BRIGHAM AND
WOMEN'S HOSPITAL

HARVARD
MEDICAL SCHOOL

De Novo Variants: Can these Inform Clinical Phenotypes?

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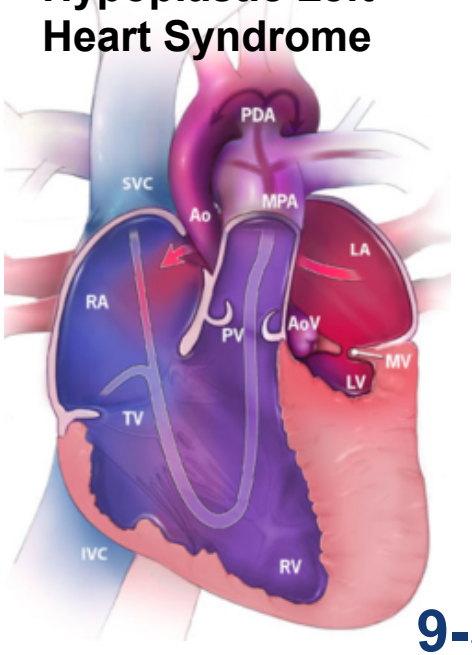
Hypoplastic Left Heart Syndrome



Critical CHD

2-3/1000 Live Births

~9% all CHD

Prevalence:1.45/1000 children

0.4 in 1000 adults

Mean Age (2000): 17 years

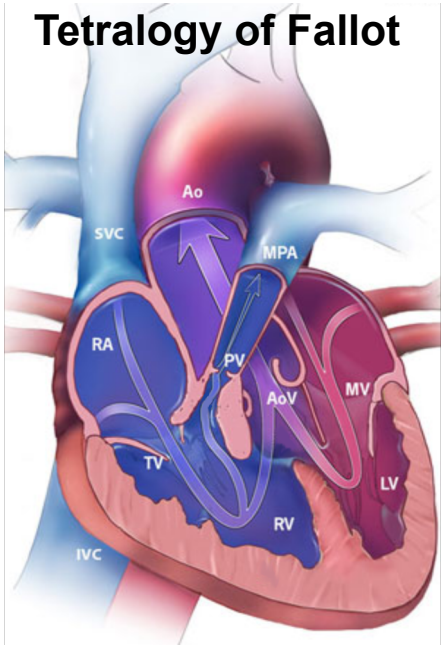
Never Familial

Life-long Health Issues:

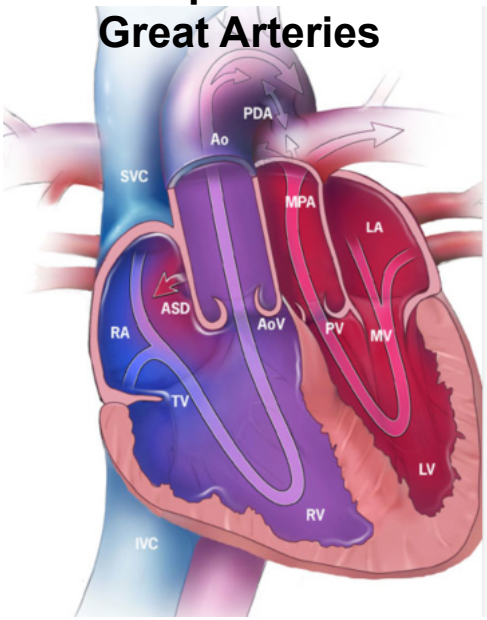
11% Congenital anomaly

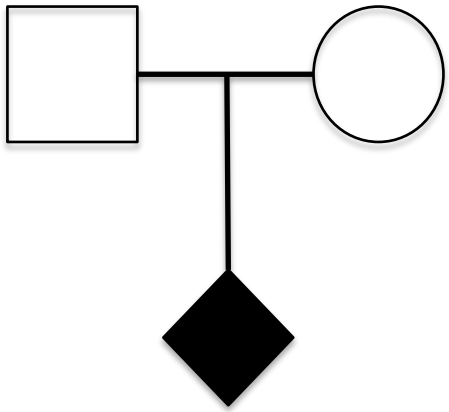
9-50% Neurodevelopmental deficits (NDD)

Tetralogy of Fallot



Transposition of Great Arteries





Schematics Courtesy of the
Center for Disease Control
National Center Birth Defects/Disabilities

Pediatric Cardiac Genomics Consortium

Collaborating Sites:

Brigham & Women's Hospital
Children's Hospital Boston
Columbia Medical Center
Children's Hospital Philadelphia
Children's Hospital Los Angeles
Mount Sinai Medical Center
Rochester Medical Center
UCLA
Yale School of Medicine
NHLBI

Hypothesis:

***De Novo* Gene Mutations Cause Critical CHD**

Mutations are Damaging

Loss of Function (LoF):

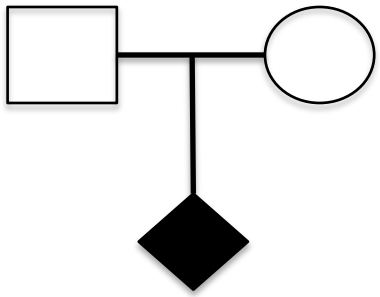
Premature Stop, Frameshift

Deleterious Missense Resides

Bench to Bassinet

National Heart Lung and Blood Institute
People Science Health

Whole Exome Sequencing of Critical CHD Trios



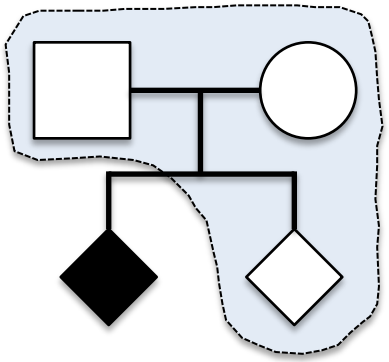
1220 critical CHD Trios
Exclude Syndromic Cases
Unaffected parents
Family history negative

vs. Expected Number per Exome

Based on the probability of mutation at each base, controlling for

a) Local sequence context
b) Depth of coverage
c) Divergences score based on human-macaque differences
d) Exclude in-frame insertions/deletions
e) Meta-SVM for Missense variants

(Samocha et al., Nature Genetics, 2014)



vs. Observed in 900 Control Trios

Unaffected parents
Unaffected sibling of Autistic Child

Simons Simplex Cohort Collaborators:

Matthew State, Michael Wigler, Eric Eichler

PCGC

Damaging *De Novo* Variants: Enriched in CHD Cases *vs* Expected or Observed

	CHD Cases, N = 1220						Controls, N = 900					
	Observed		Expected		Enrichment	p	Observed		Expected		Enrichment	p
	n	Rate	n	Rate			n	Rate	n	Rate		
All genes												
Total	1281	1.05	1320.3	1.08	1.0	0.86	925	1.03	979.7	1.09	0.9	0.96
Synonymous	279	0.23	373.5	0.31	0.7	1	229	0.25	277.4	0.31	0.8	1
Missense	850	0.70	829.6	0.68	1.0	0.24	614	0.68	615.6	0.68	1.0	0.53
D-Mis	212	0.17	133.8	0.11	1.6	**$2.9x10^{-10}$**	118	0.13	99.3	0.11	1.2	0.036
LoF	152	0.12	117.1	0.10	1.3	**0.0011**	82	0.09	86.7	0.10	0.9	0.71
Damaging	364	0.30	251.0	0.21	1.4	**$1.4x10^{-11}$**	200	0.22	186.0	0.21	1.1	0.16
Genes Highly Expressed in Developing Heart												
Total	452	0.37	374.5	0.31	1.2	**$5.6x10^{-05}$**	273	0.30	277.7	0.31	1.0	0.62
Synonymous	82	0.07	104.1	0.09	0.8	0.99	81	0.09	77.3	0.09	1.1	0.35
Missense	289	0.24	235.7	0.19	1.2	**0.00043**	164	0.18	174.7	0.19	0.9	0.8
D-Mis	99	0.08	40.9	0.03	**2.4**	**$1.1x10^{-14}$**	37	0.04	30.3	0.03	1.2	0.13
LoF	81	0.07	34.7	0.03	**2.3**	**$1.5x10^{-11}$**	28	0.03	25.7	0.03	1.1	0.35
Damaging	180	0.15	75.6	0.06	**2.4**	**$1.6x10^{-24}$**	65	0.07	55.9	0.06	1.2	0.13
LHE genes												
Total	829	0.68	945.8	0.78	0.9	1	654	0.73	702.1	0.78	0.9	0.97
Synonymous	197	0.16	269.4	0.22	0.7	1	149	0.17	200.1	0.22	0.7	1
Missense	561	0.46	593.9	0.49	0.9	0.92	451	0.50	440.9	0.49	1.0	0.32
D-Mis	113	0.09	93.0	0.08	1.2	0.024	81	0.09	69.0	0.08	1.2	0.086
LoF	71	0.06	82.4	0.07	0.9	0.91	54	0.06	61.1	0.07	0.9	0.83
Damaging	184	0.15	175.4	0.14	1.1	0.27	135	0.15	130.1	0.14	1.0	0.34

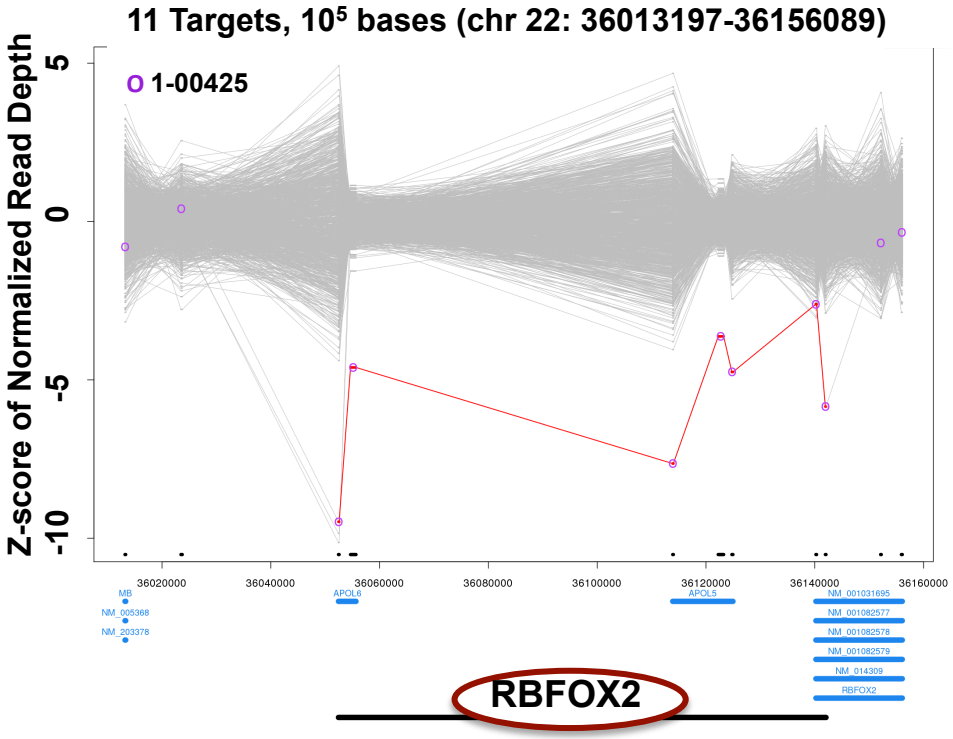
Homsy et al, Science 2015

Evidence for Pathogenicity: 21 Genes with Recurrent Damaging *De Novo* Variants

Gene	LoF	D-Mis	p
PTPN11	0	4	2.96×10^{-11}
KMT2D	4	2	4.24×10^{-09}
RBFOX2	3	0	3.46×10^{-08}
KDM5B	3	0	2.93×10^{-06}
KRT13	0	2	1.02×10^{-05}
MYH6	0	3	2.45×10^{-05}
CAD	0	3	3.80×10^{-05}
NAA15	2	0	4.72×10^{-05}
SMAD2	1	1	1.10×10^{-04}
RABGAP1L	1	1	4.05×10^{-04}
POGZ	1	1	4.38×10^{-04}
JAG1	1	1	4.52×10^{-04}
GANAB	1	1	4.57×10^{-04}
DTNA	1	1	4.73×10^{-04}
PPL	1	1	6.05×10^{-04}
CHD7	2	0	6.23×10^{-04}
ZEB2	1	1	6.25×10^{-04}
FBN1	0	2	6.86×10^{-04}
CHD4	0	2	1.17×10^{-03}
AHNAK	1	1	2.91×10^{-03}
NOTCH1	1	1	4.40×10^{-03}

De Novo Copy Number Variants Detected by WES

Glessner*, Bick,* et al, Circ Res 2014



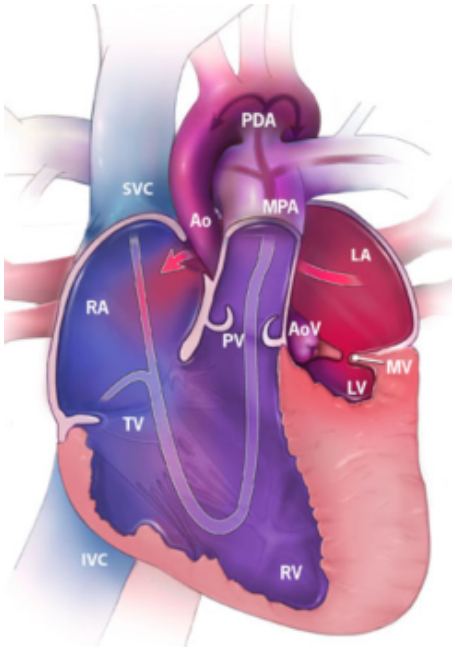
De Novo RBFOX2 LoF Variants

3 SNVs (frameshifts/stop)

1 CNV(del)

4 *De Novo* vs expected: p=1.60E-8

All with HLHS



De Novo Copy Number Variants Detected by WES

Glessner*, Bick,* et al, Circ Res 2014

RBFOX2 :

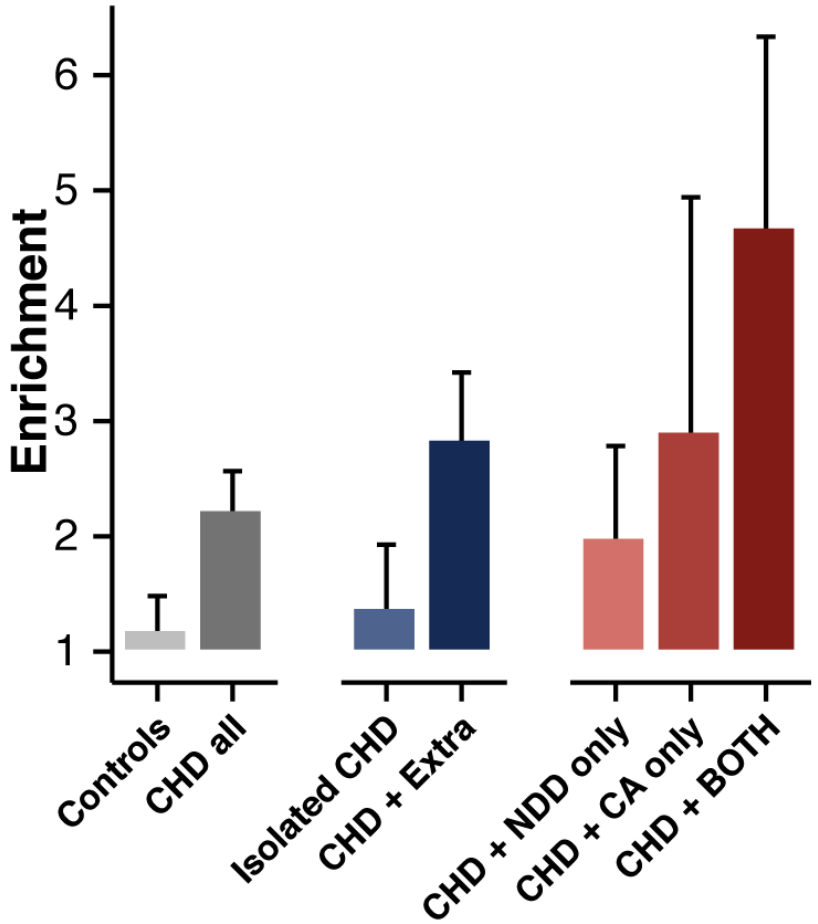
Binds mRNA splicing enhancer element (UGCAUG)
Contributes to tissue-specific exon splicing in pre-mRNAs
Highly expressed in heart (>250 reads/million)
Targets ~7% Human ES cell Genes ***(Yeo et al; Nat Struct Molec Bio, 2009)***

Table S9: *de novo* Enrichment Analysis in 2234 *RBFOX2* Target Genes

	Cases, n=1220				**Controls, n=900**			
	Observed	Expected	Enrichment	p	Observed	Expected	Enrichment	p
Total	320	203.5	1.57	**3e-14**	144	150.8	0.955	0.72
Syn	60	56.6	1.06	0.34	43	41.9	1.030	0.46
Missense	201	127.8	1.57	**1.4e-09**	79	94.7	0.834	0.96
D-Mis	57	23.2	**2.46**	**2.3e-09**	15	17.2	0.872	0.73
LoF	59	19.1	**3.08**	**2.2e-13**	22	14.2	1.550	0.032
Damaging	116	42.3	**2.74**	**9.3e-21**	37	31.4	1.180	0.18

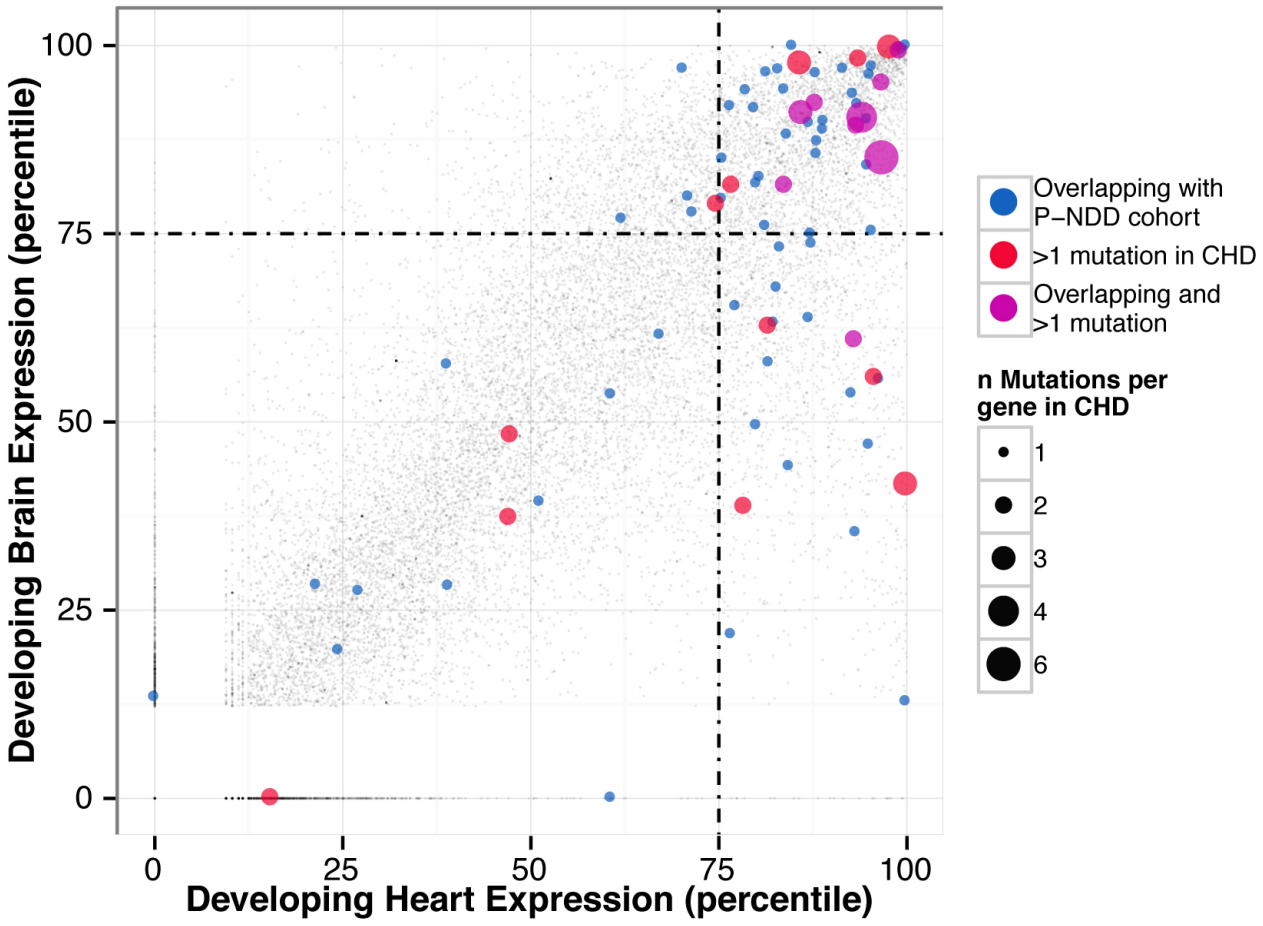
Genotype:Phenotype Assessments:
De Novo, Damaging Variants Enriched in CHD Subsets

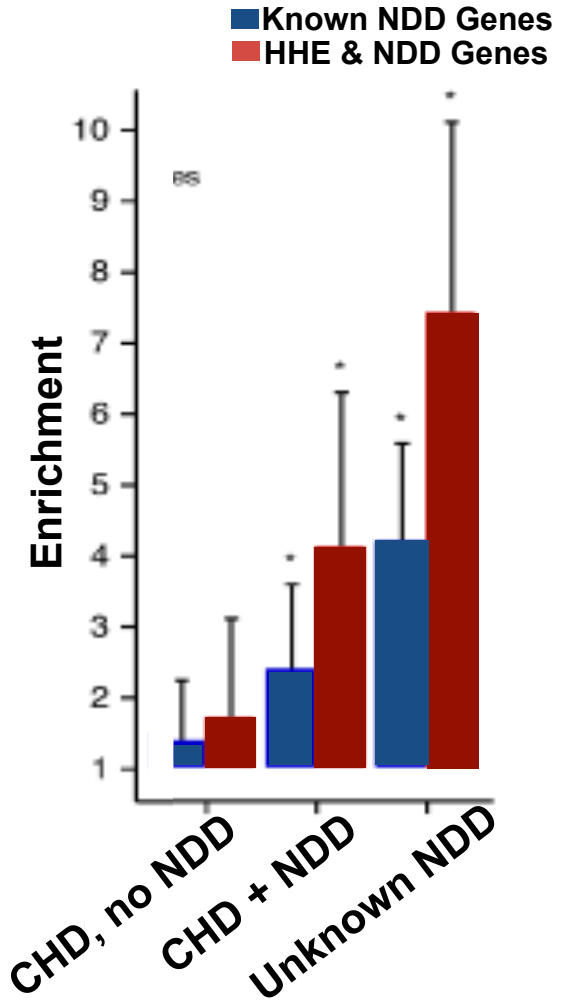
Extra: Non-cardiac Congenital Anomaly
NDD: Neurodevelopmental Delay/Deficits



Homsy et al, Science 2015

Genes Harboring *De Novo* Variants: Common to Both CHD and NDD Cohorts

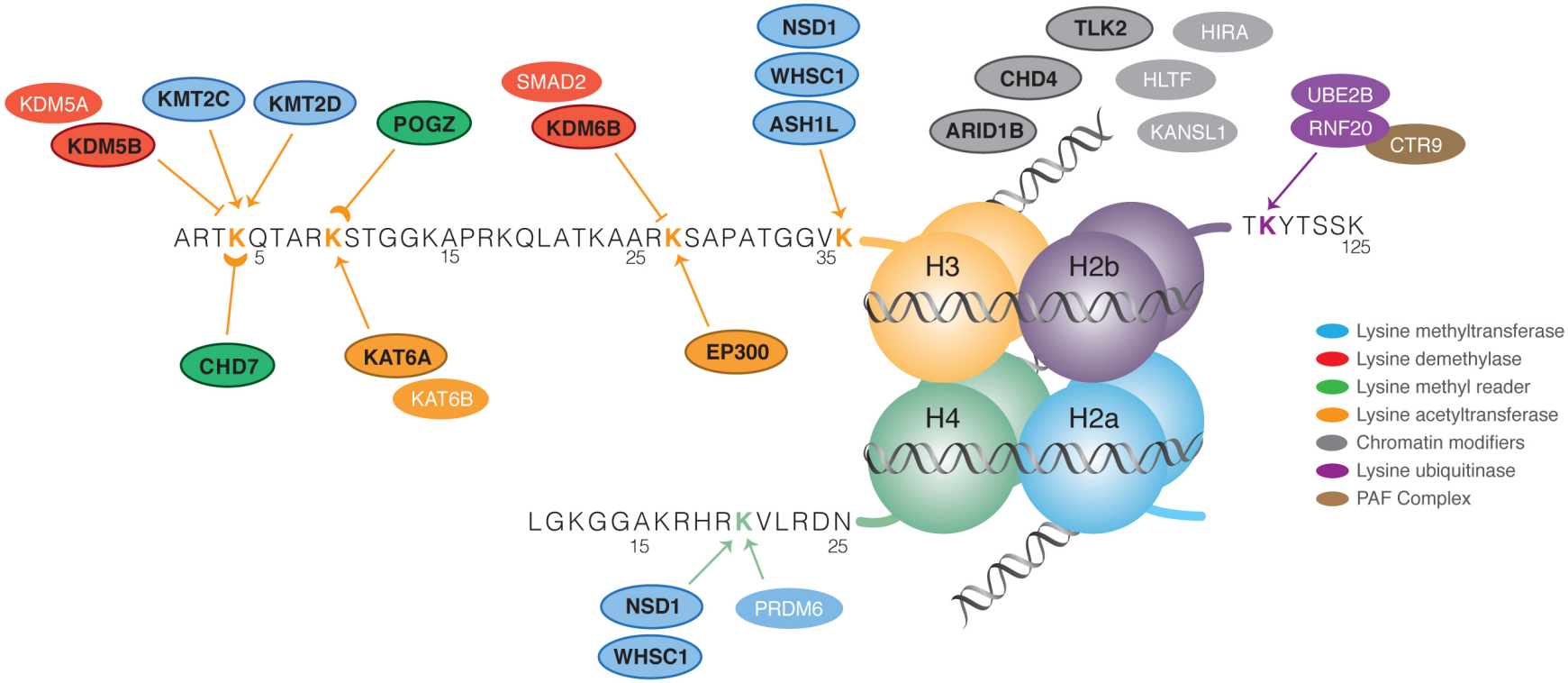




Homsy et al, Science 2015

Functional Annotation of Genes with *De Novo*, Damaging Variants

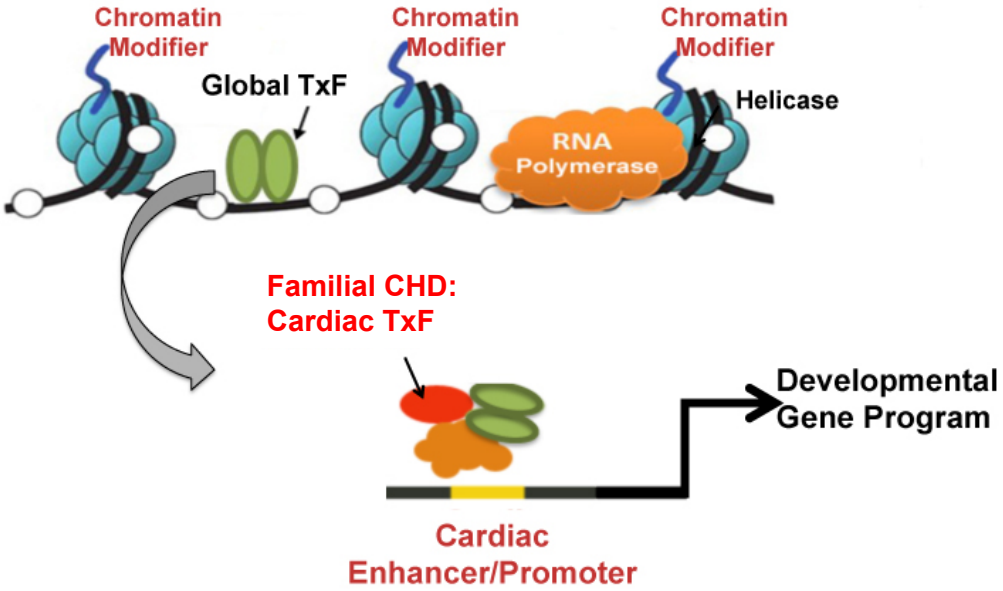
1. Neurologic Development ($p=1.8 \times 10^{-5}$)
2. Cardiovascular Developmental Processes ($p=8.6 \times 10^{-8}$)
3. Anatomic Structure Morphogenesis Modification ($p=1.1 \times 10^{-14}$)
4. Chromatin Modification ($p=7.5 \times 10^{-20}$)



Homsy et al, Science 2015

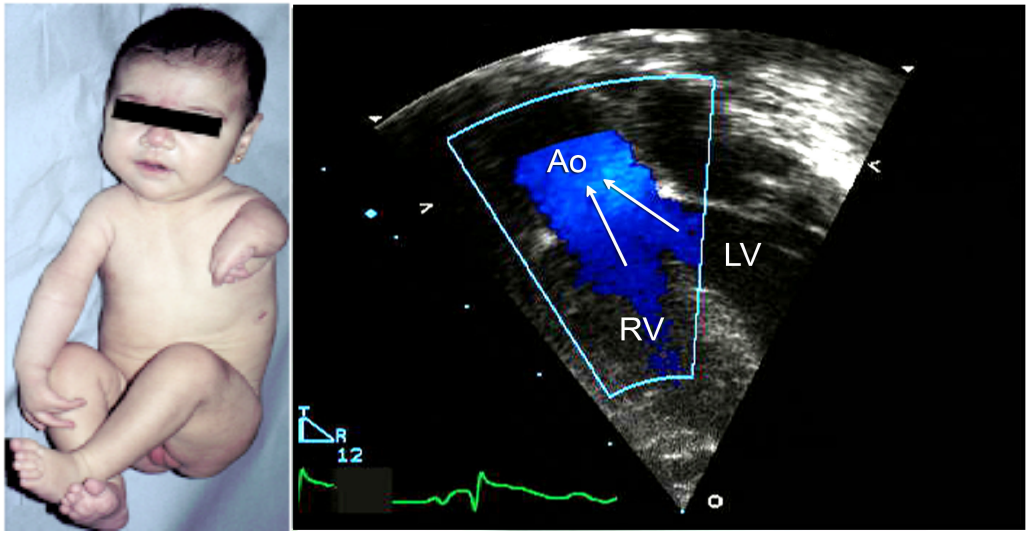
Evidence/Support that De Novo Variants are not VUS

- Unlikely Chance Co-Occurrence of *De Novo*, Damaging Mutations in "Constrained" Residues and Critical CHD
- Genes with *De Novo* Variants: Highly Expressed in Affected Tissues
- Additional (Unappreciated) Clinical Phenotypes are "Explained" by *De Novo* Variants
- Recurrent *De Novo,* Damaging Gene Variants among Probands with "Like" phenotypes
- Proteins encoded by Genes with *De Novo* Variants, like Familial Mutations Function in Related Biologic Pathways

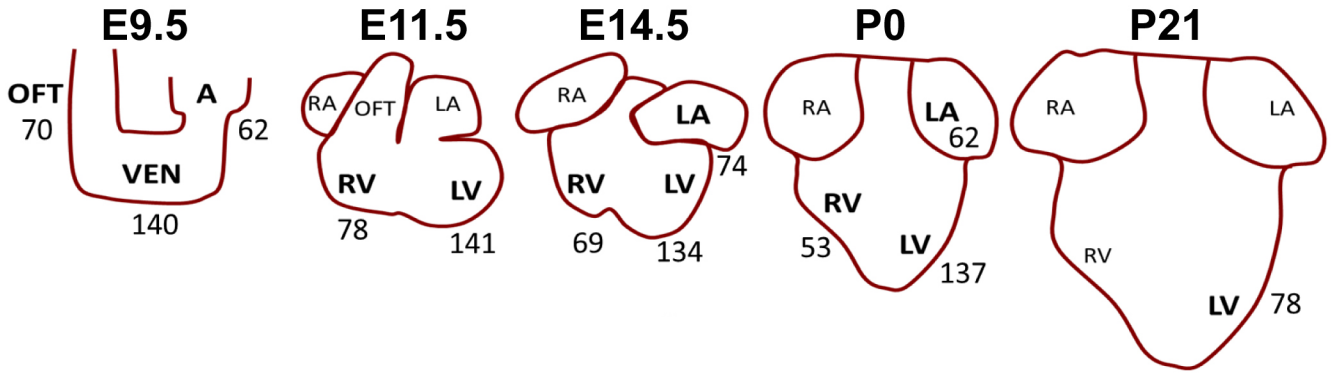


Evidence/Support that Damaging De Novo Variants do Not Fully Cause Clinical Phenotypes

- **69 Genes with *De Novo* Mutations (n=85) were shared in CHD and NDD cohorts. Highly UNLIKELY that NDD-ascertained patients with these variants had severe CHD.**
- **Not all CHD patients with *De Novo* Mutations in Genes identified in NDD-ascertained patients have NDD**
- **Mutations ≠ Definitive Causes of Disease: Asymmetric Limb Phenotypes in CHD patients**
- **Can Clinical Identification of De Novo Mutations be Diagnostic in Single Patients?**

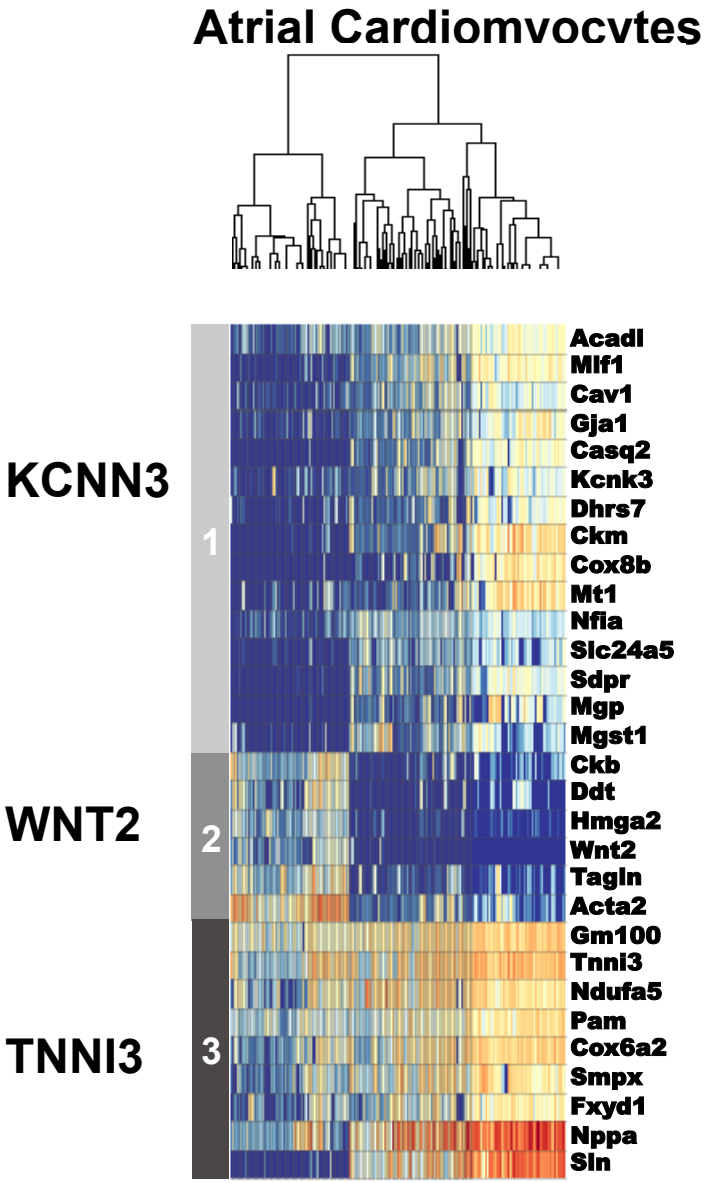


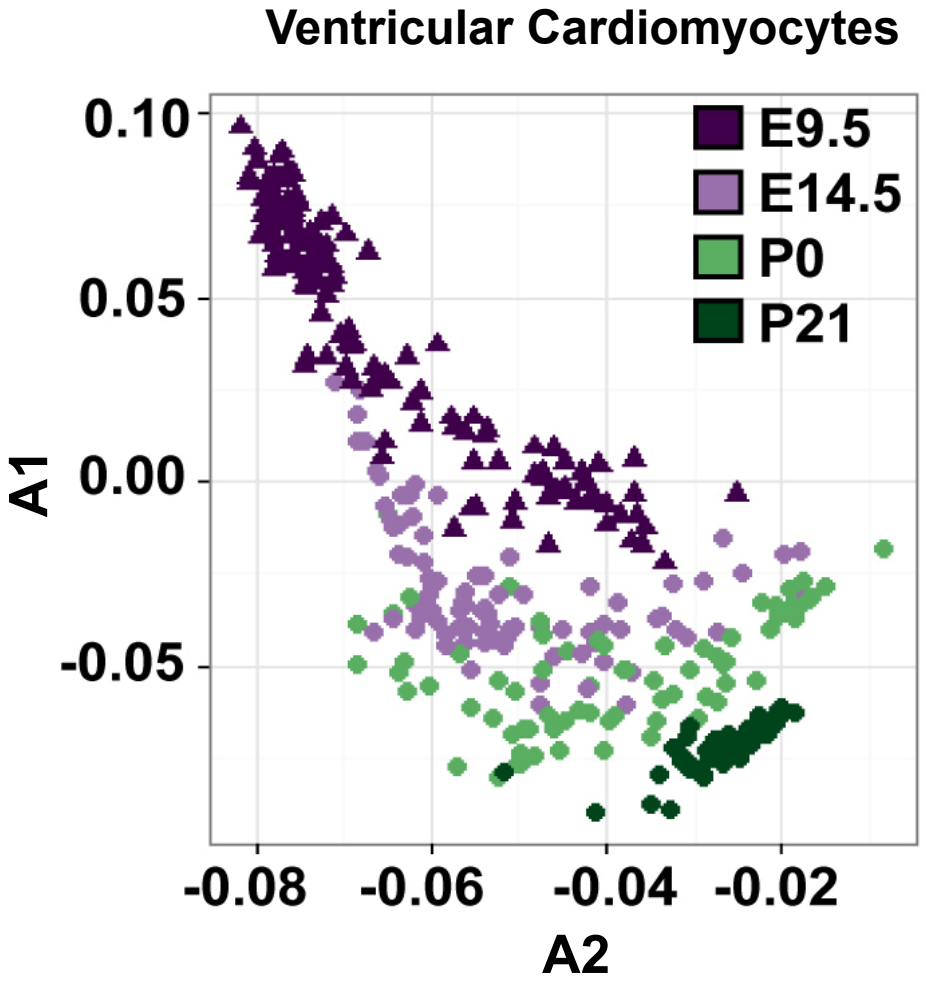
Strategies to Functionally Annotate CHD Mutations: Define Cardiac Developmental Transcription in Single Cells



Delaughter et al, in Review

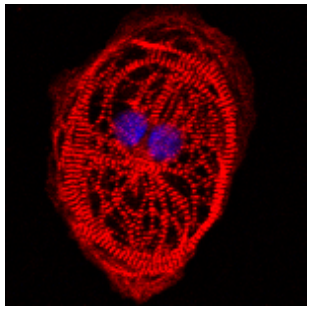
Strategies to Functionally Annotate CHD Mutations: Define Cardiac Developmental Transcription in Single Cells





Delaughter et al, in Review

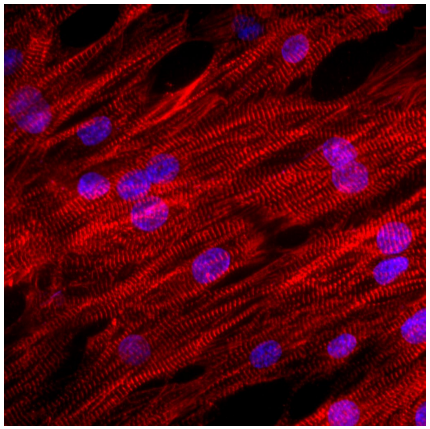
Modeling Human Mutations in Isogenic iPSC-derived Cardiomyocytes



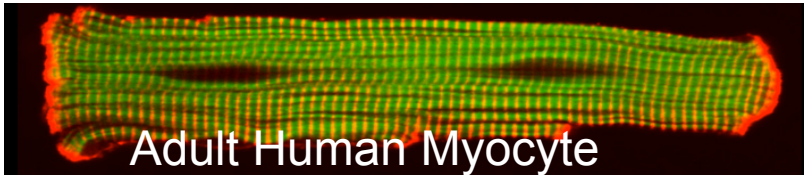
iPSC-derived Cardiomyocytes

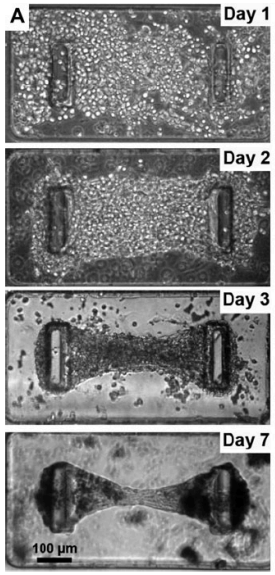
2D - patterned

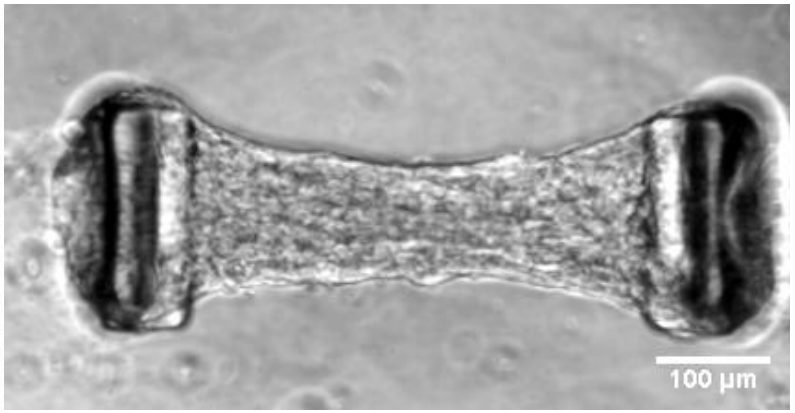
3D – Microtissue



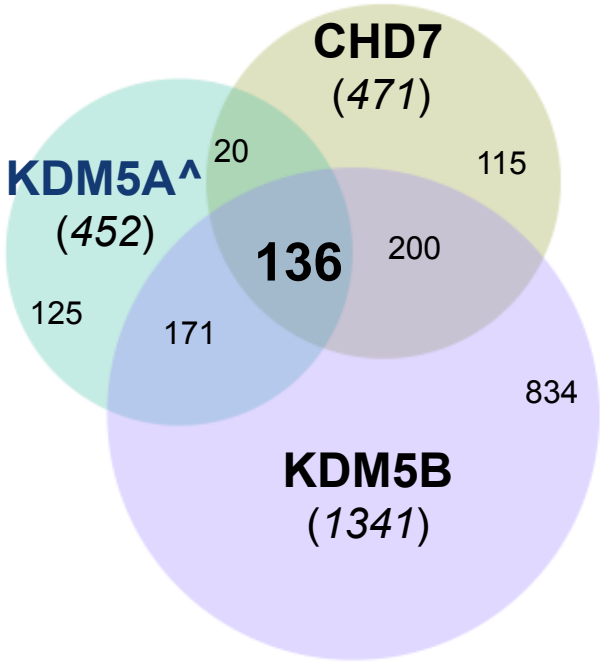
iPSC-CM



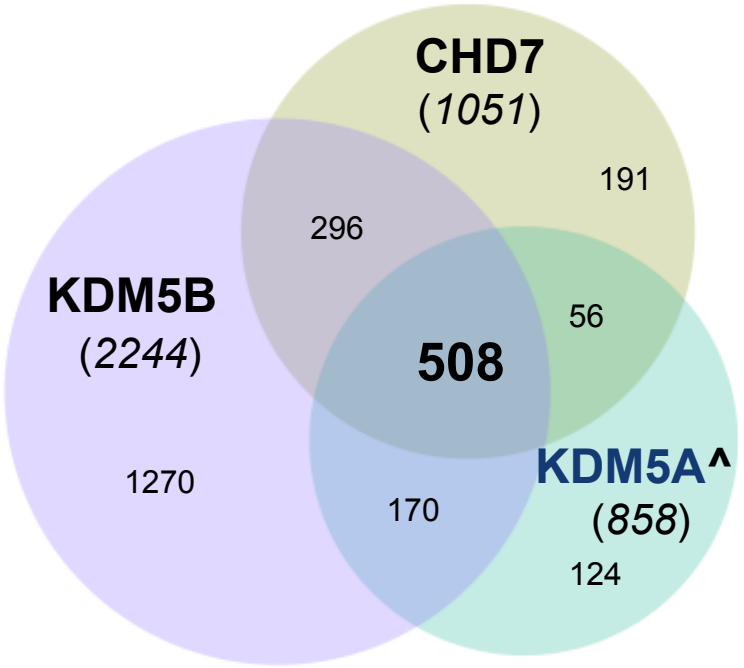




Transcriptional Differences between Isogenic WT and CHD-mutant Cardiomyocytes



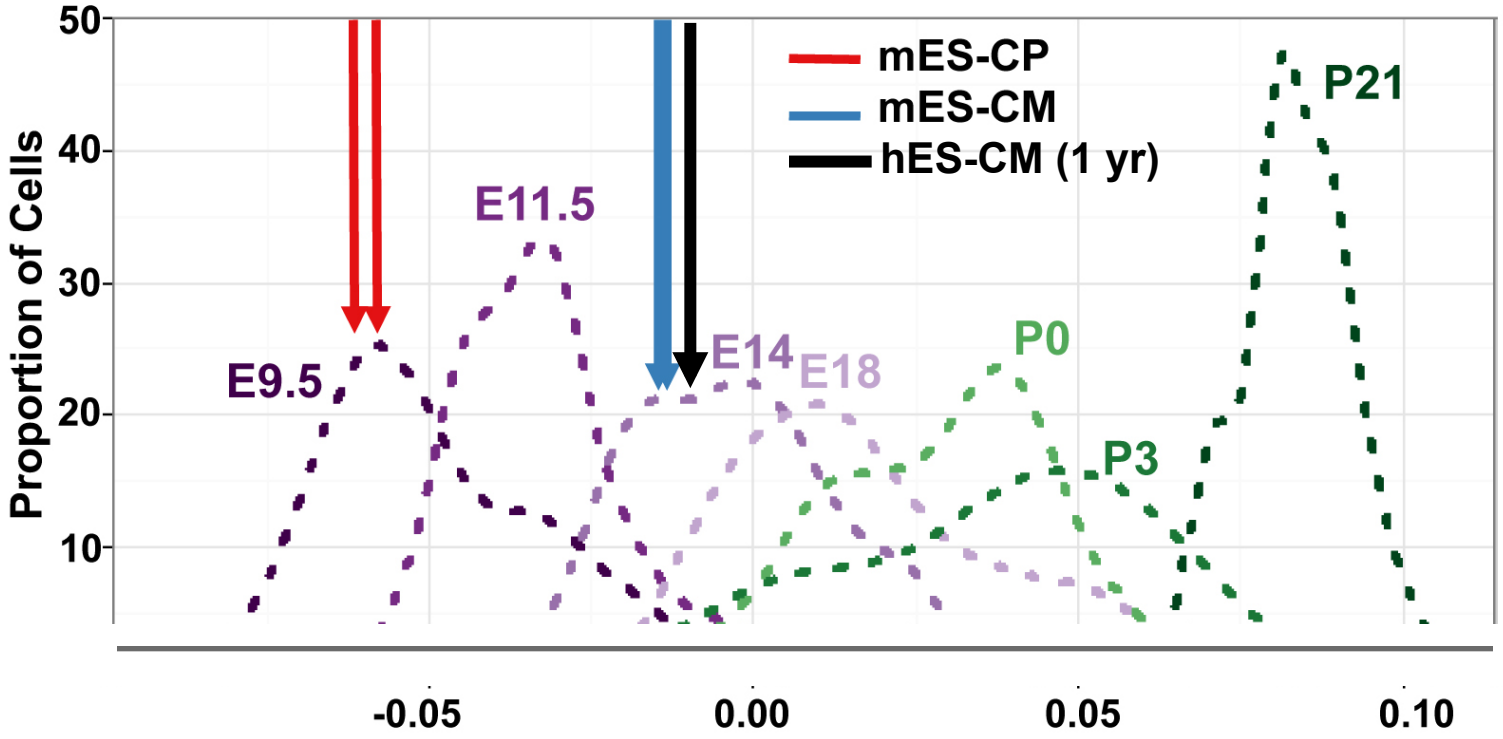
Decreased



Increased

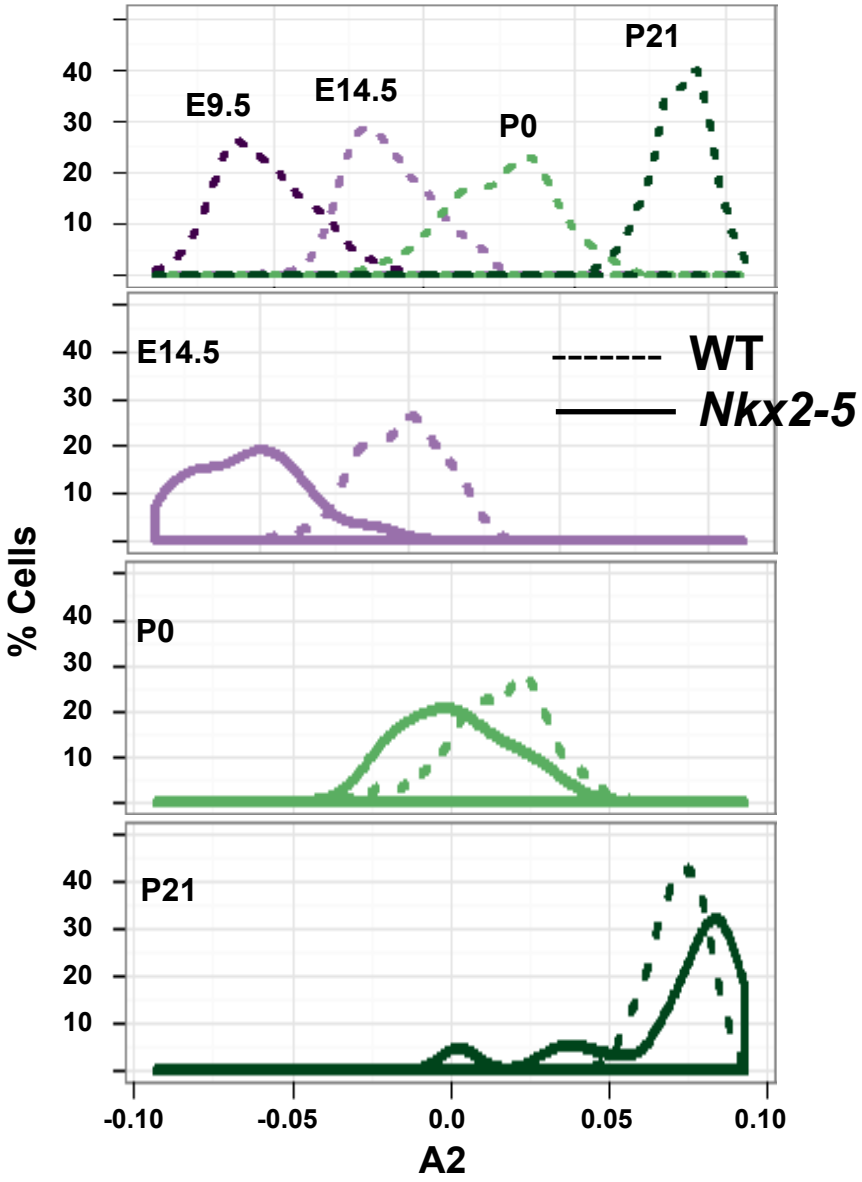
^KDM5A$^{+/R1508W}$ & KDM5A$^{+/-}$ >90%Congruent

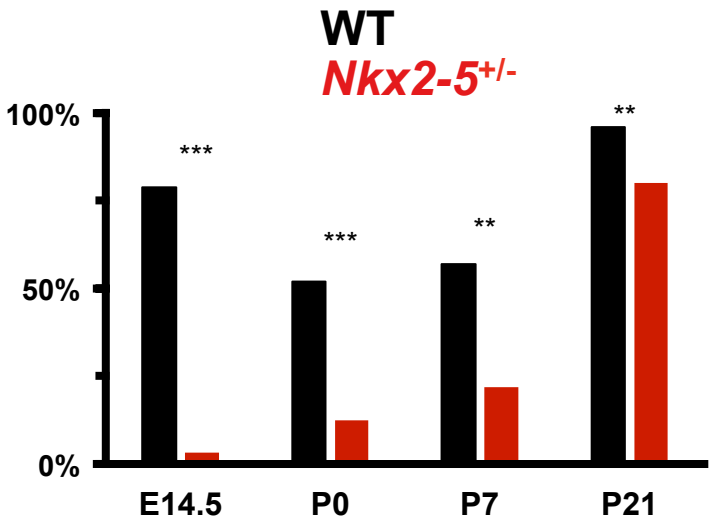
Developmental age of Cardiomyocytes Differentiated from Mouse/Human Embryonic Stem Cells



Delaughter et al, in Review

Developmental Delay in Cardiomyocytes from *Nkx2-5* Mice





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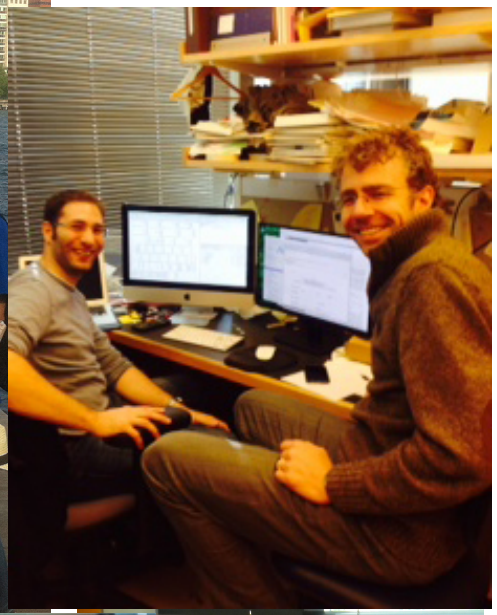

CHD Genetics

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David McKean
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Alex Bick
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Alireza Haghighi
Akl Fahed

RNAseq Studies

Dan Delaughter
Alex Bick
Arin Kim

iPS-Cardiomyocytes

Travis Hinson
Tarsha Ward
Warren Tai
Calvin Sheng
Navid Nafissi
David Conner

Pediatric CV Genetics Consortia

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Children's Hospital of Pennsylvania: Elizabeth Goldmuntz
Columbia: Wendy Chung
Icahn School of Medicine at Mt Sinai: Bruce Gelb
Yale: Martina Brueckner & Richard Lifton
NIH: Jonathan Kaltman and Charlene Shramm