Robert L. Brent Lecture – Teratology Update

Congenital Heart Defects Research: Finding the Hidden Crossroads Between Genetics and Environment

Cheryl Maslen, PhD
Professor
Knight Cardiovascular Institute
Department of Molecular and Medical Genetics
Director
Program in Enhanced Research Training
Oregon Health & Science University
How Geneticists Think About Disease

- Cystic Fibrosis
- Heart disease, diabetes, Alzheimers, etc, etc, etc

- Genetic Change(s) Severity
- Environmental Severity

- Hit by a bus
A few major genetic defects with large contributions
A large number of genes each having a minor effect

The Level of Genetic Diversity in Humans is Huge

• The average individual has:
  • ~4.2 million DNA variants in their genome
  • ~3.2 million single base variants (SNVs)
  • 850,000 insertions/deletions (copy number variants)
  • 1.3 million of these differences will be “unique”
~1 in 100 Infants is Born with a Congenital Heart Defect

~25% are in Critical Condition at Birth
CHD is a high impact problem

- 40,000 births/year in the US
- 10,000 need critical care in the first year of life
- All need some type of surveillance through life
- Over 2 million infants, children and adults with CHD in the US

Center for Disease Control

van der Linde et al, JACC, 2011
18 Distinct Types of Congenital Heart Defects

- Septal defects
- Valve abnormalities
- Outflow tract defects
Mendelian Disease
Congenital Heart Disease

Genetic Change(s) Severity
Environmental Severity

Rubella
Maternal diabetes
Maternal alcohol consumption
Maternal smoking
Maternal lithium consumption
Maternal isotretinoin exposure
Placental insufficiency

Non-genetic event

Zaidi and Brueckner, Circ Res, 2017
de Novo Chromatin SNVs?????

• Whole exome sequencing
  • 362 clinically severe CHD cases (parent-offspring trios)

• Marked excess of de novo mutations in genes involved in the production, removal or reading of H3K4 methylation (H3K4me) and the induction of methylation of H3K27 (H3K27me)

Zaidi et al, Nature 2013
Epigenetic Regulation of Gene Expression

- Unmethylated
- Methylated

Gene Expression

Gene Expression Repressed

30-nm chromatin fibril composed of nucleosomes

"Beads-on-a-string" 10-nm chromatin fibril

Naked double-helical DNA
Oct = Histone octamer

H2A
H2B
H3
H4

Octamer structure:
30-nm chromatin fibril composed of nucleosomes
“Beads-on-a-string” 10-nm chromatin fibril
Naked double-helical DNA

Inactive genes

Heterochromatin
Histone methylation
Histone deacetylation
Corepressor complexes

Coactivator complexes
Loss of H1
Histone modifications
E.g. acetylation, phosphorylation, methylation

Euchromatin
Active areas of transcription
Histone modifications
**de Novo Chromatin SNVs??**

- Genes that methylate H3K4 can no longer methylate lysine4
  - Decreased methylation = Increase expression

- Genes that remove methyl groups from H3K4 no longer do so
  - Increased methylation = Decreased expression

- Genes involved in reading methylation of H3K4 can no longer do so
  - Decreased methylation recognition = Decreased methylation = Increased expression

- Genes that induce methylation of H3K27 can no longer cause the methylation
  - Reduced methylation = Increased expression
**de Novo Chromatin SNVs?????**

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  - **Gene dosage is dysregulated**

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**How can de Novo Chromatin-Related Mutations Affect Heart Development?**

- H3K4me and H3K27me are associated with transcriptionally active genes in early development
- The mutated genes broadly regulate gene expression from the earliest stages of development
- The heart is the first organ to develop

- Dysregulation of heart development gene expression through chromatin modifications will lead to heart defects
Zaidi and Brueckner, Circ Res, 2017
Is Epigenetic Variation More Important Than We Realized?

- CHD occurs as a result of:
  - Epigenetic changes caused by mutations in histone modifying genes
- CHD is associated with:
  - Aneuploidy
  - Environmental influences
- Aneuploidy and environmental influences affect epigenetics

- Time to connect the dots!
Turner syndrome phenotype

- Complete or partial loss of the second sex chromosome (monosomy X)
- 1 in 2,000 live female births
- Short stature
- Broad chest with widely spaced nipples
- Low set ears
- Low hairline
- Premature ovarian failure
- Normal Intelligence
- Specific cognitive/visual spatial
- Webbed neck
- Lymphedema
- **Cardiovascular Defects**

![Image of a girl with Turner syndrome](image)

With Mom's Permission
Bicuspid Aortic Valve Disease (BAVD) in Turner syndrome

- Tricuspid aortic valve
- Bicuspid aortic valve (BAV)

- Thoracic aortic aneurysms

- 30% of individuals with Turner syndrome have BAVD
  - 0.5-2% of euploid individuals
  - 70% of euploid cases are in males

Epigenetic Analysis

- Widespread DNA hypomethylation and differential gene expression in Turner syndrome compared to euploid individuals (Trolle et al, 2016)
Epigenetic Analysis

Does epigenetics play a role in BAVD in Turner syndrome?

- Global DNA methylation analysis

Study Design

- Samples from BioLINCC (GenTAC registry)
- 45,X0
- Cases (5)
  - Ages 27-50 years
  - BAV
  - Aortic dilation (z-scores 3.19-5.15)
  - 2/5 had dissections
- Controls (5)
  - Ages 28-56 years
  - No BAV
  - Normal aortic dimensions (z-scores -.36 – 1.71)
DNA Methyl-Capture Sequencing Analysis

• 195 genes showed differential CpG island methylation between cases and controls

• 5 genes involved in aortic valve morphogenesis and homeostasis of the aorta were differentially methylated

Differentially Methylated Genes in NOTCH1 Pathway
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Single pass transmembrane receptor that regulates cell fate decisions during development
Differentially Methylated Genes in NOTCH1 Pathway

Mutations in NOTCH1 cause familial BAVD in humans and ascending aortic aneurysms in mice
(Garg et al, 2005, Koenig...Garg, 2017)

Hypothesis: Synergistic interaction between 5 differentially methylated interactive NOTCH1 pathway genes contributes to the etiology of BAVD in Turner syndrome.
Conclusions

• CHD is a significant health issue world-wide
• More than half of CHD is of unknown etiology
• Mutations that affect histone modification are associated with CHD
• Epigenetic differences occur in high risk populations and between sexes
• Evidence of epigenetic differences in individuals with Turner syndrome and BAVD

• Epigenetic modification should be a focus in the research on the etiology of CHD
  • Consideration for non-genetic factors that alter epigenetic marks
    • Environmental factors
    • Lifestyle choices
  • Epigenetic marks are carried across generations
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