Postnatal Screening and Testing Options

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Learning Objectives

• Understand Newborn Screening process and purpose
• Become familiar with SNP Microarray, Single Gene, Multi-Gene Panel, and Exome genetic testing
• Recognize different considerations that go into genetic test selection
Newborn screening
Newborn Screening (NBS)

- Began in 1960’s with Dr. Robert Guthrie’s test for PKU in newborns
- State-run program, Health Resources and Services Administration (HRSA) recommends screening for 35 specific conditions
- Goal is to diagnosis individuals who would benefit from early intervention

http://www.healthy.arkansas.gov
NBS Roadmap

1. Blood (from heel-prick) sent to a state-run laboratory (within 24-48 hours of life)

2. Physician is notified if any results are abnormal (no news = good news)

3. Physician informs parents of the results and discusses a follow-up plan
NBS Roadmap Example: Cystic Fibrosis

1. Newborn flags for elevated IRT levels in blood spot test

2. Newborn is brought in at 4 weeks old for a sweat chloride test

3. Confirmatory genetic testing of the CFTR gene is ordered for the newborn
What conditions should we screen for?

WHO Screening Principles

Screening Criteria

- Important Condition
- Acceptable treatment available
- Facilities for diagnosis and treatment
- Difficult to Recognize Early (but detectable)
- Suitable screening test
- Acceptable to population
- Natural history known
- Cost effective

Acceptable screening test

Natural history known

Facilities for diagnosis and treatment

Cost effective

Acceptable to population

Difficult to Recognize Early (but detectable)

Suitable screening test

Important Condition
NBS Advancements: SMA

Spinal Muscular Atrophy (SMA)

• AR disease affecting the motor neurons in the spinal cord and brainstem, resulting in progressive motor weakness and atrophy

• FDA-approved treatment
  – Spinraza (Nusinersen) FDA-approved 12/23/2016 - first disease-modifying therapy for SMA patients, all types and ages.
  – Gene therapy (Zolgensma recently approved)
    • SMN1 gene replacement therapy
States screening for SMA on NBS

www.curesma.org
OH NBS

**AA Disorders**
- Argininemia (ARG)
- Argininosuccinic Acidemia (ASA)
- Citrullinemia Type I (CIT) and Citrullinemia Type II (CIT II)
- Homocystinuria (HCY)
- Hypermethioninemia (MET)
- Maple Syrup Urine Disease (MSUD)
- Phenylketonuria (PKU)
- Tyrosinemia Type I, II, III

**Endo**
- Congenital adrenal Hyperplasia
- Primary Congenital Hypothyroidism

**FA Disorders**
- Carnitine Acylcarnitine Translocase Deficiency (CACT)
- Carnitine Palmitoyl Transferase Deficiency Type II (CPT-II)
- Carnitine Uptake Defect (CUD)
- Glutaric Acidemia Type II (GA-2)
- Long-Chain Hydroxyacyl-CoA Dehydrogenase Deficiency (LCHAD)
- Medium-Chain Acyl-CoA Dehydrogenase Deficiency (MCAD)
- Trifunctional Protein Deficiency (TFP)
- Very Long-Chain Acyl-CoA Dehydrogenase Deficiency (VLCAD)
- Krabbe Leukodystrophy
- Glycogen Storage Disease Type II (Pompe Disease)
- Mucopolysaccharidosis type I (MPS I)

**OA Disorders**
- 2-Methylbutyryl-CoA Dehydrogenase Deficiency (2MBG)
- 3-Hydroxy-3-Methylglutaryl-CoA Lyase Deficiency (HMG)
- 3-Ketothiolase Deficiency (BKT)
- 3-Methylcrotonyl-CoA Carboxylase Deficiency (3-MCC)
- Glutaric Acidemia Type I (GA-1)
- Isobutyryl-CoA Dehydrogenase Deficiency (IBG)
- Isovaleric Acidemia (IVA)
- Methylmalonic Acidemia
  - Cobalamin Disorders A and B (Cbl A,B)
  - Methylmalonyl-CoA Mutase Deficiency (MUT)
  - Methylmalonic Acidemia with Homocystinuria (Cbl C, D, F)
- Multiple CoA Carboxylase Deficiency (MCD)
- Propionic Acidemia (PROP)

**Other**
- Biotinidase Deficiency (BIOT)
- Cystic Fibrosis (CF)
- Galactosemia (GALT)
- Severe Combined Immunodeficiency (SCID)
- Sickle Cell Disease (Hb SS)
- Other Hemoglobinopathies (e.g. beta-Thalassemia)
NBS Challenges/Limitations

• Limitations to informed consent and pre-test counseling for mandatory test
• False positive results
• Patients lost to follow-up
• Some conditions screened don’t have great therapy options
  – Ex: Krabbe
• Each state is different!
Purpose of Clinical Testing

- Find a diagnosis
- Family planning or pregnancy decisions
- Treatment decisions
- Risk for future health concerns
- Risks for family members
Review/Shoutout!

FISH Test: 3 copies of chromosome 21
- Trisomy 21 (Down syndrome)
2 copies of chromosome 18

Karyotype
SNP Microarray
SNP Microarray

• Usually a front-line test in Genetics
• Detects deletions or duplications of DNA
• Can analyze which genes are in deletion or duplication
• Can identify Regions of Homozygosity (ROH)
• Limitations:
  – Cannot tell if a piece of DNA is moved to the wrong location (such as a translocation)
  – Cannot see if there is a small mutation, like a sequence change
SNP Microarray Plot

Typical chromosome
SNP Microarray Plot

- BAF
- Log R ratio
- 0 copies, double deletion
- 1 copy, single deletion
- 2 copies, neutral
- 3 copies, single duplication
- Run of homozygosity
Case Presentation

• 6 year old child presents with global developmental delay, autism, and behavioral issues
Pedigree

- 34 yrs
- 6 yrs
- Dev delay
- Autism
- Behavioral issues
SNP Microarray Plot

Result:
arr[hg19] 6p25.3(108,666-1,505,511)X1

Terminal deletion of 1.4 Mb from the short arm of chromosome 6 (6p25.3)
Sequencing
What is Sequencing?

• Looks for smaller changes in our DNA

**MISSENSE MUTATIONS** change one word or letter

THE CAR WAS RED → THE CAR WAS **HAT**
→ THE CAR WAS **RDD**

**INSERTION MUTATIONS** add one word or letter

THE CAR WAS RED → THE CAR **HAT** WAS RED
→ THE CAR **ESW ASR ED**

**NONSENSE MUTATIONS** end the instructions too soon

THE CAR WAS RED → THE CAR

**DELETION MUTATIONS**

THE CAR WAS RED → **THE WAS RED**
→ **THE RWA SRE D**
Summary of Sequencing

**Single Gene Test**

One test looks for one gene/condition
- Ex: Cystic fibrosis

**Multi-Gene Panel**

One test looks at many genes based on:
- Single condition/Dx (ex: breast cancer, Fanconi anemia)
- Group of conditions (ex: multiple cancers, autism/ID)
- Symptom (ex: epilepsy, immunodeficiency)

**Exome**

Reads through the regions of DNA (exons) that directly code for proteins
- One test looks for thousands of disorders!
### Product Comparison

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Invitae Breast and Gyn Cancers Panel - Primary + Preliminary-Evidence Genes</th>
<th>Hereditary Breast and Gynecological Cancer Panel Updated (Sequencing)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Actions</strong></td>
<td>✗ Remove From Compare</td>
<td>✗ Remove From Compare</td>
</tr>
<tr>
<td><strong>Lab Name</strong></td>
<td>✗ GeneDx</td>
<td>✗ Invitae</td>
</tr>
<tr>
<td><strong>Category</strong></td>
<td>Hereditary Breast and Ovarian Cancer Panel Tests</td>
<td>Hereditary Breast and Ovarian Cancer Panel Tests</td>
</tr>
<tr>
<td><strong>Test Code</strong></td>
<td>B273</td>
<td>01201-1</td>
</tr>
<tr>
<td><strong>Source</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Price</strong></td>
<td>$3,272.50</td>
<td>$950.00</td>
</tr>
<tr>
<td><strong>TAT</strong></td>
<td>14 days</td>
<td>10-21 days</td>
</tr>
<tr>
<td><strong>Techniques</strong></td>
<td>Deletion/Duplication, Sequencing</td>
<td>Deletion/Duplication, Sequencing</td>
</tr>
<tr>
<td><strong>Mechanisms</strong></td>
<td>MLPA, Next Generation Sequencing</td>
<td>Next Generation Sequencing</td>
</tr>
<tr>
<td><strong>Overlapping Genes</strong></td>
<td>ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, MLH1, MSH2, MSH6, MLN, NF-1, PALB2, PMS2, PTEN, RAD51C, RAD50, TFSJ</td>
<td></td>
</tr>
<tr>
<td><strong>Unique Genes</strong></td>
<td>FANCC, MUTYH, POLD1, RECDL</td>
<td>AKT1, CDC73, DICER1, FAN117R, FANCC, MRB11, BLM, DICER1, FANCM, NPB11, RAD50, RINT1, SDHB, STK11, XRCC2, SMC1A4</td>
</tr>
</tbody>
</table>
Patient name: Jane Doe
DOB: 
Sex: Female
MRN: 

Sample type: Blood
Sample collection date: 
Sample accession date: 

Report date: 
Invitae #: 
Clinical team: 

Reason for testing
Diagnostic test for personal history of disease

Test performed
Sequence analysis of the 207 genes listed in the Genes Analyzed section.
- Invitae Primary Immunodeficiency Panel

RESULT: POSITIVE

One homozygous Pathogenic variant identified in CTPS1. CTPS1 is associated with autosomal recessive CTPS1 deficiency.

One Pathogenic variant identified in PMM2. PMM2 is associated with an autosomal recessive congenital disorder of glycosylation.

Additional Variant(s) of Uncertain Significance identified.

<table>
<thead>
<tr>
<th>GENE</th>
<th>VARIANT</th>
<th>ZYGOSITY</th>
<th>VARIANT CLASSIFICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTPS1</td>
<td>c.1692-1G&gt;C (Splice acceptor)</td>
<td>homozygous</td>
<td>PATHOGENIC</td>
</tr>
<tr>
<td>PMM2</td>
<td>c.422G&gt;A (p.Arg141His)</td>
<td>heterozygous</td>
<td>PATHOGENIC</td>
</tr>
<tr>
<td>MOGS</td>
<td>c.721C&gt;T (p.Arg241Cys)</td>
<td>heterozygous</td>
<td>Uncertain Significance</td>
</tr>
</tbody>
</table>

About this test
This diagnostic test evaluates 207 gene(s) for variants (genetic changes) that are associated with genetic disorders. Diagnostic genetic testing, when combined with family history and other medical results, may provide information to clarify individual risk, support a clinical diagnosis, and assist with the development of a personalized treatment and management strategy.

Report Example
CCHMC Approach

• If child presents with global developmental delay and autism, at CCHMC our standard of care is to order:
  1. SNP Microarray
  2. Fragile X
  3. Autism/ID panel*
Whole Exome Sequencing

- Sequencing of Exons
- Proband Only vs. Trio approach
- Answer found only ~25% for Trios
- Primary vs. Secondary Findings
Whole Genome vs. Whole Exome

**Genome**

A genome is like watching a football game from beginning to end.

**Exome**

An exome is like reading about the game highlights the next day.
Exome Result Example

Results Summary

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Inheritance</th>
<th>Characterized/Uncharacterized Gene*</th>
<th>Protein Change</th>
<th>Nucleotide Change</th>
<th>Genotype</th>
<th>Alteration Type</th>
<th>Alteration Classification</th>
<th>Clinical Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMPCA</td>
<td>Autosomal recessive</td>
<td>Characterized</td>
<td>p.A377V</td>
<td>c.1130C&gt;T</td>
<td>Homozygous, biparental</td>
<td>Missense</td>
<td>Likely pathogenic</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Patient's likely diagnosis based on molecular results:
Spinocerebellar ataxia, autosomal recessive 2 (OMIM_213200)
Case Examples:
Single gene vs. panel vs. Exome

1. 15-year-old adolescent presents with hypertrophic cardiomyopathy
Case Examples:

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2. 3-year-old presents with a family history of cardiomyopathy, and his mother has a known mutation in *EMD* gene
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Single gene vs. panel vs. Exome

1. 15-year-old adolescent presents with hypertrophic cardiomyopathy

2. 3-year-old presents with a family history of cardiomyopathy, and his mother has a known mutation in *EMD* gene

3. 5-day-old presents with dysmorphic facial features, failure to thrive, multiple congenital anomalies, and ataxia with no recognizable condition. All frontline testing is normal
Predictive or Predisposition Testing

• Used to determine a healthy person’s predisposition to develop disease
• Testing usually sought based on family or medical history
• Should only be done in minors if there are medical interventions that would be recommended as children, if the testing is positive
Predictive or Predisposition Testing

• Treatments/Screening available
  – Cardiac Genetic Panels
  – Cancer Genetic Panels

• **Treatment not available**
  – AD Alzheimer’s Disease
  – Huntington’s Disease
Proactive Genetic Testing

• Marketed to healthy adults who want to understand their DNA and focus on prevention

• For example: Three testing options that analyze up to 147 genes that are well-established indicators of a significantly increased risk of developing hereditary cancers, cardiovascular conditions, and other medically important disorders
Testing Considerations
Test Interpretation

• Our technology is improving faster than our ability to correctly interpret results

• Types of Results:
Test Interpretation

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• Types of Results:
  – Positive
    • Accurate recurrence risks
    • Discover other medical concerns that should be evaluated
    • Possibly direct treatment
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  – Negative
    • Can rule out some conditions
    • Can make other conditions less likely
    • Doesn’t take away a clinical diagnosis for some conditions
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  – Negative
    • Can rule out some conditions
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    • Doesn’t take away a clinical diagnosis for some conditions
  – Variant of Uncertain Clinical Significance (VUS)
    • Does not change medical management
    • Could be reclassified in the future
Other Considerations

• Picking the best test for your patient
  – Not every test is equal!

• Insurance
  – Will obtaining a diagnosis change management?
  – Will insurance company cover cost of testing?
  – GINA (Genetic Information Nondiscrimination Act)

• Incidental findings
  – Find Dx you weren’t looking for
  – Parental relationships that weren’t disclosed
Questions?
lauren.hsuan@cchmc.org
References

- http://genotopia.scienceblog.com/
- http://www.cdc.gov/newbornscreening/
- http://www.savebabies.org/
- http://www.sequenom.com/
- www.babysfirsttest.org/home
- NIH Promoting Safe and Effective Genetic