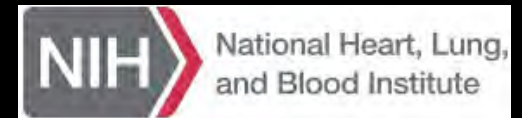


Leveraging Congenital Heart Disease Mouse Model Findings to Improve Clinical Outcome



GENOME Medicine IX

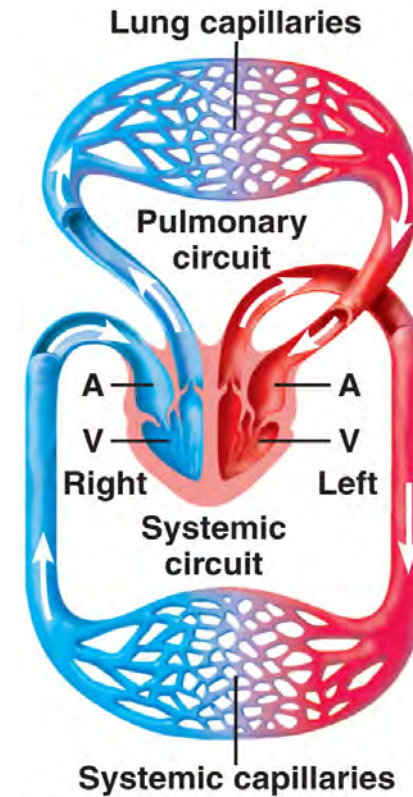
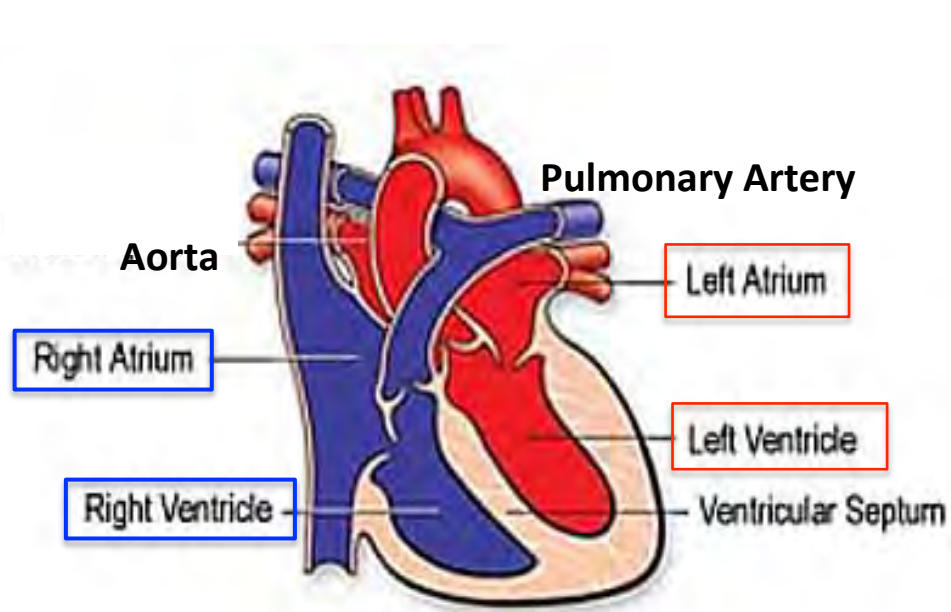


Cecilia Lo, Ph.D.
Department of Developmental Biology
University of Pittsburgh School of Medicine

Congenital Heart disease

- **One of the most common birth defects**
- **Characterized by abnormalities in cardiovascular structures**

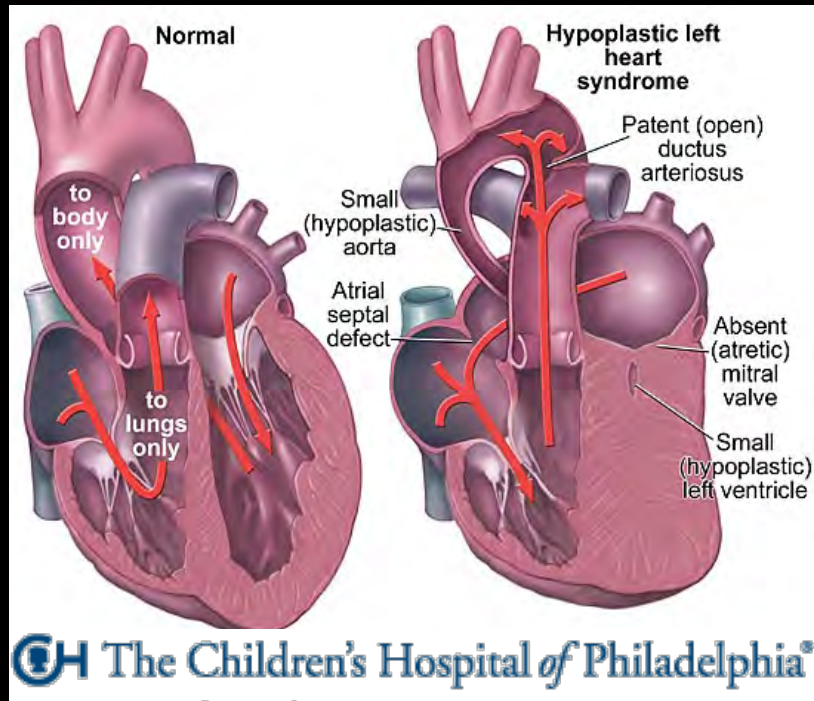
Four-Chamber Heart with Separate Systemic-Pulmonary Circulation



Congenital Heart Disease

- **Advances in surgical palliation allows most CHD patients to survive their structural heart defects**
- **Patients with the same structural heart defect can have very different outcome.**

Hypoplastic Left Heart Syndrome (HLHS)



- Aortic Atresia/Stenosis
- Hypoplastic LV
- Mitral Valve Atresia/Stenosis

Patient intrinsic factors play a significant role in determining the long term outcome of patients with HLHS and other CHD.

Genetic Etiology of Congenital Heart Disease

MOUSE

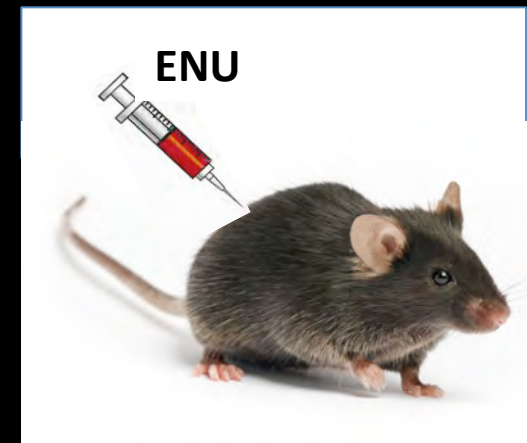


- Mice have same 4-chamber cardiac anatomy as human
- Inbred mice avoid genetic heterogeneity in human studies

SYSTEMS GENETICS APPROACH

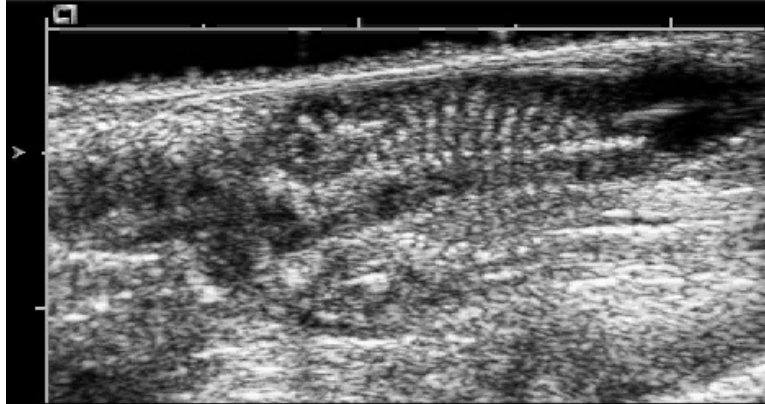
A large scale **forward genetic screen** to interrogate the genetic etiology of **congenital heart disease**

- **Phenotype driven approach without a *priori* gene bias**
- **Identify genes and pathways driving CHD pathogenesis**
- **Insights into the genomic context for disease pathogenesis**



In Utero Ultrasound Screen

020050604-18.5-2: : L1
NIH-MIF 27 Aug 02



9:47:15 pm
15L8-S 66Hz
14.0MHz 31mm
Mouse-Rat 15L8
Fetal-2
Pwr= 0dB MI=49
60dB S1/+3/2/5
Gain= 2dB Δ=2
Store in progress

Exit Res Box

020050604-18.5-3: 200-1: R4
NIH-MIF



15L8
14.0
Mou
Fete
Pwr
60d
Gain
Stor

2D/COLOR 3000msec/1500msec

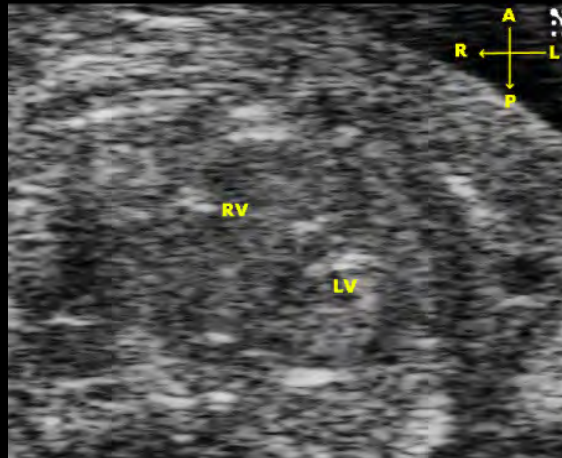
Frontal View

Sagittal View

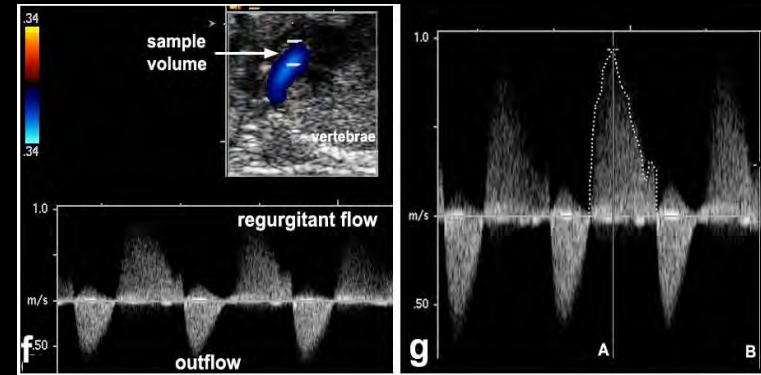
Cardiac Phenotyping by Noninvasive Fetal Ultrasound

High Throughput and High Detection Sensitivity/Specificity for CHD

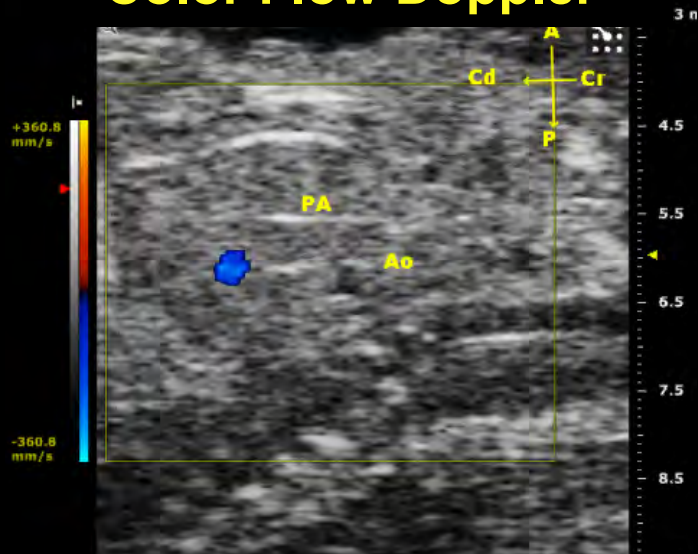
2D Imaging



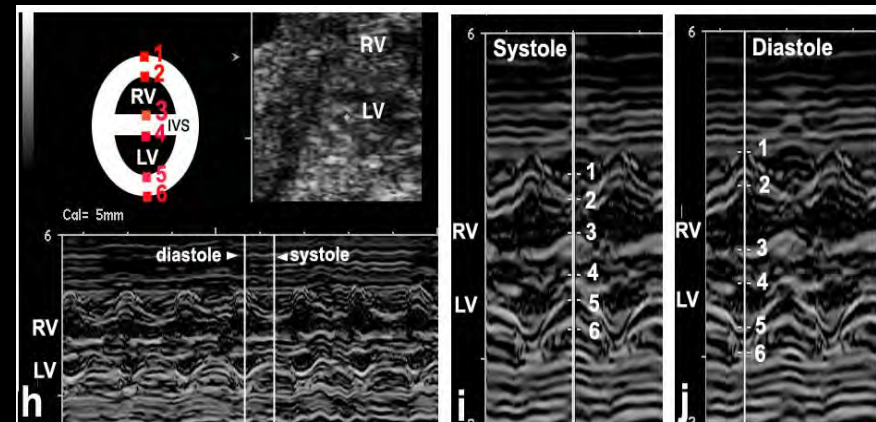
Pulsed Wave Doppler



Color Flow Doppler



M-Mode Imaging



Summary of Ultrasound Screen

	Pedigrees	G2 Females	Total Fetuses	With Cardiac Anomalies
Total Screened	3007	12377	100,057	
Cardiac Anomalies	1220 (40.6%)	2091 (16.9%)	3290 (3.3%)	
Prenatal Lethality	823 (27.4%)	1178 (9.5%)	1631 (1.6%)	306 (18.8%)
Growth Retarded	211 (7.0%)	400 (3.2%)	642 (0.6%)	552 (86.0%)
Hydrops	745 (24.8%)	1176 (9.5%)	1811 (1.8%)	1228 (67.6%)
Craniofacial/ Limb Defects	178 (5.9%)	354 (2.9%)	625 (0.6%)	466 (74.6%)
Body Wall Defects	36 (1.2%)	42 (0.3%)	56 (0.1%)	45 (80.4%)
Laterality Defects	54 (1.8%)	78 (0.6%)	101 (0.1%)	96 (95.0%)

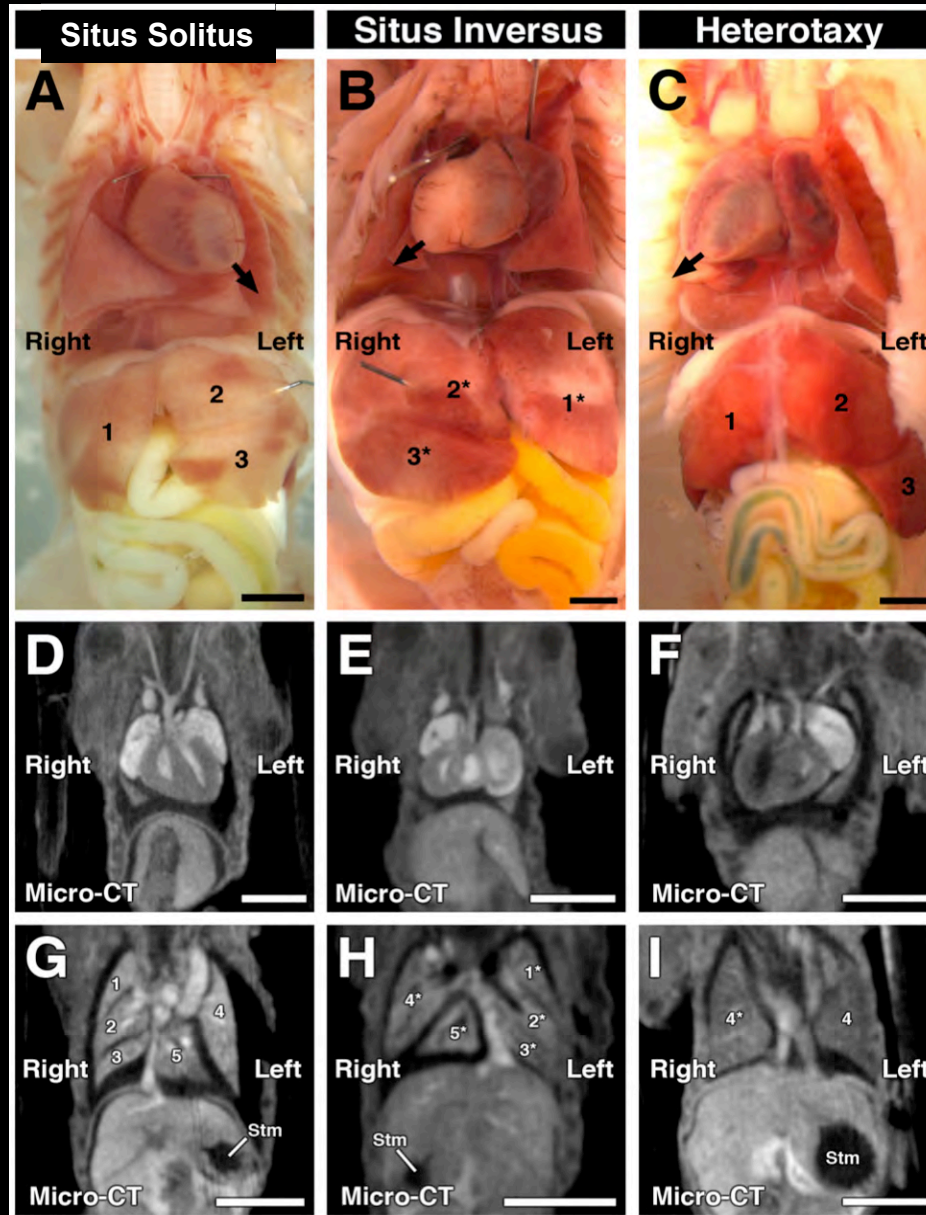
Noncardiac defects highly associated with CHD

**~300 Mutant Mouse Lines Recovered
Wide Spectrum CHD Phenotypes**

PHENOTYPE	No. Mutant Lines
Laterality defects	71
Great artery anomalies	79
ASD/VSD/AVSD	64
Aortic arch anomalies	25
Left heart obstructive lesions	11
Right heart obstructive lesions	11
Myocardial anomalies	18
Craniofacial defects	45
Kidney defects	40
TOTAL LINES	>200

25% of the CHD mutants exhibit laterality defects

Complex CHD Highly Associated with Heterotaxy



>300 Mutant Mouse Lines Sperm Cryopreserved at JAX

The screenshot shows the MGI website search results for the term 'CVDC'. The page header includes the MGI logo and navigation links. The search results are displayed in a table under the heading 'Genome Features'. The table has columns for Score, Type, Symbol, and Name. The results list 17 entries, all with a score of three stars (***). The symbols range from b2b508.1Clo to b2b251Clo. The names describe various mutant mouse lines, such as 'Bench to Bassinet Program (B2B/CVDC), mutation 508, subline 1 Cecilia Lo'.

Quick Search Results for:

Examples: embryo* develop* NM_013627 MGI:97490 Fas<pr> Pax* axial skeletal dys

⚠ Could not find the independent term(s): cvdc. See [details](#) for this search.

Genome Features

Sorted by best match, showing 1 - 49 of 49

Score	Type	Symbol	Name
***	heritable phenotypic marker	b2b508.1Clo	Bench to Bassinet Program (B2B/CVDC), mutation 508, subline 1 Cecilia Lo
***	DNA segment	b2b508.2Clo	Bench to Bassinet Program (B2B/CVDC), mutation 508, subline 2 Cecilia Lo
***	Chemically induced allele	b2b012Clo	Bench to Bassinet Program, mutation 012 Cecilia Lo; Bench to Bassinet Program (B2B/CVDC), mutation 012 Cecilia Lo
***	Chemically induced allele	b2b019Clo	Bench to Bassinet Program, mutation 019 Cecilia Lo; Bench to Bassinet Program (B2B/CVDC), mutation 019 Cecilia Lo
***	Chemically induced allele	b2b1035Clo	Bench to Bassinet Program, mutation 1035 Cecilia Lo; Bench to Bassinet Program (B2B/CVDC), mutation 1035 Cecilia Lo
***	Chemically induced allele	b2b1116Clo	Bench to Bassinet Program, mutation 1116 Cecilia Lo; Bench to Bassinet Program (B2B/CVDC), mutation 1116 Cecilia Lo
***	Chemically induced allele	b2b1134Clo	Bench to Bassinet Program, mutation 1134 Cecilia Lo; Bench to Bassinet Program (B2B/CVDC), mutation 1134 Cecilia Lo
***	Chemically induced allele	b2b1154Clo	Bench to Bassinet Program, mutation 1154 Cecilia Lo; Bench to Bassinet Program (B2B/CVDC), mutation 1154 Cecilia Lo
***	Chemically induced allele	b2b1247Clo	Bench to Bassinet Program, mutation 1247 Cecilia Lo; Bench to Bassinet Program (B2B/CVDC), mutation 508 Cecilia Lo
***	Chemically induced allele	b2b134Clo	Bench to Bassinet Program, mutation 134 Cecilia Lo; Bench to Bassinet Program (B2B/CVDC), mutation 134 Cecilia Lo
***	Chemically induced allele	b2b145Clo	Bench to Bassinet Program, mutation 145 Cecilia Lo; Bench to Bassinet Program (B2B/CVDC), mutation 145 Cecilia Lo
***	Chemically induced allele	b2b183Clo	Bench to Bassinet Program, mutation 183 Cecilia Lo; Bench to Bassinet Program (B2B/CVDC), mutation 183 Cecilia Lo
***	Chemically induced allele	b2b191Clo	Bench to Bassinet Program, mutation 191 Cecilia Lo; Bench to Bassinet Program (B2B/CVDC), mutation 191 Cecilia Lo
***	Chemically induced allele	b2b220Clo	Bench to Bassinet Program, mutation 220 Cecilia Lo; Bench to Bassinet Program (B2B/CVDC), mutation 220 Cecilia Lo
***	Chemically induced allele	b2b246Clo	Bench to Bassinet Program, mutation 246 Cecilia Lo; Bench to Bassinet Program (B2B/CVDC), mutation 246 Cecilia Lo
***	Chemically induced allele	b2b251Clo	Bench to Bassinet Program, mutation 251 Cecilia Lo; Bench to Bassinet Program (B2B/CVDC), mutation 251 Cecilia Lo

<http://www.informatics.jax.org>
Search term: B2B

Detailed Phenotype Annotation in MGI

MGI has a [job opening](#) for a biologist.

Keywords, Symbols, or IDs Quick Search

Home Genes Phenotypes Expression Recombinases Function Pathways Strains / SNPs Orthology Tumors

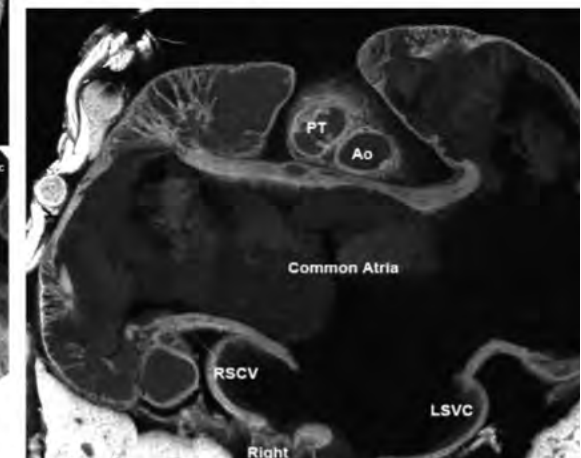
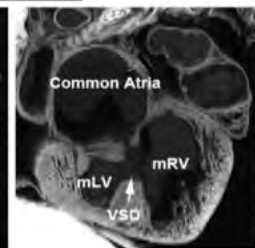
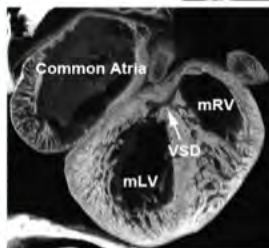
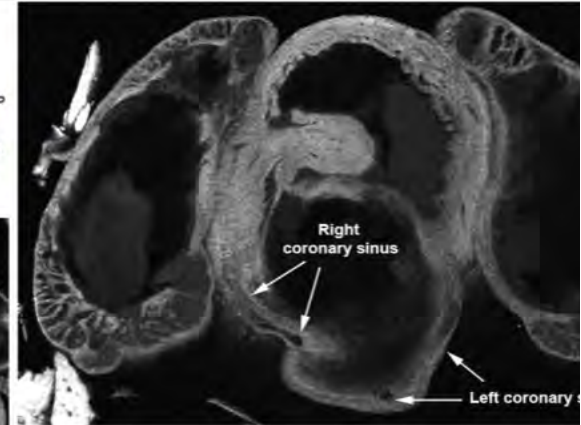
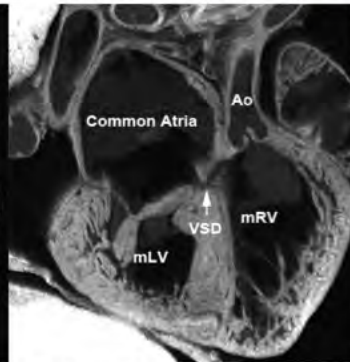
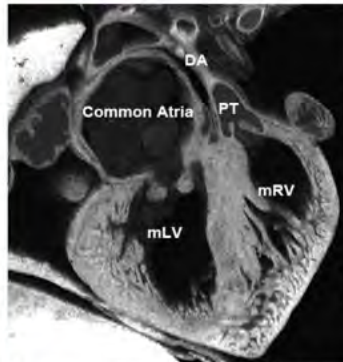
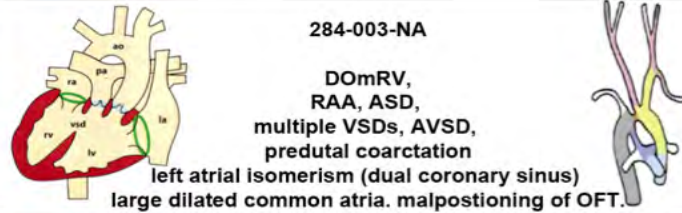
Search Download More Resources Submit Data Find Mice (IMSR) Analysis Tools Contact Us

Dync2h1^{b2b414Clo} Your Input Welcome

Chemically induced Allele Detail

Nomenclature | Mutation origin | Mutation description | Find Mice (IMSR) | Phenotype summary | Phenotypes by genotype | Disease models | Notes | References

Nomenclature	<p>Symbol: Dync2h1^{b2b414Clo}</p> <p>Name: dynein cytoplasmic 2 heavy chain 1; Bench to Bassinet Program (B2B/CVDC), mutation 414 Cecilia Lo Pulmonary atresia with MAPCAs and duplicated IVC.</p> <p>MGI ID: MGI:5311370</p> <p>Synonyms: Dync2h1^{p.V234E}, Lucifer</p> <p>Gene: Dync2h1 Location: Chr9:6928503-7184446 bp, - strand Genetic Position: Chr9, 2.46 cM</p>	
Mutation origin	Strain of Origin: C57BL/6J	
Mutation description	<p>Allele Type: Chemically induced (ENU)</p> <p>Mutation: Single point mutation</p> <p>This ENU-induced mutation replaces valine to glutamate change at position 234.</p>	
Find Mice (IMSR)	<p>Mouse strains and cell lines available from IMSR</p> <p>Carrying this Mutation: MGI:5311370</p> <p>Carrying any Dync2h1 Mutation: 4</p>	
Phenotype summary	<p>Phenotype Summary by Mammalian</p> <p>(show or hide all annotated terms)</p> <p>Genotypes are listed in the next section</p> <p>Affected Systems</p> <p>cardiovascular system</p> <p>aorta pulmonary collateral artery abnormal inferior vena cava morphology abnormal coronary vessel morphology coronary fistula common atrium complete atrioventricular septal defect</p>	

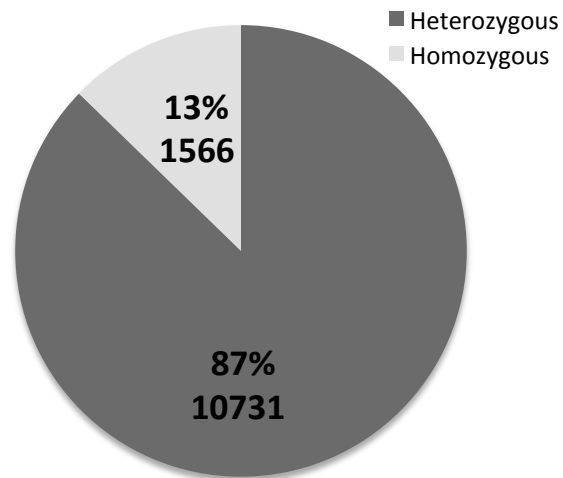


MPO
Fyler Codes

Mutation Recovery by Exome Sequencing

All Coding/Splicing Mutations

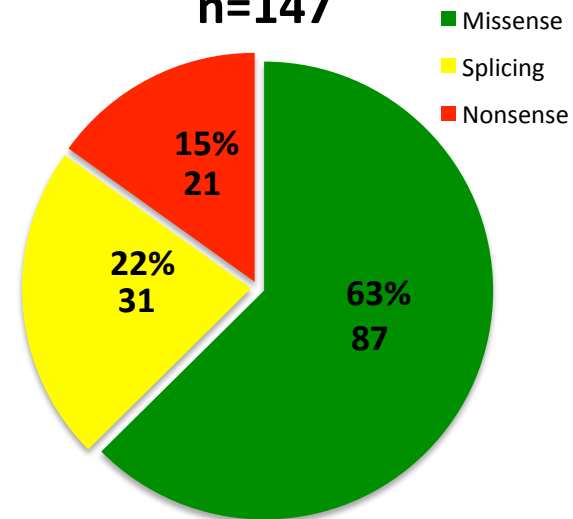
n=12,297



Mutations in 7,235 genes

Pathogenic Mutations


n=147






Mutations in 98 genes

Suggests Screen at 30% Saturation

Library of >12,000 Mouse Mutations Available


B**B**
Bench to Bassinet

ABOUT FOR FAMILIES CENTERS **FOR RESEARCHERS** PUBLICATIONS 

Home > For Researchers > Model Organism Search PRINT  SHARE 

Model Organism Search

Search Type:

- Model Organisms
- Mouse Mutations 

Congenital Heart Disease Mouse Mutation Database

Mouse lines with congenital heart defects (CHD) have been recovered from a large-scale ultrasound screen of C57BL6/J mice mutagenized with ethylnitrosourea (ENU). Analysis by whole exome sequencing enables recovery of the ENU-induced mutations, both disease causing mutation(s) and also other incidental mutations not known to contribute to the disease phenotype. The totality of these mutations is searchable in the CvDC Mouse ENU-Induced Mutation Database. All lines have been sperm cryopreserved at the Jackson Laboratory, and are available to interested investigators who wish to re-animate a line. Phenotype information associated with each mutant line is annotated in the [Mouse Genome Informatics \(MGI\) database](#), and genotype information is also curated if the disease-causing mutation has been identified and confirmed.

Search by:

- Gene
- B2B Mutant Line
- Phenotype term or corresponding human disease

Gene:

<http://benchtobassinet.com/ForResearchers/ModelOrganismSearch>

CHD Mutation Recovery

- **98** genes with 147 pathogenic mutations
- **23** genes with multiple alleles.
- **47** novel CHD genes

Estimating Number of CHD Genes in Mouse Genome

Unseen Species Method

$$C = c/u + (g^2)*d*(1-u)/u$$

c = number of observed CHD genes (97);

c_1 = number of CHD genes with 1 mutation (74);

d = total number of CHD mutations (141);

$u = 1 - c_1/d$ (0.419)

probability that newly added mutation hits a previously mutated gene;

g = the coefficient of variation of probability that one or more mutations would fall in each gene (averaged by 10,000 simulations)

Sanders et al., Nature 485:273-41, 2012

Zaidi et al., Nature 498:220-223, 2013

Chao and Lee, J. Am. Statistical Association 87(417):210-217, 1992

Estimated No. CHD Genes: ~272

Suggests screen is at ~35% saturation

Dan Weeks & Ying Shan
Graduate School of Public Health
University of Pittsburgh

Homozygote Null Mutations

151 Homozygote Null Mutations

KOMP suggests 30%
embryonic lethals
expected

108 in Genes with Known KO Mouse Model

4 genes exhibit early
embryonic lethality

104 viable to weaning

3.7%*

96.3%

Genetic Resiliency

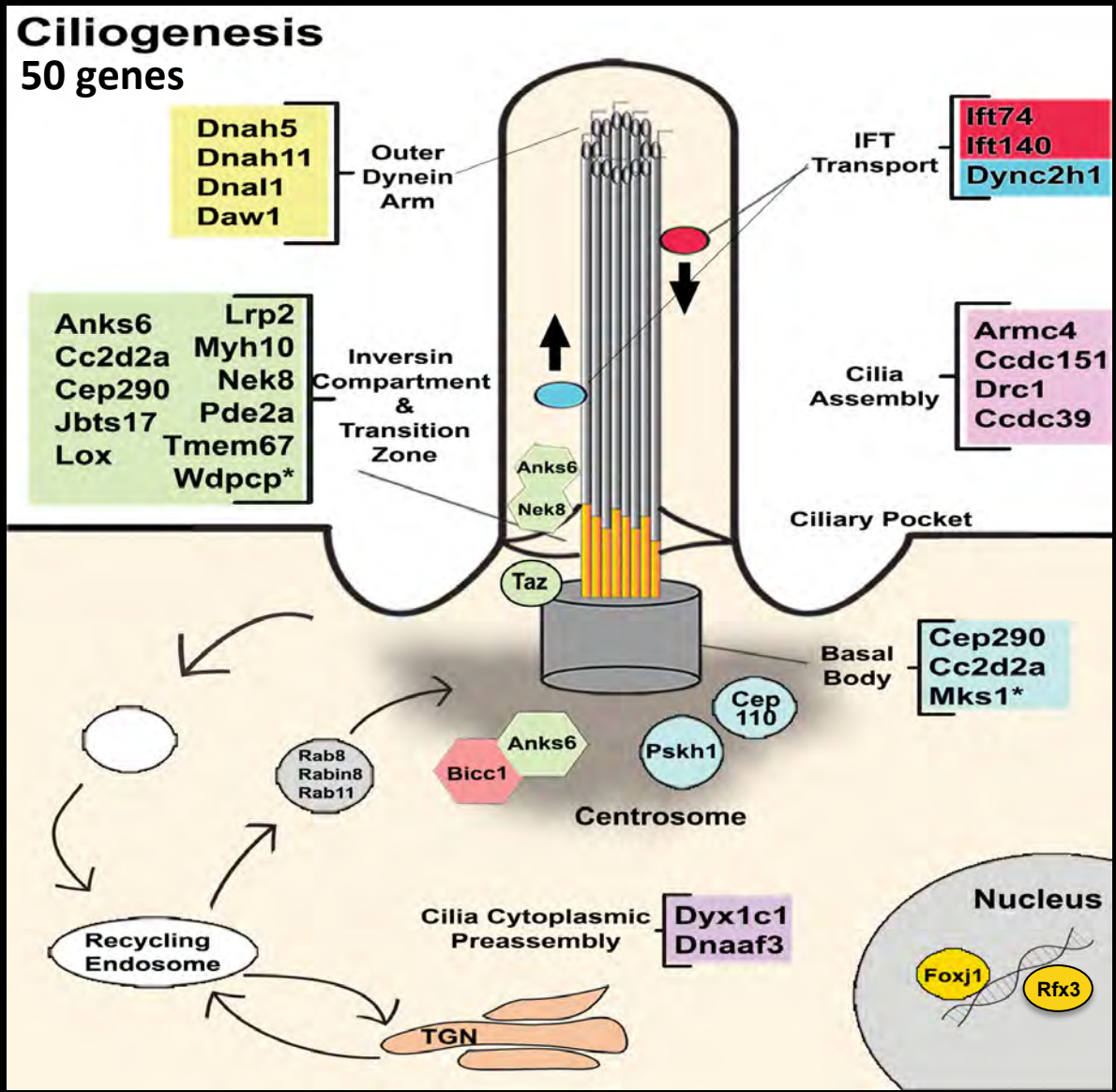
Chen et al., Nat Biotech 2016

CHD Genes

- **Cilia Related (50)**
- **Cilia Transduced Cell Signaling (21)**
- **Endocytic/Vesicular Trafficking (15)**

Disturbance of cilia and cilia related function plays an important role in the pathogenesis of CHD

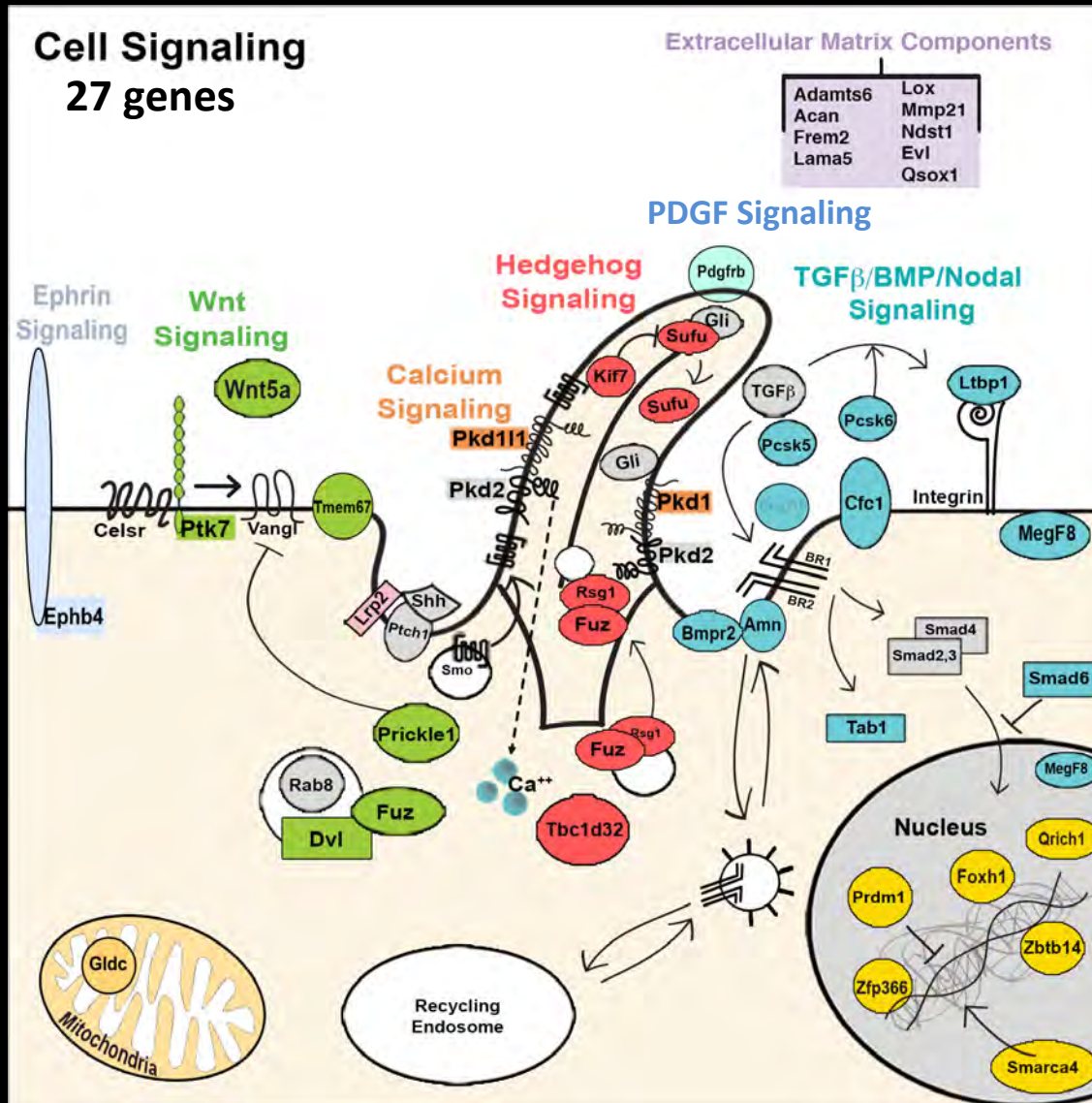
Ciliome CHD Genes



- Armc4**
- Cc2d2a**
- Ccdc151**
- Ccdc39**
- Dnaaf3**
- Dnah5**
- Dnah11**
- Dnai1**
- Drc1**
- Dyx1c1**
- Anks6**
- Nek8**
- IFT140**
- Dync2h1**
- Cep290**
- Jbts17**
- Tmem67**
- Wdpcp**
- Mks1**

50% of Ciliome Mutations in Non-Laterality Mutant Lines

Cilia Transduced Cell Signaling Genes



Dvl3
 Ptk7
 Prickle1
 Wnt5a
 Pkd111
 Pkd1
 Fuz
 Kif7
 Lrp2
 Rsg1
 Sufu
 Tbc1d32
 Bmpr2
 Pcsk5
 Pcsk6
 Cfc1
 Ltpb1
 Megf8
 Smad6
 Tab1
 Pdgfrb

De Novo Pathogenic Mutations Recovered in CHD Patients from PCGC Exome Analysis

11 of 27 (41%) in pathways identified by mouse CHD screen

Table 1: Functional annotation for PCGC patients with de novo mutations

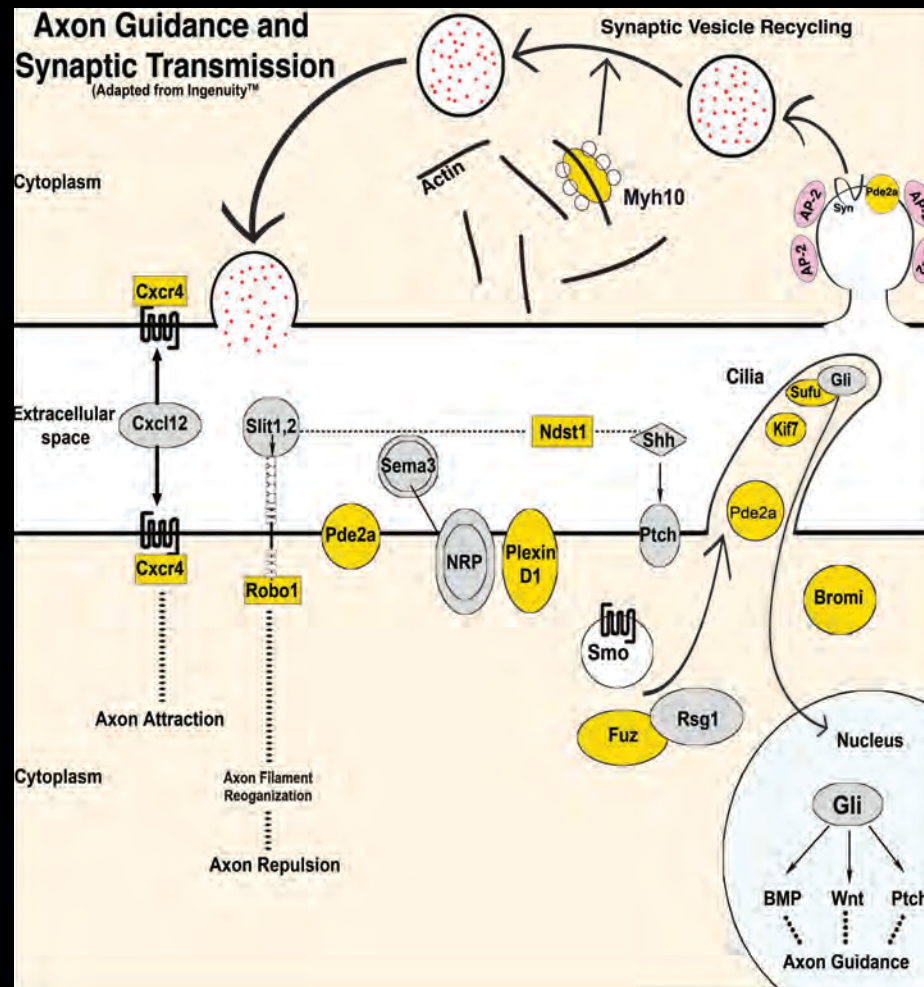
Patient ID	CHD [§]	Gene	Mutation	Gene Function
1-00638	CTD	FBN2	p.D2191N	TGFβ signaling
1-02020	HTX	SMAD2	p.IVS12+1G>A	TGFβ signaling
1-02621	HTX	SMAD2	p.W244C	TGFβ signaling
1-00197	LVO	BCL9	p.M1395K	WNT signaling
1-01828	CTD	DAPK3	p.P193L	WNT signaling
1-01138	LVO	USP34	p.L432P	WNT signaling
1-00802	LVO	PTCH1	p.R831Q	SHH signaling/Ciliome
1-02598	HTX	LRP2*	p.E4372K	SHH signaling/Endocytic trafficking
1-01913	Other	RAB10	p.N112S	Endocytic trafficking
1-00750	LVO	HUWE1	p.R3219C	Ciliome
1-01151	CTD	SUV420H1	p.R143C	Ciliome
1-00853	CTD	WDR5	p.K7Q	Ciliome
1-02952	LVO	PITX2	p.A47V	Laterality related

*LRP2 is an endocytic gene also recovered from our mouse screen.
[§]CTD: conotruncal defect; HTX: heterotaxy; LVO: left ventricular obstruction.



**Pediatric Cardiac Genomics Consortium
(Zaidi et al., Nature 498: 220-223,2013)**

Axon Guidance, Neurogenesis, and Synaptic Transmission



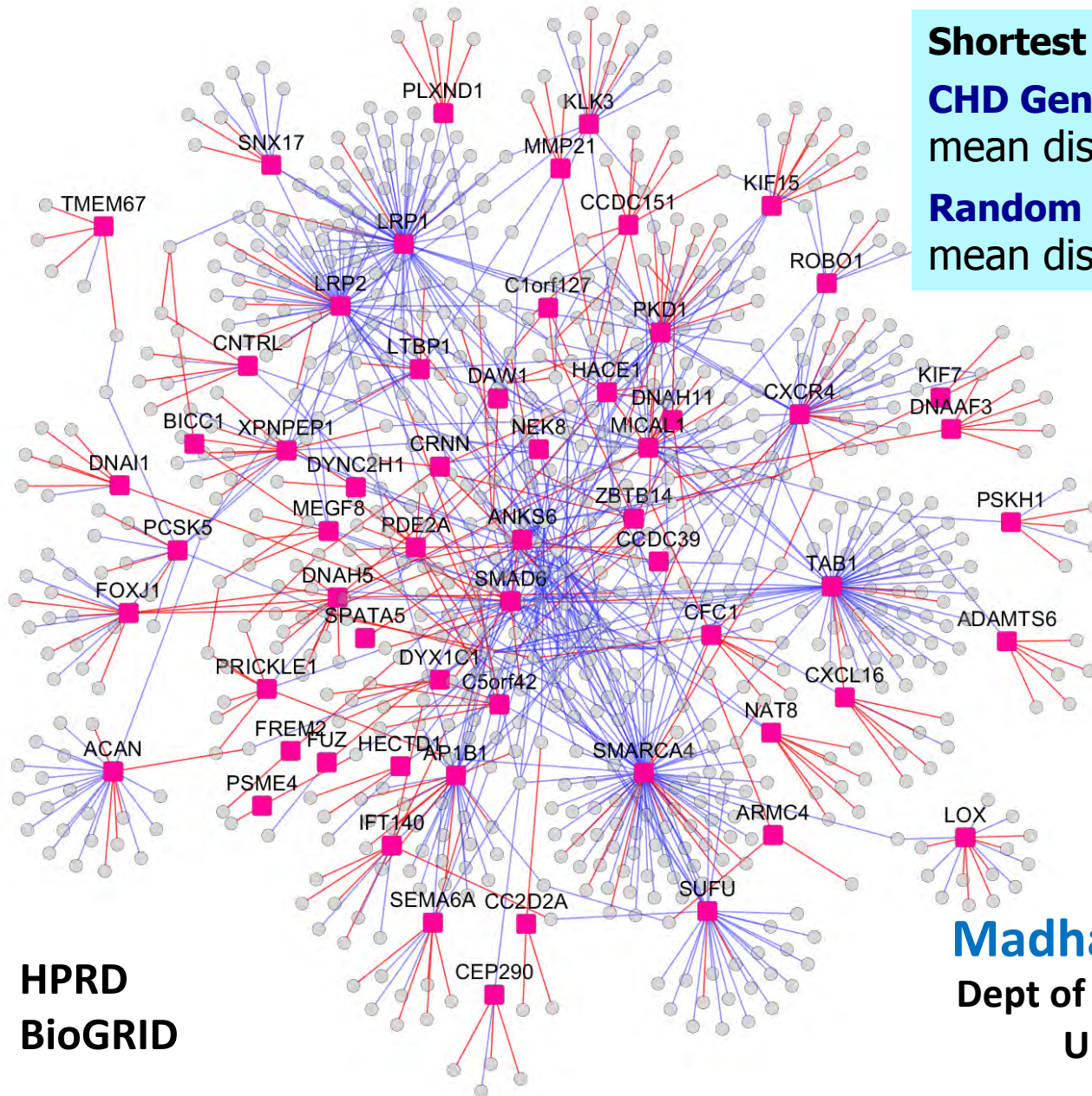
Pathogenic CHD Mutations in Interacting Proteins

- *Anks6-Nek8*
- *Bicc1-Ank6*
- *Nek8-TAZ*
- *Cep110-Cep290*
- *Snx17-Lrp1*

- *Transition Zone Complex*

- *CPLANE Proteins: Wdpcp, Jbts17, Fuz, Rsg1*

Interactome Network Generated by Mouse CHD Genes



Shortest Paths Between Genes
CHD Genes: $p < 0.00114$
mean distance: 4.7 ± 3.3
Random Genes:
mean distance: 14.9 ± 4.9

■ CHD Gene

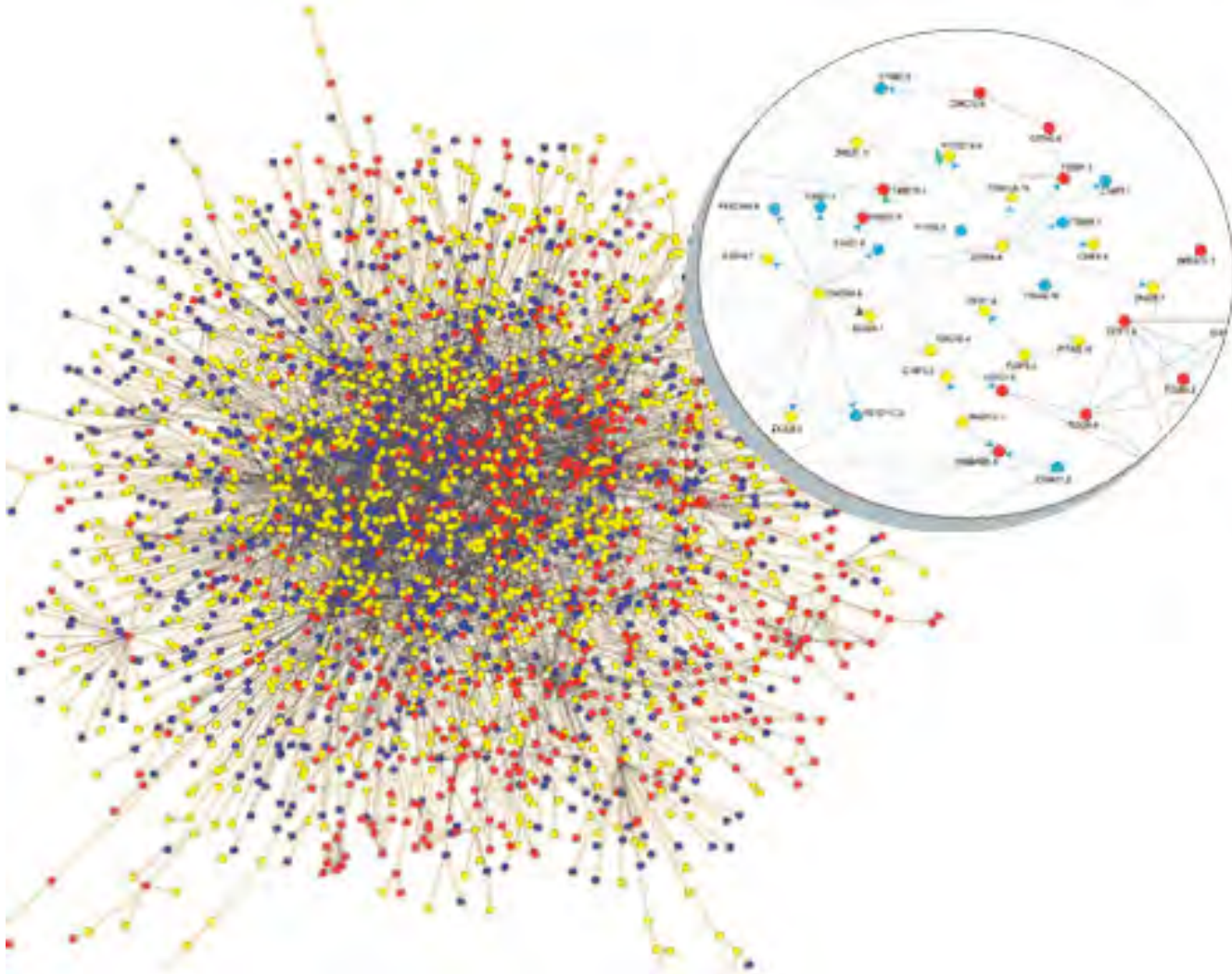
— Experimentally Known Interaction

— Computationally Predicted Interaction

HPRD
BioGRID

Madhavi Ganapathiraju
Dept of Biomedical Informatics
Univ. of Pittsburgh

Interactome network may provide the genomic context contributing to the complex genetics of CHD



Experimental evidence for complex genetic interactions causing CHD?

ARTICLE

Received 15 May 2014 | Accepted 2 Dec 2014 | Published 20 Jan 2015

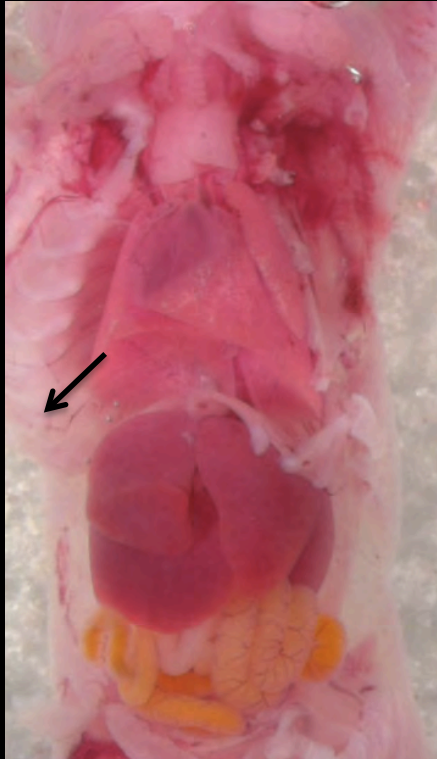
DOI: [10.1038/ncomms7023](https://doi.org/10.1038/ncomms7023)

ANKS6 is the critical activator of NEK8 kinase in embryonic *situs* determination and organ patterning

Peter G. Czarnecki^{1,2,3,*}, George C. Gabriel^{4,*}, Danielle K. Manning^{5,*}, Mikhail Sergeev^{1,2}, Kristi Lemke⁴, Nikolai T. Klena⁴, Xiaoqin Liu⁴, Yu Chen⁴, You Li⁴, Jovenal T. San Agustin⁶, Maija K. Garnaas⁵, Richard J. Francis⁴, Kimimasa Tobita⁴, Wolfram Goessling⁵, Gregory J. Pazour⁶, Cecilia W. Lo⁴, David R. Beier^{5,7} & Jagesh V. Shah^{1,2}

Anks6-Nek8 Exhibit Epistasis

Anks6 +/m, Nek8 +/m



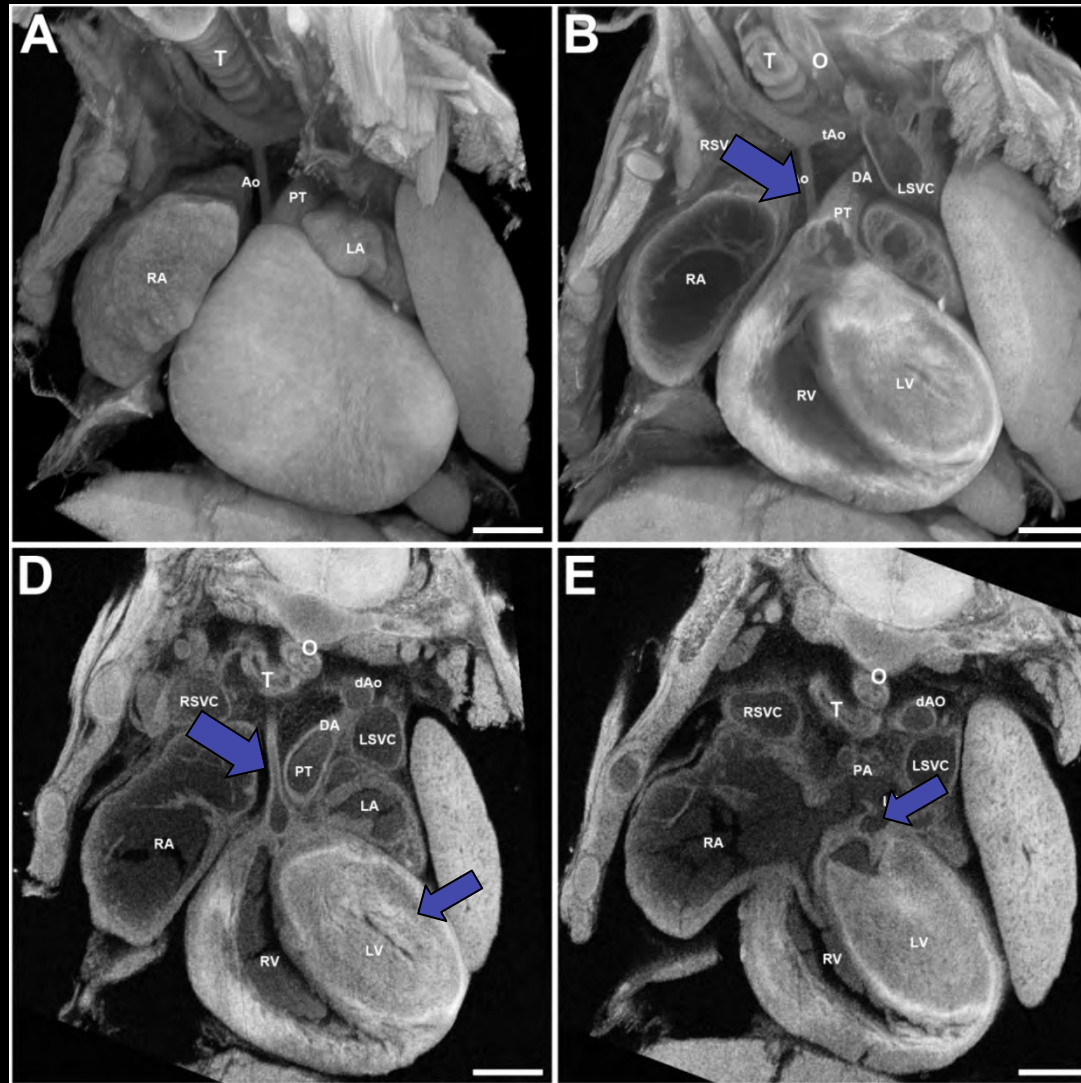
Anks6 +/m X Nek8 +/m

Genotype	# Embryos
Anks6 +/+, Nek8 +/+	47 (29%)
Anks6 +/m, Nek8 +/+	45 (27%)
Anks6 +/+, Nek8 +/m	42 (26%)
Anks6 +/m, Nek8 +/m	27 (16%)
TOTAL	161

- 17/27 (62%) Anks6/Nek8 double heterozygote mice have same phenotypes as homozygote mutants

Anks6/Nek8 digenic interactions can yield same phenotype as *Ank6* or *Nek8* homozygote mutants

Hypoplastic Left Heart Syndrome



Hypoplastic Aorta

Hypoplastic Mitral Valve

Multigenic etiology indicated with no mutations shared in common among 8 lines

Systems Genetics with Mutagenesis to Interrogate the Complex Genetics of Human Diseases

- **Mendelian genetic contribution to disease**
- **Complex genetics of disease**
- **Genomic context of disease pathogenesis**
genetic resiliency, protective vs. pathogenic alleles, penetrance
- **Potential value of a mutagenesis database to query sequence variants**

Animal Modeling of Human Diseases

- **Animal model should have similar anatomy/physiology relevant to human disease**
- **Availability of inbred strains important for genetic analysis**
- **Phenotype ontology should parallel the human phenotype ontology**
- **Disseminate phenotype and genotype data in public databases**
- **Animal model validation of human sequence variants**

University of Pittsburgh

Developmental Biology

Xiaoqin Liu

Yu Chen

Richard Francis

You Li

Kimimasa Tobita

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Dennis Kostka

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Deciphering Developmental Disorder

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