Robert L. Brent Lecture – Teratology Update

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

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No Disclosures

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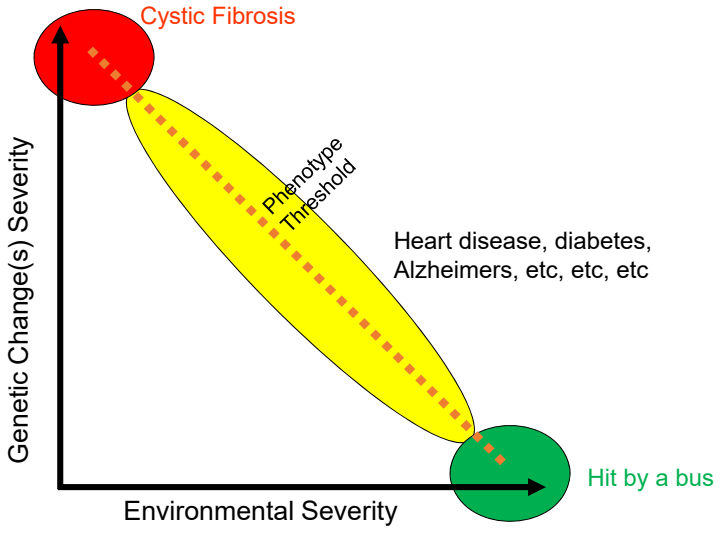
How Geneticists Think About Disease



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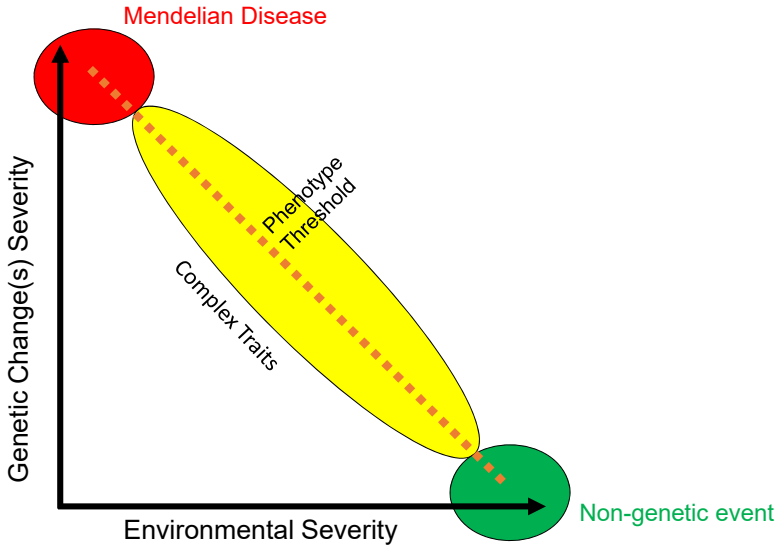

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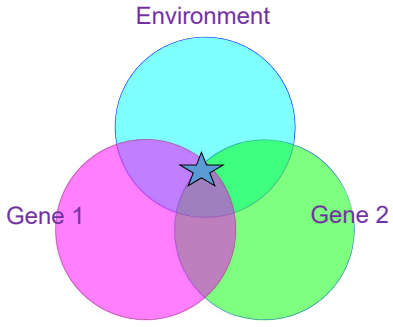
A few major genetic defects with large contributions



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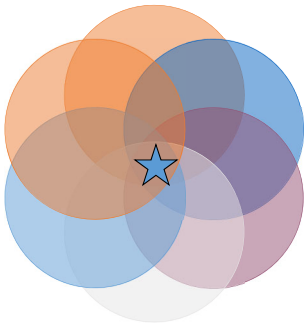
A large number of genes each having a minor effect



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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”

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~1 in 100 Infants is Born with a Congenital Heart Defect

9

~25% are in Critical Condition at Birth

10

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

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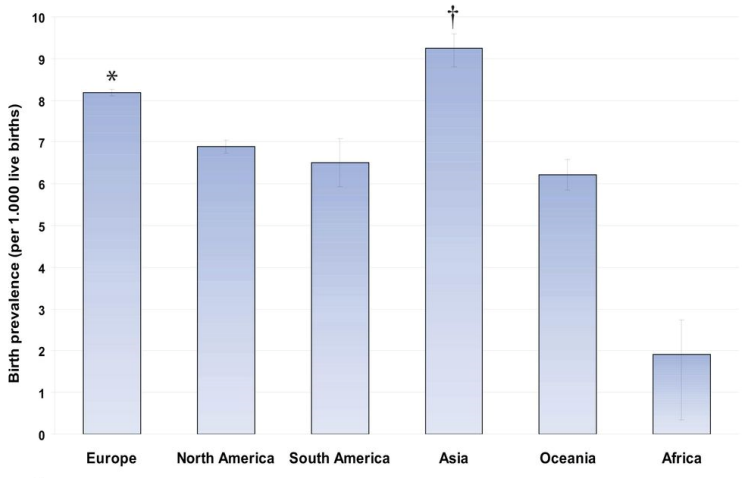

van der Linde et al, JACC, 2011

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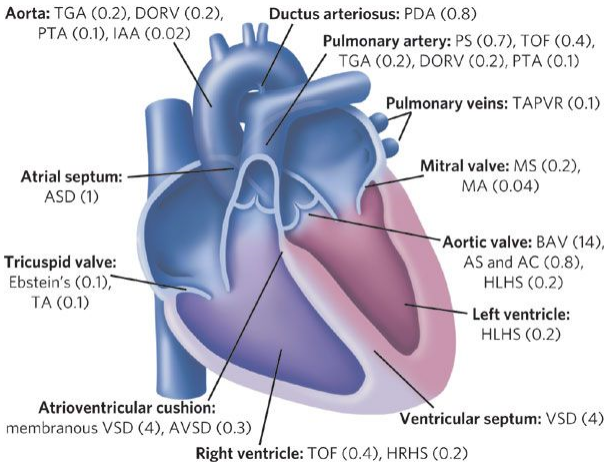
18 Distinct Types of Congenital Heart Defects



(incidence/1,000 live births)

Bruneau, Nature 2008

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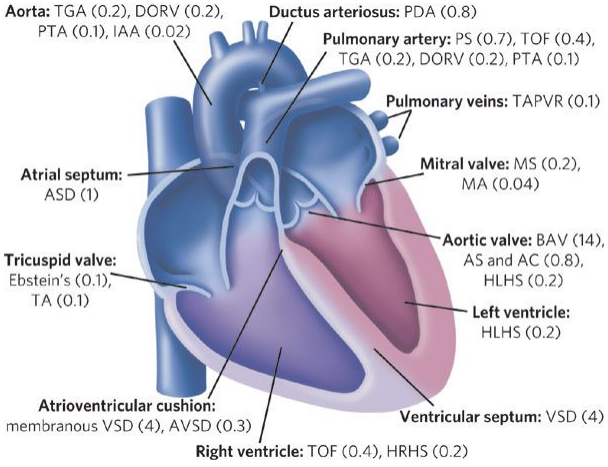
18 Distinct Types of Congenital Heart Defects



- Septal defects
- Valve abnormalities
- Outflow tract defects

Bruneau, Nature 2008

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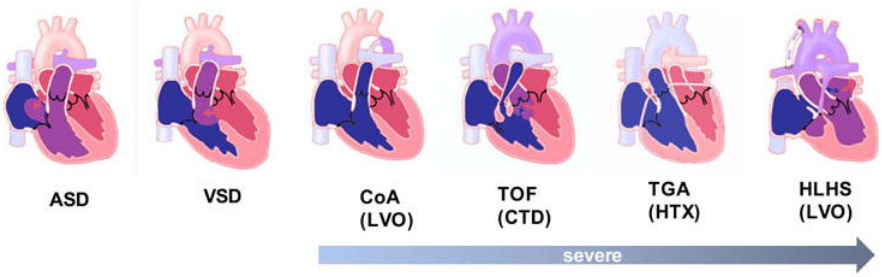

Zaidi and Brueckner, Circ Res, 2017

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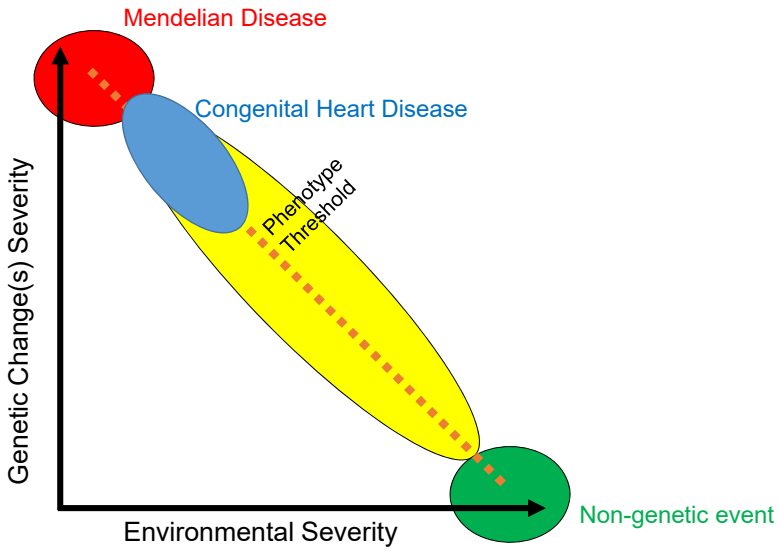

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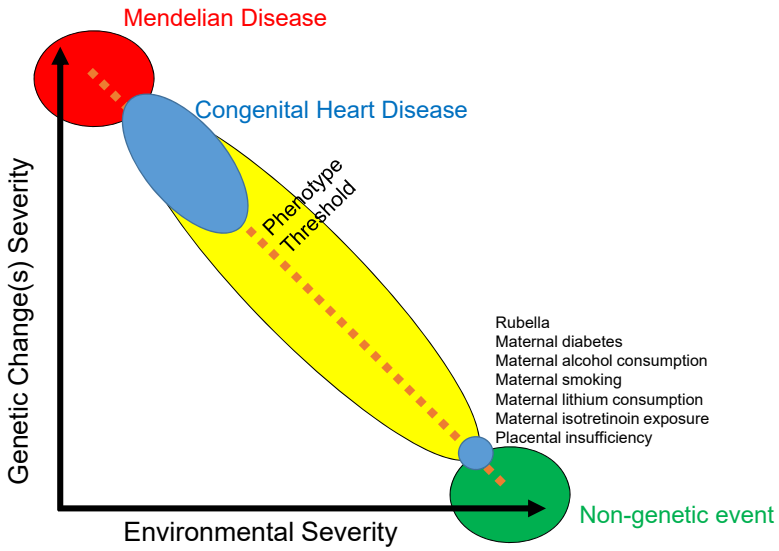

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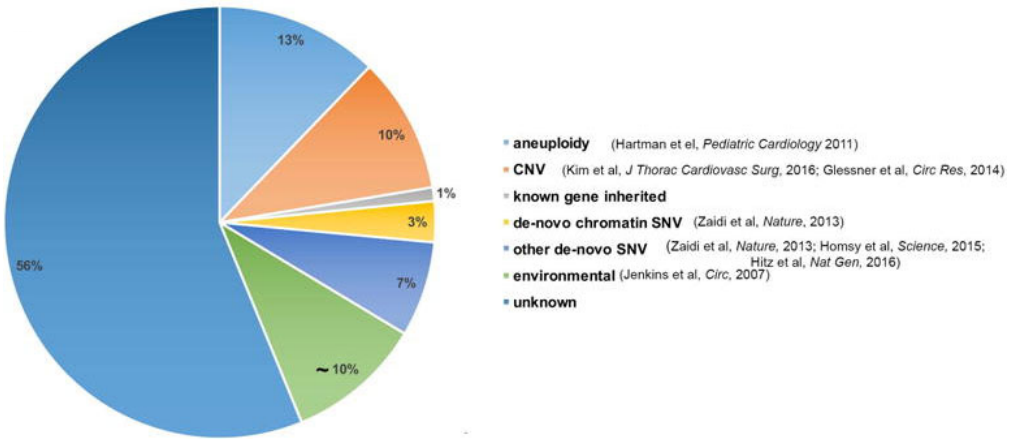

Zaidi and Brueckner, Circ Res, 2017

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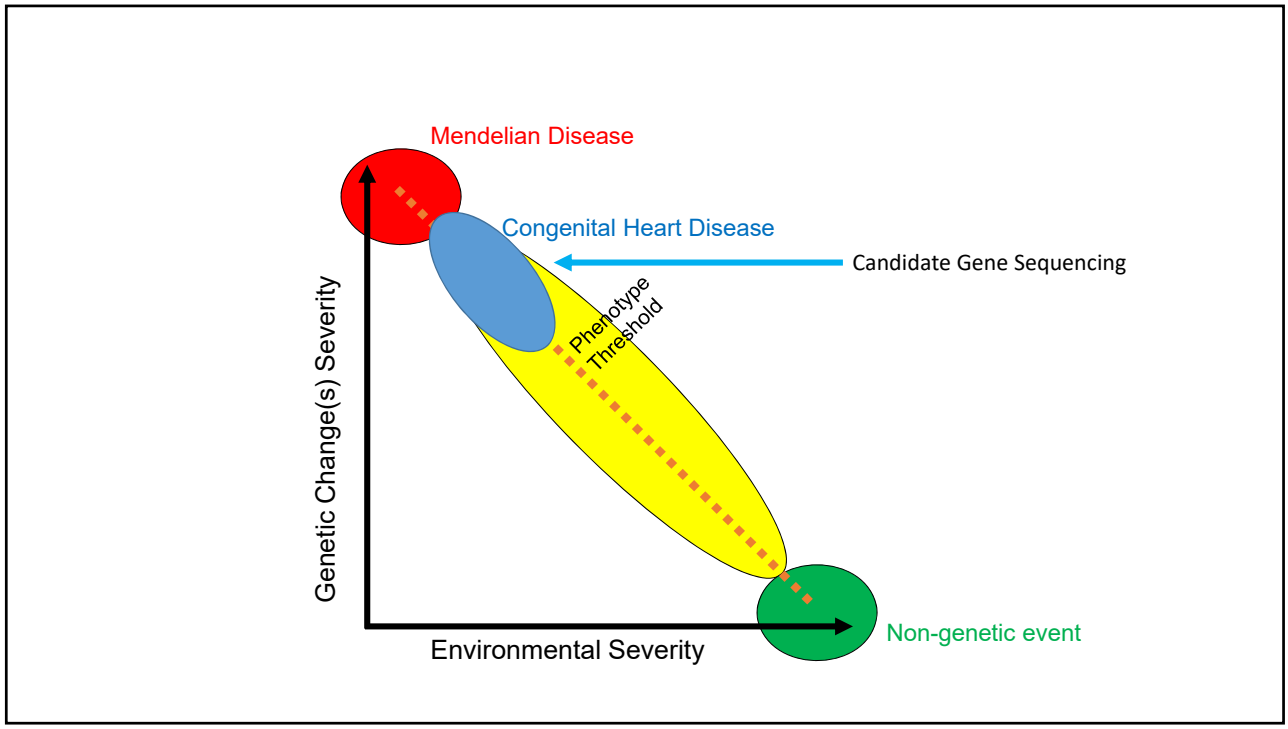
Mendelian Disease
Congenital Heart Disease
Candidate Gene Sequencing
Phenotype Threshold
Genetic Change(s) Severity
Environmental Severity
Non-genetic event

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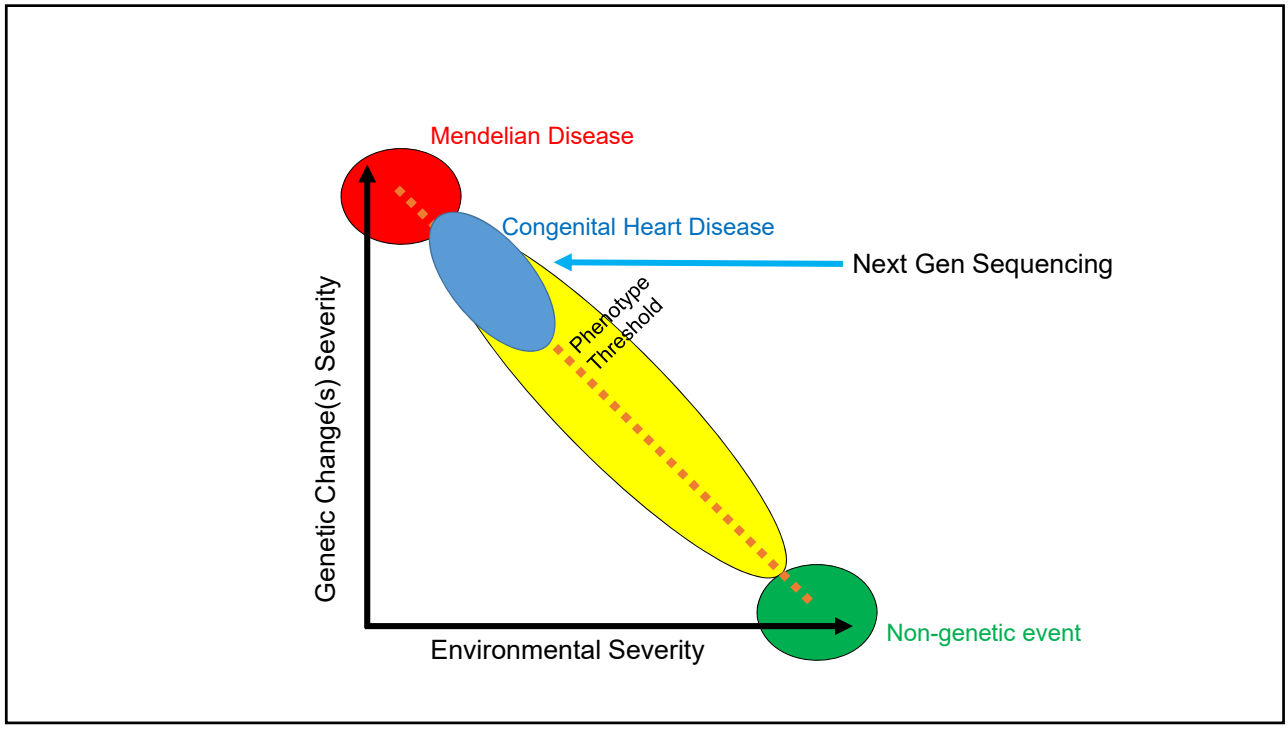
Mendelian Disease
Congenital Heart Disease
Next Gen Sequencing
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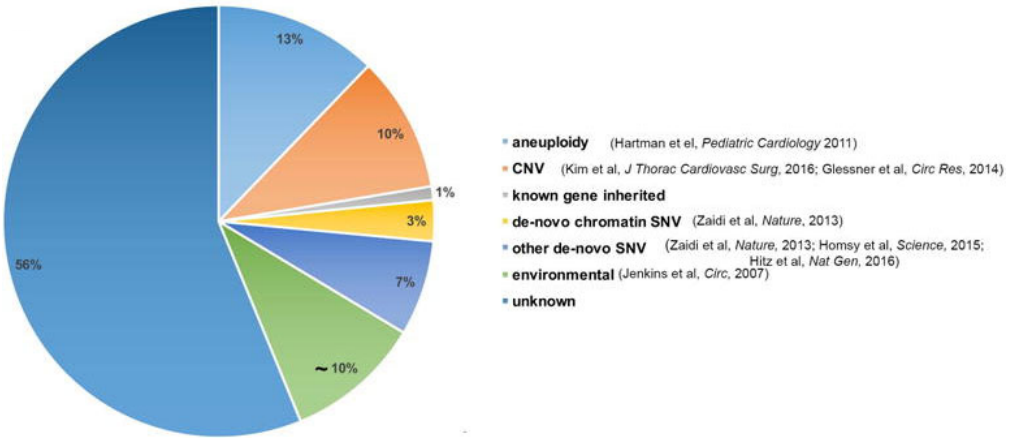

Zaidi and Brueckner, Circ Res, 2017

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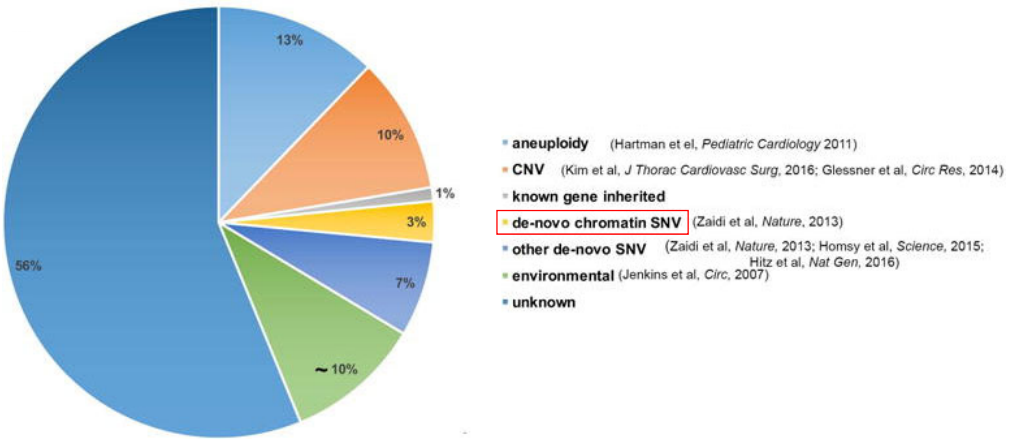

Zaidi and Brueckner, Circ Res, 2017

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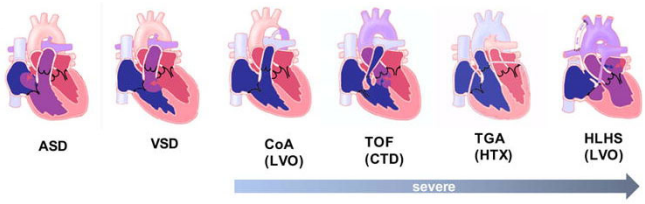
de Novo Chromatin SNVs?????

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



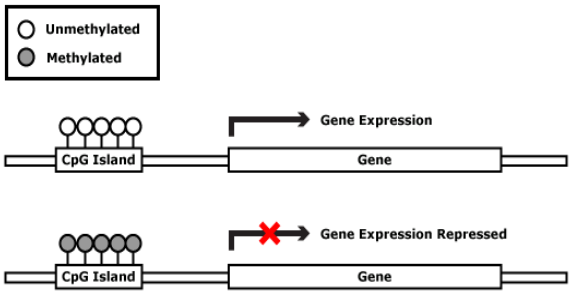
  - 264 controls (parent-offspring trios)
- Are there new mutations that occur in cases but not controls?

Zaidi et al, Nature 2013

de Novo Chromatin SNVs?????

- 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)

Epigenetic Regulation of Gene Expression



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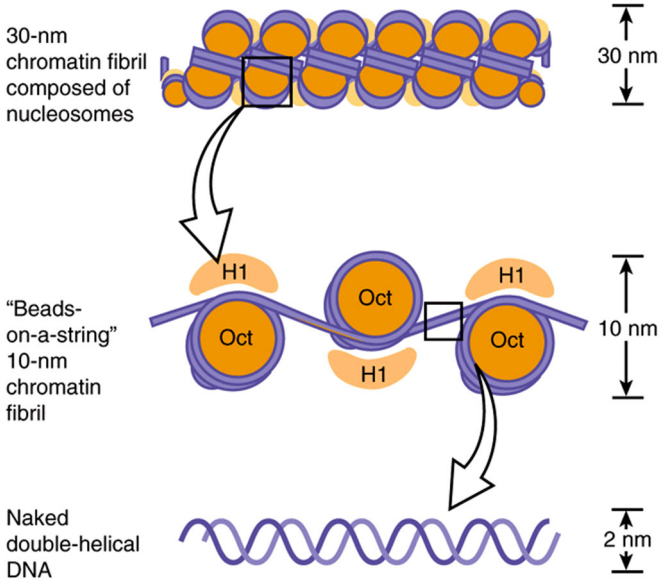

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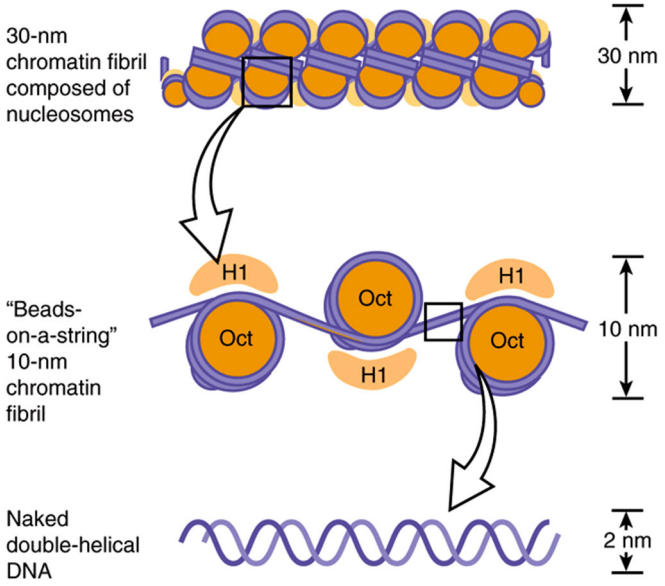

Oct = Histone octamer

H2A
H2B
H3
H4

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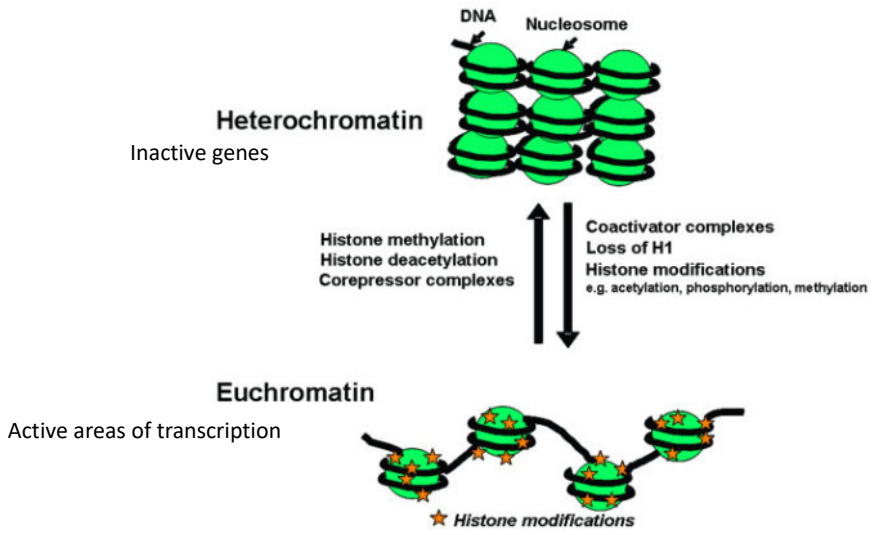

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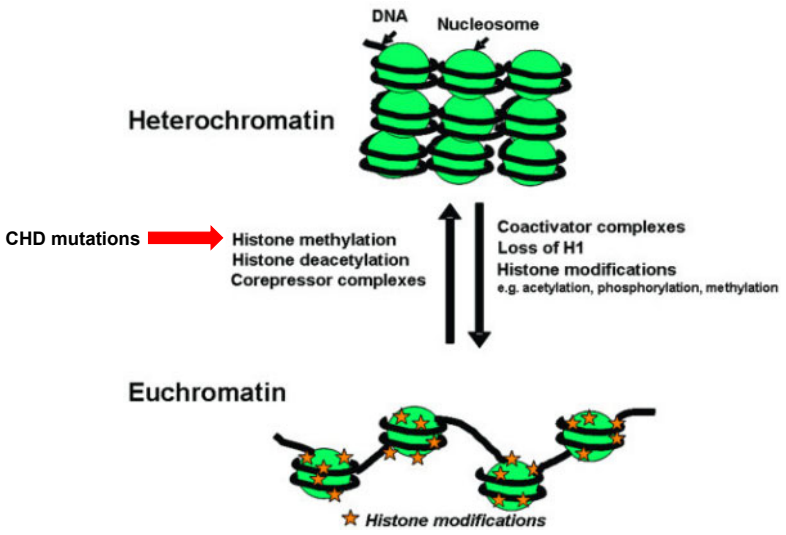

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de Novo Chromatin SNVs?????

- 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

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- Genes that induce methylation of H3K27 can no longer cause the methylation
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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

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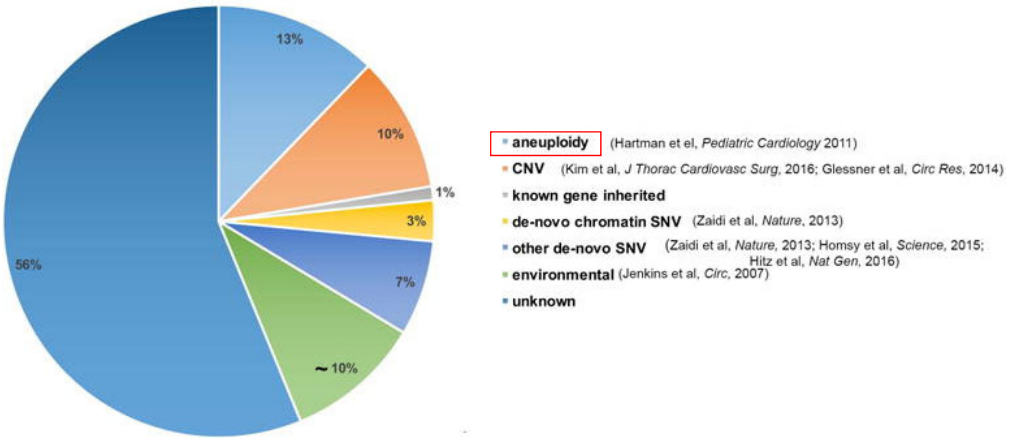

Zaidi and Brueckner, Circ Res, 2017

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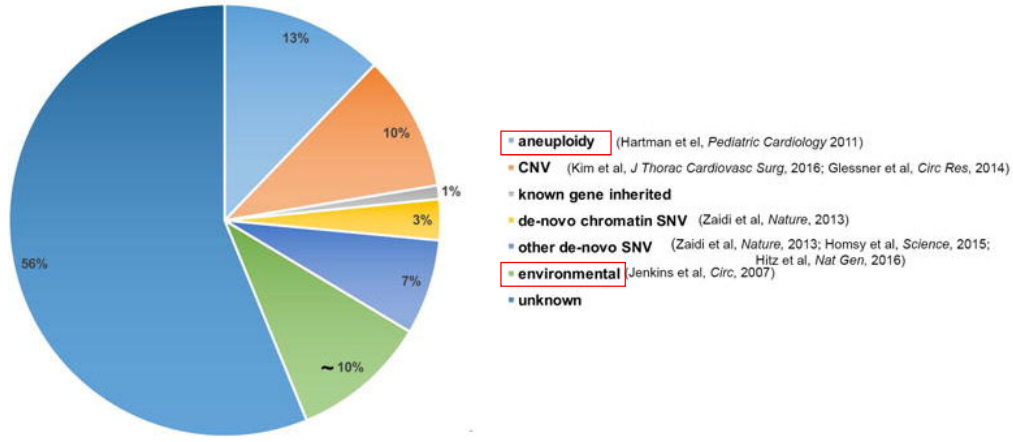

Zaidi and Brueckner, Circ Res, 2017

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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

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Is Epigenetic Variation More Important Than We Realized?

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  - Environmental influences
- Aneuploidy and environmental influences affect epigenetics

- Time to connect the dots!

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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**

With Mom's Permission

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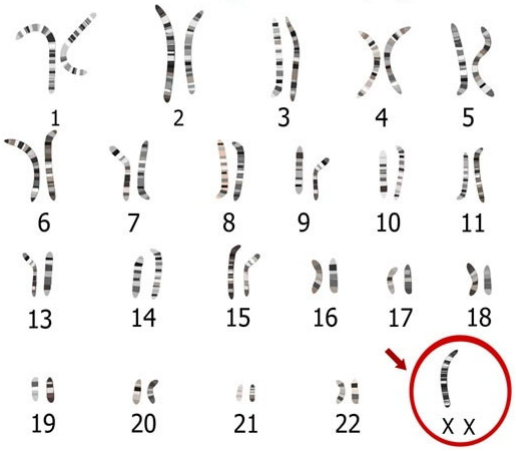
Turner syndrome karyotype



Deficiency of a second sex chromosome

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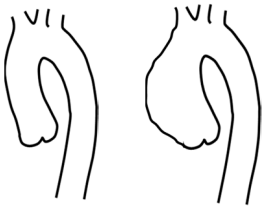
Bicuspid Aortic Valve Disease (BAVD) in Turner syndrome

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

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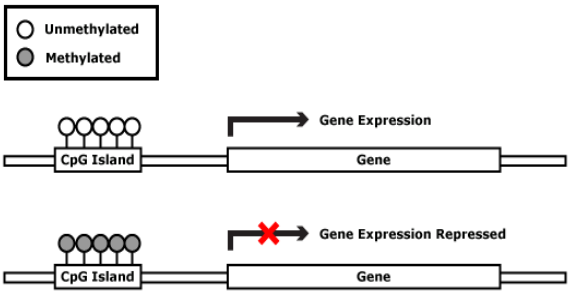
Epigenetic Analysis

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



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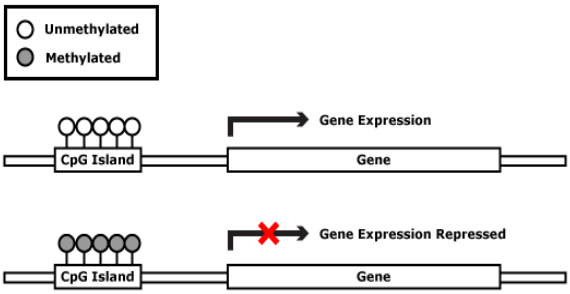
Epigenetic Analysis

Does epigenetics play a role in BAVD in Turner syndrome?

- Global DNA methylation analysis



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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)

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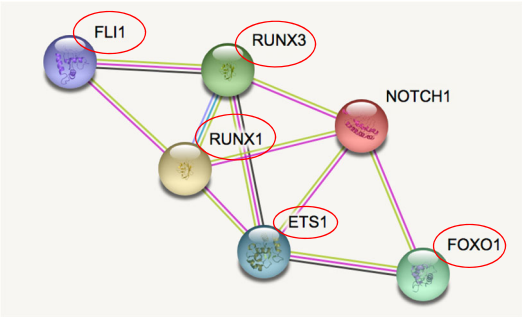
DNA Methyl-Capture Sequencing Analysis

- 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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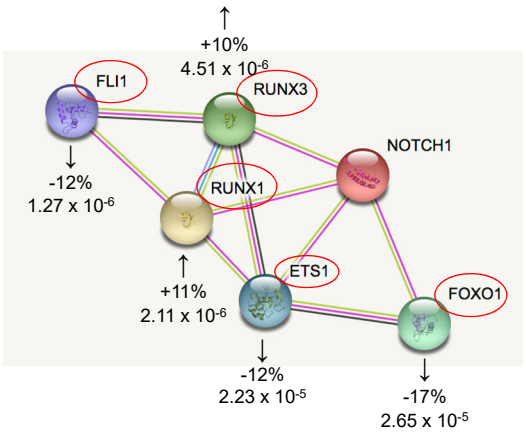

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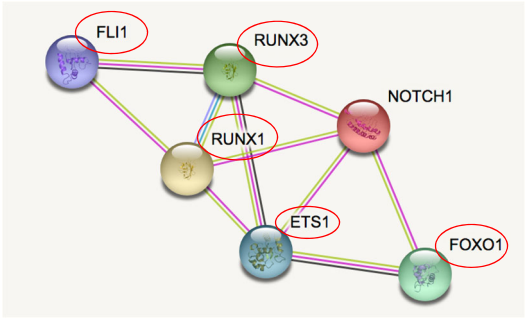
Differentially Methylated Genes in NOTCH1 Pathway



Single pass transmembrane receptor that regulates cell fate decisions during development

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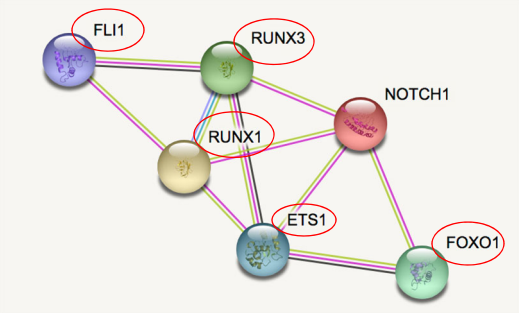
Differentially Methylated Genes in NOTCH1 Pathway



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

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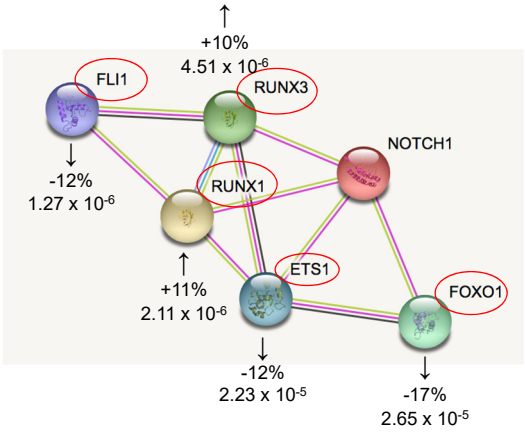
Differentially Methylated Genes in NOTCH1 Pathway



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

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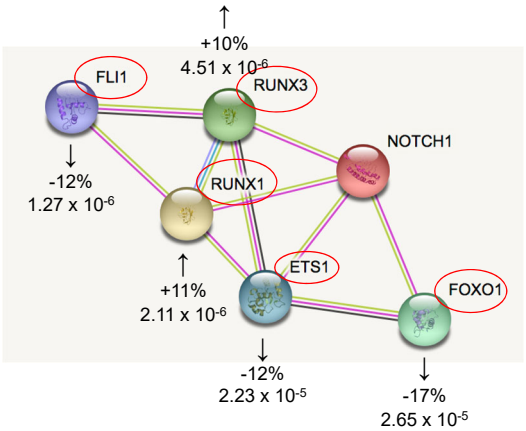
Differentially Methylated Genes in NOTCH1 Pathway



Do epigenetic differences account for the sex bias of BAVD towards males?

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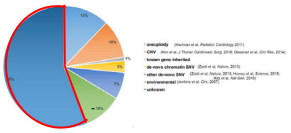
Conclusions



- 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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Acknowledgments

Collaborators

Turner syndrome study subjects and their families

TSSUS

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Jessica Kushner

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Shane Morris (Baylor College of Medicine)
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genTAC

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