De Novo Variants: Can these Inform Clinical Phenotypes?

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Investigator, Howard Hughes Medical Institutes
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)

Hypoplastic Left Heart Syndrome

Transposition of Great Arteries

Tetralogy of Fallot

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
Whole Exome Sequencing of Critical CHD Trios

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

1220 critical CHD Trios
Exclude Syndromic Cases
Unaffected parents
Family history negative
### Damaging De Novo Variants: Enriched in CHD Cases vs Expected or Observed

<table>
<thead>
<tr>
<th>Genes Highly Expressed in Developing Heart</th>
<th>CHD Cases, N = 1220</th>
<th>Controls, N = 900</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CHD Cases</strong></td>
<td>Observed</td>
<td>Expected</td>
</tr>
<tr>
<td>n</td>
<td>Rate</td>
<td>n</td>
</tr>
<tr>
<td>---</td>
<td>------</td>
<td>---</td>
</tr>
<tr>
<td>All genes</td>
<td></td>
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</tr>
<tr>
<td>Total</td>
<td>1281</td>
<td>1.05</td>
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<tr>
<td>Synonymous</td>
<td>279</td>
<td>0.23</td>
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<tr>
<td>Missense</td>
<td>850</td>
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<tr>
<td>D-Mis</td>
<td>212</td>
<td>0.17</td>
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<tr>
<td>LoF</td>
<td>152</td>
<td>0.12</td>
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<tr>
<td>Damaging</td>
<td>364</td>
<td>0.30</td>
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</table>

<table>
<thead>
<tr>
<th>Genes Highly Expressed in Developing Heart</th>
<th>CHD Cases, N = 1220</th>
<th>Controls, N = 900</th>
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<tbody>
<tr>
<td><strong>LHE genes</strong></td>
<td>Observed</td>
<td>Expected</td>
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<tr>
<td>n</td>
<td>Rate</td>
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<tr>
<td>Total</td>
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<td>0.68</td>
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<tr>
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<tr>
<td>Missense</td>
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<td>D-Mis</td>
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<td>0.09</td>
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<td>LoF</td>
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<td>0.06</td>
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<tr>
<td>Damaging</td>
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<td>0.15</td>
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</table>

*Homsy et al, Science 2015*
Evidence for Pathogenicity: 21 Genes with Recurrent Damaging *De Novo* Variants

<table>
<thead>
<tr>
<th>Gene</th>
<th>LoF</th>
<th>D-Mis</th>
<th>p</th>
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<tr>
<td><em>PTPN11</em></td>
<td>0</td>
<td>4</td>
<td>2.96 x 10^{-11}</td>
</tr>
<tr>
<td><em>KMT2D</em></td>
<td>4</td>
<td>2</td>
<td>4.24 x 10^{-9}</td>
</tr>
<tr>
<td><strong>RBFOX2</strong></td>
<td>3</td>
<td>0</td>
<td>3.46 x 10^{-8}</td>
</tr>
<tr>
<td><em>KDM5B</em></td>
<td>3</td>
<td>0</td>
<td>2.93 x 10^{-6}</td>
</tr>
<tr>
<td><em>KRT13</em></td>
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<td>2</td>
<td>1.02 x 10^{-5}</td>
</tr>
<tr>
<td><em>MYH6</em></td>
<td>0</td>
<td>3</td>
<td>2.45 x 10^{-5}</td>
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<tr>
<td><em>CAD</em></td>
<td>0</td>
<td>3</td>
<td>3.80 x 10^{-5}</td>
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<tr>
<td><em>NAA15</em></td>
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<td><em>SMAD2</em></td>
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<tr>
<td><em>RABGAP1L</em></td>
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<td>1</td>
<td>4.05 x 10^{-4}</td>
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<td><em>POGZ</em></td>
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<tr>
<td><em>JAG1</em></td>
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<td>4.52 x 10^{-4}</td>
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<tr>
<td><em>GANAB</em></td>
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<td>4.57 x 10^{-4}</td>
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<td><em>DTNA</em></td>
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<tr>
<td><em>PPL</em></td>
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<tr>
<td><em>ZEB2</em></td>
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<td>1</td>
<td>6.25 x 10^{-4}</td>
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<td><em>FBN1</em></td>
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<td>6.86 x 10^{-4}</td>
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<td><em>CHD4</em></td>
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<td>1.17 x 10^{-3}</td>
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<td><em>AHNAK</em></td>
<td>1</td>
<td>1</td>
<td>2.91 x 10^{-3}</td>
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<td><em>NOTCH1</em></td>
<td>1</td>
<td>1</td>
<td>4.40 x 10^{-3}</td>
</tr>
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De Novo Copy Number Variants Detected by WES


11 Targets, 10^5 bases (chr 22: 36013197-36156089)

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


**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

<table>
<thead>
<tr>
<th></th>
<th>Cases, n=1220</th>
<th>Controls, n=900</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>Expected</td>
</tr>
<tr>
<td>Total</td>
<td>320</td>
<td>203.5</td>
</tr>
<tr>
<td>Syn</td>
<td>60</td>
<td>56.6</td>
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<tr>
<td>Missense</td>
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<tr>
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<td>59</td>
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<tr>
<td><strong>Damaging</strong></td>
<td>116</td>
<td>42.3</td>
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</table>
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 \textit{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 ($p=7.5 \times 10^{-20}$)

\textit{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
Transcriptional Differences between Isogenic WT and CHD-mutant Cardiomyocytes

^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

Delaughter et al, in Review
CHD Genetics

Jason Homsy
David McKean
Steve DePalma
Alex Bick
Karou Ito

James Ware
Michael Parvenof
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

Boston Children’s Hospital: Jane Newburger, Amy Roberts
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