Genome Sequencing: Applications and Newborn Screening

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When DNA is Replicated for Cell Division, Errors can Occur

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

Normal - no changes
Mutations in the Cystic Fibrosis Gene

Case I – unrecognizable

CAWPR Mutations in the Cystic Fibrosis Gene
Mutations in the Cystic Fibrosis Gene

- **Normal**
- **Case I**
- **Case II** – typo
- **Case III**

**COLAR**
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 2014 and Beyond

The Broad Institute of MIT and Harvard large-scale Sanger DNA sequencing center

Radioactive Sanger Sequencing
Next Generation Sequencing


http://www.uvm.edu/~cgep/Ed ucation/Sequence.html

Life Technologies Ion Torrent Sequencer
HiSeq 2500 Sequencer

Sanger DNA sequencing center
Sequencing the Genome

- U.S. Human Genome Project began in 1989 and was completed by 23 collaborating laboratories in 13 years (cost ~$2.7 billion)

- Latest DNA sequencers can sequence a human genome in a few days for less than $5,000

- “2013 is likely to be the year where we see the $1000 genome.” - Daniel Franklin, executive editor of The Economist
Will Genome Sequencing be like a Healthcare Tsunami?

Or Will We Ride the Wave?

Either way, we are going to get wet…
“…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
Sanger Sequencing Technology

Cost per Genome

Advent of Next Generation Sequencing Technology

Moore's Law

NIH National Human Genome Research Institute

genome.gov/sequencingcosts
Sequencing the Human Genome

- Genome is comprised of 3 billion bases
- Exome is approximately 1% of the genome
  - Includes DNA segments that contain genes
  - Genes hold the recipe for all the body’s proteins

 genome vs. exome

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Clinical Whole-Exome Sequencing for the Diagnosis of Mendelian Disorders

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Molecular Diagnosis using Genome Sequencing

- Exome sequencing to achieve a clinical diagnosis
- Recent NEJM study made a molecular diagnosis in 62 of 250 cases (25%)
- Marked improvement over testing single genes or gene panels currently used
Limitations of Sequencing
No Method is Perfect

- Deletions removing large segments of DNA, rearrangements or copy number variations will not be detected
  - Examples of newborn diseases resulting from large deletions: Cystic fibrosis and Congenital Adrenal Hyperplasia

- Person to person DNA variation can give inaccurate results due to mis-priming

- Traditional sequencing has an error rate of 1/10,000 to 1/100,000 - Next Gen sequencing is considerably higher
Challenges of Genome/Exome Sequencing

- **Major Challenge:** Determining whether any given variant is pathogenic
- **ACMG defined 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
Genome Sequencing Results

- What data should be returned to patients and physicians?
- Factors to consider
  - Patient autonomy
  - Patient privacy
  - Physician liability
  - Clinical laboratory guidelines for reporting
Characteristics of Newborn Disorders Include

- Significant disease
- Prevention possible
- Not evident until harm is done
- Mass testing methods available
- Benefits justify costs

Slide Courtesy of Mike Glass
Washington State Dept of Health
Molecular Testing in Newborn Screening Laboratories

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

- **Primary molecular test**
  - When no other assay is available – Severe Combined Immunodeficiency (SCID)
NBS Molecular Testing Status: 2014

41 states offer a molecular test
3 states use targeted DNA sequencing
Future of Sequencing in Newborn Screening…

- Targeted gene sequencing to identify mutations associated with diseases that have treatable or preventable outcomes

- Mutation specific drugs
  - Kalydeco – treats Cystic Fibrosis patients with specific mutations

- Exome and Genome sequencing will be explored over the next few years by NICHD grant recipients
Translating Research to Medicine and Public Health
Thank you!

Newborn Screening

Saving Lives.

Promoting Healthier Babies.

Protecting our Future.

For more information please contact Centers for Disease Control and Prevention
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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.