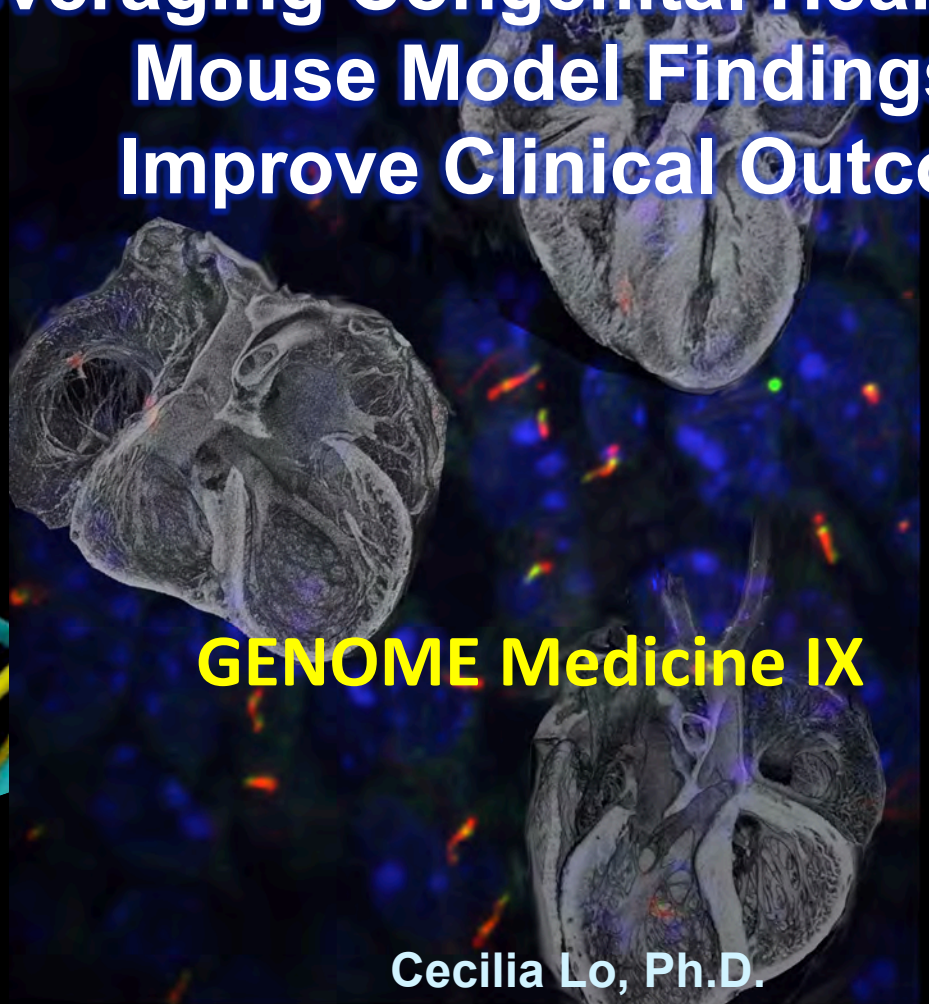
Leveraging Congenital Heart Disease Mouse Model Findings to Improve Clinical Outcome



GENOME Medicine IX

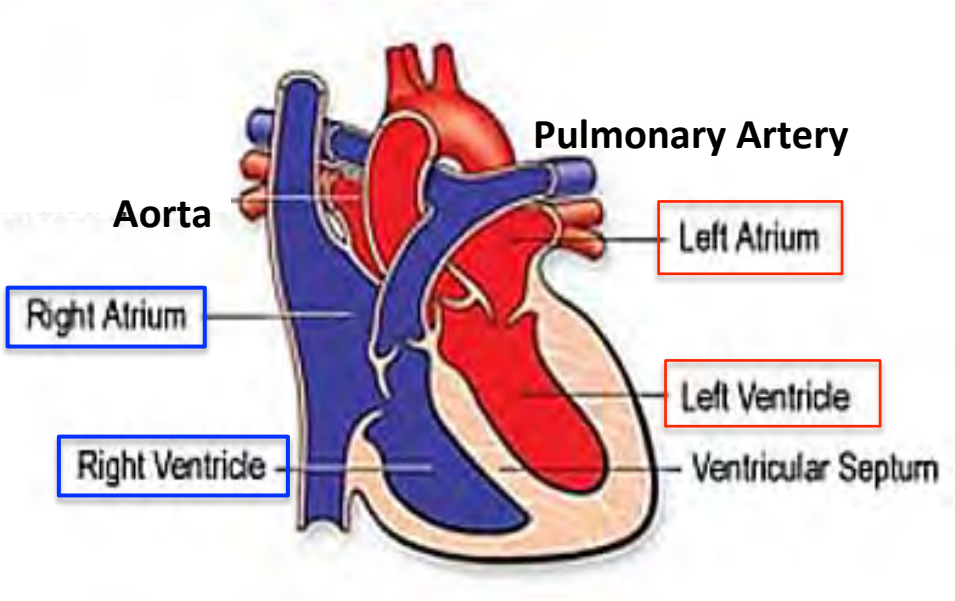
NIH National Heart, Lung, and Blood Institute

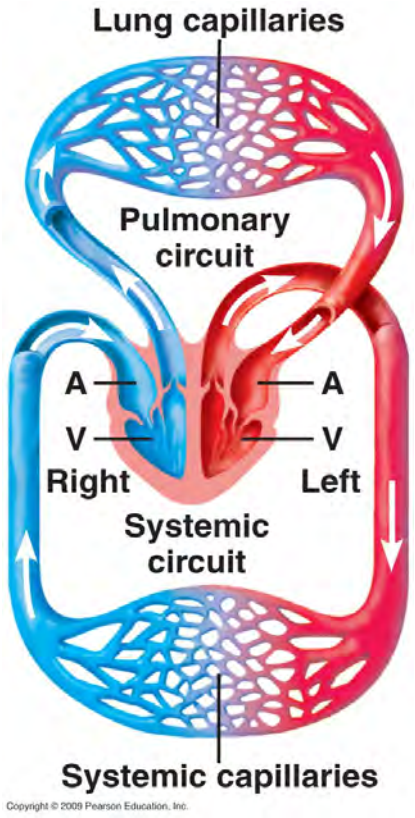
Cecilia Lo, Ph.D.
Department of Developmental Biology
University of Pittsburgh School of Medicine

Congenital Heart disease

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

Four-Chamber Heart with Separate Systemic-Pulmonary Circulation



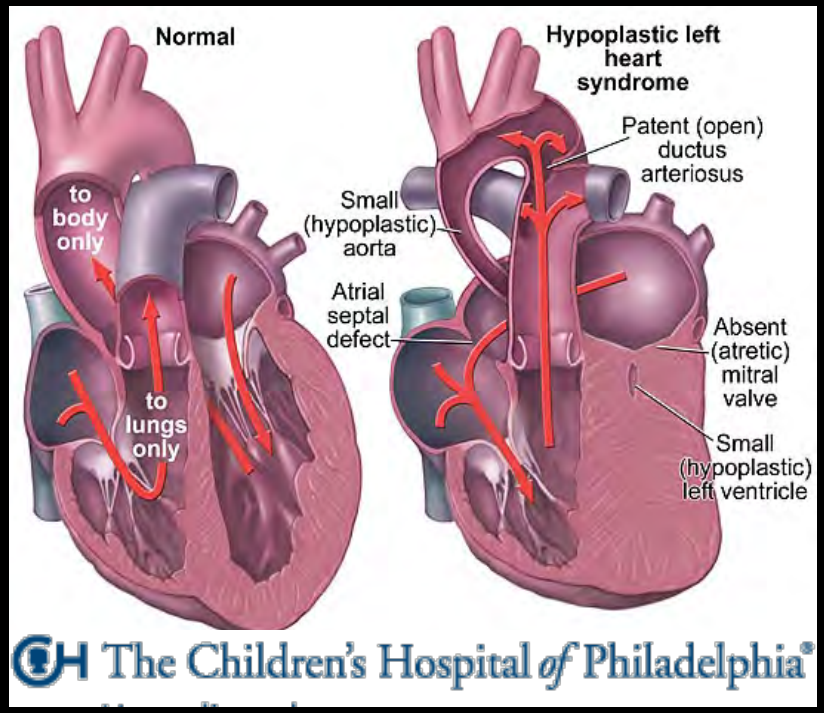


Congenital Heart Disease

- **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)

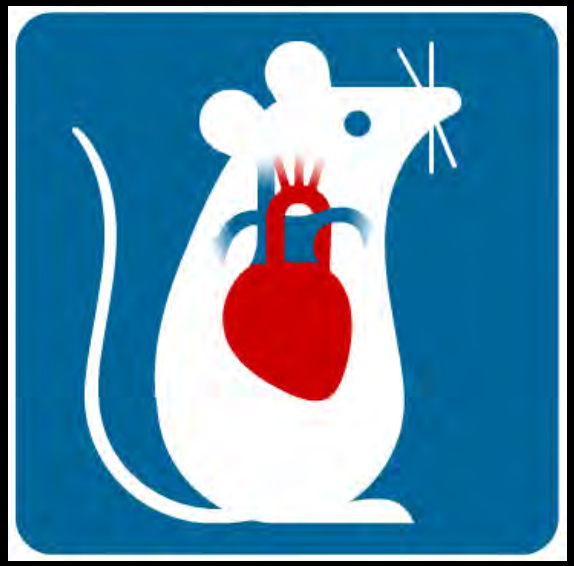


- Aortic Atresia/Stenosis
- Hypoplastic LV
- Mitral Valve Atresia/Stenosis

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

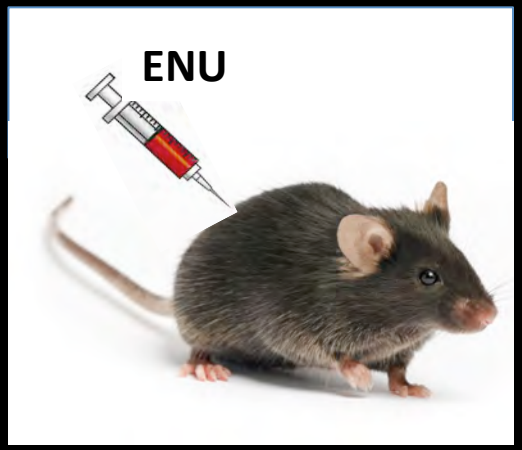
MOUSE



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

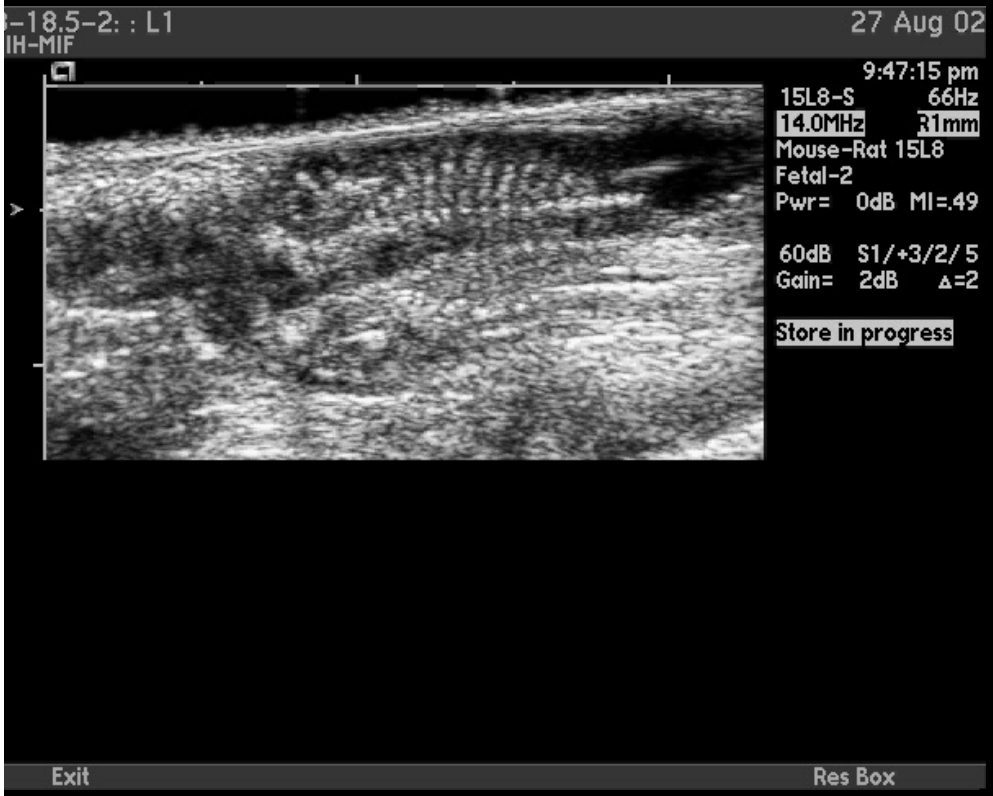
SYSTEMS GENETICS APPROACH

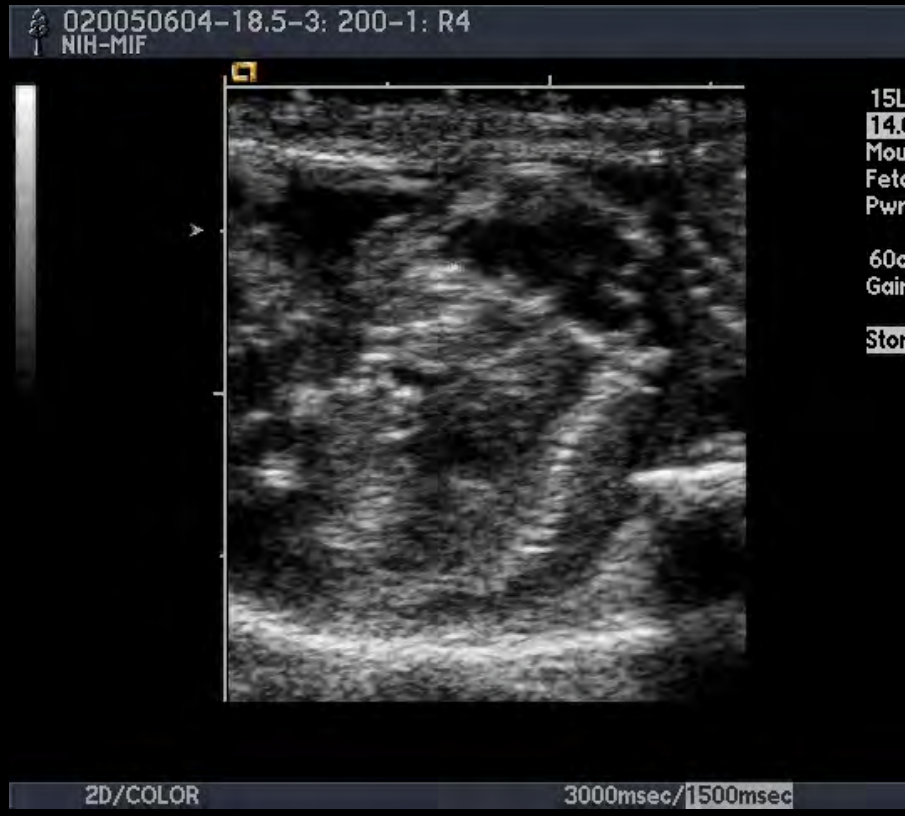
A large scale forward genetic screen to interrogate the genetic etiology of congenital heart disease



- **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





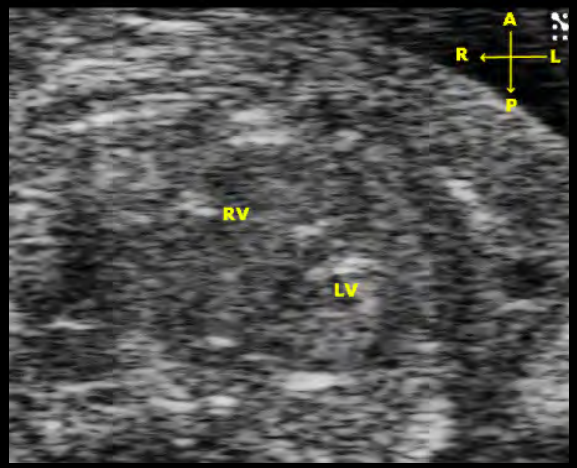
Frontal View

Sagittal View

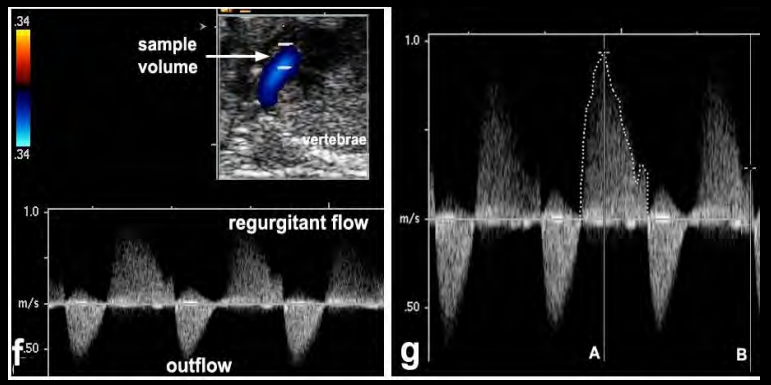
Cardiac Phenotyping by Noninvasive Fetal Ultrasound

High Throughput and High Detection Sensitivity/Specificity for CHD

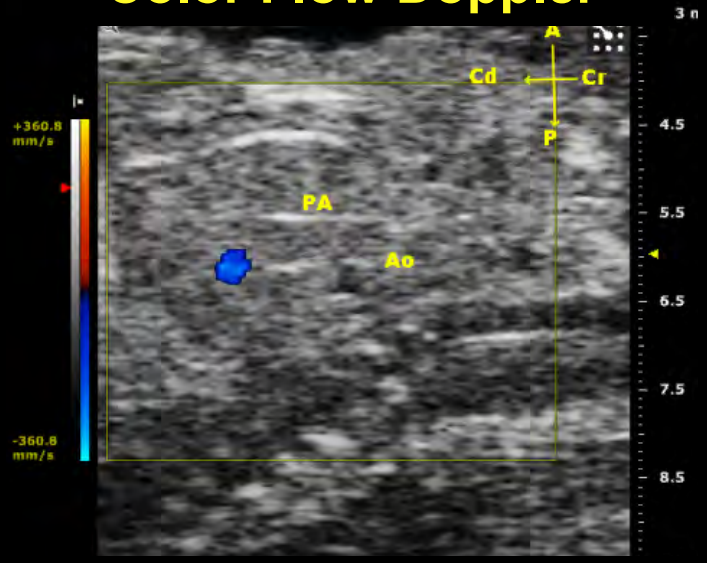
2D Imaging



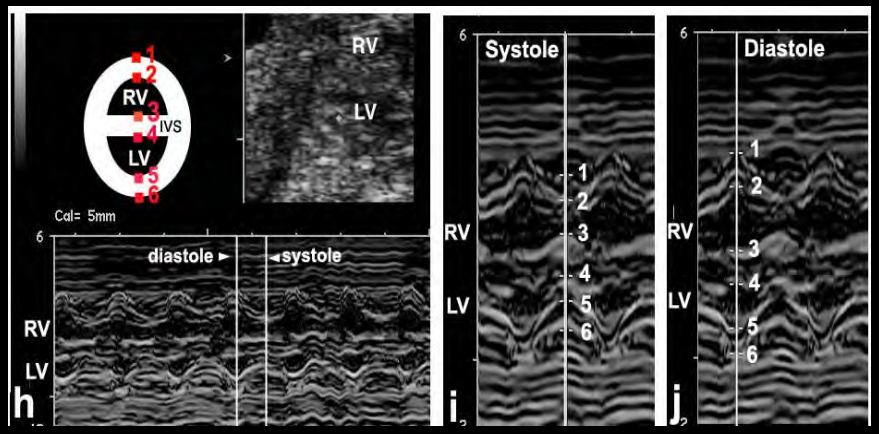
Pulsed Wave Doppler



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

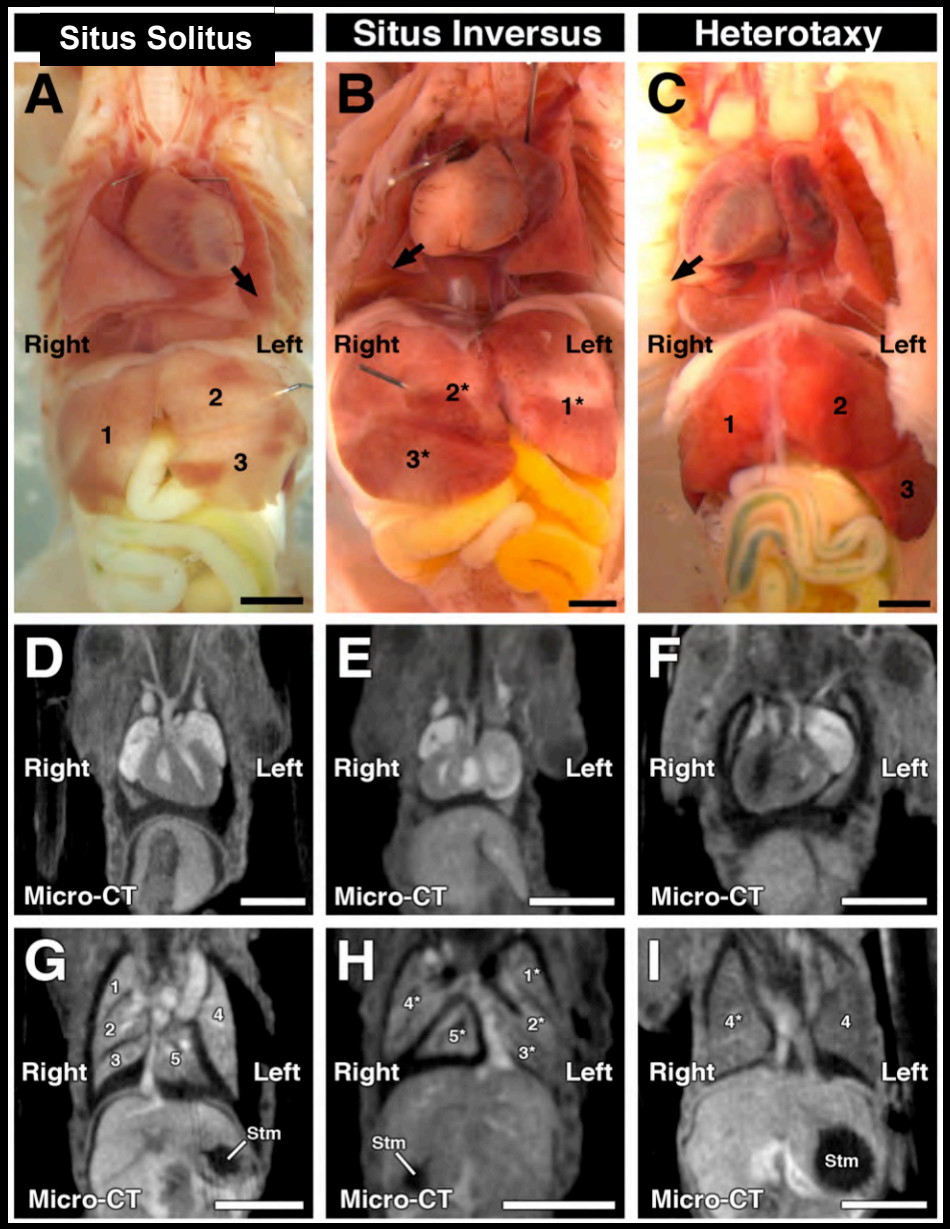
~300 Mutant Mouse Lines Recovered

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

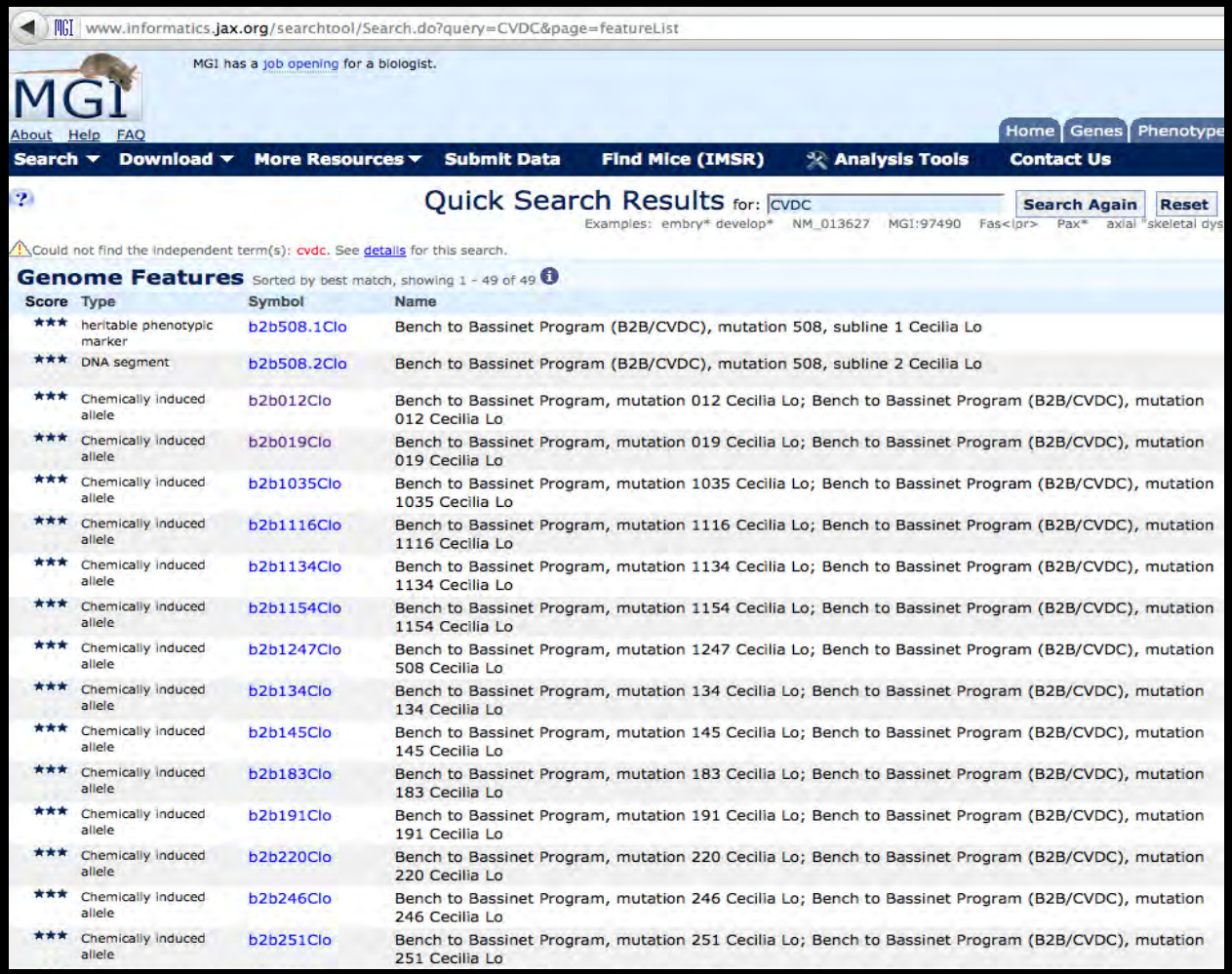
25% of the CHD mutants exhibit laterality defects

Complex CHD Highly Associated with Heterotaxy



>300 Mutant Mouse Lines

Sperm Cryopreserved at JAX



www.informatics.jax.org/searchtool/Search.do?query=CVDC&page=featureList

MGI has a job opening for a biologist.

MGI

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Quick Search Results for: CVDC Search Again Reset

Examples: embry* develop* NM_013627 MGI:97490 Fas<lpr> 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

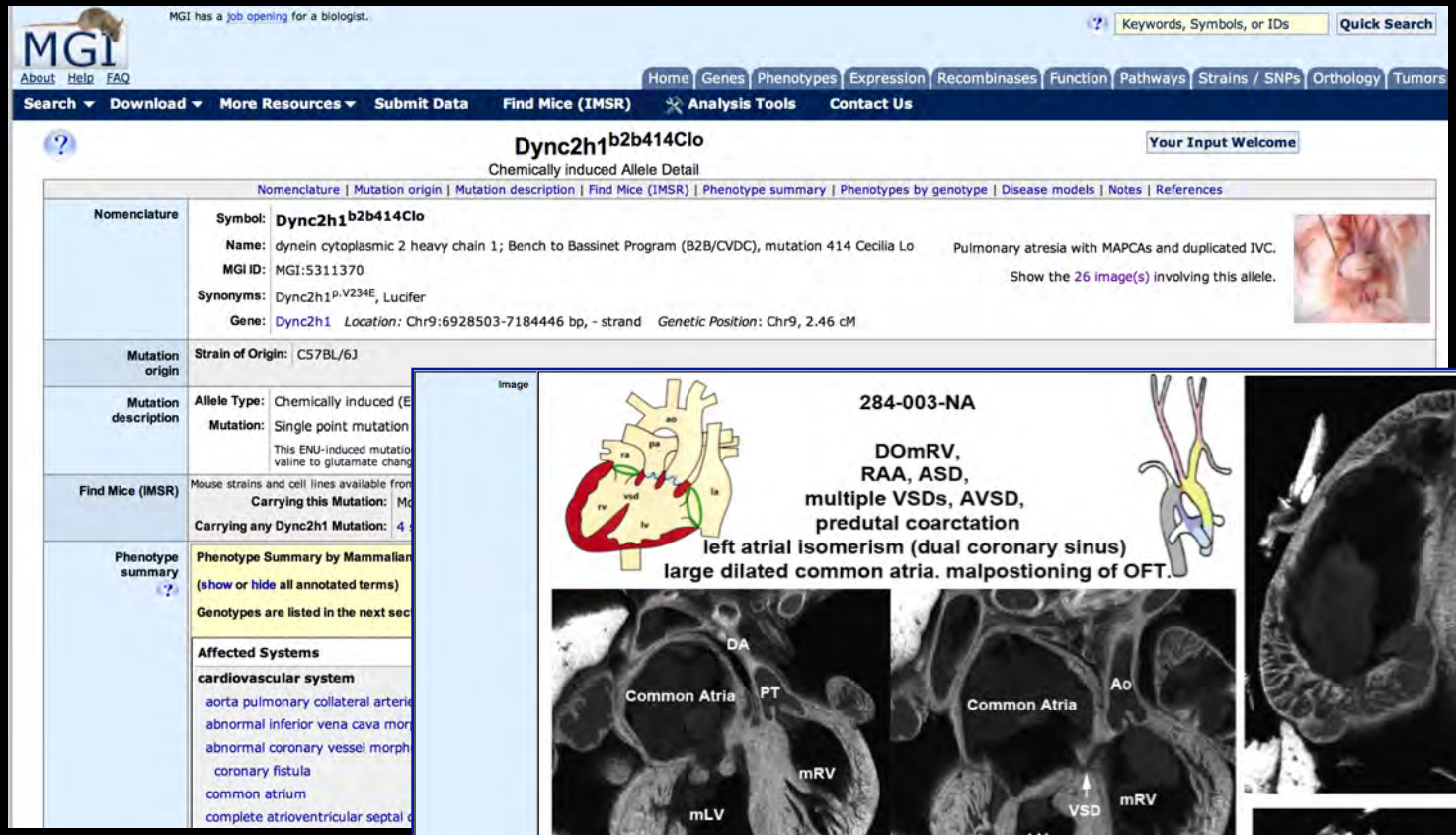
Score	Type	Symbol	Name
***	heritable phenotypic marker	b2b508.1Clo	Bench to Bassinet Program (B2B/CVDC), mutation 508, subline 1 Cecilia Lo
***	DNA segment	b2b508.2Clo	Bench to Bassinet Program (B2B/CVDC), mutation 508, subline 2 Cecilia Lo
***	Chemically induced allele	b2b012Clo	Bench to Bassinet Program, 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

www.informatics.jax.org

Detailed Phenotype Annotation in MGI

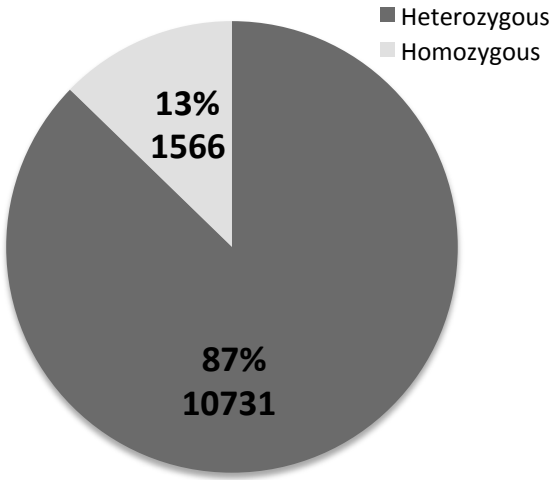


MPO
Fyler Codes

Mutation Recovery by Exome Sequencing

All Coding/Splicing Mutations

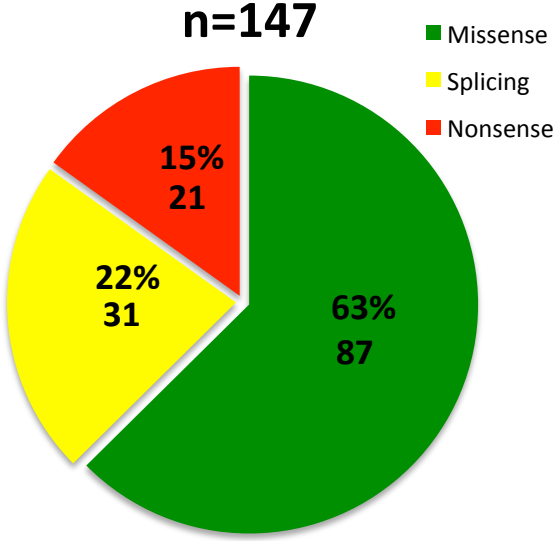
n=12,297



Mutations in 7,235 genes

Pathogenic Mutations

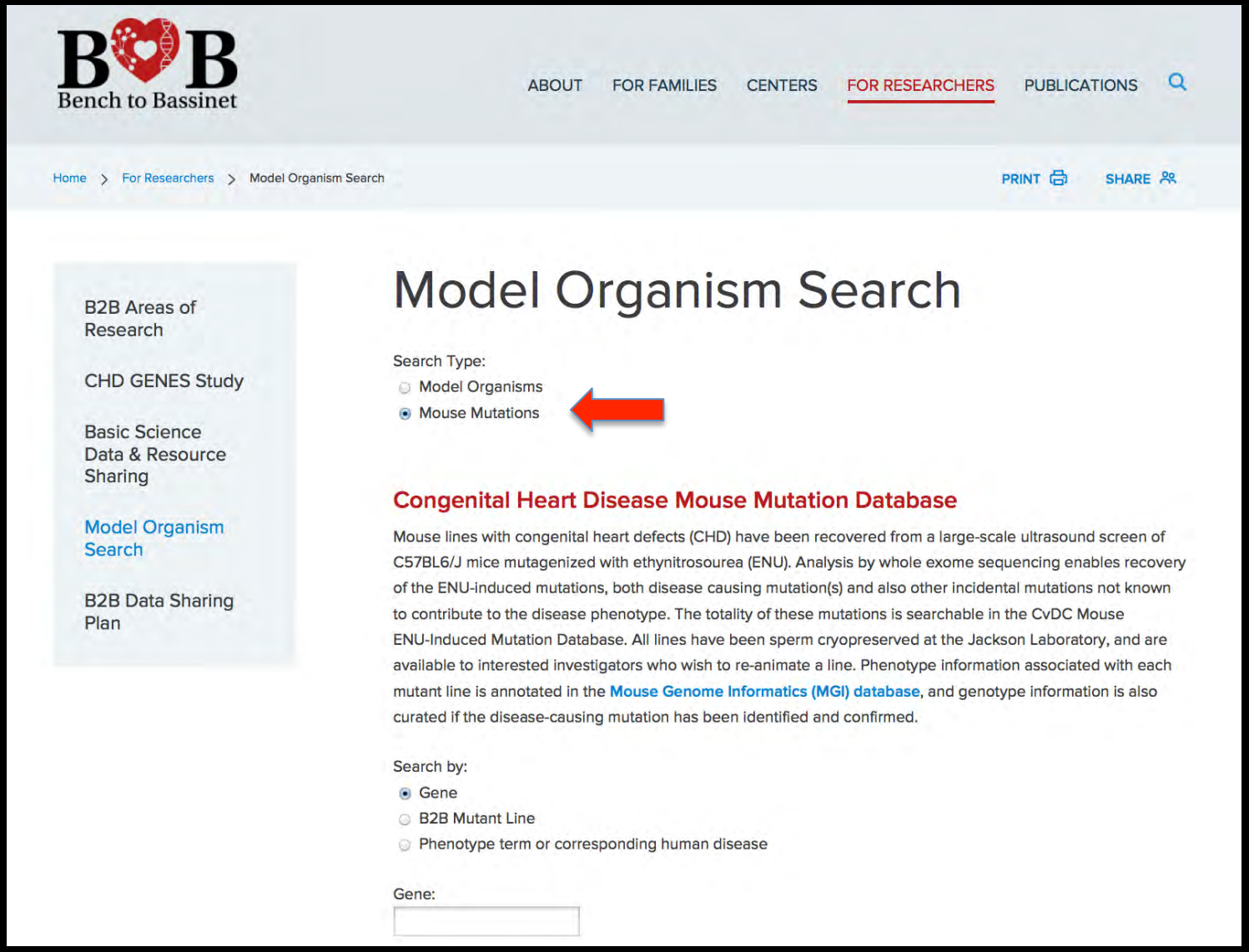
n=147



Mutations in 98 genes

Suggests Screen at 30% Saturation

Library of >12,000 Mouse Mutations Available



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);
c1= **number of CHD genes with 1 mutation** (74);
d= **total number of CHD mutations** (141);
u=1 – c1/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)**

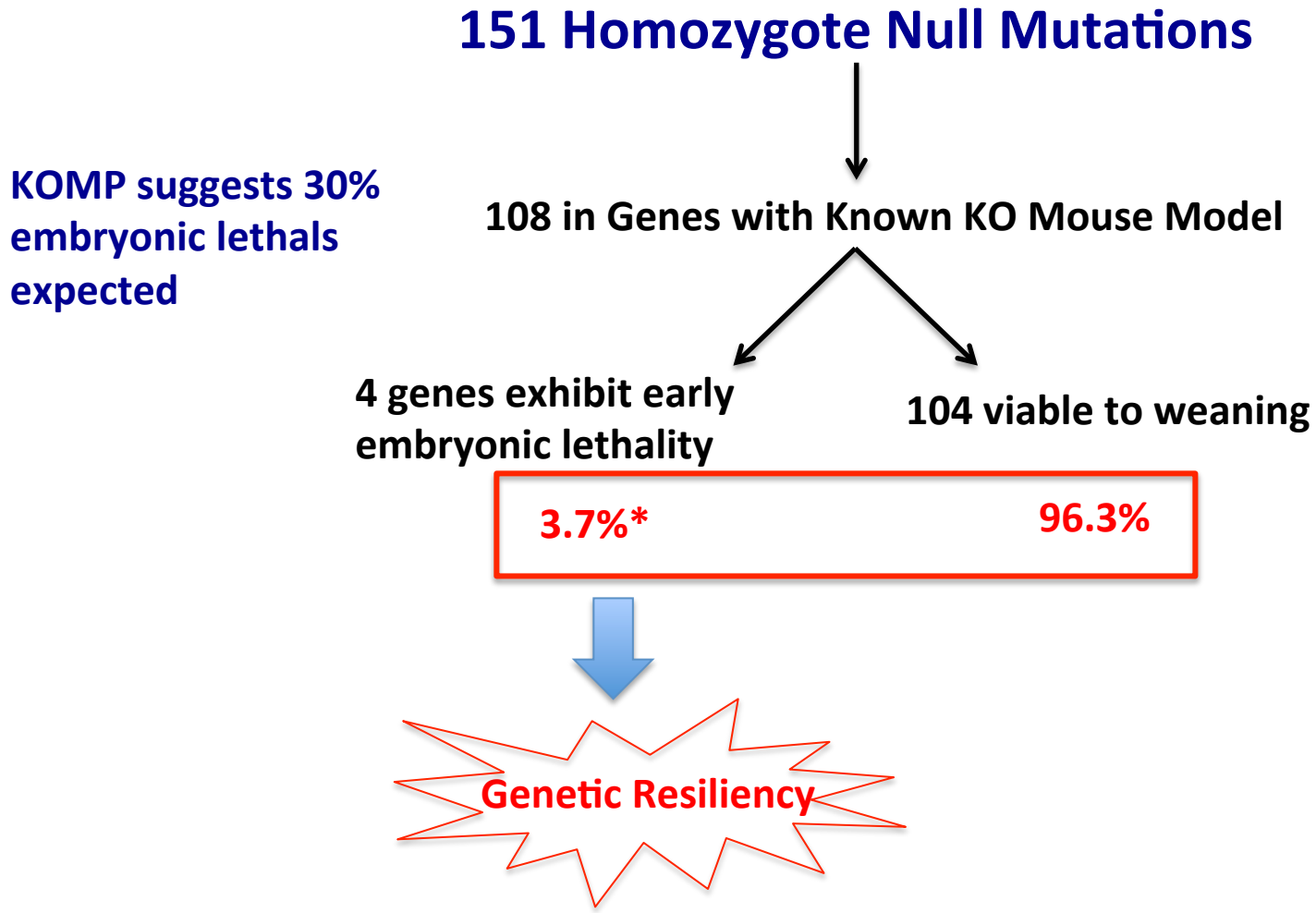
Sanders et al., Nature 485:273-41, 2012
Zaidi et al., Nature 498:220-223, 2013
Chao and Lee, J. Am. Statistical Association 87(417):210-217, 1992

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



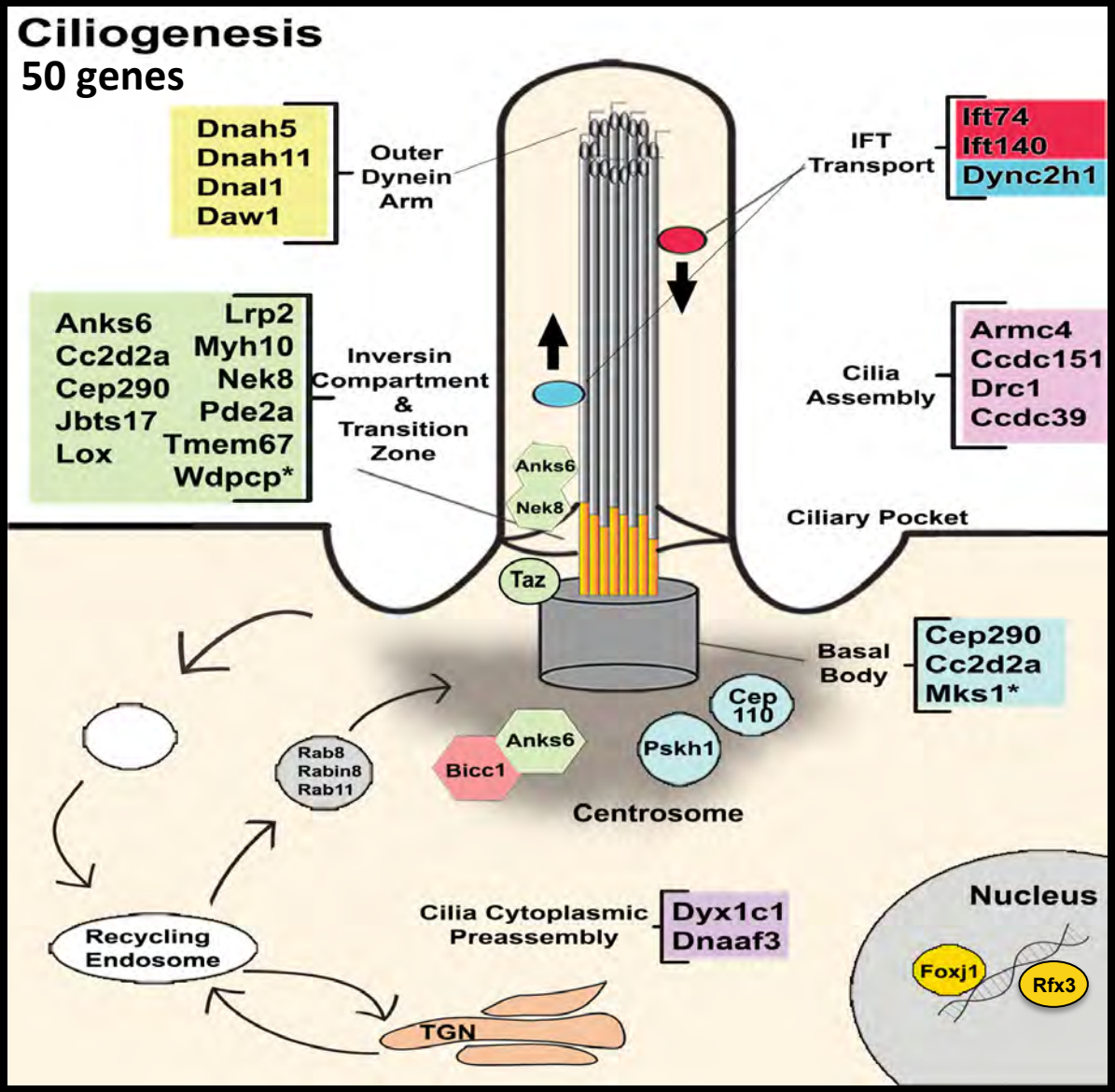
Chen et al., Nat Biotech 2016

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

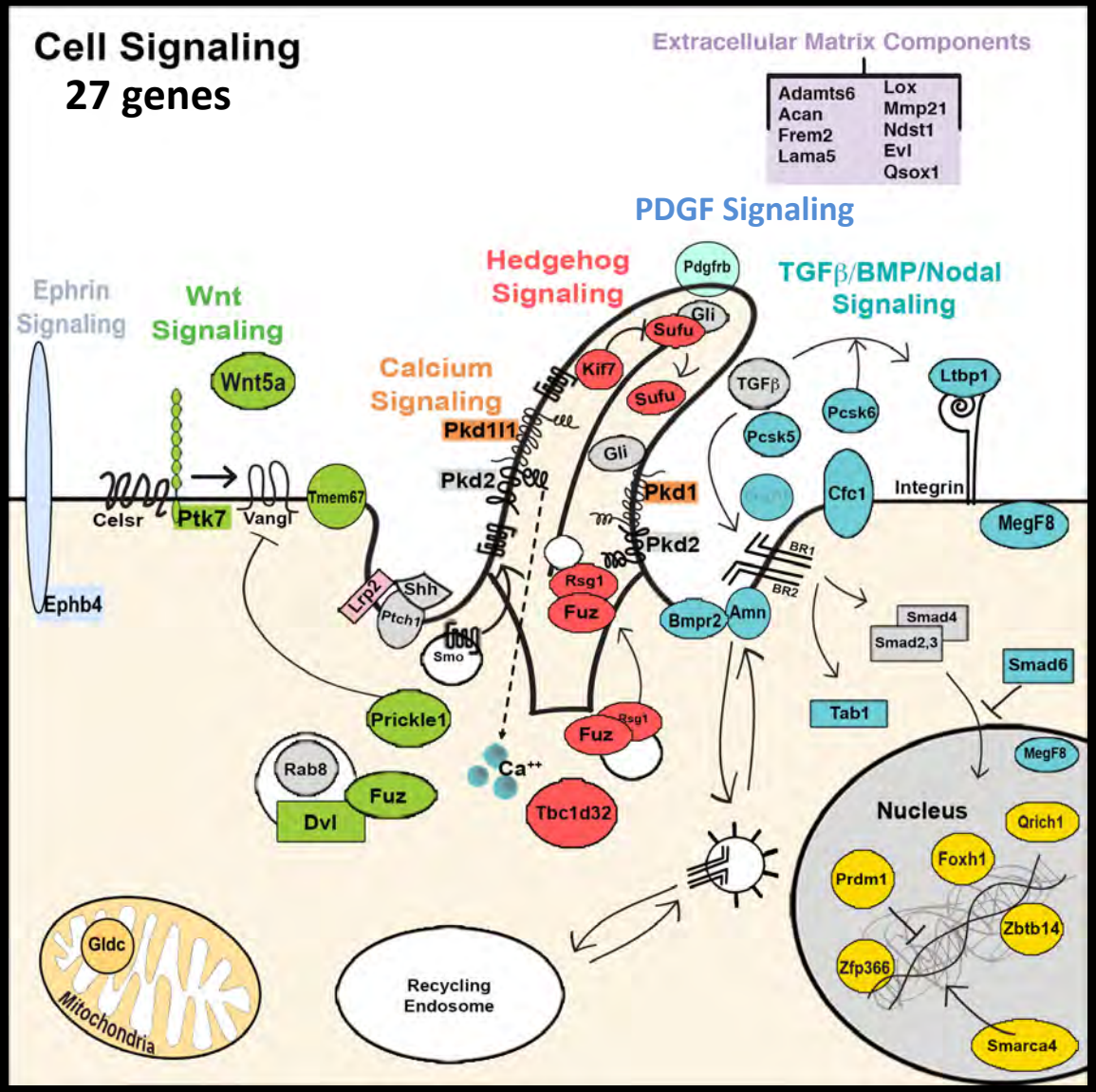
Ciliome CHD Genes



50% of Ciliome Mutations in Non-Laterality Mutant Lines

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

Cilia Transduced Cell Signaling Genes



Dvl3
Ptk7
Prickle1
Wnt5a
Pkd1l1
Pkd1
Fuz
Kif7
Lrp2
Rsg1
Sufu
Tbc1d32
Bmpr2
Pcks5
Pcsk6
Cfc1
Ltbp1
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

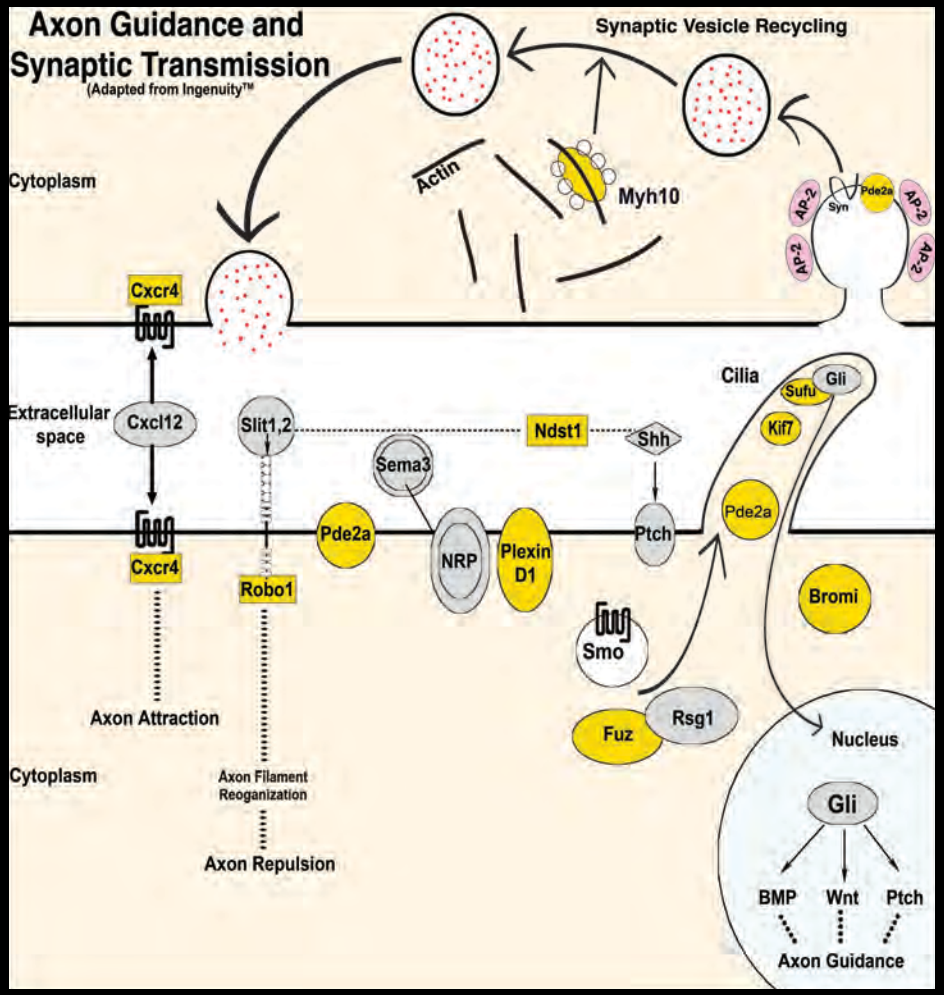
Patient ID	CHD§	Gene	Mutation	Gene Function
1-00638	CTD	FBN2	p.D2191N	TGFβ signaling
1-02020	HTX	SMAD2	p.IVS12+1G>A	TGFβ signaling
1-02621	HTX	SMAD2	p.W244C	TGFβ signaling
1-00197	LVO	BCL9	p.M1395K	WNT signaling
1-01828	CTD	DAPK3	p.P193L	WNT signaling
1-01138	LVO	USP34	p.L432P	WNT signaling
1-00802	LVO	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)

B2B Bench to Bassinet
Funded by NHLBI

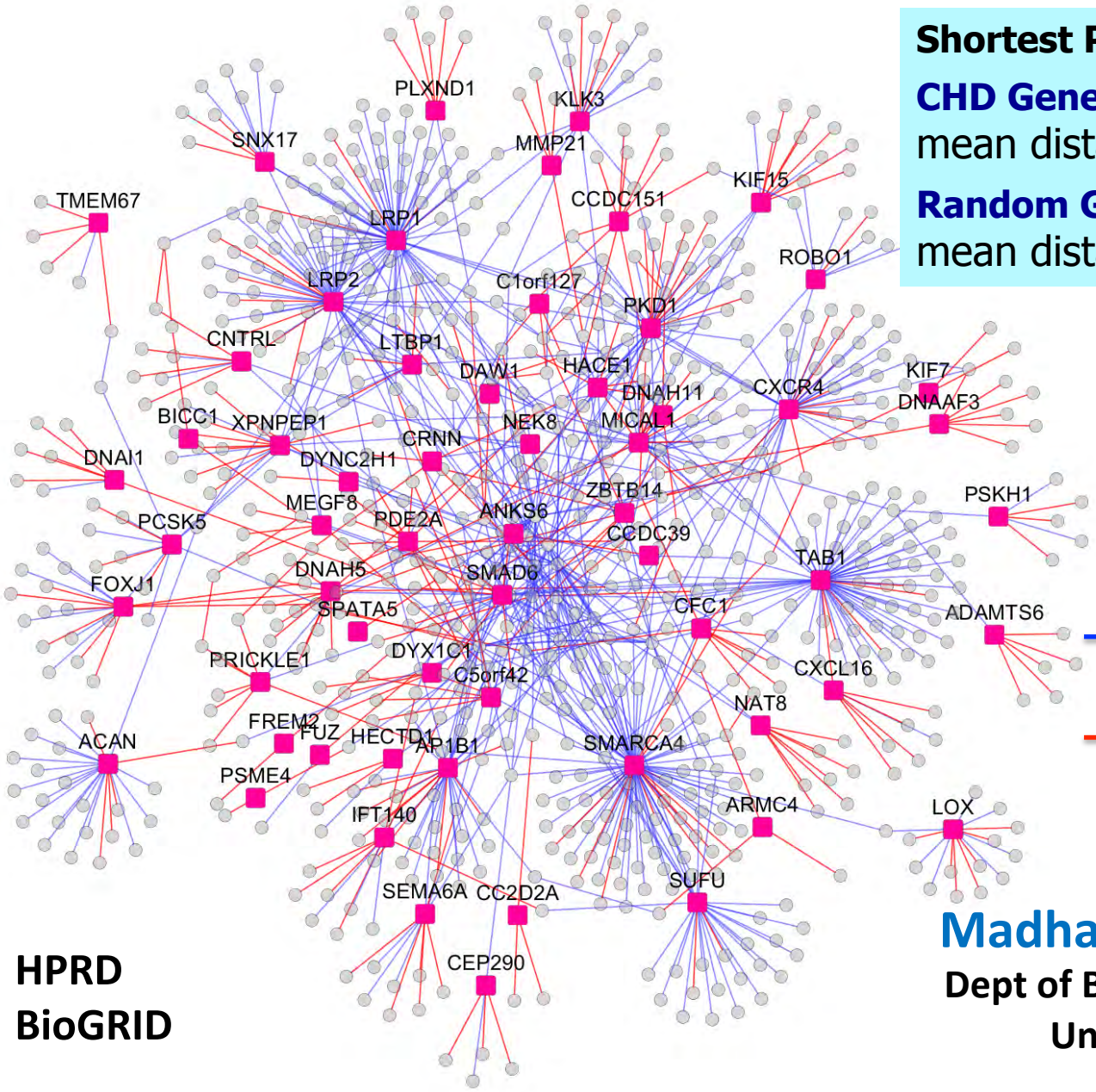
Axon Guidance, Neurogenesis, and Synpatic Transmission



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

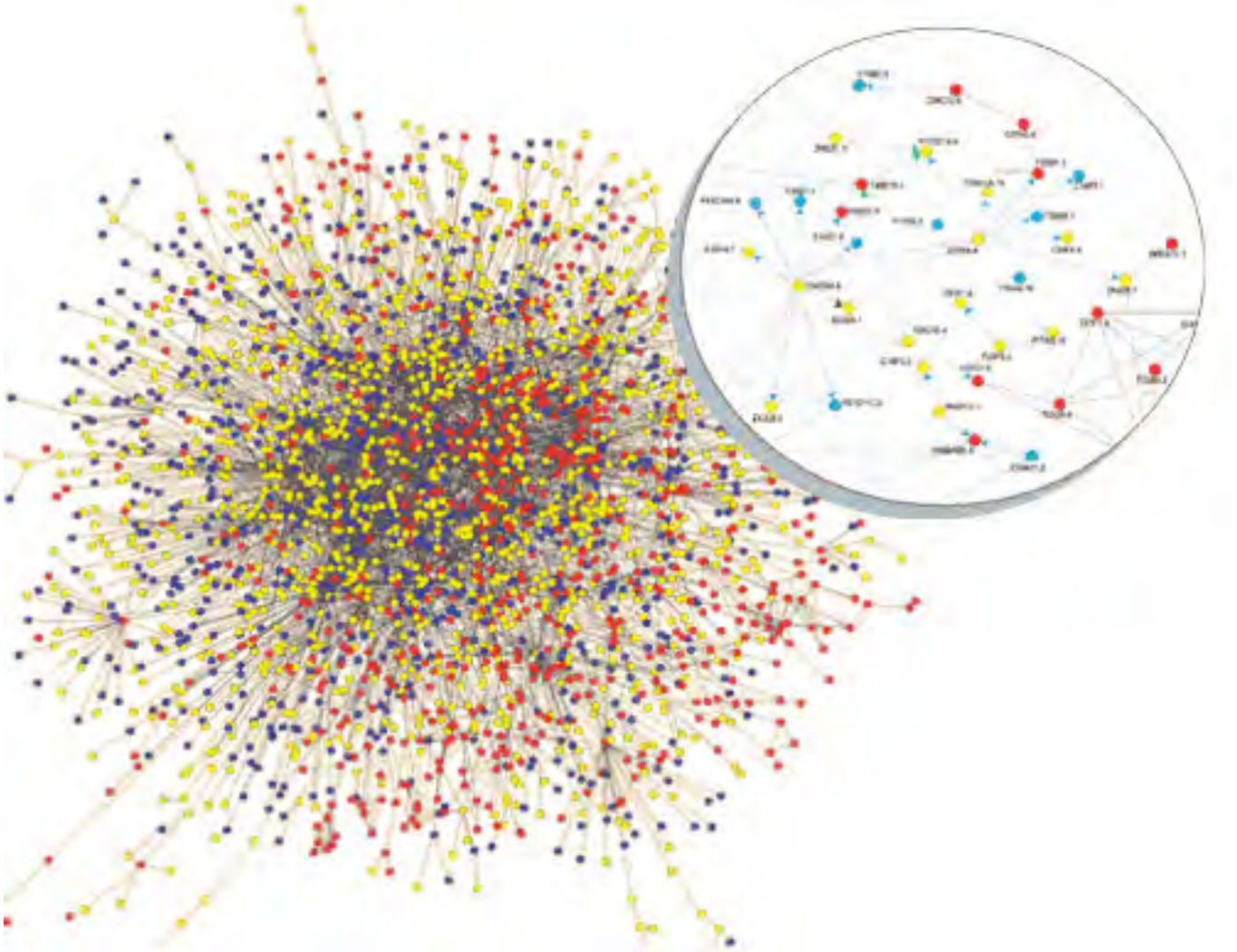
BioGRID

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

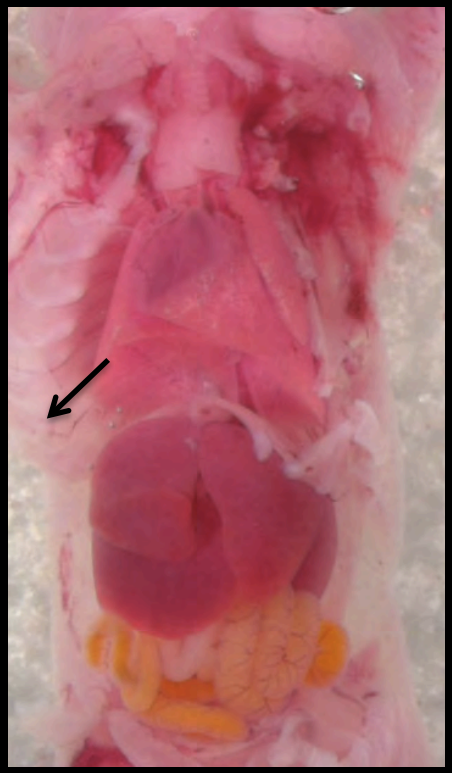
DOI: 10.1038/ncomms7023

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 Lemke[4], Nikolai T. Klena[4], Xiaoqin Liu[4], Yu Chen[4], You Li[4], Jovenal T. San Agustin[6], Maija K. Garnaas[5], Richard J. Francis[4], Kimimasa Tobita[4], Wolfram Goessling[5], Gregory J. Pazour[6], Cecilia W. Lo[4], David R. Beier[5,7] & Jagesh V. Shah[1,2]

Anks6-Nek8 Exhibit Epistasis

Anks6 +/m, Nek8 +/m



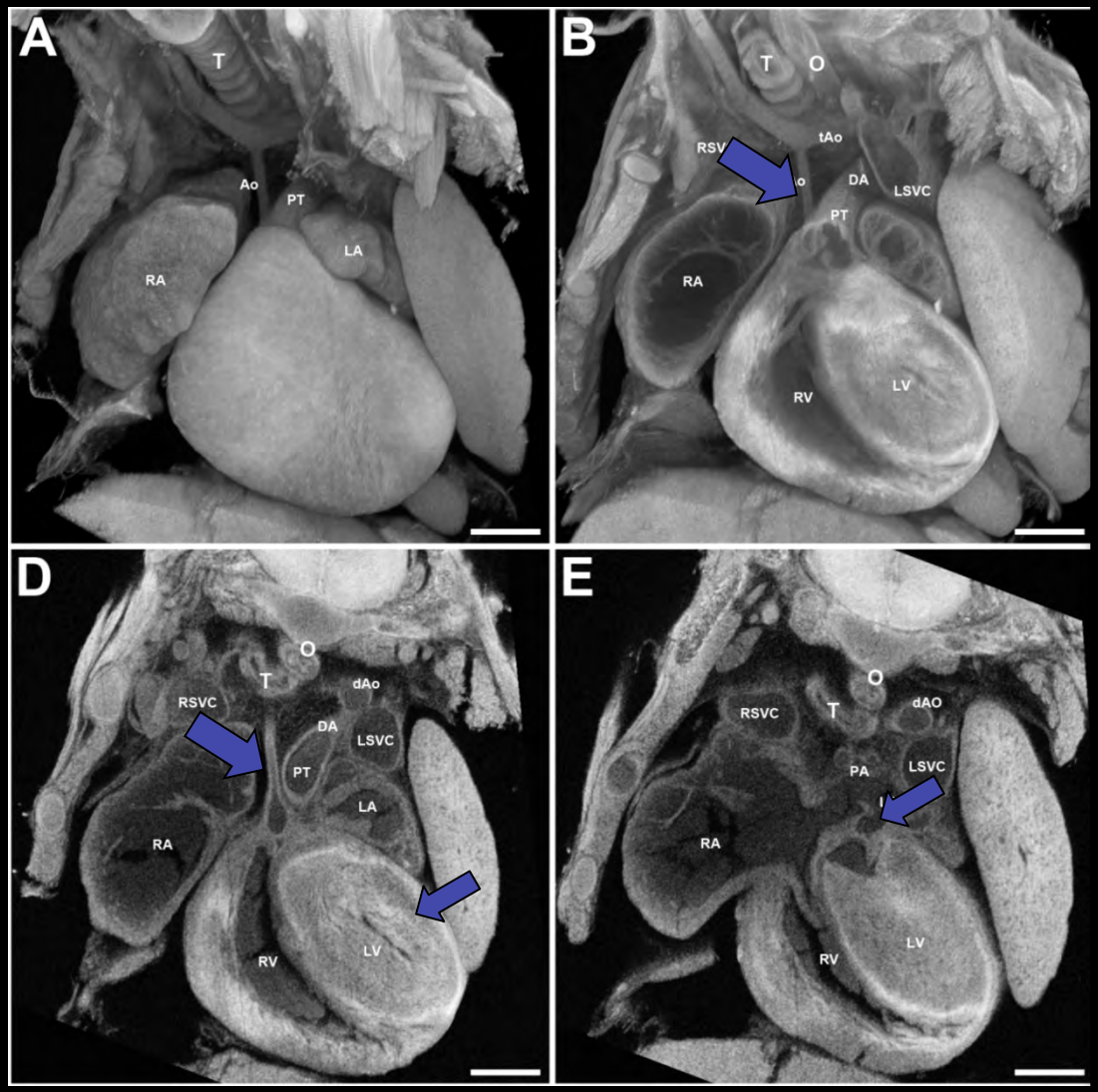
Anks6 +/m X Nek8 +/m

Genotype	# Embryos
Anks6 +/+, Nek8 +/+	47 (29%)
Ansk6 +/m, Nek8 +/+	45 (27%)
Ansk6 +/+, Nek8 +/m	42 (26%)
Ansk6 +/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

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