The Promise of Genetic Research in Congenital Heart Disease in Children

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Disclosures

Ariosa Diagnostics, acquired by Roche 2015 (Cofounder)

TAI Diagnostics (Cofounder, CSO, Board Member)

*Any financial conflicts of interest that may be related to the work presented here have been disclosed to The Medical College of Wisconsin as required per Federal Regulation(s) 42 CFR Part 50, Subpart F and 45 CFR Part 94*
Hypoplastic left heart syndrome (HLHS) a complex form of CHD

Treatment requires a minimum of three surgeries, starting in the neonatal period

Three decades ago, almost all died

Today at major centers, >90% survive surgery

The clinical goal has become achieving normal neurological and functional outcomes which last a normal lifetime
Background: Congenital Heart Disease (CHD)

- **Incidence**: CHD affects ~1% of all live births \(^1,2\)
  - 130,000 hospitalizations per year \(^3\)
  - Most frequent cause of infant death from birth defects \(^4\)

- **Prevalence**: The number of adults surviving with CHD is growing rapidly as therapy becomes increasingly effective. There are >1 million adults living in the US with CHD. More adults with CHD than children.

- **Survival outcomes** for the most complex lesions have improved significantly

- **Neurocognitive** and **long term outcomes**, are more variable

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\(^1\) Hoffman JIE, Kaplan S. JACC 2002  
\(^2\) CDC 2004  
\(^3\) Healthcare Cost and Utilization Project (HCUP) 2000  
\(^4\) Gilboa, Circulation 2010.
What causes Congenital Heart Disease?

• Environmental Risk Factors
  – Maternal Conditions/Exposures
    – diabetes, infections
    – folate intake

• Genetic causes
  – Single gene variants
  – Association with genetic syndromes and chromosomal abnormalities (cytogenetically visible)
  – Gene Duplications or Deletions (Copy Number Variants or CNVs)

• Unknown
  Complex Disease (incomplete penetrance)
  – Genetic susceptibility (not everyone will have a heart problem)
  – Genetic/environmental interaction

1Baltimore Washington Infant Study 1981-1989
2Reviewed in Pierpont, Circulation, 2007
Hypoplastic Left Heart Syndrome (HLHS)

- Affects ~ 1/5000 live births
- Hypoplasia of the left ventricle
- Hypoplastic ascending aorta
- Stenosis or atresia of the mitral and/or aortic valves

https://www.cdc.gov/ncbddd/heartdefects/hlhs.html
Evidence supporting a genetic basis for HLHS:

1) Family clustering and high heritability\(^1\),

2) Occurrence with specific chromosomal disorders e.g. Turner syndrome and Jacobsen syndrome as well as rare copy number variants (CNVs) in cardiogenic genes\(^2,3\),

3) Increased frequency of HLHS-related heart defects including Bicuspid Aortic Valve (BAV) and Coarctation of the Aorta (CoA) in relatives of HLHS patients\(^1,-4,5\).

However for the most part, HLHS etiology is thought to be complex and largely unknown (95% cases)

A Family Pedigree to Identify HLHS Genes

LVOTO: LV outflow tract obstruction
DORV: double outlet RV
CoA: coarctation of aorta
VSD: ventricular septal defect

Physiol Genomics. 2016 Dec 1;48(12):912-921.
NGS & Filtering Identified 20 Variants

Filtering Criteria
1. NGS artifacts
2. not susceptible to indels
3. novel or rare
4. conserved
5. coding region
6. protein dysfunction

Physiol Genomics. 2016 Dec 1;48(12):912-921.
Among these 20 candidate genes, only **MYH6** is highly expressed in the heart.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>TPM Atrium</th>
<th>TPM Ventricle</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABHD4</td>
<td>Lipid metabolism</td>
<td>17.8</td>
<td>24.1</td>
</tr>
<tr>
<td>ANXA4</td>
<td>Prostaglandin Synthesis and Regulation</td>
<td>23.2</td>
<td>12.1</td>
</tr>
<tr>
<td>BCLAF1</td>
<td>Apoptotic process</td>
<td>19.4</td>
<td>18.2</td>
</tr>
<tr>
<td>BIVM-ERCC5</td>
<td>DNA repair</td>
<td>7.6</td>
<td>7.6</td>
</tr>
<tr>
<td>BRD8</td>
<td>Chromatin organization</td>
<td>25.4</td>
<td>22.7</td>
</tr>
<tr>
<td>CNTNAP2</td>
<td>Membrane protein</td>
<td>0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>DIAPH3</td>
<td>Regulation of actin cytoskeleton</td>
<td>3.3</td>
<td>0.7</td>
</tr>
<tr>
<td>FAR1P1</td>
<td>Regulation of GTPase activity</td>
<td>52.4</td>
<td>25.7</td>
</tr>
<tr>
<td>GPR1</td>
<td>G Protein coupled Receptor</td>
<td>1.1</td>
<td>0.8</td>
</tr>
<tr>
<td>GXYLT1</td>
<td>Integral component of membrane</td>
<td>1.6</td>
<td>1.3</td>
</tr>
<tr>
<td>LAG3</td>
<td>Immunoglobin superfamily</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>LGI1</td>
<td>regulating postnatal glutamatergic synapse development</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>MYH6</strong>*</td>
<td><strong>Sarcomere organization, contraction</strong></td>
<td>**6,190.8</td>
<td><strong>978.8</strong></td>
</tr>
<tr>
<td>NRL</td>
<td>Regulation of photoreceptor</td>
<td>1.2</td>
<td>1.4</td>
</tr>
<tr>
<td>PSMB10</td>
<td>Proteosome subunit</td>
<td>44.7</td>
<td>51.8</td>
</tr>
<tr>
<td>RAB36</td>
<td>GTPase</td>
<td>2.5</td>
<td>1.6</td>
</tr>
<tr>
<td>SLC16A14</td>
<td>Transmembrane transport</td>
<td>1.5</td>
<td>2.8</td>
</tr>
<tr>
<td>SLC5A12</td>
<td>Transmembrane transport</td>
<td>4.2</td>
<td>0.0</td>
</tr>
<tr>
<td>ZAN</td>
<td>Integral component of membrane</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>ZZEF1</td>
<td>Zinc, calcium ion binding protein</td>
<td>12.5</td>
<td>15.4</td>
</tr>
</tbody>
</table>

Are other rare \textit{MYH6} variants associated with HLHS?

- Case-Control Association Study
- 190 HLHS patients (unrelated) were compared with the 1,000 Genomes Project Database.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Variants in Cases</th>
<th>Variants in controls</th>
<th>Fisher Exact Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{ABHD4}</td>
<td>Lipid metabolism</td>
<td>1 / 170</td>
<td>2 / 1063</td>
<td>6.96 x 10^{-1}</td>
</tr>
<tr>
<td>\textit{ANXA4}</td>
<td>Prostaglandin Synthesis and Regulation</td>
<td>2 / 170</td>
<td>6 / 1063</td>
<td>3.04 x 10^{-1}</td>
</tr>
<tr>
<td>\textit{BCLAF1}</td>
<td>Apoptotic process</td>
<td>3 / 170</td>
<td>3 / 1063</td>
<td>4.48 x 10^{-2}</td>
</tr>
<tr>
<td>\textit{BIVM-ERCC5}</td>
<td>DNA repair</td>
<td>4 / 170</td>
<td>18 / 1063</td>
<td>3.60 x 10^{-1}</td>
</tr>
<tr>
<td>\textit{BRD8}</td>
<td>Chromatin organization</td>
<td>1 / 170</td>
<td>17 / 1063</td>
<td>5.35 x 10^{-1}</td>
</tr>
<tr>
<td>\textit{CNTNAP2}</td>
<td>Membrane protein</td>
<td>7 / 170</td>
<td>22 / 1063</td>
<td>9.22 x 10^{-2}</td>
</tr>
<tr>
<td>\textit{DDMH3}</td>
<td>Regulation of actin cytoskeleton</td>
<td>11 / 170</td>
<td>20 / 1063</td>
<td>1.73 x 10^{-2}</td>
</tr>
<tr>
<td>\textit{FARP1}</td>
<td>Regulation of GTPase activity</td>
<td>6 / 170</td>
<td>32 / 1063</td>
<td>4.29 x 10^{-1}</td>
</tr>
<tr>
<td>\textit{GPR1}</td>
<td>G Protein coupled Receptor</td>
<td>1 / 170</td>
<td>11 / 1063</td>
<td>8.33 x 10^{-1}</td>
</tr>
<tr>
<td>\textit{GXYLT1}</td>
<td>Integral component of membrane</td>
<td>0 / 170</td>
<td>3 / 1063</td>
<td>1.00 x 10^{0}</td>
</tr>
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<td>\textit{LAG3}</td>
<td>Immunoglobulin superfamily</td>
<td>4 / 170</td>
<td>3 / 1063</td>
<td>8.72 x 10^{-1}</td>
</tr>
<tr>
<td>\textit{LG11}</td>
<td>regulating postnatal glutamatergic synapse development</td>
<td>0 / 170</td>
<td>2 / 1063</td>
<td>1.00 x 10^{0}</td>
</tr>
<tr>
<td>\textit{MYH6*}</td>
<td>Sarcomere organization, contraction</td>
<td>20 / 190*</td>
<td>31 / 1063</td>
<td>5.66 x 10^{-6}</td>
</tr>
<tr>
<td>\textit{NRL}</td>
<td>Regulation of photoreceptor</td>
<td>5 / 170</td>
<td>2 / 1063</td>
<td>7.84 x 10^{-4}</td>
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<td>Proteosome subunit</td>
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<td>0 / 1063</td>
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</tr>
<tr>
<td>\textit{RAB36}</td>
<td>GTPase</td>
<td>3 / 170</td>
<td>0 / 1063</td>
<td>2.58 x 10^{-2}</td>
</tr>
<tr>
<td>\textit{SLC16A14}</td>
<td>Transmembrane transport</td>
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<td>3 / 1063</td>
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<td>\textit{SLC5A12}</td>
<td>Transmembrane transport</td>
<td>5 / 170</td>
<td>7 / 1063</td>
<td>1.65 x 10^{-2}</td>
</tr>
<tr>
<td>\textit{ZAN}</td>
<td>Integral component of membrane</td>
<td>11 / 170</td>
<td>18 / 1063</td>
<td>9.31 x 10^{-4}</td>
</tr>
<tr>
<td>\textit{ZUFZ1}</td>
<td>Zinc, calcium ion binding protein</td>
<td>13 / 170</td>
<td>35 / 1063</td>
<td>9.81 x 10^{-3}</td>
</tr>
</tbody>
</table>

Physiol Genomics. 2016 Dec 1;48(12):912-921.
Rare *MYH6* variants were significantly (P<10^{-5}) enriched in >10% of HLHS Subjects.

These rare *MYH6* variants include R443P.
What is MYH6? MYH6 is cardiomyocyte-specific

- MYH6 encodes the α-Myosin Heavy Chain (α-MHC).
- A major isoform of α-MHC is β-MHC, which is encoded by MYH7.
- α-MHC is expressed in the atria at embryonic and adult stages.
- β-MHC is the major isoform in the ventricles.
- Myosin Heavy Chains comprise the thick filaments of the sarcomere.
- MYH6 variants are associated with cardiomyopathy (dilated and hypertrophic) and cardiac defects.


The sarcomere is the basic unit of striated muscle contraction.
**MYH6 (α-MHC)**

- **In human & zebrafish:**
  - α-MHC is major isoform in atria.
  - β-MHC is major isoform in ventricles.

- **In mice this is reversed:**
  - α-MHC is major isoform in ventricles.
  - β-MHC is major isoform in atria.
Do *MYH6* variants have clinical impact?

HLHS patients with a *MYH6* variant (n=10) had significantly lower cardiac transplant-free survival than HLHS patients without a variant (n=62) (p=0.006).

*Physiol Genomics.* 2016 Dec 1;48(12):912-921.
Are other genes mis-expressed in patients who carry MYH6 variants (including R443P)?

Transcriptome Sequencing “RNAseq”

• MYH6 variants are accompanied by differential expression of 22 genes (p<0.01).
• Among these, 5 genes encoding sarcomeric proteins are strongly up-regulated, including MYH7.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Expression in MYH6 Variant Carriers</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNNT2</td>
<td>1,231% ↑</td>
<td>2.81 X 10^{-3}</td>
</tr>
<tr>
<td>MYL2</td>
<td>366% ↑</td>
<td>3.94 X 10^{-3}</td>
</tr>
<tr>
<td>MYH7</td>
<td>346% ↑</td>
<td>7.06 X 10^{-4}</td>
</tr>
<tr>
<td>ACTA1</td>
<td>315% ↑</td>
<td>5.13 X 10^{-3}</td>
</tr>
<tr>
<td>TPM2</td>
<td>221% ↑</td>
<td>8.61 X 10^{-4}</td>
</tr>
</tbody>
</table>
MYH7 RNA expression and β-MHC protein, are increased in HLHS hearts with R443P & other MYH6 variants.

RNAseq Western Blot

Physiol Genomics. 2016 Dec 1;48(12):912-921.
Can iPSC-derived Cardiomyocytes (CMs) model HLHS?

Three iPSC lines were re-programmed from 3 individuals in the Family Pedigree:
- III:1 CoA parent with $R443P$ variant
- III:2 Heart healthy parent (WT)
- IV:3 HLHS proband with $R443P$ variant

Cardiomyocytes were induced using the “Palecek” Protocol.

Induction:
- D-4 D-1 D0 D1 D2 D3 D4 D5 D6 D7 D8 D9 D10
  - D0: 10 ng/ml A-A
  - D1: 9 µM CHIR
  - D3: 5 µM IWP
  - D6: rhythmic beating
  - MF20 staining & Flow Cytometry with cTnT

Physiol Genomics. 2016 Dec 1;48(12):912-921.
CM differentiation is inhibited in iPSCs from the Proband and the Carrier parent, both of which contain the \textit{R443P} variant in \textit{MYH6}. 

\textbf{CMs at Differentiation Day 10} 

\textbf{Flow Cytometry} 

\begin{itemize} 
\item Heart Healthy Parent (+/+) 
\item Carrier Parent (+/-) 
\item HLHS proband (+/-) 
\end{itemize} 

\textbf{Immunostaining} 

\begin{itemize} 
\item Heart Healthy Parent (+/+) 
\item Carrier Parent (+/-) 
\item HLHS proband (+/-) 
\end{itemize} 

\textit{Physiol Genomics}. 2016 Dec 1;48(12):912-921.
Also, iPSC-derived CMs from two unrelated *MYH6* variant families phenocopy the up-regulated expression of *MYH7* seen *in vivo*.

**RNA-seq**

<table>
<thead>
<tr>
<th>Heart healthy parent (+/+</th>
<th>HLHS proband (+/-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLHS Family R443P</td>
<td></td>
</tr>
</tbody>
</table>

**qRT-PCR**

<table>
<thead>
<tr>
<th>Normal Parent (+/+)</th>
<th>HLHS proband (+/-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLHS Family R443P</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Normal Parent (+/+)</th>
<th>HLHS proband (+/-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLHS Family D588A</td>
<td></td>
</tr>
</tbody>
</table>

*p* = 2.4x10^-3

*p* = 5x10^-4

*Physiol Genomics.* 2016 Dec 1;48(12):912-921.
CMs containing R443P variants exhibit dysmorphic sarcomeres.

CMs in Mass Culture at Differentiation Day 62
α-actinin

Heart Healthy Parent (+/+)
Carrier Parent (+/-)
HLHS proband (+/-)

Low-Density CMs at Differentiation Day 68
Red: α-actinin  Green: MF20  Blue: Dapi

Heart Healthy Parent (+/+)
Carrier Parent (+/-)
HLHS proband (+/-)

Physiol Genomics. 2016 Dec 1;48(12):912-921.
1. We identified a novel rare MYH6 variant -- R443P -- in a multi-generational family with a high prevalence of CHD/HLHS.

2. A case-control association study revealed that rare MYH6 variants are highly enriched in HLHS (~10%).

3. HLHS subjects with MYH6 variants have reduced cardiac transplant-free survival.

4. MYH6 variant carriers exhibit increased expression of sarcomere genes, including MYH7.

5. In iPSC-CMs derived from MYH6 HLHS probands & carrier parents:
   - cardiomyogenic differentiation is reduced.
   - MYH7 expression is increased.
   - sarcomere structure is dysmorphic.
At least one model suggests that *myh6* variants are causative of HLHS-like phenotype.

Zebrafish mutation of *myh6* (weak atrium or “wea” mutant)
- atrial defects (dysmorphic sarcomere structure)
- ventricle: compact, a thickened myocardial wall, a narrow lumen and changes in myocardial gene expression (very similar to HLHS phenotype)

**Summary:** *Myh6* is expressed only in the atrium, the ventricular phenotypes represent a secondary response to atrial dysfunction.
Biomechanical effects of variants on cardiac tissue and iPSC derived cardiomyocytes

Understanding etiology (cause) is key to designing potential effective therapies.

Figure 2. Fiber microsystem A) steel troughs connected to motor arm and force transducer and B) attached cardiomyofibrils photographed at x 800 magnification (Stiffness, velocity, peak power, and cross bridge reattachment rate).
Future work

• Second family pedigree analysis

• Echocardiogram analysis (longitudinal atrial strain analysis)
  • How do MYH6 variants impact atrial function in HLHS?

• Ex vivo tissue
  • Is force affected in CMs from human atrial septal & ventricular ex vivo tissue of MYH6 variant carriers?

• Do patient specific variants model HLHS in zebrafish?

• Using in vitro CMs derived from HLHS families
  • Do CMs with MYH6 variants have contractile defects?
  • Are contractile defects ameliorated by drugs?

  Cardiac myosin compounds
MYH6 Study Team Members - Thank you!
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The Department of Surgery

MYH6 Collaborating Investigators

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Brian Link, Ph.D.
Robert Fitts, Ph.D. (MU)
Jeanne James, M.D.
Elle Geddes, M.D.
Aron Geurts, Ph.D.
Sara Creighton, M.D.
Pippa Simpson, Ph.D.

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