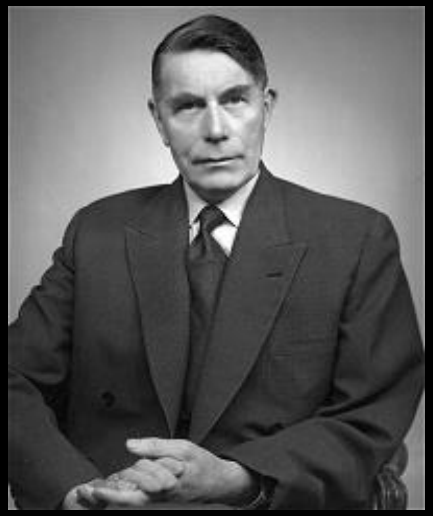
Overview Molecular Newborn Screening

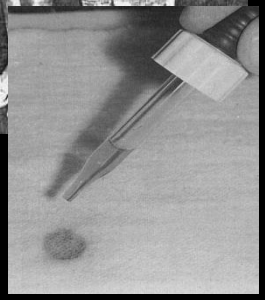
Michele Caggana, Sc.D.
Director, Newborn Screening Program
New York State Department of Health
Wadsworth Center

A Disorder, Treatment and Diagnostic Test



Dr. Asbjorn Folling

Dr. George Jervis



Dr. Willard Centerwall

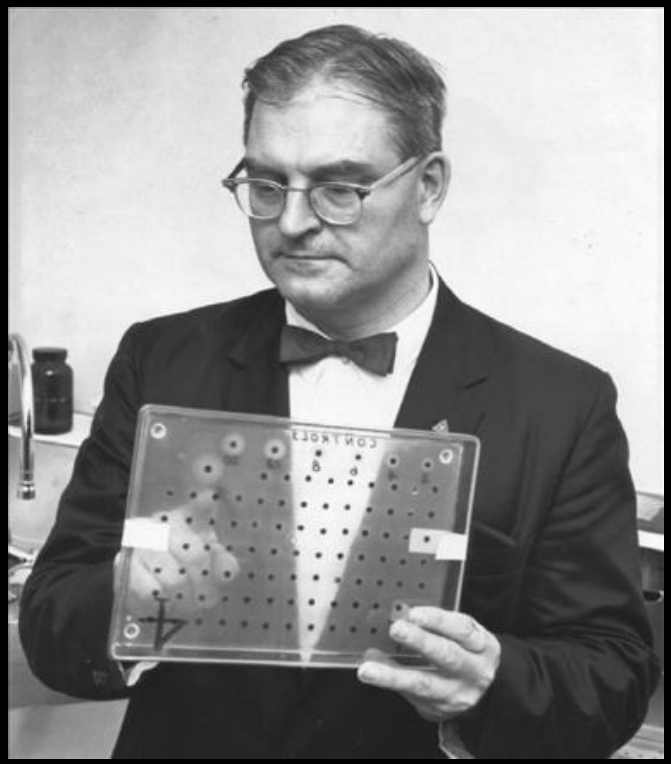
Dr. Horst Bickel

The World of PKU www.pkuworld.org

SA Centerwall, WR Centerwall (2000) The discovery of Phenylketonuria: The story of a young couple, two retarded children, and a scientist. Pediatrics 105: 89-103.

Wadsworth Center
NEW YORK STATE DEPARTMENT OF HEALTH

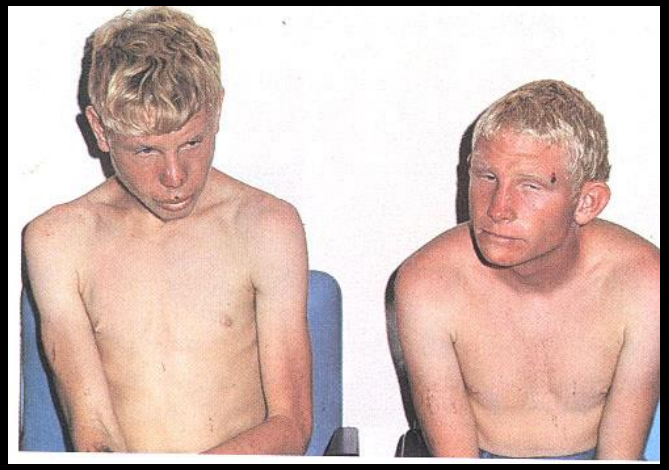
The Perfect Storm

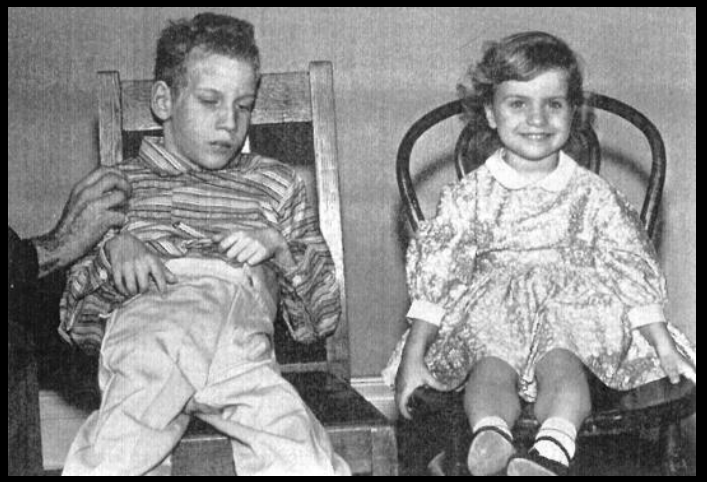


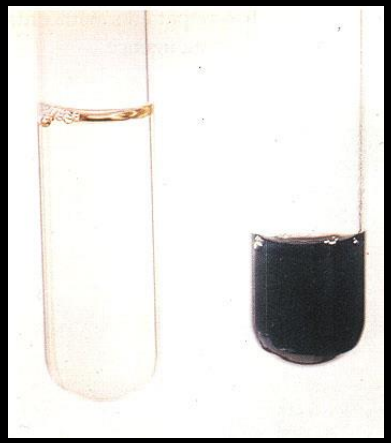
Dr. Robert Guthrie

Pictures Courtesy of Dr. Kenneth Pass

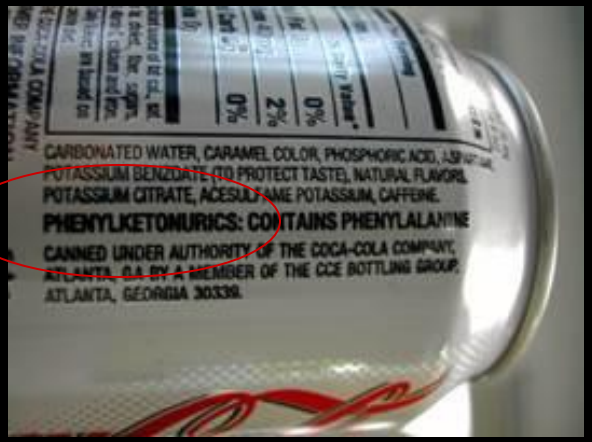
Phenylketonuria

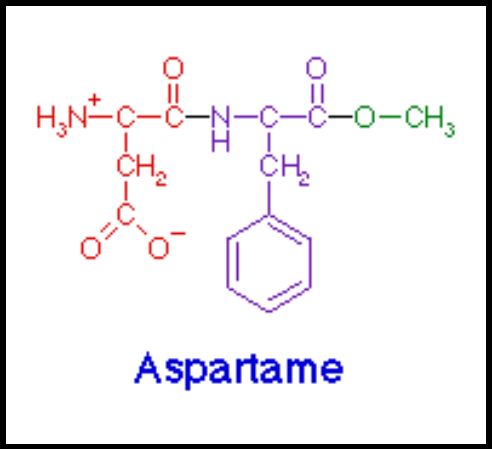






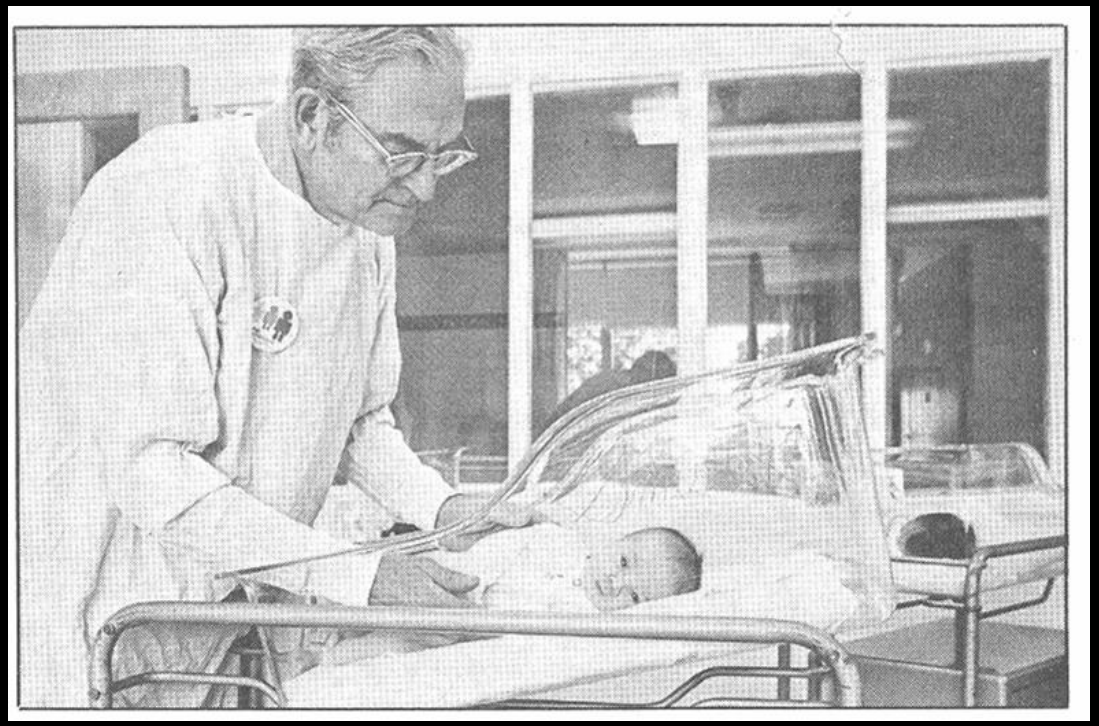
Phenylpyruvic acid
FeCl3 test

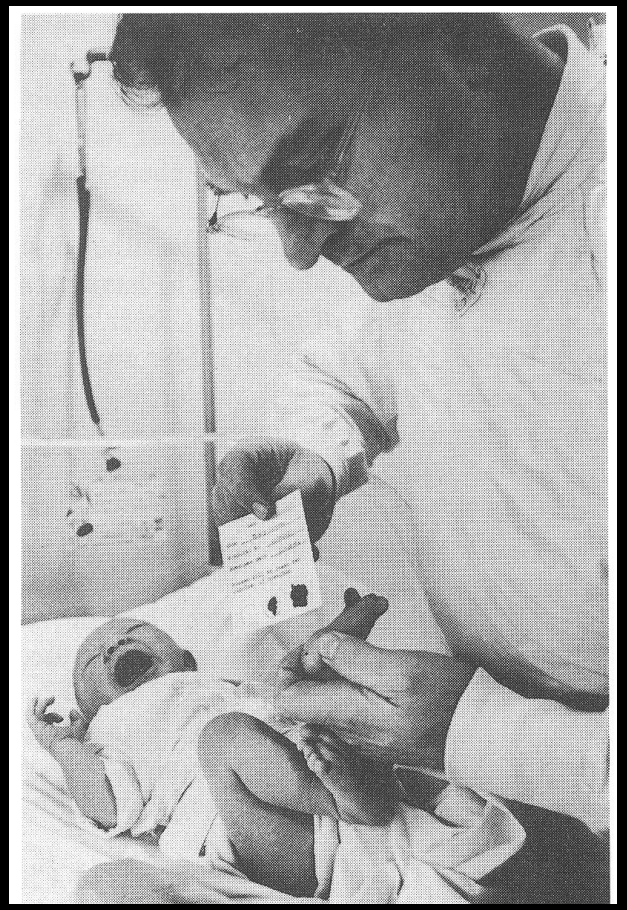




http://138.192.68.68/bio/Courses/biochem2/AminoAcids/AminoAcidCarbonDeg.html

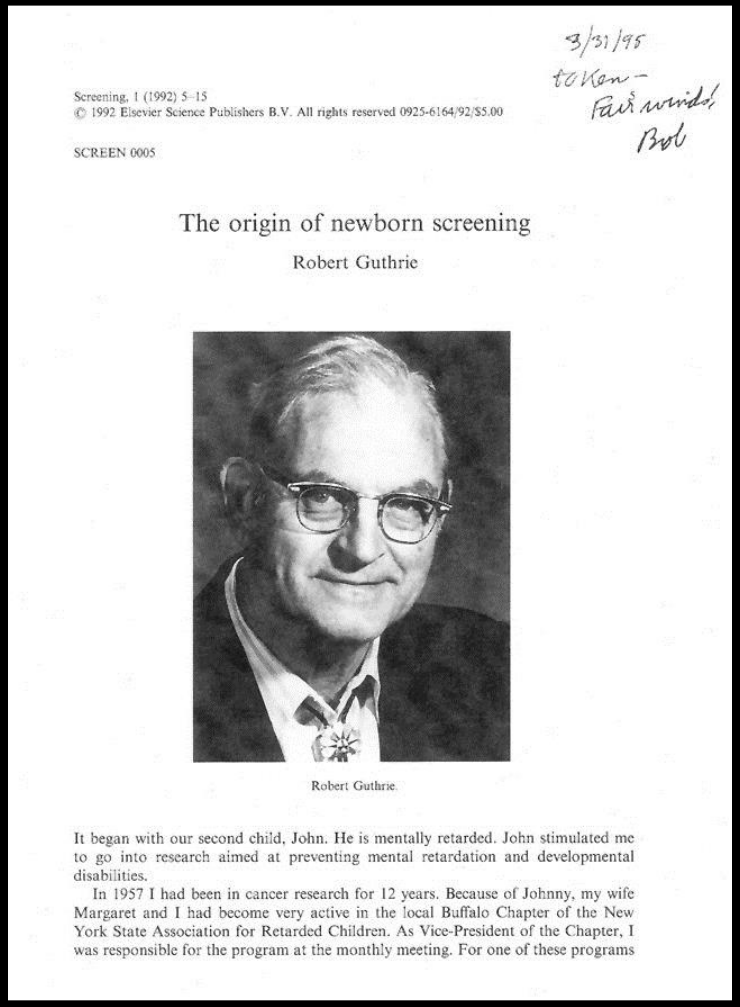
The Perfect Storm Continues





Pictures Courtesy of Dr. Kenneth Pass

Jamestown, New York



3/31/95
to Ken –
Fair winds!
Bob

Screening, 1 (1992) 5–15
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SCREEN 0005

The origin of newborn screening

Robert Guthrie

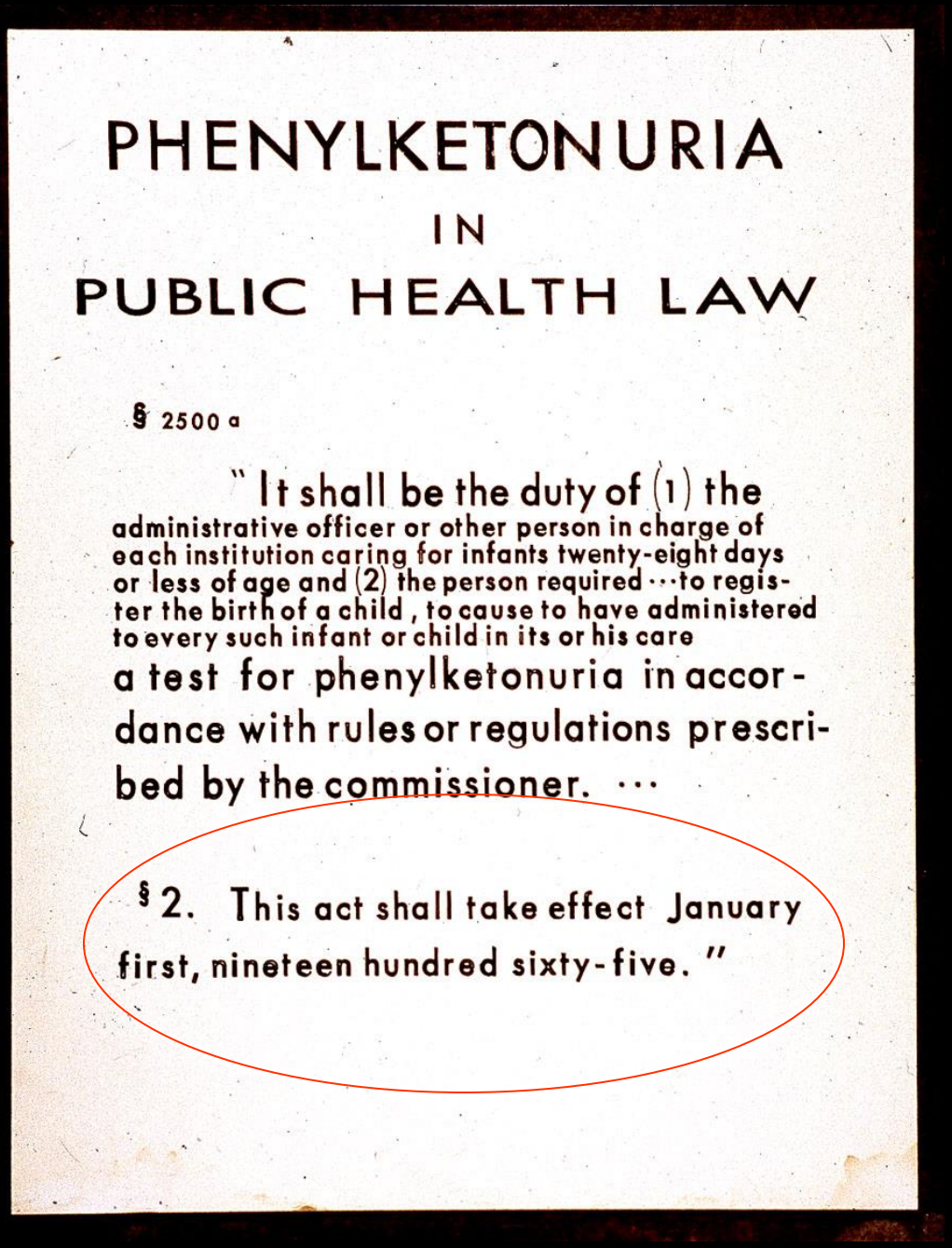
Robert Guthrie

It began with our second child, John. He is mentally retarded. John stimulated me to go into research aimed at preventing mental retardation and developmental disabilities.

In 1957 I had been in cancer research for 12 years. Because of Johnny, my wife Margaret and I had become very active in the local Buffalo Chapter of the New York State Association for Retarded Children. As Vice-President of the Chapter, I was responsible for the program at the monthly meeting. For one of these programs

Picture Courtesy of Dr. Kenneth Pass

- 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”


PHENYLKETONURIA
IN
PUBLIC HEALTH LAW

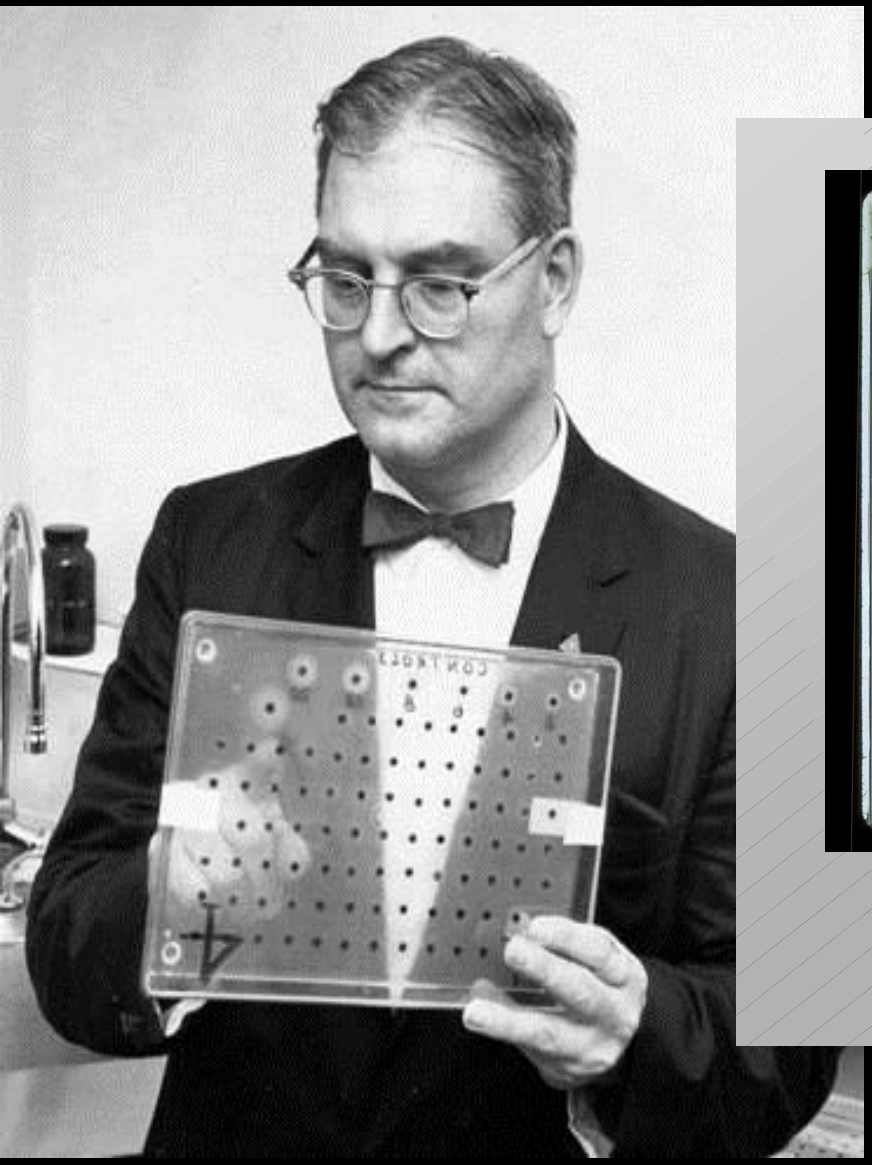
§ 2500 a

"It shall be the duty of (1) the administrative officer or other person in charge of each institution caring for infants twenty-eight days or less of age and (2) the person required ··· to register the birth of a child, to cause to have administered to every such infant or child in its or his care a test for phenylketonuria in accordance with rules or regulations prescribed by the commissioner. ···

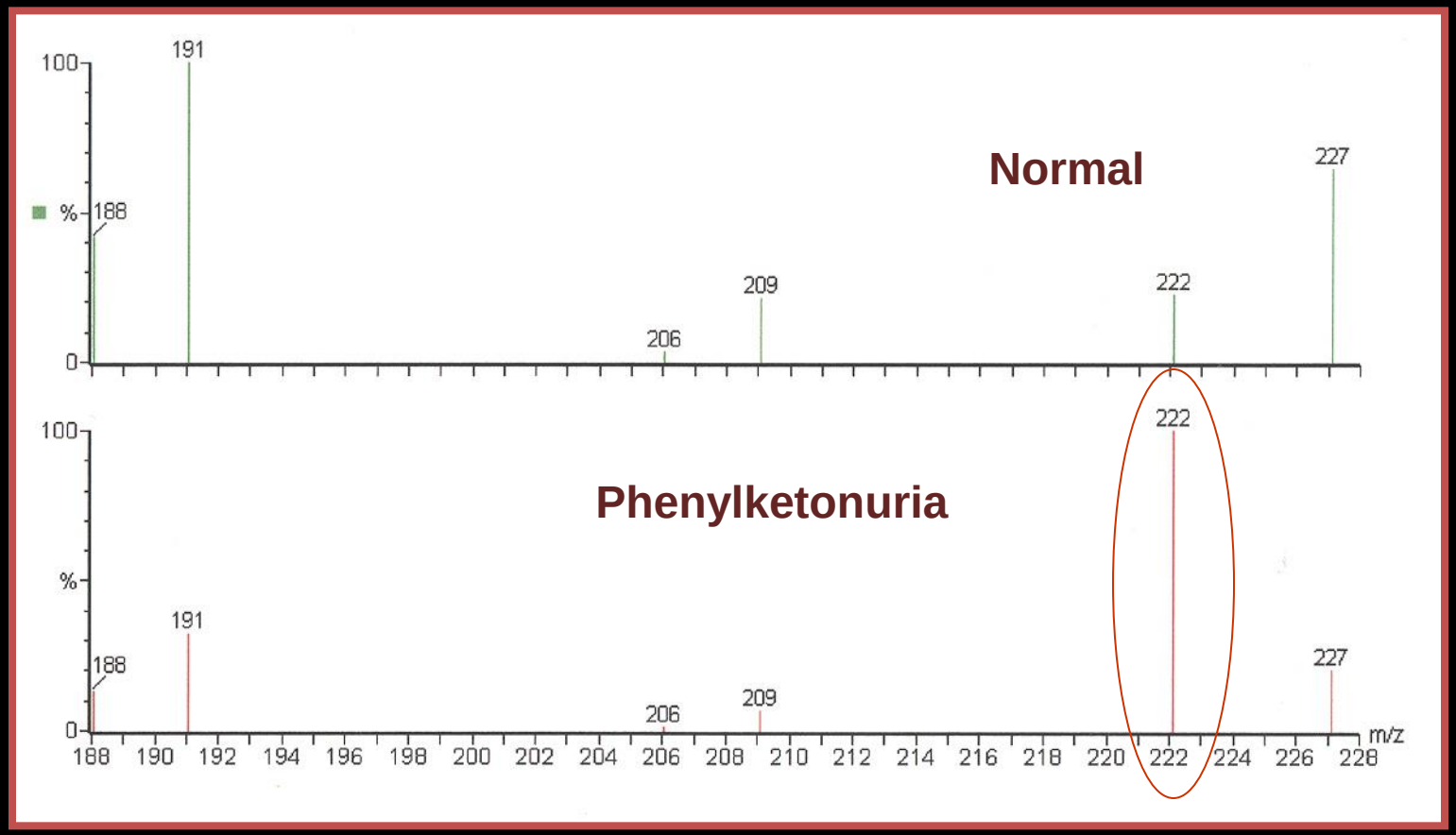
§ 2. This act shall take effect January first, nineteen hundred sixty-five."

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
- 1994 Molecular in Washington (2002 in NY)

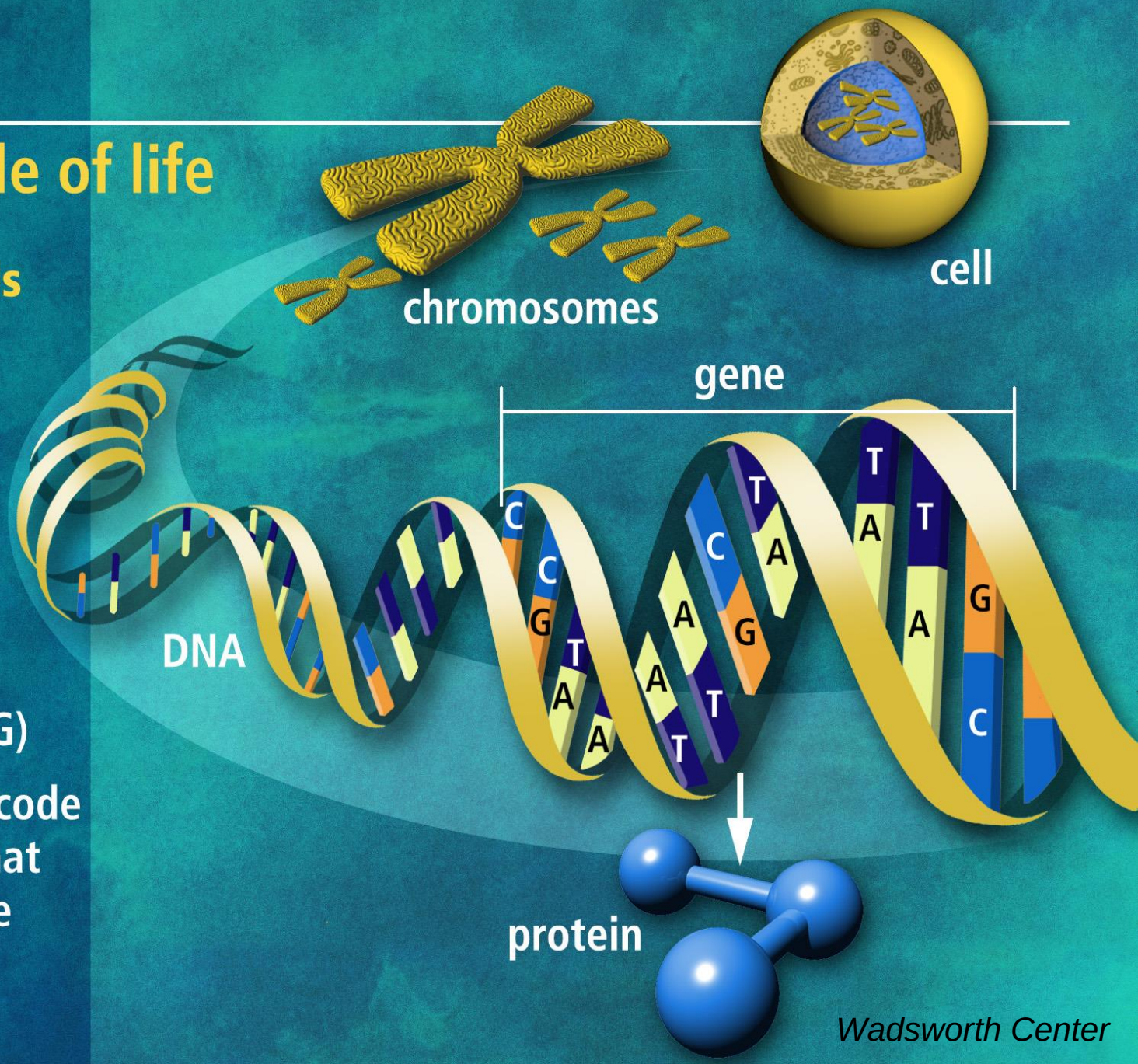
DNA

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



Y-GA 98-090R ORNL

Wadsworth Center

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

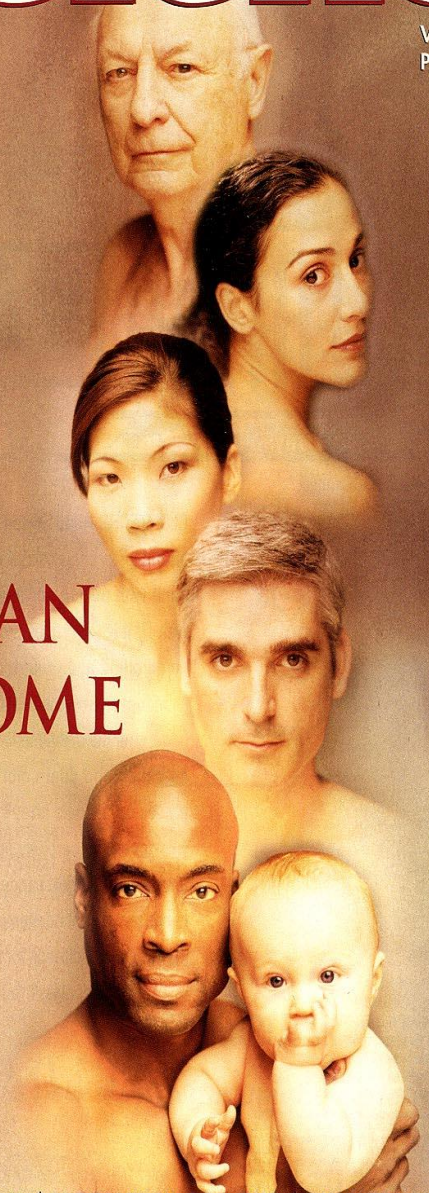
Accurate Sequence Data:

- 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
  chromosome 21 reported 5/00
- Projected finish 2003, original 2005

16 February 2001

Science

Vol. 291 No. 5507
Pages 1145–1434 $9



THE HUMAN GENOME

American Association for the Advancement of Science

15 February 2001

nature

$10.00 www.nature.com

the human genome

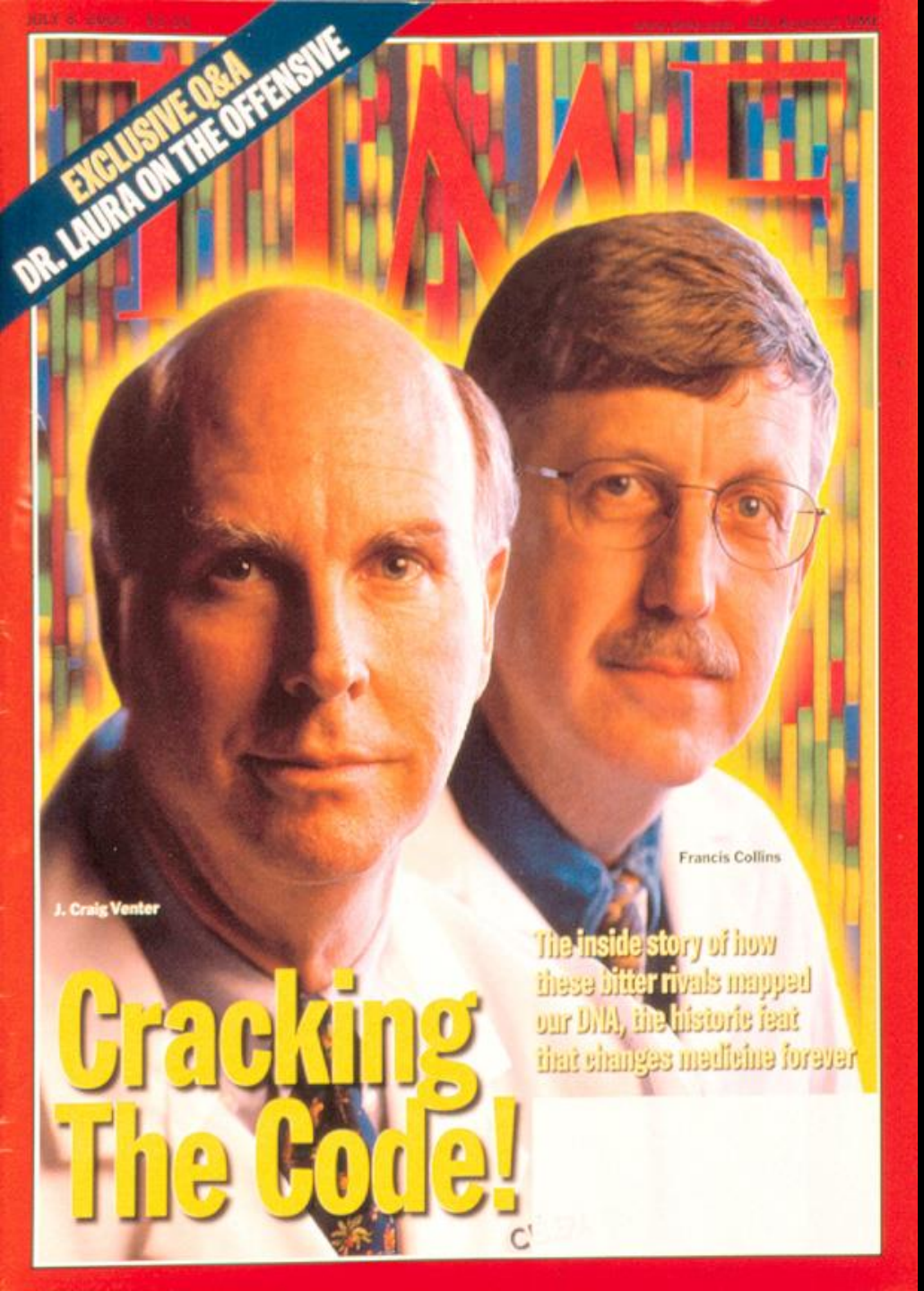
Nuclear fission
Five-dimensional energy landscapes

Seafloor spreading
The view from under the Arctic ice

Career prospects
Sequence creates new opportunities

naturejobs

Wadsworth Center



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

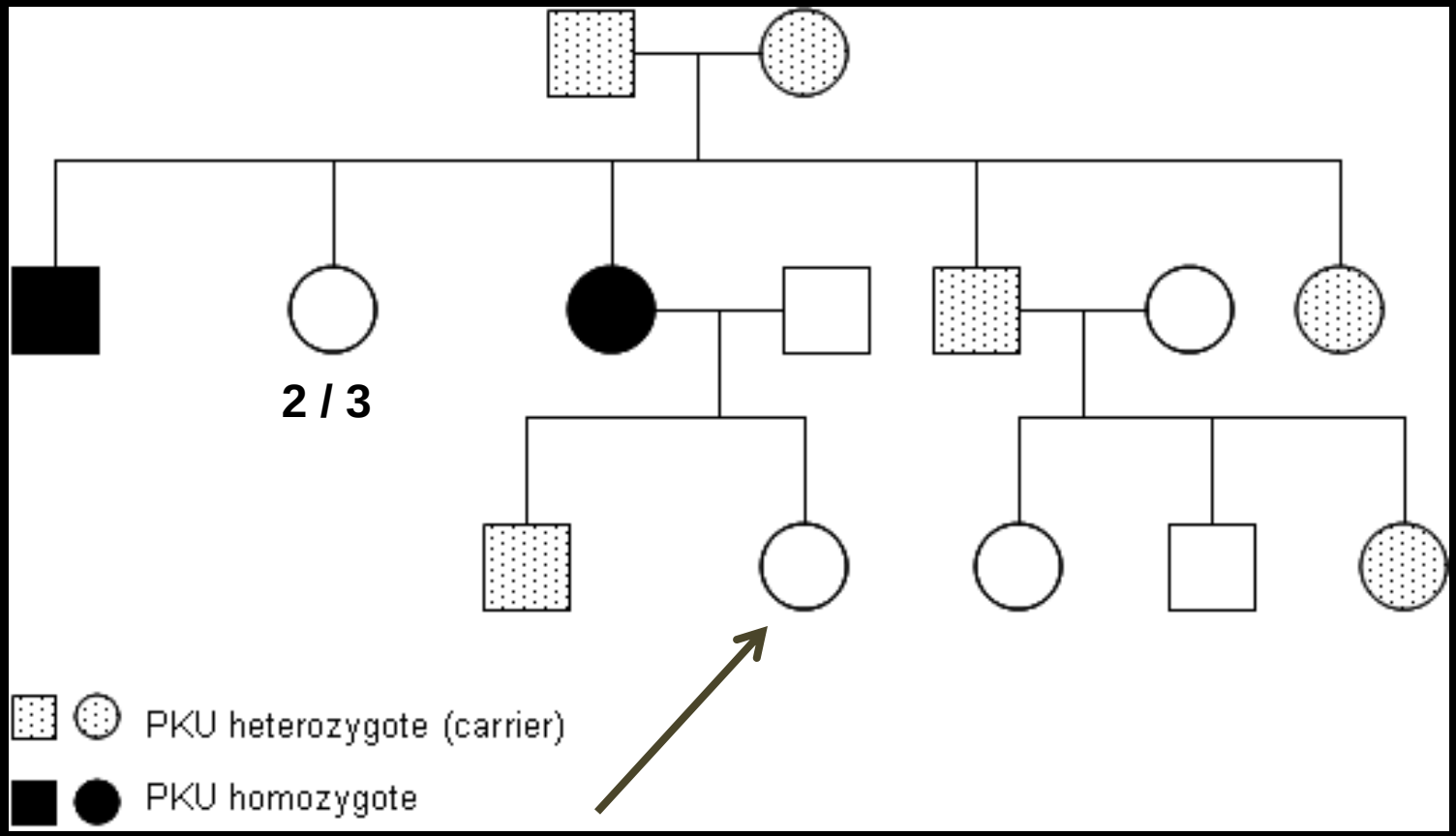
Classes of Single Gene Disorders

- **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

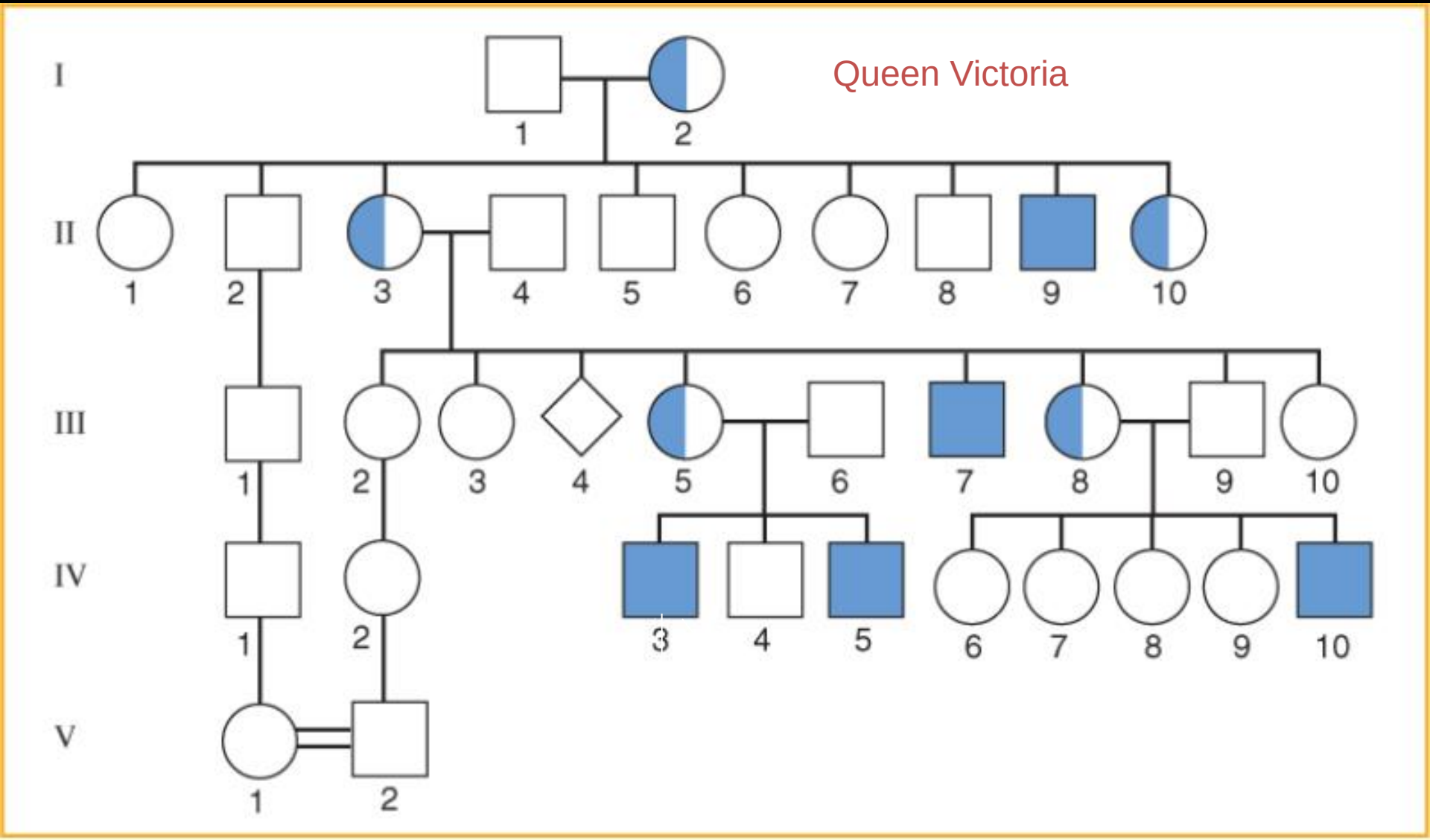
Classes of Single Gene Disorders

- 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



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Molecular Testing for Genetic Diseases

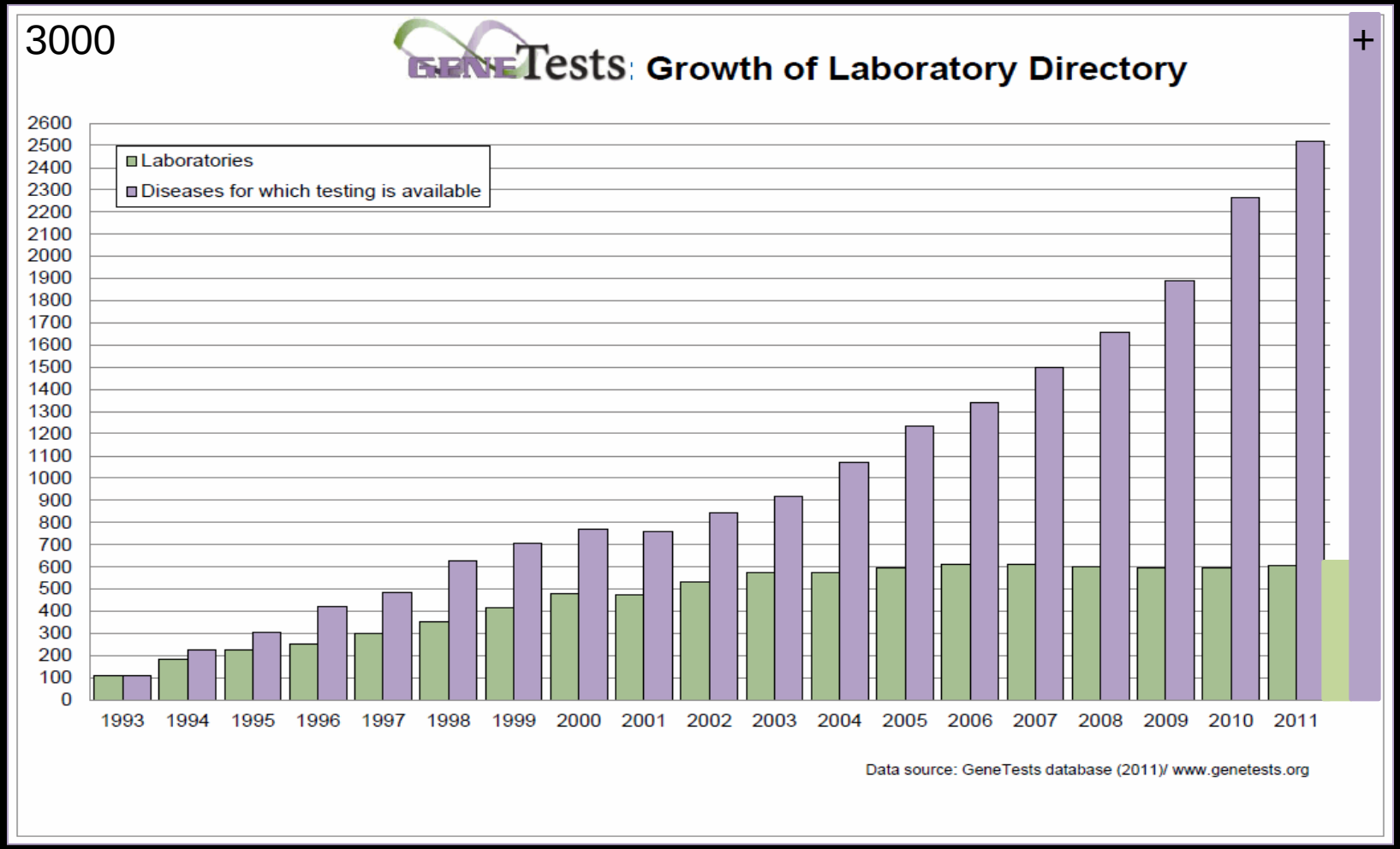
- Enabled by gene mapping to identify location of genes on chromosomes AND ability to differentiate between harmful and neutral mutations
- Identification of disease-causing mutations for:
  - Diagnosis
  - Predictive testing
  - Carrier detection
  - Prenatal screening
  - Preimplantation testing
 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

Availability of Genetic Tests

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



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
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??

OR

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

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 hemoglobinpathies & 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 / Why Use a Molecular Test?

- 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

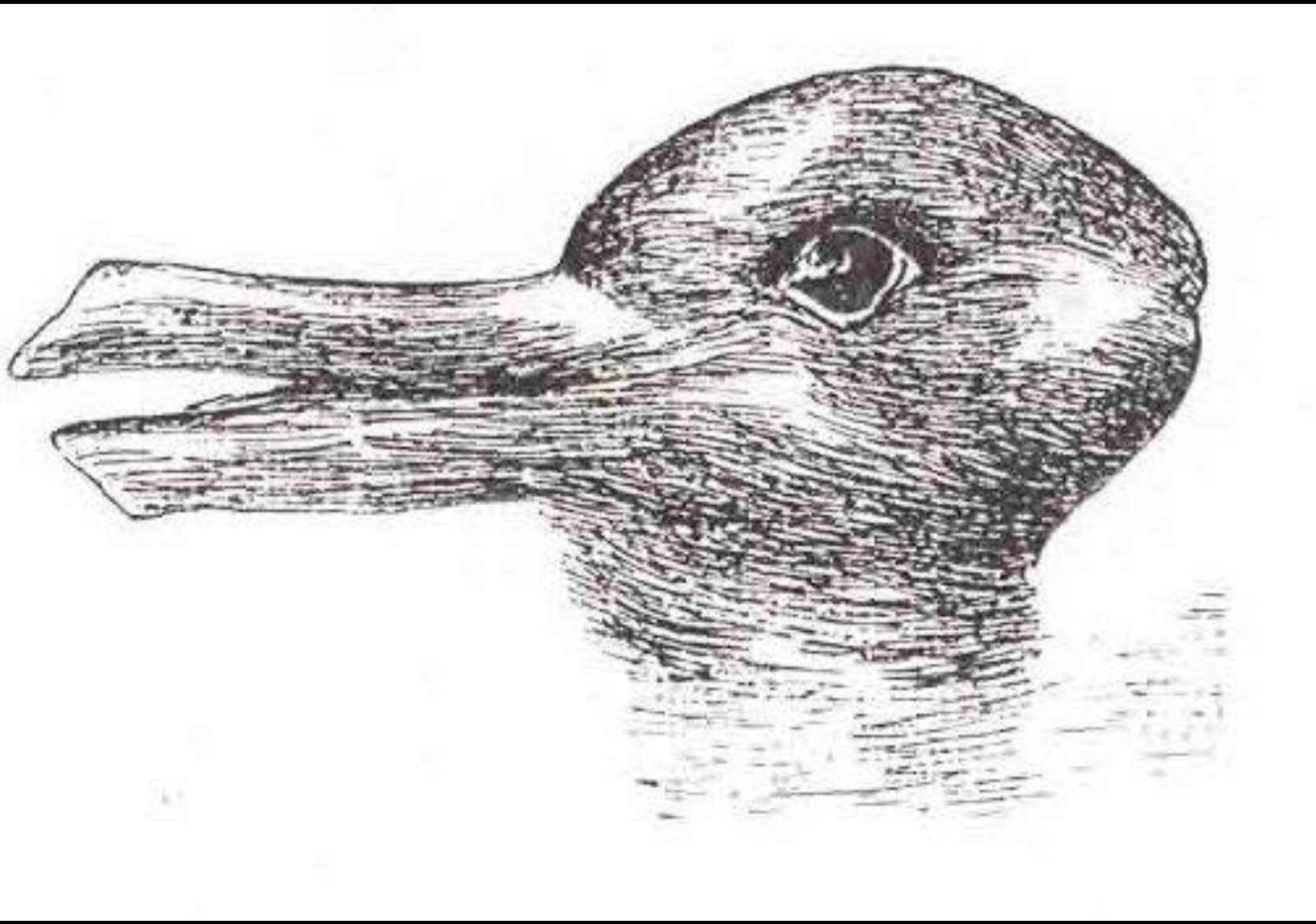
- Which tests will have a molecular component?
- Specimen transport
- Screening or confirmatory?
- Timing and prioritization for contract lab
- Systems integration
- Follow-up integration

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

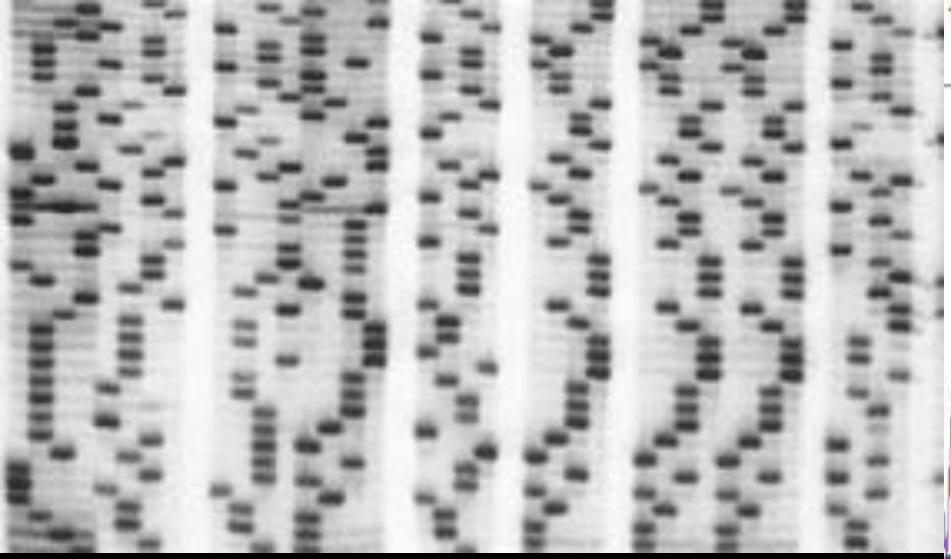


Potential Future Applications of Molecular Testing in NBS

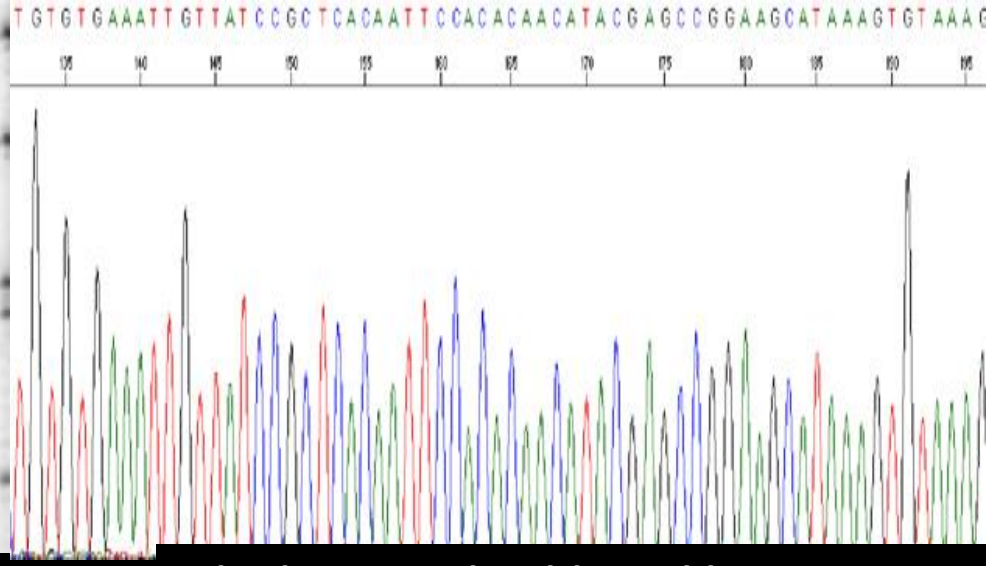
- Expansion to other existing or potential NBS disorders
  - Congenital adrenal hyperplasia (CAH)
  - Biotinidase deficiency
  - Ornithine transcarbamylase deficiency (OTC)
  - Cytomegalovirus
  - Fragile X syndrome
  - Spinal muscular atrophy
  - Duchenne muscular dystrophy (DMD)
  - Other lysosomal 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



Mix deoxynucleotides with
ddA, ddT, ddC*, ddG
1 scan per person/fragment
~800 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: Scientific American - Windows Internet Explorer
http://www.scientificamerican.com/article.cfm?id=perils-of-newborn-screening
File Edit View Favorites Tools Help Convert Select
Favorites Suggested Sites Free Hotmail Web Slice Gallery
turtle - Google Search Perils of Newborn Screen...
Page Safety Tools

50 Like
6 Tweet
1 +1
1 Share
submit reddit

Perils of Newborn Screening

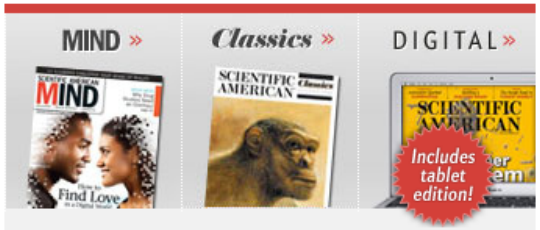
Doctors may be testing infants for too many diseases

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

More from Scientific American



Done Internet 100%

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 gen
- ~30 nov etected in screenir
- Commo
- Variants ce
- One mut ty
- Two mut
- Two mut types
- Parental

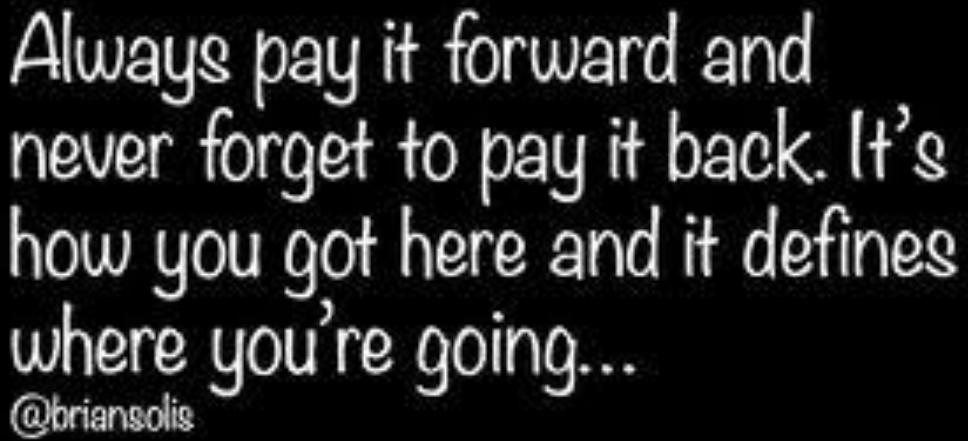
Krabbe Today Mimics CF Yesterday

Improvement of the Literature

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



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


Thank You
Mahalo
Kiitos
Tack
Toda
Grazie
Obrigado
Thanks
Takk
Merci
Gracias

CNN THE WALL STREET JOURNAL. WSJ

Anne Brown worries that someone could gain access to the DNA sample from her daughter Isabel with Isabel's name attached

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??