Leveraging Congenital Heart Disease Mouse Model Findings to Improve Clinical Outcome

GENOME Medicine IX

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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

- Aorta
- Pulmonary Artery
- Left Atrium
- Left Ventricle
- Right Atrium
- Right Ventricle
- Ventricular Septum
- Lung capillaries
- Pulmonary circuit
- Systemic circuit
- Systemic capillaries

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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

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

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

Noncardiac defects highly associated with CHD
~300 Mutant Mouse Lines Recovered
Wide Spectrum CHD Phenotypes

<table>
<thead>
<tr>
<th>PHENOTYPE</th>
<th>No. Mutant Lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laterality defects</td>
<td>71</td>
</tr>
<tr>
<td>Great artery anomalies</td>
<td>79</td>
</tr>
<tr>
<td>ASD/VSD/AVSD</td>
<td>64</td>
</tr>
<tr>
<td>Aortic arch anomalies</td>
<td>25</td>
</tr>
<tr>
<td>Left heart obstructive lesions</td>
<td>11</td>
</tr>
<tr>
<td>Right heart obstructive lesions</td>
<td>11</td>
</tr>
<tr>
<td>Myocardial anomalies</td>
<td>18</td>
</tr>
<tr>
<td>Craniofacial defects</td>
<td>45</td>
</tr>
<tr>
<td>Kidney defects</td>
<td>40</td>
</tr>
<tr>
<td>TOTAL LINES</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

25% of the CHD mutants exhibit laterality defects
Complex CHD Highly Associated with Heterotaxy

**Situs Solitus**

**Situs Inversus**

**Heterotaxy**

**A**

**B**

**C**

**D**

**E**

**F**

**G**

**H**

**I**
>300 Mutant Mouse Lines
Sperm Cryopreserved at JAX

Detailed Phenotype Annotation in MGI

MPO
Fyler Codes
Mutation Recovery by Exome Sequencing

All Coding/Splicing Mutations
n=12,297

- Heterozygous: 13% (1,566)
- Homozygous: 87% (10,731)

Mutations in 7,235 genes

Pathogenic Mutations
n=147

- Missense: 63% (90)
- Splicing: 22% (31)
- Nonsense: 15% (21)

Mutations in 98 genes

Suggests Screen at 30% Saturation
Library of >12,000 Mouse Mutations Available

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 C57BL/6J mice mutagenized with ethynitrosourea (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:
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

\[ C = \frac{c}{u} + (g^2)d(1-u)/u \]

- \( c \) = 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)


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

- 108 in Genes with Known KO Mouse Model
- 4 genes exhibit early embryonic lethality
- 104 viable to weaning

KOMP suggests 30% embryonic lethals expected

3.7%* 96.3%

Genetic Resiliency

Chen et al., Nat Biotech 2016
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
Cilia Transduced Cell Signaling Genes

27 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

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>CHD*</th>
<th>Gene</th>
<th>Mutation</th>
<th>Gene Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-00638</td>
<td>CTD</td>
<td>FBN2</td>
<td>p.D2191N</td>
<td>TGFβ signaling</td>
</tr>
<tr>
<td>1-02020</td>
<td>HTX</td>
<td>SMAD2</td>
<td>p.IVS12+1G&gt;A</td>
<td>TGFβ signaling</td>
</tr>
<tr>
<td>1-02621</td>
<td>HTX</td>
<td>SMAD2</td>
<td>p.W244C</td>
<td>TGFβ signaling</td>
</tr>
<tr>
<td>1-00197</td>
<td>LVO</td>
<td>BCL9</td>
<td>p.M1395K</td>
<td>WNT signaling</td>
</tr>
<tr>
<td>1-01828</td>
<td>CTD</td>
<td>DAPK3</td>
<td>p.P193L</td>
<td>WNT signaling</td>
</tr>
<tr>
<td>1-01138</td>
<td>LVO</td>
<td>USP34</td>
<td>p.L432P</td>
<td>WNT signaling</td>
</tr>
<tr>
<td>1-00802</td>
<td>LVO</td>
<td>PTCH1</td>
<td>p.R831Q</td>
<td>SHH signaling/Ciliome</td>
</tr>
<tr>
<td>1-02598</td>
<td>HTX</td>
<td>LRP2*</td>
<td>p.E4372K</td>
<td>SHH signaling/Endocytic trafficking</td>
</tr>
<tr>
<td>1-01913</td>
<td>Other</td>
<td>RAB10</td>
<td>p.N112S</td>
<td>Endocytic trafficking</td>
</tr>
<tr>
<td>1-00750</td>
<td>LVO</td>
<td>HUWE1</td>
<td>p.R3219C</td>
<td>Ciliome</td>
</tr>
<tr>
<td>1-01151</td>
<td>CTD</td>
<td>SU420H1</td>
<td>p.R143C</td>
<td>Ciliome</td>
</tr>
<tr>
<td>1-00853</td>
<td>CTD</td>
<td>WDR5</td>
<td>p.K7Q</td>
<td>Ciliome</td>
</tr>
<tr>
<td>1-02952</td>
<td>LVO</td>
<td>PITX2</td>
<td>p.A47V</td>
<td>Laterality related</td>
</tr>
</tbody>
</table>

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

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

Madhavi Ganapathiraju
Dept. of Biomedical Informatics
Univ. of Pittsburgh

**Shortest Paths Between Genes**

**CHD Genes:**
- mean distance: 4.7 ± 3.3

**Random Genes:**
- mean distance: 14.9 ± 4.9

### Experimentally Known Interaction

### Computationally Predicted Interaction

CHD Gene

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HPRD
BioGRID

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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?

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

Peter G. Czarnecki,2,3,*, George C. Gabriel4,*, Danielle K. Manning5,*, Mikhail Sergeev1,2, Kristi Lemke4, Nikolai T. Klena4, Xiaoxin 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. Beier5,7 & Jagesh V. Shah1,2
**Anks6-Nek8 Exhibit Epistasis**

<table>
<thead>
<tr>
<th>Genotype</th>
<th># Embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anks6 +/+ , Nek8 +/+</td>
<td>47 (29%)</td>
</tr>
<tr>
<td>Anks6 +/m, Nek8 +/+</td>
<td>45 (27%)</td>
</tr>
<tr>
<td>Anks6 +/+ , Nek8 +/m</td>
<td>42 (26%)</td>
</tr>
<tr>
<td>Anks6 +/m, Nek8 +/m</td>
<td>27 (16%)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>161</strong></td>
</tr>
</tbody>
</table>

- 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

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