Overview Molecular Newborn Screening

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A Disorder, Treatment and Diagnostic Test


The World of PKU www.pkuworld.org

The Perfect Storm

Dr. Robert Guthrie

Pictures Courtesy of Dr. Kenneth Pass
Phenylketonuria

Phenylpyruvic acid
FeCl3 test

http://138.192.68.68/bio/Courses/biochem2/AminoAcids/AminoAcidCarbonDeg.html
The Perfect Storm Continues

Pictures Courtesy of Dr. Kenneth Pass
Jamestown, New York

- Fall 1961 talk for The Association for Retarded Children
- Began to receive newborn filter-paper specimens
- “Thus, screening had its start in Jamestown, New York in 1961”
BUT
Newborn screening
Is more than a “PKU test”.

It is a comprehensive, free, public health system provided to identify infants at risk for devastating conditions.
MS/MS Phenylketonuria

Data from Dr. Mark Morrissey
Chronology of NBS

- 1957  Diaper Test for PKU in California
- 1958  Phenistix Used in Europe
- 1963  Guthrie and Susi – Bacterial Inhibition Assay
- 1964  Universal Screening in Massachusetts
- 1978  Radioimmunoassay Introduced
- 1994  MS / MS Used
DNA
the molecule of life

Trillions of cells

Each cell:
- 46 human chromosomes
- 2 m of DNA
- 3 billion DNA subunits (the bases: A, T, C, G)
- 80,000 genes code for proteins that perform all life functions
Human Genome Project

- Proposed by Victor McKusick in 1968
  *(when did newborn screening start)??

- DOE and NIH, 15 years, 30 billion dollars

- James Watson original head then Francis Collins

- International effort

- ELSI budget
Human Genome Project

Five Main Objectives:

1. Generate genetic and physical maps
2. Develop new DNA technologies
3. Accurately sequence the human genome
4. Develop informatics
5. Sequence model organisms
Human Genome Project

**Accurate Sequence Data:**

- 3,000,000,000 bases; haploid
- Rough draft / 90%, summer 2000, 2/01 “finished”
- Highly accurate (1 error in 100,000 bases) no gaps or ambiguities by 2003
- First chromosome 22 reported 12/99
- First chromosome 21 reported 5/00
- Projected finish 2003, original 2005
Venter & Collins
Private vs. Public

1000 Genomes Project
Genetic Disorders

- Caused by mutations in genes or chromosomes
- Mutations may occur on:
  -- An autosome (autosomal)
  -- A sex chromosome (X-linked or Y-linked)
  -- Multiple genes
- Disease expression may be impacted by environmental factors
Single Gene Disorders

- Caused by mutations in one gene
- Generally follow Mendelian inheritance patterns
  -- Dominant vs. Recessive
  -- Expression may be impacted by genomic imprinting or penetrance
- Includes most inborn errors of metabolism

Most “single gene disorders” are probably influenced by multiple genes / DNA
Classes of Single Gene Disorders

- **Autosomal Dominant**
  - One copy of a mutated allele results in affected individual
  - aka: AA or Aa
  - Heterozygous and homozygous individuals are affected
  - e.g. achondroplasia, Huntington disease

- **Autosomal Recessive**
  - Both copies of the gene must be mutated to be affected
  - aka: aa
  - Only homozygous individuals are affected.
  - e.g. Sickle cell anemia, cystic fibrosis, galactosemia
Classes of Single Gene Disorders

- **X-linked Recessive**
  - Males affected if X chromosome is mutated
  - Females affected only if both X chromosomes are mutated; e.g. Duchenne muscular dystrophy & hemophilia and ALD

- **X-linked Dominant**
  - Individuals with 1 mutant copy of X chromosome are affected; e.g. Rett syndrome

- **Y-linked**
  - Individuals with a mutated Y chromosome are affected
  - Rare
Autosomal Recessive Inheritance
X-Linked Recessive Inheritance

[Genetic diagram showing inheritance patterns with Queen Victoria highlighted as an affected individual.]

Molecular Testing for Genetic Diseases

- Enabled by gene mapping to identify location of genes on chromosomes AND ability to differentiate between harmful and neutral mutations
- Identification of disease-causing mutations for:
  - Diagnosis
  - Predictive testing
  - Carrier detection
  - Prenatal screening
  - Preimplantation testing
  - Pharmacogenetics
## Availability of Genetic Tests

<table>
<thead>
<tr>
<th>GeneTESTS: Availability of Genetic Tests</th>
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**GeneTESTS: Availability of Genetic Tests**

- >600 Laboratories offering in-house molecular genetic testing, specialized cytogenetic testing, and biochemical testing for inherited disorders
- >3000 Diseases
Availability of Genetic Tests

GeneTests: Growth of Laboratory Directory

Distinction Between Mutations and SNPs

**Mutations:**
Changes in the DNA, which are ‘rare’; can be private; newer

**SNPs/Polymorphisms:**
Changes in the DNA occurring at a higher frequency, usually greater than 1%; may start as mutations and reach a higher frequency; older changes.

Both are inherited and can be used to track DNA changes

cSNPs are in the coding region

- **synonymous:** no change to the amino acid (silent)
- **non-synonymous:** change to the amino acid

Non-coding SNPs:

- promoter, splice sites, stability, other regulatory changes
## TYPES OF MUTATIONS

### Normal
```
CCG GGA AGC AAU
Pro  Gly  Ser  Asn
```

### Missense
```
CCG GCA AGC AAU
Pro  Val  Ser  Asn
```

### Nonsense
```
CCG UGA AGC AAU
Pro  STOP
```

### Frameshift (insertion)
```
CCG AGG AAG CAA
Pro  Arg  Lys  Gln
```

### Frameshift (deletion)
```
CCG GAA GCA AUG
Pro  Glu  Asp  Met
```

### Trinucleotide
```
CAG CAG CAG CAG CAG
Gln  Gln  Gln  Gln  Gln
```

---

Mutations can be helpful – camouflage; selection
Mutations can be silent – markers, forensics, mapping, population studies
Mutations can be harmful – sickle cell, PKU, CF and other diseases
History of Molecular Testing in Newborn Screening

- **1994**
  - Washington – hemoglobin confirmatory testing (Hb S, C, E by RFLP)
  - Wisconsin – CFTR mutation analysis for ΔF508

- **1998**
  - New England – 2 GALT mutations (Q & N) by RFLP

- **1999**
  - New England – MCADD (c.985A>G) by RFLP
History of NBS Molecular Testing

- **2005**
  - Wisconsin – MSUD (p.Y438N)

- **2006**
  - New York – Krabbe disease (3 polymorphisms & 5 mutations; full gene DNA sequence analysis)

- **2008**
  - Wisconsin – SCID – TREC analysis
    - *1st use of molecular test as a primary full population screen*

- **2010**
  - 37 NBSPs in US use molecular testing for CF
Things for Programs to Consider

- Which tests will have a molecular component?
- DNA extraction methods; (cost/labor)
- Degree of automation; vendors and contracts
- Manipulation (single tube? 96-well? 384-well?)
- # Instruments, data collection, interpretation
- Staff training (lab and follow-up)
Uses of Molecular Tests in NBS

- **Primary Screening Test**
  - TREC analysis for detection of SCID; SMA

- **Second-Tier Test**
  - DNA test results provide supplemental information to assist with diagnosis
    - Often provided in separate report
    - β-globin and GALT mutation analysis

- **Genotypic information is required for interpretation of the screen result**
  - Cystic fibrosis mutation analysis
NBS Molecular Tests in US

- Primary screen -- SCID
- Second-tier screen
  -- Hemoglobinopathies
  -- Galactosemia
  -- Cystic fibrosis
  -- MCAD and other FAOs (VLCAD?)
  -- Phenylketonuria
  -- Krabbe disease; Pompe disease
  -- Maple syrup urine disease
  -- Adrenoleukodystrophy (FYI)
Does Molecular Testing Add Value??

- Increase in sensitivity of a primary test, effect on specificity?
- Identification of carriers; teaching moments
- Predictions regarding phenotype
- Clinicians’ perception, diagnostic tool

OR
When / Why Use a Molecular Test?

- To increase sensitivity without compromising specificity
  -- Lower IRT cutoff to avoid missing CF cases

- To increase specificity of a complex assay
  -- Allow differentiation of hemoglobinopathies & thalassemias (e.g. Hb S/b-thalassemia)
When / Why Use a Molecular Test?

- When the primary analyte is transient
  - The primary analyte is present for only a limited time after birth and analysis of a second specimen could result in a false negative. (e.g. VLCAD / CPT2)

- To speed diagnosis in order to avoid serious medical consequences
  - GALT enzyme activity is decreased by heat & humidity, increase in false positive screens
  - Genotyping helps sort out the true positives for faster diagnosis.
When / Why Use a Molecular Test?

- When there are significant founder mutations in a population

- Due to high frequency (1 in 176 live births) of MSUD in Mennonite population in WI, mutation analysis for p.Y438N serves as primary screen for MSUD for Mennonites.

- CPT1a in Alaskan Inuit (p.P479L) & Hutterite populations (p.G710E)
When diagnostic testing is slow and/or invasive

-- Traditional confirmatory testing for VLCAD & CPT1a involves skin biopsy (invasive to collect and slow to grow)

When no other test exists for the analyte

- SCID, SMA, FRAX
Things for Programs to Consider By Contract

- Which tests will have a molecular component?
- Specimen transport
- Screening or confirmatory?
- Timing and prioritization for contract lab
- Systems integration
- Follow-up integration
Things for Programs to Consider In-House 1

- Volume / quality of specimens
- Cost ($$$$) per sample
- “Simple test” mentality
- Public health infrastructure
  -- Equipment
  -- Space
- ELSI
- Have test, no Tx
Things for Programs to Consider In-House 2

- Capacity – Throughput -- Automation?
- IVD v. ASR / LDT;
- Expertise / Interpretation
- Methods / Manipulation – single tube? 96-well or 384-well plates
- Control Materials
- Integration into Program / LIMs / Follow-up / TAT
Potential Future Applications of Molecular Testing in NBS

Expansion to other existing or potential NBS disorders

- Congenital adrenal hyperplasia (CAH)
- Biotinidase deficiency
- Ornithine transcarbamylase deficiency (OTC)
- Cytomegalovirus
- Fragile X syndrome
- Spinal muscular atrophy
- Duchenne muscular dystrophy (DMD)
- Other lysosomal storage disorders (LSD)
Potential Future Applications of Molecular Testing in NBS

- Genome-wide association studies
- Susceptibility testing (heart disease, cancer, obesity, diabetes)
- Next generation sequencing - exome, genome and transcriptome
- Pharmacogenetics and NBS
  -- Drugs in clinical trials to treat specific CF causing mutations (VX-770/G551D and VX-890 / DF508)
  -- Ataluren (formerly PTC124) is an investigational drug that reads through nonsense or STOP mutations
Mix deoxynucleotides with ddA, ddT, ddC*, ddG
4 lanes per person/fragment
~200 readable bases

Chop up the human genome
Make a library of fragments
Sequence billions of bases
Multiplexing multiple people
Millions of ‘reads’
Perils of Newborn Screening

Doctors may be testing infants for too many diseases

By Ariel Bleicher

The first symptoms often appear a month or two after birth. The babies’ muscles stiffen. They lose their hearing and vision, stop sleeping and scream in pain. Some develop seizures. By the time many parents learn that their children have Krabbe disease—a rare genetic disorder that degrades nerve cells—it is too late for the only viable treatment, a transfusion of umbilical cord blood stem cells from healthy donors. Children with full-blown Krabbe who do not receive medical treatment, as well as many who do get treated, usually die by age two.

In some cases, doctors can prevent this grim outcome by screening infants at birth for genetic harbingers of disease. Right now such tests are mandatory in only a few
Challenges of 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
Evolution of Krabbe Disease Screening

- Pan-ethnic
- Frequency 1:100,000 worldwide
- Gene described in 1993
- Prenatal screening 80’s by enzyme, molecular 90’s
- New York 8/7/06; 2+ million screened; MO 2.5 years
- Legislation/lobbying (NM, IL, NJ, MD, PA, TN, MI……..)
State of NBS for Krabbe Disease

- Full gene sequence required
- ~30 novel variants have been detected in screening
- Common complex genotypes
- Variants of unknown significance
- One mutation, no enzyme activity
- Two mutations, asymptomatic
- Two mutations, different phenotypes
- Parental anxiety
Krabbe Today Mimics CF Yesterday

- No population / carrier screening
- Molecular data from symptomatic, infantile
- No common panel, except 30Kb deletion
- No natural history from a screened population
- Information will drive treatment
- Information will develop evidence base
- Policy will follow
- Will we ever get to the "common" mutation panel?
Improvement of the Literature

Collect data, clinical followup

http://www.miragebookmark.ch/images/astronomy-library-utrecht.jpg
Learning Points

- Newborn screening has accepted new technology and evolved over time
- Molecular NBS began in 1994 and continues to include more testing
- Almost all NBS invokes genetics and thus the family
- Programs need to address utility and laboratory needs for molecular NBS
- Molecular testing will continue to enter NBS algorithms and sequencing poses challenges for Programs to consider
Always pay it forward and never forget to pay it back. It’s how you got here and it defines where you’re going...

@briansolis

Thanks to Suzanne Cordovado, Ph.D. and Co., Susan Tanksley, Ph.D. and Rachel Lee, Ph.D. for slides
Thank You

Mahalo

Kiitos

Toda

Gracias

Obrigado

Merci

Grazie

Takk

Thanks
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