(Cardiac) Genetics: Present, Near-present and Future

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Olson Center Brown Bag Lecture series
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Disclosures

I have no financial or conflict of interest disclosures.

I do plan to discuss and reference investigational or off-label use of therapeutic agents or products in this presentation.
Talk Objectives

• Outline the importance of familial gene testing and cascade screening
• Discuss currently available genetic tests, including direct-to-consumer test options
• Provide a basic biological framework for understanding genetic therapy

Talk Outline

I. (Cardiac) Genetics: Present
   Basics of genetic disease
   Discuss currently available genetic tests
   Outline the importance of familial gene testing and cascade screening
   Brief discussion of direct-to-consumer genetic testing

II. (Cardiac) Genetics: Near-Present
   Genome/Genetic risk scores
   Gene specific therapy

III. (Cardiac) Genetics: Future
   Gene editing – CRISPR-Cas9
Part I.
(Cardiac) Genetics: Present

The Human Genome
- Human genome first published in 2001; final version published 2006
- 3 billion total base pairs with 1-2% actually encoding ~21,000 genes
- 99.9% of the genome is identical person-to-person
- Less than half of identified genes have known function.
Central Dogma of Biology

- DNA ~20,000 genes
- mRNA ~17 copies/cell
- Protein ~50,000 copies/cell

~400 cardiac genes linked with pathology

McManus et al. Mol Biosyst 2015

Disease from a genetic perspective...

- Cystic Fibrosis
- Familial Hyperlipidemia
- Coronary Artery Disease
- Diabetes

Gene effect size (impact)

Gene frequency (common)

Monogenic
- single gene
- Rare (<1%)

Polygenic
- many genes
- Common (>1%)
Genome Terminology

Genome
3 billion bp

Exome
1-2% of genome

Genes

Current genetic testing options

Gene Panel tests
- Sequencing of genes (up to ~200 genes for cardiac) known to cause the disease
- Coverage and depth enriched for genes of interest (often 100x coverage)

Exome sequencing
- Sequencing focused on the exome (gene coding regions, splicing sites)
- Lower sequencing coverage and depth

Whole genome sequencing
- Sequencing of the entire genome
- Most expensive and time consuming
- Lowest sequence coverage and depth
Types of Genetic Mutations

DNA: ATG TGC CAT CAT TAA
RNA: MET CYS HIS HIS STOP
Protein: MET CYS HIS HIS STOP

- Missense Mutation

DNA: ATG TGC CAT CAA CAT TAA
RNA: MET CYS GLN HIS STOP
Protein: MET CYS GLN HIS STOP

- Nonsense Mutation
Types of Genetic Mutations

DNA
ATG TGC CAT CAT TAA
RNA
MET CYS HIS HIS STOP
Protein

Frameshift Mutation

DNA
ATG TGC CAC ATT AAC
RNA
MET CYS HIS ILE ASN
Protein

Understanding genetic test results

Benign ↔ Likely Benign ↔ Variant of Unknown Significance ↔ Likely Pathogenic ↔ Pathogenic

• Genetic testing results are **probabilistic** not **deterministic**.

• Penetrance can vary for the same genetic variant within the same family.

Richards et al. *Genetics in Medicine* 2015
Reasons for genetic testing

Diagnosis (or clarification of diagnosis)

Cascade (family) screening

Hypertrophic Cardiomyopathy: What is it?

Normal Hypertrophic Cardiomyopathy

Genetics Home Reference, NIH website
Nishimura et al. Circulation 2003, HCM Registry
Hypertrophic Cardiomyopathy
Why does it occur?

- Affects ~1 in 500 individuals, autosomal dominant
- Caused of gene mutations impacting the sarcomere, the contractile element in myocardium
- ~15-20 implicated genes

Clinical manifestations of Hypertrophic Cardiomyopathy

Symptoms
- Dyspnea with exertion
- Chest pain

Sudden death
- Ventricular arrhythmias

Maron BJ. *NEJM* 2018
Treatment of Hypertrophic Cardiomyopathy mitigates risk.

Maron BJ. *NEJM* 2018

**Mortality**

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<table>
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<tbody>
<tr>
<td>HCM</td>
<td>2%</td>
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<tr>
<td>HCM + therapy</td>
<td>0.55%</td>
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<tr>
<td>US population</td>
<td>0.80%</td>
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Who should get genetic testing?

The patient with the genetic disease:

If a pathogenic variant is identified:

All first degree relatives of anyone with a known pathogenic variant should be offered cascade genetic testing.
Direct to Consumer genetic testing

- Provide a wide range of genetic testing – from SNPs to exome to whole genome
- Oversight is limited
- Here to stay

Direct to Consumer genetic testing – how accurate?

Abnormal DTC tests (n=49) referred for confirmation (2014-2016)

40% of findings were false positives.
  - incorrect DNA sequence
  - incorrect variant interpretation

Tandy-Connor et al. Genetics in Medicine 2018
Interpretation of results: Absolute vs Relative Risk

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Relative Risk Reduction</th>
<th>Absolute Risk Reduction</th>
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<tbody>
<tr>
<td>Red</td>
<td>50%</td>
<td>6%</td>
</tr>
<tr>
<td>Black</td>
<td>50%</td>
<td>0.3%</td>
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Part II.
(Cardiac) Genetics: Near-Present
Premature Coronary Artery disease is caused by Familial Hyperlipidemia (FH)

- FH is caused by gene mutations (LDLR, PCSK9…) resulting in markedly increased serum cholesterol.
- Affects ~1 in 200
- Increase CAD risk ~3x
- Most FH is untreated

Khera et al. JACC 2016

Disease from a genetic perspective...

- Monogenic: single gene, Rare (<1%)
- Polygenic: many genes, Common (>1%)
Genome Polygenic Risk score for CAD

- Utilized the UK Biobank (~400k patients) and ~6.6 million variants
- Identify individuals with CAD risk similar to FH mutation carriers (>3x)
- Identified 8% of the population with >3x CAD risk
- Only 20% had high cholesterol vs 13% of the rest of the population.
- Can be obtained from genetic test results like 23andMe

Khera AV et al. Nature Genetics 2018

*Expected Benefits of Genome Polygenic Risk scores*

- Sums risk from thousands of individual minimal risk variants
- Does NOT change with age
- Measures risk from currently unknown factors
- Applicable to common diseases (diabetes, breast cancer…)

Khera AV et al. Nature Genetics 2018
Can I alter my genetic destiny?

Yes…to an extent.

Modified from Khera AV et al. *NEJM* 2016

Cardiac Amyloidosis

- Prototype of restrictive cardiomyopathy
- Extracellular deposits of misfolded amyloid protein into organs (heart, kidney)
- CHF symptoms with normal EF and LVH, and often have preceding arrhythmias

Dungu et al. *Heart* 2012
Falk et al. *JACC* 2016
Mutations in the same gene cause different disease manifestations.

Rapezzi et al. EHJ 2012, Semigran JACC 2016

Gene specific therapy for amyloidosis

Ruberg et al. JACC 2019
Part III. (Cardiac) Genetics: Future

Duchenne Muscular Dystrophy

- X-linked dystrophinopathy
- 1 in 3,500 to 5,000 boys
- No cure
- Cardiac disease is the primary mode of death

Fairclough et al. Nature Review Genetics 2013

“Shock absorber” for the cell
CRISPR-Cas9

Jiang and Doudna. *Annual Rev Biophys* 2017

CRISPR-Cas9 Genome Editing

Restoration of dystrophin in DMD mouse model

a) IP injection
   AAV9-Cas9
   AAV9-sgRNA
   4 days old
   Bioluminescence measurements
   3 weeks
   10 weeks

b) 3 weeks post injection
   10 weeks post injection

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<thead>
<tr>
<th>WT-Dmd-Luc</th>
<th>ΔEx50-Dmd-Luc</th>
<th>ΔEx50-Dmd-Luc</th>
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<tbody>
<tr>
<td>Control</td>
<td>Control</td>
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<tr>
<td>AAV9-Cas9</td>
<td>AAV9-Cas9</td>
<td>AAV9-Cas9</td>
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Amoasii et al., *Nature Communications* 2019

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Restoration of dystrophin in DMD mouse

d) Diaphragm | Heart | Tibialis anterior | Triceps

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Amoasii et al., *Nature Communications* 2019
Challenges to gene editing

• Delivery of CRISPR-Cas9 to specific tissue types
• Correction of large mutations (insertion/deletion changes)
• Off target gene editing >> increased risk of malignancy?
• Ethics and oversight. International moratorium on germline gene editing for clinical purposes is currently in place.

Chinese scientist He Jiankui announced that he had used CRISPR to edit the embryos of human twins to reduce the siblings’ risk of acquiring HIV infection in 2018. He has not been seen publically since and was reportedly jailed for 3 years.

Dennis Normile, Scienecmag.org, 12/30/19

Actively recruiting clinical trials utilizing CRISPR-Cas9

<table>
<thead>
<tr>
<th>First posted</th>
<th>Disease</th>
<th>ClinicalTrials.gov #</th>
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<tbody>
<tr>
<td>August 2018</td>
<td>B-thalassemia</td>
<td>NCT03655678</td>
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<tr>
<td>November 2018</td>
<td>Sickle cell anemia</td>
<td>NCT03745287</td>
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<tr>
<td>January 2020</td>
<td>Refractory multiple myeloma</td>
<td>NCT04244656</td>
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<tr>
<td>July 2019</td>
<td>Refractory B cell malignancies</td>
<td>NCT04035434</td>
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<tr>
<td>March 2019</td>
<td>Blindness</td>
<td>NCT03872479</td>
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ClinicalTrials.gov, accessed April 19, 2020
Alternative strategy: generate resistance to disease

Protective genetic profile for CAD.

Points to remember

- Family cascade testing is a powerful tool for reducing disease risk.
- Accurate interpretation of genetic results is vital.
- The role of genetics in medicine will continue to expand.
Thank you

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