Tips and Tricks for Genetic Testing

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Section Chief, Medical Genetics and Genomics
September 25, 2020
Objectives

- Review and reinforce genetic concepts
- Discuss genetic testing options
- Understand the risks and benefits
- Become aware of next steps
Disclosures

- Grant funding from Children’s Foundation
- Helen DeVos Children’s Hospital Foundation support
‘You are my sunshine, my only sunshine’

Born with an ultra-rare condition that caused her brain to outgrow her skull, little Kenzie Bennett is “still smiling at life.”

You’re one in a billion, baby boy

Little Hayden, born with two rare disorders, might be the first of his kind. His parents treasure his uniqueness.

Haylie’s mystery, solved

The Schneider family faced profound questions about their daughter’s development differences. Advances in genetic testing finally revealed answers.

Merritt’s mystery

Merritt Smith experienced seizures and missed developmental milestones. Genetic testing revealed the cause—a missing chunk of a chromosome.
Smith’s Recognizable Patterns of Human Malformation, 7th Edition
THE BASICS
Chromosome to Gene to Protein

Cell
Each chromosome is composed of one large continuous DNA molecule.

Chromosomes
Gene
A gene is a segment of DNA that encodes a protein product.

Protein
A protein is a complex organic compound composed of hundreds or thousands of amino acids.

DNA
Nucleotides
- Adenine
- Thymine
- Guanine
- Cytosine
Normal Female - 46,XX

Chromosomes artificially straightened for illustrative purposes causing some apparent discrepancies in banding patterns of chromosome pairs.
Normal Male - 46,XY

Chromosomes artificially straightened for illustrative purposes causing some apparent discrepancies in banding patterns of chromosome pairs.
THE TESTS
Trisomy 21 Karyotype - Down Syndrome 47,XX,+21

Chromosomes artificially straightened for illustrative purposes causing some apparent discrepancies in banding patterns of chromosome pairs.
Unbalanced 14;21 Translocation - Down Syndrome - 46,XY, der(14;21)(q10;q10),+21
FISH Analysis of Chromosomal Trisomy

Normal Interphase

Normal Metaphase

Trisomy Interphase

Trisomy Metaphase
FISH

Patient DNA

Clone DNA (1 probe)

Control DNA

aCGH

Patient DNA

Microarray with Genomic Clones (probes)

aCGH = hundreds of FISH probes
Patient DNA is added to the microarray chip.
Microarray chip is scanned by instrument.

The data is transferred to computer for interpretation.

Loss  Gain

Gain  Loss
Sequencing Methodologies

Single gene sequencing

The car was red.
The car was red.

The train was black.
The __ was black.

Look for errors in a single sentence in the book

Targeted gene panel sequencing

The car was red.
The car was red.

The boat was blue.
The boat was blue.

Look for errors in a specific group of sentences in the book

Exome sequencing

Look for errors in the most important chapters in the book

Whole Genome sequencing

Look for errors in every single word in the book
Frameshift Mutation

Normal Sequence

DNA: ATG AAG TTT GGC GCA TTG AAA
Protein: Met Lys Phe Gly Ala Leu Lys

Abnormal Sequence

DNA: ATG AAT TTG GCG CAT TGA AA
Protein: Met Lys Leu Ala His STOP
FMR1-Related Disorders

Normal
- less than 55 CGG repeats
  - Transcription (gene activity)
  - FMR1 mRNA
  - Translation (protein production)
  - FMRP
  - Normal

Premutation
- between 55 and 200 repeats
  - Transcription (gene activity)
  - FMR1 mRNA
  - Translation (protein production)
  - FMRP
  - Fragile X-associated Tremor/Ataxia Syndrome
  - Primary Ovarian Insufficiency

Full Mutation
- greater than 200 repeats
  - Transcription (gene activity)
  - FMR1 mRNA
  - Translation (protein production)
  - FMRP
  - Fragile X syndrome
Uniparental Disomy (UPD)

- Paternal UPD
- Normal
- Maternal UPD
Inheritance of Prader-Willi Syndrome

- Active PWS-related genes
- Inactive PWS-related genes

Prader-Willi Syndrome

- Paternal Deletion 70%
- Maternal UPD 25%
- Imprinting Defect <1%
- Unknown ~4%

Adapted from Journal of the American Academy of Child and Adolescent Psychiatry, 2000;39:388
Inheritance of Angelman Syndrome

- Active AS-related gene (UBE3A)
- Inactive AS-related gene (UBE3A)
- UBE3A Mutation

Angelman Syndrome

- Maternal Deletion ~68%
- Paternal UPD ~7%
- Imprinting Defect ~3%
- UBE3A Mutation ~11%
- Unknown ~11%

Adapted from Journal of the American Academy of Child and Adolescent Psychiatry, 2000;39:388
X Inactivation

- Both X chromosomes active
- Maternal X chromosome active
- Paternal X chromosome active

Fertilized egg

Early embryo

Random X chromosome inactivation in each cell

Fixed X chromosome inactivation in all descendant cells

Random X chromosome inactivation

Skewed X chromosome inactivation
THE RISKS
Distribution of Cancer

Hereditary
- Gene mutation is inherited in family
- Significantly increased cancer risk

Familial
- Multiple genes & environmental factors may be involved
- Some increase in cancer risk

Sporadic
- Cancer occurs by chance or related to environmental factors
- General population cancer risk
Cancer Syndromes by Primary Cancer Site

**Thyroid Cancer**
- Cowden syndrome
- Multiple Endocrine Neoplasia, Type 1
- Multiple Endocrine Neoplasia, Type 2
- Peutz-Jeghers syndrome
- Familial Adenomatous Polyposis

**Breast Cancer**
- Hereditary Breast-Ovarian Cancer
- Cowden syndrome
- Li-Fraumeni syndrome
- Peutz-Jeghers syndrome

**Colon Cancer**
- Hereditary Nonpolyposis Colon Cancer/Lynch syndrome
- Familial Adenomatous Polyposis
- MUTYH-associated Polyposis
- Cowden syndrome

**Ovarian Cancer**
- Hereditary Breast-Ovarian Cancer
- Hereditary Nonpolyposis Colon Cancer/Lynch syndrome
- Cowden syndrome
- Multiple Endocrine Neoplasia, Type 1

**Uterine Cancer**
- Hereditary Nonpolyposis Colon Cancer/Lynch syndrome
- Cowden syndrome
- Li-Fraumeni syndrome
- Peutz-Jeghers syndrome

**Sporadic Cancer**

- One Copy of Gene with Mutation → Both Copies of Gene with Mutation → Tumor Develops

**Hereditary Cancer**

- One Copy of Gene with Mutation in All Cells → Both Copies of Gene with Mutation in One Cell → Tumor Develops
Variants of Unknown significance!
Mutation

DISEASE

HEALTHY

??????
<table>
<thead>
<tr>
<th>GENE</th>
<th>GENOTYPE</th>
<th>PREDICTED PHENOTYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9/KORC1</td>
<td>*1/*1; A/A</td>
<td>Increased Sensitivity (IS)</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>*4/*4</td>
<td>Poor Metabolizer (PM)</td>
</tr>
<tr>
<td>HTR2C</td>
<td>C/T</td>
<td>Variant Expressor (VX)</td>
</tr>
<tr>
<td>OPRM1</td>
<td>G/G</td>
<td>Reduced Expressor (RE)</td>
</tr>
<tr>
<td>UGT2B15</td>
<td>*1/*2</td>
<td>Intermediate Metabolizer (IM)</td>
</tr>
<tr>
<td>COMT</td>
<td>A/G</td>
<td>Normal Activity (NA)</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>*1/*1</td>
<td>Extensive (Normal) Metabolizer (EM)</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>*1/*1</td>
<td>Extensive (Normal) Metabolizer (EM)</td>
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<tr>
<td>CYP2C9</td>
<td>*1/*1</td>
<td>Extensive (Normal) Metabolizer (EM)</td>
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<tr>
<td>CYP3A4/CYP3A5</td>
<td>*1/*1; *3/*3</td>
<td>Intermediate Metabolizer (IM)</td>
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<tr>
<td>DRD2</td>
<td>INS/INS</td>
<td>Normal Responder (NR)</td>
</tr>
<tr>
<td>HLA-B*15:02</td>
<td>Negative</td>
<td>Typical Risk of Hypersensitivity (TR)</td>
</tr>
<tr>
<td>MTHFR</td>
<td>C/C (C677T); A/A (A1298C)</td>
<td>Normal Activity (NA)</td>
</tr>
</tbody>
</table>

**CURRENT REPORTED MEDICATIONS**

Celecoxib, Fluoxetine, Hydrocodone, Omeprazole, Warfarin
**MEDICATION SELECTION GUIDE (based on potential genetic impact)**

### LOW GENETIC IMPACT

<table>
<thead>
<tr>
<th>Antidepressants, SSRI / SNRI</th>
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</thead>
<tbody>
<tr>
<td>Citalopram (Celexa&lt;sup&gt;®&lt;/sup&gt;)&lt;sup&gt;DDI&lt;sup&gt;1,2&lt;/sup&gt;&lt;/sup&gt;</td>
</tr>
<tr>
<td>Desvenlafaxine (Pristiq&lt;sup&gt;®&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Escitalopram (Lexapro&lt;sup&gt;®&lt;/sup&gt;)&lt;sup&gt;DDI&lt;sup&gt;1,2&lt;/sup&gt;&lt;/sup&gt;</td>
</tr>
<tr>
<td>L-Methylfolate (Deplin&lt;sup&gt;®&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Levomilnacipran (Fetzima&lt;sup&gt;®&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Milnacipran (Savella&lt;sup&gt;®&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Sertraline (Zoloft&lt;sup&gt;®&lt;/sup&gt;)&lt;sup&gt;DDI&lt;sup&gt;1,2&lt;/sup&gt;&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

### MODERATE GENETIC IMPACT

<table>
<thead>
<tr>
<th>Antidepressants, SSRI / SNRI</th>
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</thead>
<tbody>
<tr>
<td>Fluoxetine (Prozac&lt;sup&gt;®&lt;/sup&gt;)</td>
</tr>
</tbody>
</table>

### HIGH GENETIC IMPACT

<table>
<thead>
<tr>
<th>Antidepressants, SSRI / SNRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paroxetine (Paxil&lt;sup&gt;®&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Venlafaxine (Effexor XR&lt;sup&gt;®&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Vortioxetine (Brintellix&lt;sup&gt;®&lt;/sup&gt;)</td>
</tr>
</tbody>
</table>
Mutations in the DNA can lead to genetic conditions.

Central Dogma of Biology:

- DNA to RNA: Transcription
- RNA to Protein: Translation
- RNA to Protein: Transport to cytoplasm for protein synthesis

Genetic Condition
Genetic Predisposition

Polymorphism

Central Dogma of Biology

DNA

RNA

RNA

Protein

Transcription

Transport to cytoplasm for protein synthesis

Translation
Polymorphism

Central Dogma of Biology

DNA

RNA

Transport to cytoplasm for protein synthesis

Translation

Protein

?Genetic Predisposition?
Multifactorial Causation

Genetic Factors

Affected

Environmental Factors
23andMe provides ancestry-related genetic reports and uninterpreted raw genetic data. We no longer offer our health-related genetic reports. If you are a current customer please go to the health page for more information. Close alert.

Status of our health-related genetic reports.

We no longer offer our health-related genetic reports to new customers to comply with the U.S. Food and Drug Administration’s directive to discontinue new consumer access during our regulatory review process.

At this time, we do not know the timeline as to which health reports might be available in the future or when they might be available.
## Wellness reports

5+ reports
- Alcohol Flush Reaction
- Caffeine Consumption
- Deep Sleep
- Lactose Intolerance
- Muscle Composition
- Saturated Fat and Weight
- Sleep Movement

### Traits reports
19+ traits
- Asparagus Odor Detection
- Bald Spot (available for men only)
- Bitter Taste Perception
- Cheek Dimples
- Cleft Chin
- Earlobe Type
- Earwax Type
- Eye Color
- Finger Length Ratio
- Freckles
- Hair Curliness
- Light or Dark Hair
- Male Hair Loss (available for men only)
- Newborn Hair Amount
- Photic Sneze Reflex
- Red Hair
- Skin Pigmentation
- Sweet Taste Preference
- Toe Length Ratio
- Unibrow
- Widow's Peak

### Carrier Status reports
35+ reports

<table>
<thead>
<tr>
<th>REPORT</th>
<th>GENE</th>
<th>VARIANTS</th>
<th>RELEVANT ETHNICITIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARSACS</td>
<td>SACS</td>
<td>1 Variant</td>
<td>French Canadian</td>
</tr>
<tr>
<td>Agenesis of the Corpus Callosum with Peripheral Neuropathy</td>
<td>SLC12A6</td>
<td>1 Variant</td>
<td>French Canadian</td>
</tr>
<tr>
<td>Autosomal Recessive Polycystic Kidney Disease</td>
<td>PKHD1</td>
<td>3 Variants</td>
<td>N/A</td>
</tr>
<tr>
<td>Beta Thalassemia and Related Hemoglobinopathies</td>
<td>HBB</td>
<td>10 Variants</td>
<td>Cypriot, Greek, Italian, Sardinian</td>
</tr>
<tr>
<td>Bloom Syndrome</td>
<td>BLM</td>
<td>1 Variant</td>
<td>Ashkenazi Jewish</td>
</tr>
<tr>
<td>Canavan Disease</td>
<td>ASPA</td>
<td>3 Variants</td>
<td>Ashkenazi Jewish</td>
</tr>
<tr>
<td>Congenital Disorder of Glycosylation Type 1a (PMM2-CDG)</td>
<td>PMM2</td>
<td>2 Variants</td>
<td>Danish</td>
</tr>
<tr>
<td>Cystic Fibrosis</td>
<td>CFTR</td>
<td>28 Variants</td>
<td>European, Hispanic/Latino, Ashkenazi Jewish</td>
</tr>
<tr>
<td>D-Bifunctional Protein Deficiency</td>
<td>HSD17B4</td>
<td>2 Variants</td>
<td>N/A</td>
</tr>
<tr>
<td>Dihydrolipoamide Dehydrogenase Deficiency</td>
<td>DLD</td>
<td>1 Variant</td>
<td>Ashkenazi Jewish</td>
</tr>
</tbody>
</table>
How To Use This Test

This test does not diagnose any health conditions. Please talk to a healthcare professional if this condition runs in your family, you think you might have this condition, or you have any concerns about your results.

Intended Uses

- Tests for multiple variants in the CFTR gene.
- To identify carrier status for cystic fibrosis.

Limitations

- Does not test for all possible variants for the condition.
- Does not report if someone has two copies of a tested variant.

Important Ethnicities

- This test is most relevant for people of European, Hispanic/Latino, and Ashkenazi Jewish descent.
How To Use This Test

This test does not diagnose Alzheimer's disease or any other health conditions. Please talk to a healthcare professional if this condition runs in your family, you think you might have this condition, or you have any concerns about your results.

Review the Genetic Health Risk tutorial
See Scientific Details
See Frequently Asked Questions

Intended Uses

- Tests for the ε4 variant in the APOE gene.
- Identifies if someone has the ε4 variant associated with an increased risk of developing late onset Alzheimer's disease.

Limitations

- Does not include all possible variants or genes associated with late onset Alzheimer's disease.
- Does not include any variants or genes linked to early onset Alzheimer's disease.
- Does not determine a person's full APOE genotype.

Important Ethnicities

- The ε4 variant included in this test is found and has been studied in many ethnicities. Detailed risk estimates have been studied the most in people of European descent.
THE FUTURE
Epigenetics and Methylation

Epigenetics
The study of changes in gene function that are stable and heritable but do not involve a change in DNA sequence. One example is the process of DNA methylation.

Methylation
The addition of a chemical (methyl group) usually to cytosine in DNA. This modification to DNA is important in regulating gene expression and in imprinting.
Cell-free Fetal DNA

Blood sample from pregnant mother

Cell-free DNA fragments from the mother and fetus (placenta)

Plasma

White Blood Cells

Red Blood Cells
Preimplantation Genetic Diagnosis

- Egg
- Sperm
- Polar Body

Standard In Vitro Fertilization

Embryo

Genetic Testing

Intracytoplasmic Sperm Injection (ICSI)
New GUINNESS WORLD RECORDS™ Title Set for Fastest Genetic Diagnosis

Innovations in whole genome sequencing speed answers and hope for newborns and children with rare, genetic diseases.

San Diego—Feb. 12, 2018—Scientists at the Rady Children’s Institute for Genomic Medicine (RCIGM) have compressed the time needed to decode rare genetic disorders in newborns through DNA sequencing to less than a day.

Through close collaboration with leading technology and clinical partners, the Rady Children’s Technology and Innovation Center (TCIC) has set the GUINNESS WORLD RECORD™ for the fastest whole genome sequence on newborns.
Ineffective Current Standard of Care for Babies Presenting with Disease of Unknown Etiology

- Disease of Unknown Etiology
- Search for etiological diagnosis
- Interim empirical treatment
- Improvement or worsening
- Genetic Testing 6 weeks
- Treatment Modification
- Discharged Home
- Palliative care
- Death
- Anxiety, Suffering
- Cost

Courtesy: Vermont Oxford Network
rWGS-based Genomic Medicine Improves Outcomes for Children in ICUs

- Search for etiological diagnosis with rWGs
- Interim empirical treatment
- 35% Genetic Disease Diagnosis
- 25% Precision Medicine
- Genetic Testing 1.5–10 days
- ~50% Disease of Unknown Etiology
- 20% improved outcomes Less cost and suffering

~50% Disease of Unknown Etiology

70

Courtesy: Vermont Oxford Network
<table>
<thead>
<tr>
<th>ODC1</th>
<th>None currently described</th>
<th>Unknown</th>
<th>p.K448X</th>
<th>c.1342A&gt;T</th>
<th>Heterozygous</th>
<th>De Novo</th>
<th>Variant of Uncertain Significance</th>
</tr>
</thead>
</table>

**Discover New Diseases**
Michigan girl's disorder was discovered through genetic testing

Medical puzzle: It's 1st of its kind

3 DMC doctors demoted; 1 resigns

Doctors puzzled by toddler's mystery condition discover she's the first to have it

She's one of a kind

Groundbreaking research unravels a mysterious genetic mutation affecting little Marley Berthoud. And offers hope for her future.
Lasting Impact