Next Generation Sequencing and Newborn Screening

Suzanne Cordovado, PhD
Molecular Quality Improvement Program
Centers for Disease Control and Prevention

2016 Molecular Training Workshop
April 28, 2016
Errors can Occur when DNA is Replicated for Cell Division

These errors can result in disease
Mutations in the Cystic Fibrosis Gene

Normal - no changes

Case I: Severe Symptoms
Case II: Mild Symptoms
Case III: No Symptoms
Mutations in the Cystic Fibrosis Gene

Case I – unrecognizable

Case I

Normal

Case I

Mutation 1

Severe Symptoms

Case II

Mutation 2

Mild Symptoms

Case III

Mutation 3

No Symptoms
Mutations in the Cystic Fibrosis Gene

Case I

Case II – simple error

Case III
Mutations in the Cystic Fibrosis Gene

Case III – American vs. British
Molecular Genetics Milestones

1952: Described DNA as a double helix (using x-ray diffraction)

1975: Devised techniques for DNA sequencing

1985: Conducted first polymerase chain reaction (PCR) experiments to amplify specific gene regions

2003: Human Genome Sequencing Project is completed
DNA Sequencing
1975 to 2015 and Beyond

Radioactive Sanger Sequencing

http://www.uvm.edu/~cgep/Education/Sequence.html
DNA Sequencing
1975 to 2015 and Beyond

The Broad Institute of MIT and Harvard large-scale Sanger DNA sequencing center
DNA Sequencing
1975 to 2015 and Beyond

Next Generation Sequencing

Roughly 50% of the sequences have a 3 bp deletion (TCT)
Mix deoxynucleotides with ddA, ddT, ddC*, ddG
4 lanes per person/fragment
~200 readable bases

Chop up the human genome or amplify regions of interest
Make a library of fragments
Sequence billions of bases
Multiplex multiple people
- Millions of ‘reads’

Mix deoxynucleotides with ddA, ddT, ddC*, ddG
1 scan per person/fragment
~800 readable bases
Sequencing a Human Genome

- **U.S. Human Genome Project**
  - 23 laboratories
  - ~$3 billion

- **Next Generation sequencing**
  - Several weeks
  - ~$10,000

- **Latest DNA sequencers**
  - Several days
  - ~$1,000 assay costs
“…it may soon be easier and cheaper to sequence an entire genome than to test for a number of known mutations.”

- Foundation for Genomics and Population Health
Will Genome Sequencing Take the Healthcare System by Storm?

Or Will We Ride the Wave?

Either way, we are going to get wet...
Advent of Next Generation Sequencing Technology

Sanger Sequencing Technology

Cost per Genome

NIH National Human Genome Research Institute

genome.gov/sequencingcosts
The Declining Cost of Genome Sequencing

- Produces 16 human genomes in 3 days at 30x coverage
- Projected costs per genome
  - Reagents $797
  - Machine depreciation $137
  - Technician $55–65
- Does not include overhead, infrastructure and analysis costs
- Instrument cost $10 Million USD

HiSeq X Ten Next Gen Sequencer

http://www.nature.com/news/technology-the-1-000-genome-1.14901
Sequencing the Human Genome

- Genome is comprised of 3 billion bases
- Exome is approximately 1% of the genome
  - Includes DNA segments containing protein coding regions of genes

Earth’s Land Mass ~ 57 million square miles
Alaska Land Mass ~ 663 thousand square miles
Genome Sequencing for Clinical Diagnosis

The NEW ENGLAND JOURNAL of MEDICINE

Original Investigation

Molecular Findings Among Patients Referred for Clinical Whole-Exome Sequencing

Yaping Yang, PhD; Donna M. Muzny, MS; Fan Xia, PhD; Zhiyv Niu, PhD; Richard Person, PhD; Yan Ding, MD; Patricia Ward, MS; Alicia Braxton, MS; Min Wang, PhD; Christian Buhay, BS; Narayanan Veeraraghavan, PhD; Alicia Hawes, BS; Theodore Chiang, MS; Magalie Leduc, PhD; Joke Beuten, PhD; Jing Zhang, PhD; Weimin He, PhD; Jennifer Scull, PhD; Alecia Willis, PhD; Megan Landsverk, PhD; William J. Craigie, MD, PhD; Mir Reza Bekheirnia, MD; Asbjorg Stray-Pedersen, MD, PhD; Pengfei Liu, PhD; Shu Wen, PhD; Wendy Alcaraz, PhD; Hong Cui, PhD; Magdalena Walkiewicz, PhD; Jeffrey Reid, PhD; Matthew Bainbridge, PhD; Ankita Patel, PhD; Eric Boerwinkle, PhD; Arthur L. Beaudet, MD; James R. Lupski, MD, PhD; Sharon E. Plon, MD, PhD; Richard A. Gibbs, PhD; Christine M. Eng, MD

Richard A. Gibbs, Ph.D., and Christine M. Eng, M.D.

Oct 2013

Nov 2014
Molecular Diagnosis using Genome Sequencing

- Exome sequencing to achieve a clinical diagnosis
- Initial NEJM (2013) and follow up JAMA (2014) study found that ~25% of previously undiagnosed patients were given a molecular diagnosis
- Marked improvement over testing single genes or gene panels currently used

http://www.jsonline.com
Limitations of PCR Based Sequencing
No Method is Perfect

- Deletions removing large segments of DNA are not readily detected
  - Example of newborn diseases resulting from large deletions: CF, CAH and DMD
- Rearrangements or copy number variations will not be detected
- Person to person DNA variation can give inaccurate results due to mis-priming
- Error rate of traditional sequencing is lower than Next Gen sequencing – although this is improving!
Challenges of Genome/Exome Sequencing

- **Major Challenge:** Determining whether any given variant is pathogenic
- **ACMG determined 5 categories to classify variants:**
  - Known pathogenic
  - Likely to be pathogenic
  - Unknown significance
  - Likely to be benign
  - Benign

- Knowledge accruing daily, however the medical impact of most variants is unknown
What data should be returned to patients and physicians?

Factors to consider
- Patient autonomy
- Patient privacy
- Physician liability
- Clinical laboratory guidelines for reporting
Research on Utility of Genomic Sequencing in Newborn Screening

- NIH awarded $25M to 4 clinical labs to perform research on genome/exome sequencing in newborns
  - Goal 1 - Explore medical utility beyond what is already delivered by current NBS
  - Goal 2 - Understand diseases identified in the newborn period
  - Goal 3 - Research the ethical, legal and social implications of genomic sequencing of newborns
**Newborn Sequencing in Genomic Medicine and Public Health: NSIGHT**

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Institution</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robert Green and Alan Beggs</td>
<td>Brigham and Women’s Hospital</td>
<td>Genome Sequence-Based Screening for Childhood Risk and Newborn Illness</td>
</tr>
<tr>
<td>Stephen Kingsmore</td>
<td>Children’s Mercy Hospital</td>
<td>Clinical and Social Implications of 2-day Genome Results in Acutely III Newborns</td>
</tr>
<tr>
<td>Robert Nussbaum</td>
<td>University of California, San Francisco</td>
<td>Sequencing of Newborn Blood Spot DNA to Improve and Expand Newborn Screening</td>
</tr>
<tr>
<td>Cynthia Powell and Jonathan Berg</td>
<td>University of North Carolina at Chapel Hill</td>
<td>NC NEXUS, North Carolina Newborn Exome Sequencing for Universal Screening</td>
</tr>
</tbody>
</table>
NSIGHT Project:

**The Genomic Oracle**

If your DNA is sequenced at birth, how would it affect your life? A new project aims to find out.

By Carl Zimmer

Genome Sequence-Based Screening for Childhood Risk and Newborn Illness

“If you have your genome readily available from birth, how is that likely to influence your life?”

Robert C. Green, principal investigator of BabySeq describes program goal

http://www.slate.com/articles/health_and_science/human_genome/2013/10/baby_seq_genome_study_will_sequencing_dna_at_birth_change_someone_s_life.html
Characteristics of Newborn Disorders Include

- Significant disease
- **Treatment possible**
- Not evident until harm is done
- Mass testing methods available
- Benefits justify costs
Current Molecular Testing in Newborn Screening Laboratories

- **Second tier molecular tests**
  - Increase sensitivity or specificity of primary assay
    - Cystic Fibrosis (CF)
  - Clarify an ambiguous result
    - Hemoglobinopathies
  - Supplemental “Just in Time” assay
    - Galactosemia

- **Primary molecular test**
  - When no other assay is available – Severe Combined Immunodeficiency (SCID)
49 states or territories use at least one molecular test
2 states perform targeted DNA sequencing
23 Countries that Participate in CDC’s Molecular PT Programs in 2015

- Countries Participating only in CF PT (14)
- Countries Participating only in SCID PT (3)
- Countries Participating in CF & SCID PT (6)

Note that not all NSQAP PT participating countries offer universal screening.
Next Gen Sequencing and Cystic Fibrosis Newborn Screening

Issue:
Many referred CF screens are clinical false positives

- Screen positive – ↑IRT and at least 1 CF causing mutation
  - Most assays detect a panel of mutations that cause CF
  - >2000 known mutations/variants in CFTR gene
- Next Gen sequencing screen positive – ↑IRT and 2 CF causing mutations
  - Preliminary data indicates CF screening referral rate would drop from 900 to 100 annually in New York, U.S. (annual birth rate 250,000) - M. Caggana personal communication
- Not all CFTR mutations cause classic CF
  - Will identify CF related metabolic syndrome or unknown variants
  - Can limit sequence detection to known mutations but will miss cases
Next Gen Sequencing and SCID Newborn Screening

Issue:
SCID is a spectrum of disorders that can only be differentiated by identifying causative mutations

- >25 genes involved in SCID
- Immunologists can provide better care when SCID causative mutations are known quickly
- Screening labs can provide timely mutation analysis
- When public health provides mutational analysis, ensures health equality
Molecular Analysis in Newborn Screening
A Staged Approach

- Genotyping Panel of Mutations
- Sequencing Single Gene
- Sequencing Panel of Genes
- Sequencing of NBS Genes
- Genome
- Exome

Ongoing in routine NBS
Experimental in NBS
Offered clinically and research outside NBS
Future of Genome Sequencing and Newborn Screening...

- **Targeted sequencing to identify diseases that have treatable or preventable outcomes**
  - CDC funding New York State to develop Next Gen Sequencing SCID gene panel to identify causative mutations

- **Exome/Genome sequencing that detects only newborn disease causing mutations**

- **Mutation specific drugs**
  - Kalydeco (ivacaftor) – treats Cystic Fibrosis patients with one of nine specific mutations including G551D (2012)
  - Orkambi (lumacaftor & ivacaftor) – treats Cystic Fibrosis patients with F508del
Translating Research to Medicine and Public Health