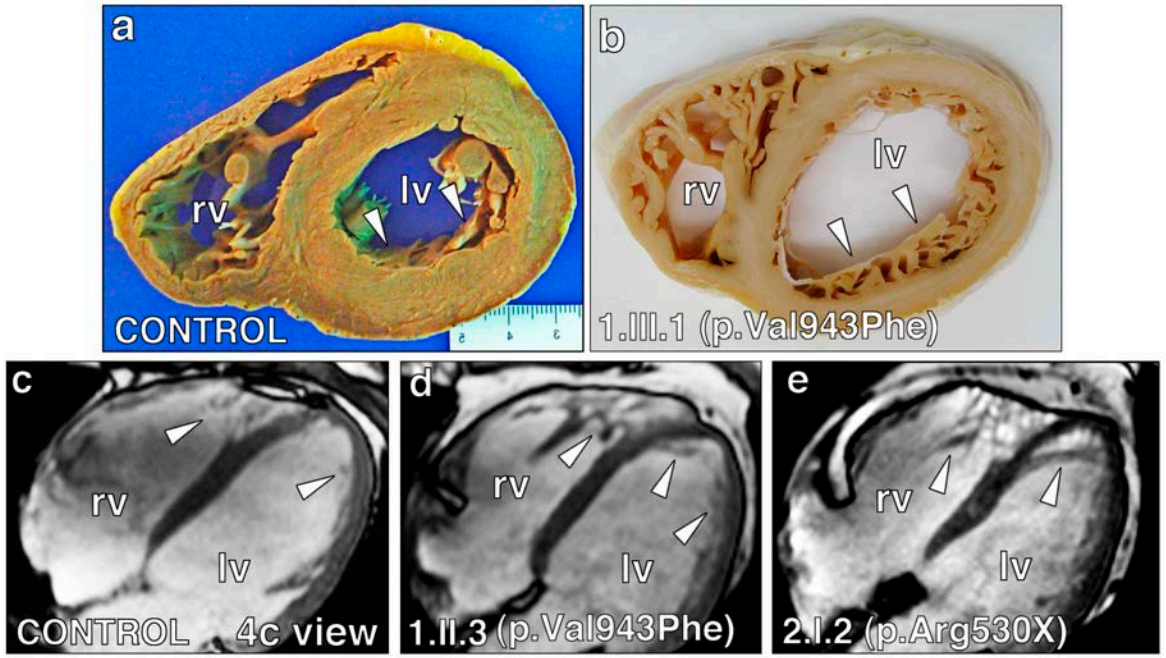
-LVNC cohort with 100 cases: 50% familial







Autosomal dominant inheritance



Luxán et al. *Nat Med* (2013) García-Pavía and de la Pompa *JACC* (2014)

Recent work. Ventricular chamber development: Trabeculation & Compaction

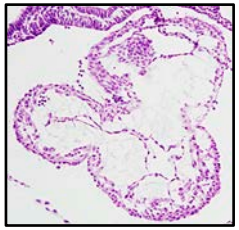
Trabeculation

Compaction

WT

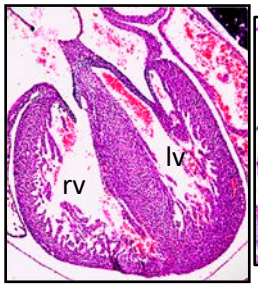
Dll4^{flox};Tie2-Cre

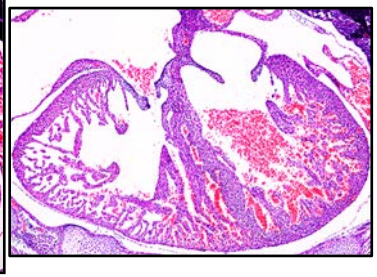
E9.5



Mib1^{flox};cTnT-Cre or
Jag1^{flox};Jag2^{flox};cTnT-Cre

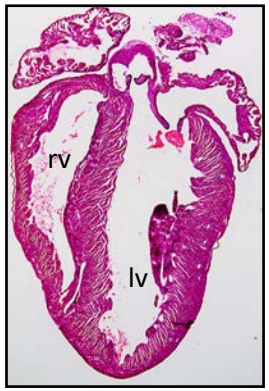
E16.5

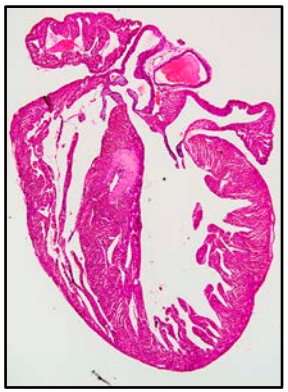




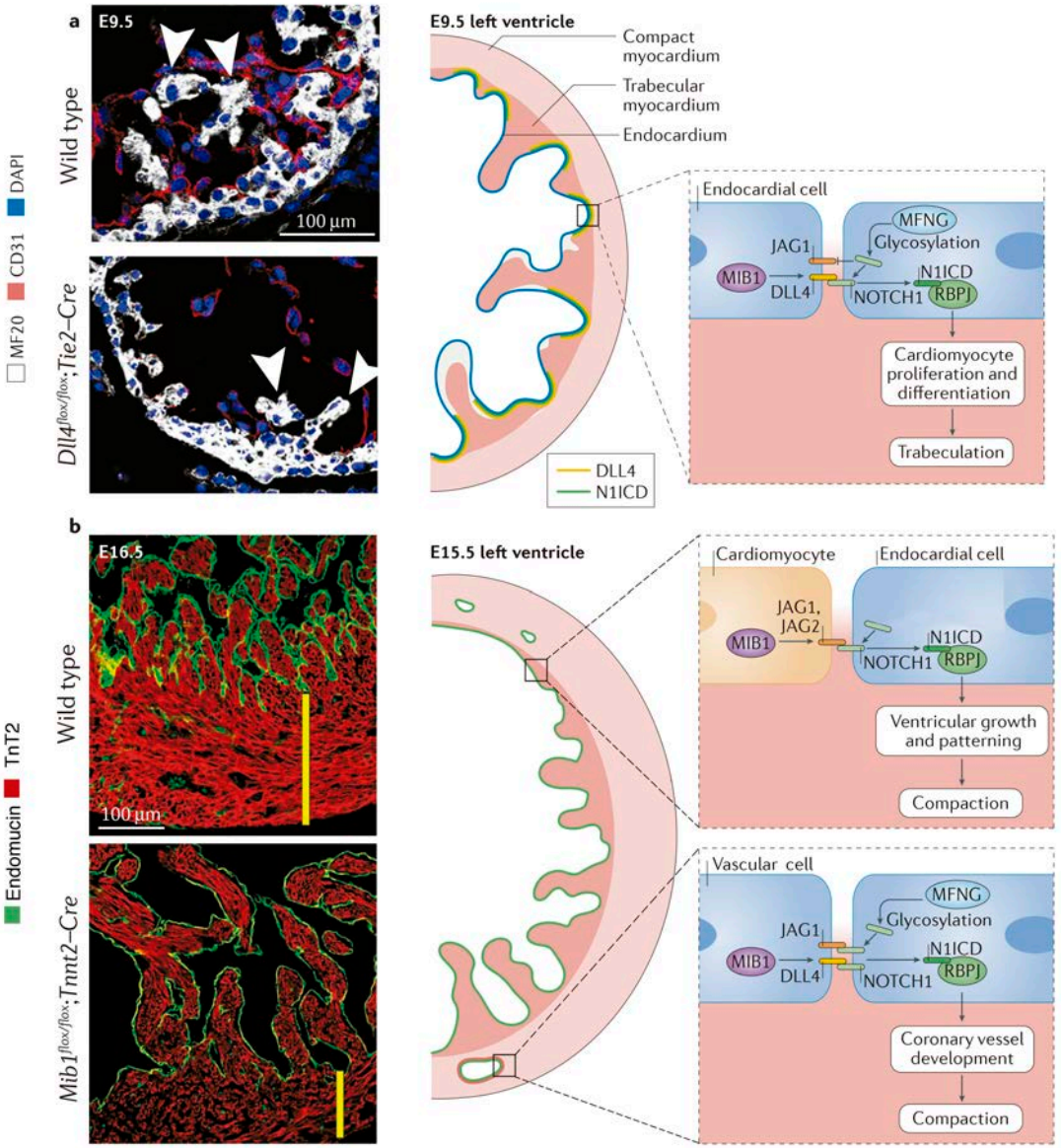
Mib1^{flox};cTnT-Cre or
Jag1^{flox};Jag2^{flox};
cTnT-Cre

P3





Endocardium/Myocardium dependent



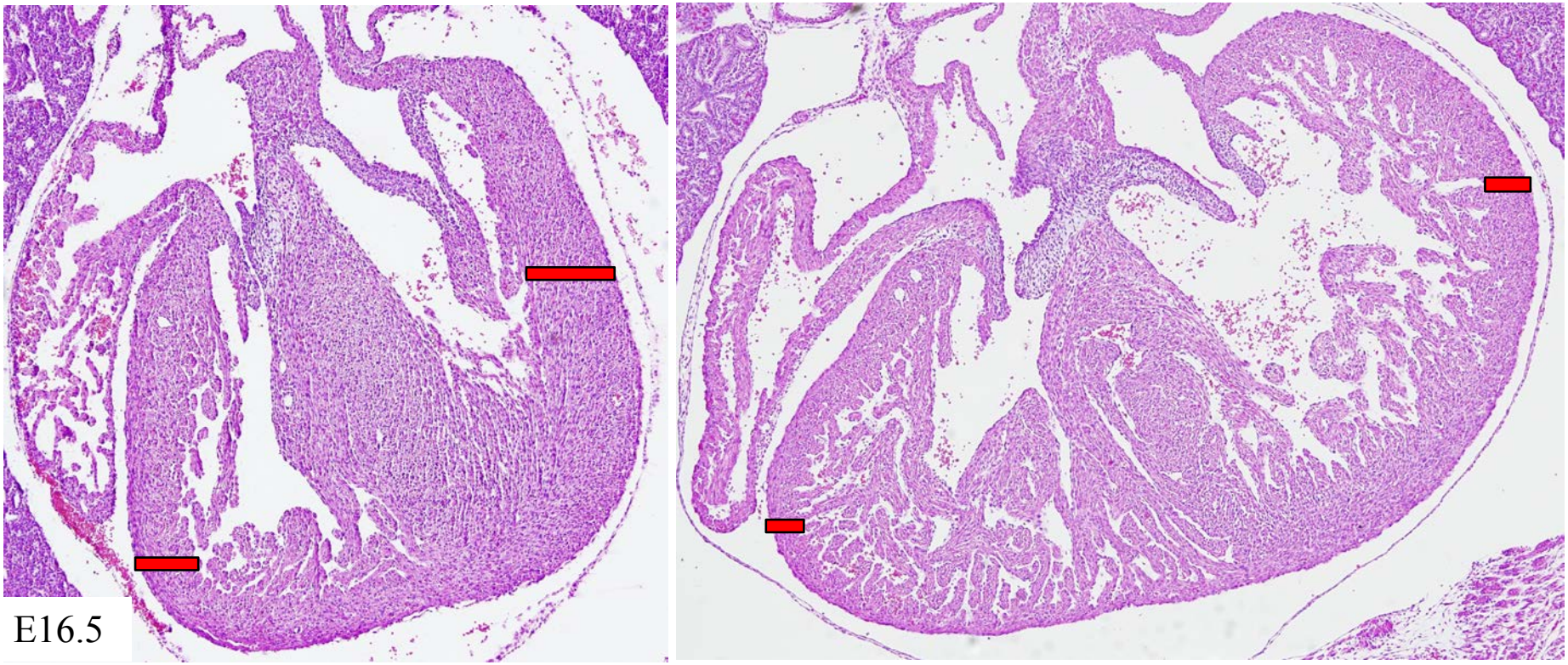
- Luxán et al. *Nat Med* (2013)
- D'Amato et al. *Nat Cell Biol* (2016)
- MacGrogan et al. *Nat Rev Cardiol* (2018)
- VanDusen et al. *Cell Reports* (2013)
- Münch et al. *Development* (2017)
- Travisano et al. (submitted)

- ***Ongoing:*** **Functional analysis of *Mib1* alleles found in LVNC families**

Generation of *MIB1*^{R530X} and *MIB1*^{V943F} alleles in mice using Crispr-Cas9 gene edition

Mib1$^{flox/+}$*; cTnT-Cre/+*

Mib1$^{R530X/flox}$*; cTnT-Cre/+*



1. *Mib1* inactivation in the myocardium (*Mib1*$^{R530X/flox}$*;cTnT-Cre*) leads to LVNC. Similar to *Mib1*$^{flox/flox}$*;cTnT-Cre*.

   Mib1$^{R530X/+}$: No LVNC. BAV and VSD when combined with *Notch1* LOF.

2. *Mib1*^{V943F}: No LVNC phenotype in heterozygous or homozygous conditions. BAV and VSD when combined with *Notch1* LOF.

- Modifiers? Oligogenic inheritance? Exome

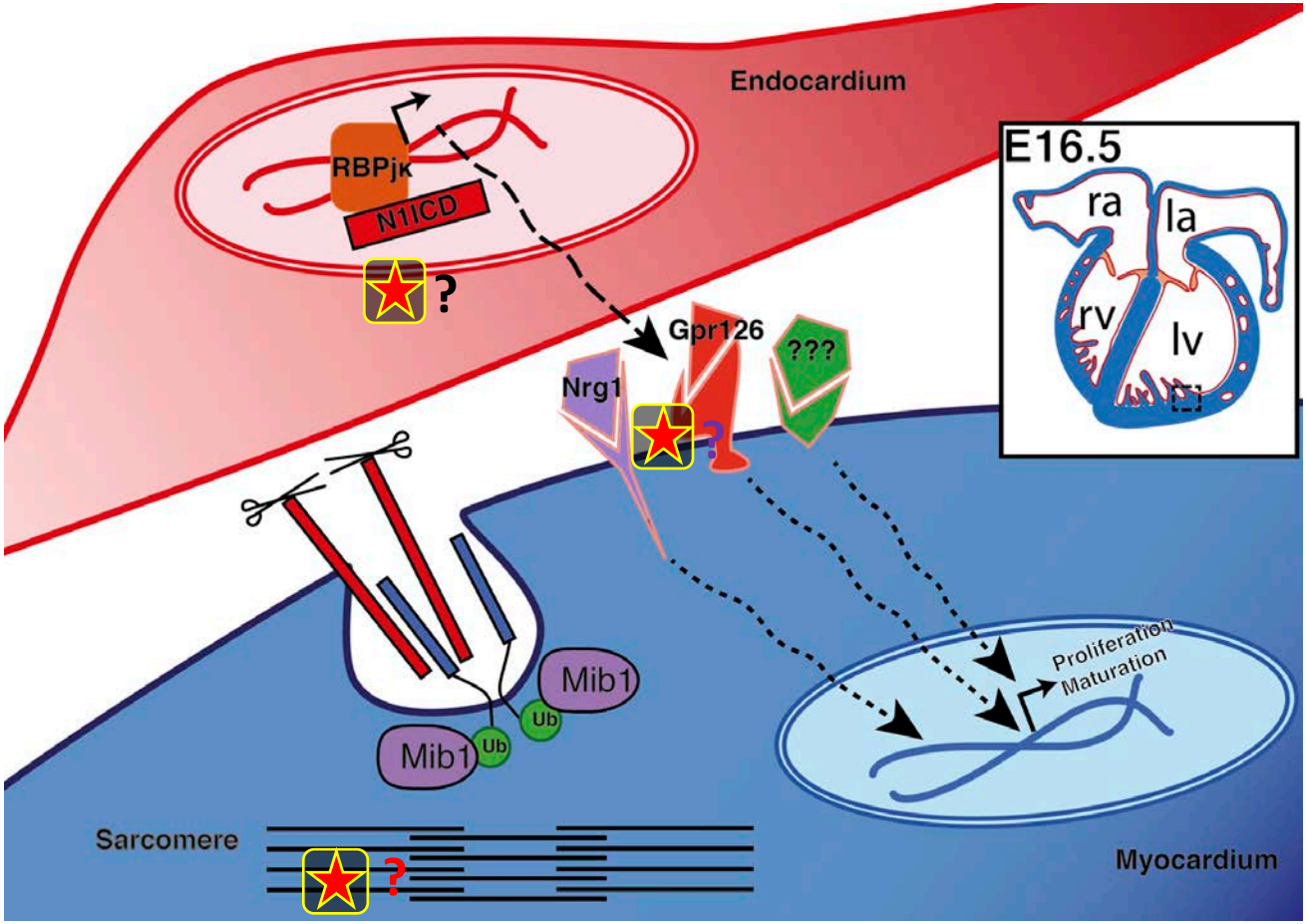
Exome sequencing: 84 individuals (24 families, including healthy controls and at least two LVNC samples)

Different possibilities:

1. Mutations affecting the NOTCH pathway

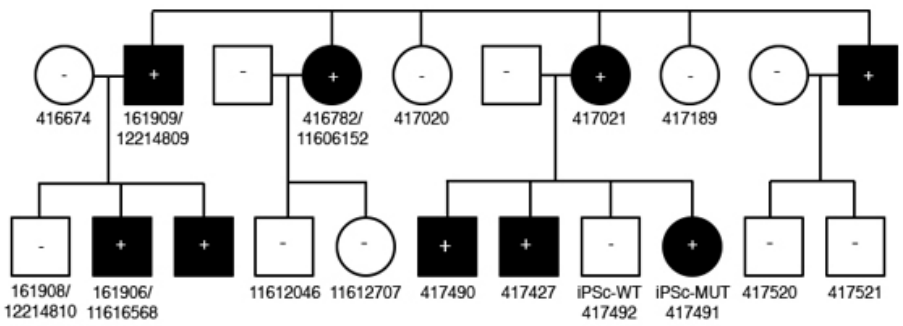
2. Mutations in mediators of endocardium-myocardium communication

3. Mutations in structural/sarcomeric/ ion channels/metabolic genes



- All these possibilities could be intervening alone or in combination to cause LVNC

Arg530X (H862) family



CHROM	SYMBOL	Comment	Protein_position	Amino_acids	Existing_variation
18	*APCDD1*	Wnt inhibitor, expressed during heart development		150 V/I	rs3748415
18	***MIB1***			***530 R/****	***rs201850378&CM130893***
18	*ASXL3*	Homeotic genes, polycomb TF. Expressed in heart. Bainbridge-Ropers		1415 M/V	rs181303838

SYMBOL	416675	S11606152	S11616568	S12214809	S417190	S417491	S417021	S417490	S11612046	S12214810	S417020	S417189	S417492	S417520	S417521
APCDD1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MIB1	***0/1***	***0/1***	***0/1***	***0/1***	***0/1***	***0/1***	***0/1***	***0/1***	***0/0***	***0/0***	***0/0***	***0/0***	***0/0***	***0/0***	***0/0***
ASXL3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0

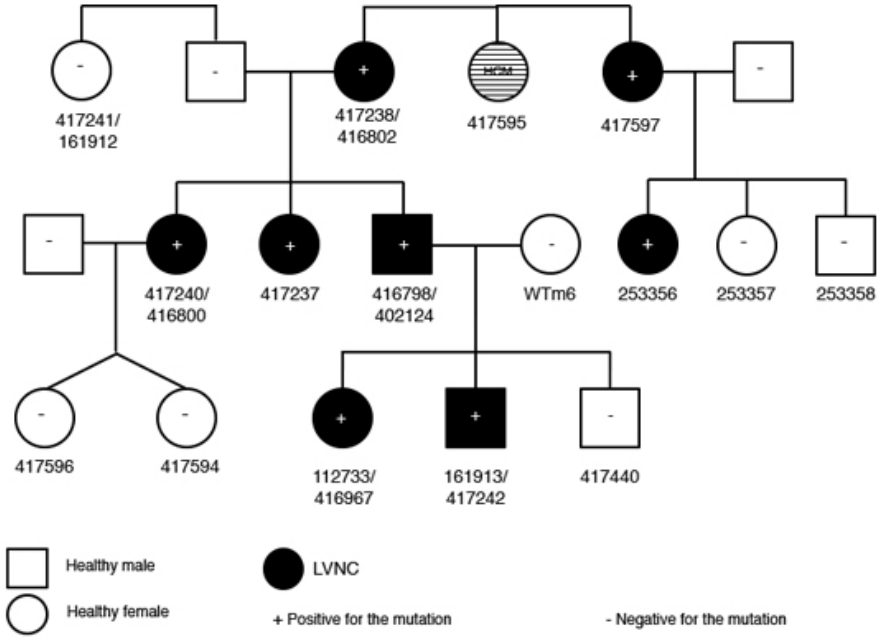
Proband

8 affected | 7 healthy

SYMBOL	Conserved	gnomAD_AF	gnomAD_NFE_AF	LoFtool	CADD_PHRED
APCDD1	Conserved in mammals	0.1156	0.1289	0.555	12.86
MIB1		***0.00003657***	***0.00006273***	***0.882***	***37***
ASXL3	Poorly conserved in mammals	0.0001097	0.00008956	0.222	0.003

- LVNC due to the presence of *R530X APCDD1 ASXL3* heterozygous mutations? Mouse models by Crispr-Cas9 gene edition

V943F (H243) family



CHROM	SYMBOL	Comentarios	Protein_position	Amino_acids	Existing_variation
18	*CEP192*	Centromeric protein	951	T/M	rs141331552
18	***MIB1***	***V943F Variant***	***943***	***V/F***	***rs200035428***
12	*BCL7A*	Intronic variants affecting expression. Burkitt. Exp. in developing heart	Intronic		rs884785&rs884786
18	*TMX3*	Found in haplotypes. Expressed in heart, 2 isoforms. Protein stabilization	Intronic (191?)	F/X	rs143627864

Tmx3. Disulfide isomerase (PDI) family of endoplasmic reticulum (ER) proteins that catalyze protein folding and thiol-disulfide interchange reactions.

SYMBOL	S416967	S253356	S416798	S417237	S417238	S417240	S417241	S417440	S253357	S253358	S417594	S417596	SWTm6	S417595
CEP192	0/1	0/1	0/1	0/1	0/1	0/1	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0
MIB1	***0/1***	***0/1***	***0/1***	***0/1***	***0/1***	***0/1***	***0/0***	***0/0***	***0/0***	***0/1***	***0/0***	***0/0***	***0/0***	***0/0***
BCL7A	0/1	0/1	0/1	0/1	0/1	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/1
TMX3	0/1	0/1	0/1	0/1	0/1	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

Proband

6 affected | 7 healthy | 1 HCM

SYMBOL	Conservation	gnomAD_AF	LoFtool	CADD_PHRED
CEP192	Not conserved	0.00002439	0.85	18.45
MIB1	***Highly conserved***	***0.0001625***	***0.882***	***19.75***
BCL7A	Conserved in mammals		0.379	1.525
BCL7A	Conserved in most mammals.		0.379	6.117
TMX3	Conserved in mammals.	0.09514	0.726	1.023

- LVNC due to the presence of *V943F CEP192 TMX3; BCL7A* heterozygous mutations? Mouse models

Future work:

1. Valves

-Mechanisms regulating valve mesenchyme proliferation downstream of NOTCH: *Hbegf GOF , Cxcl12 LOF.* Impact in BAV?

-*Mib1-Gata6* and BAV

-*Mib1; TercKO* and CAVD

-Gene profiling of human valve development and CAVD disease: Early disease marker identification & validation

2. Ventricles/cardiomyopathies

-Role of *Gpr126, Nrg1/ErbB2 & EphrinB2* (Notch targets) in ventricular wall development: Live imaging and functional studies

-scRNA-seq: Genetic transitions in compaction

-NOTCH-dependent transcriptional regulatory landscape during ventricular wall development (ATAC-seq)

-CM differentiation of $MIB1^{R530X}$ and $MIB1V^{943F}$ hiPSC

-Oligogenic inheritance of LVNC: *MIB1-TMX3-CEP192* and *MIB1-APCDD1-ASXL3* mouse models

-Sarcomeric genes (*MYBPC3*), HCM and LVNC: $MYBPC3^{R887A\,fs*160}$, $MYBPC3^{c.26\text{-}2A>G}$ and $MYBPC3^{P108A\,fs*9}$

RELEVANCE AND TRANSLATIONAL IMPACT:

- Understand the mechanisms controlling valve mesenchyme proliferation
- Understand the mechanisms underlying BAV (affects 1-2% of the population: Lewin and Otto, *Circulation* 2005)
- Identification of early valve disease markers
- Understand the molecular and celular bases of ventricular chamber development
- Contribute to understand the genetics of LVNC (prevalence 0.05–0.3%, Towbin et al., *Lancet* 2015) and its relation with HCM
- Identify the developmental and/or structural bases of LVNC