Exome/Genome Sequencing and Newborn Screening

Advisory Committee on Heritable Disorders of Newborns and Children
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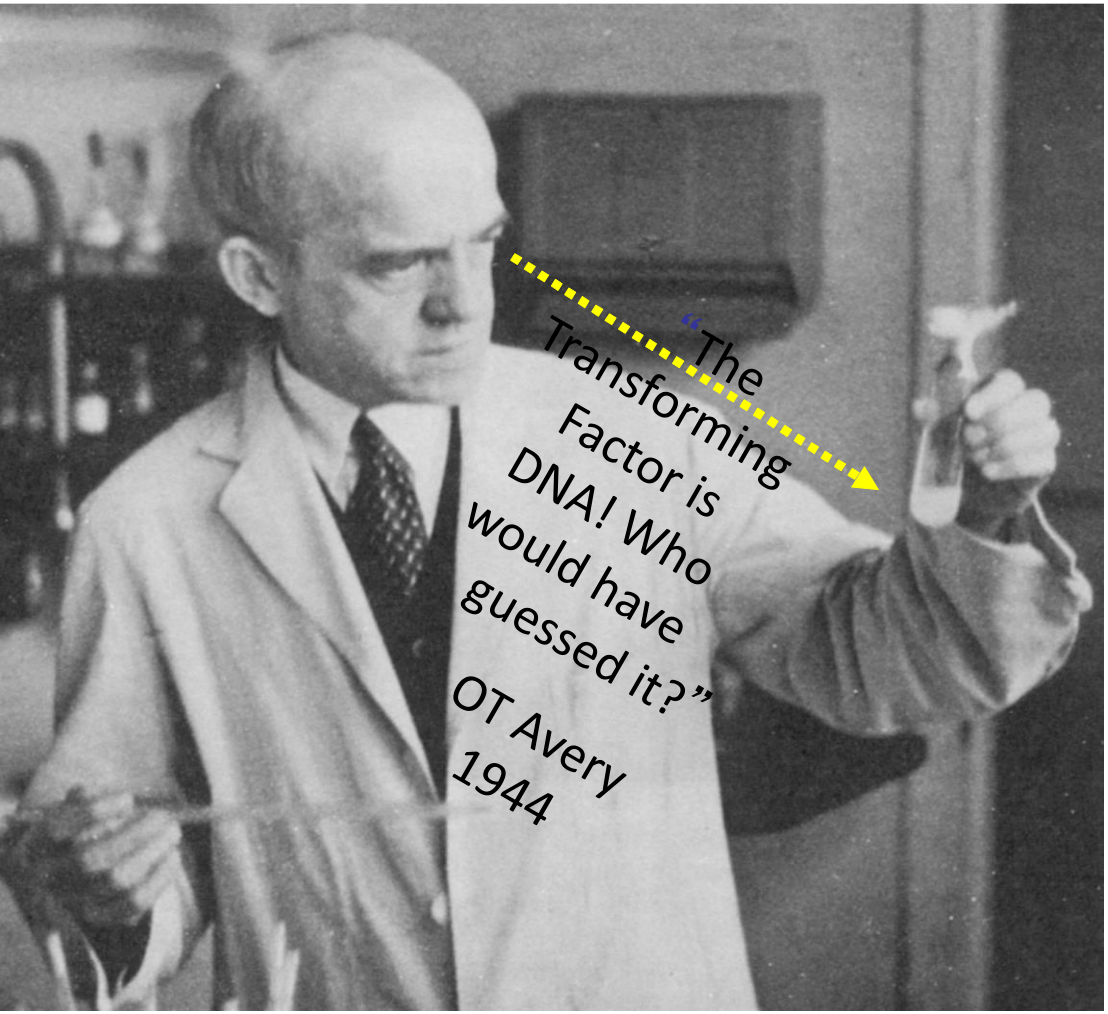
American College of Medical Genetics
20th Anniversary 1991-2011

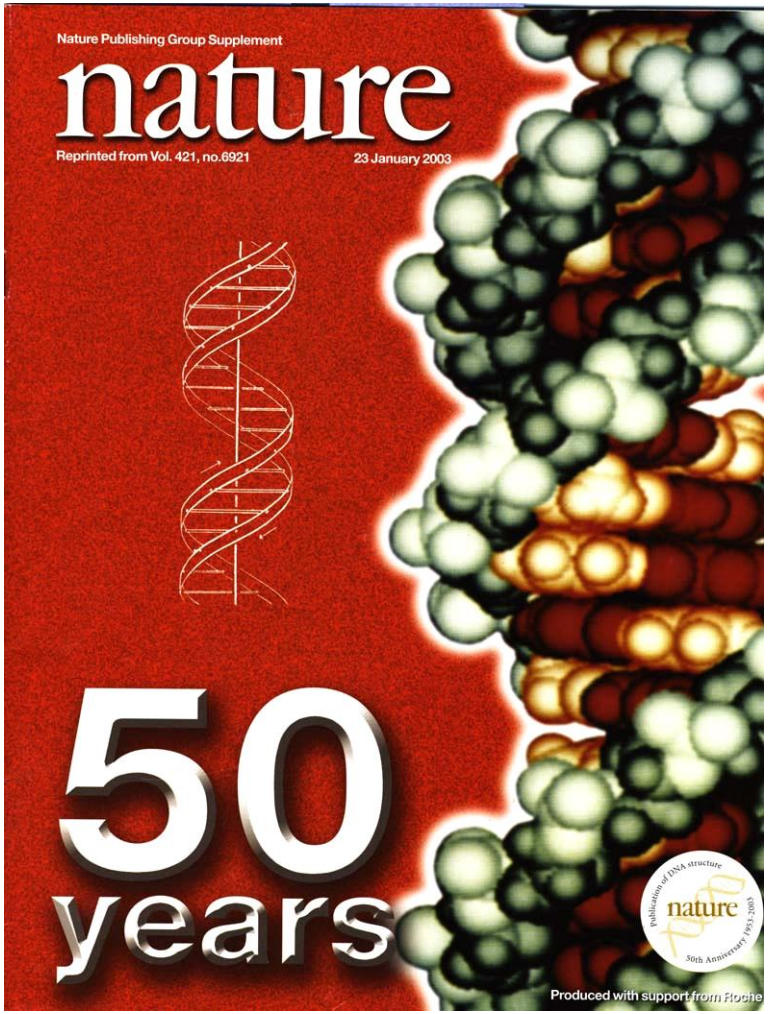
ACMG
American College of Medical Genetics and Genomics
Translating Genes Into Health®

Overview

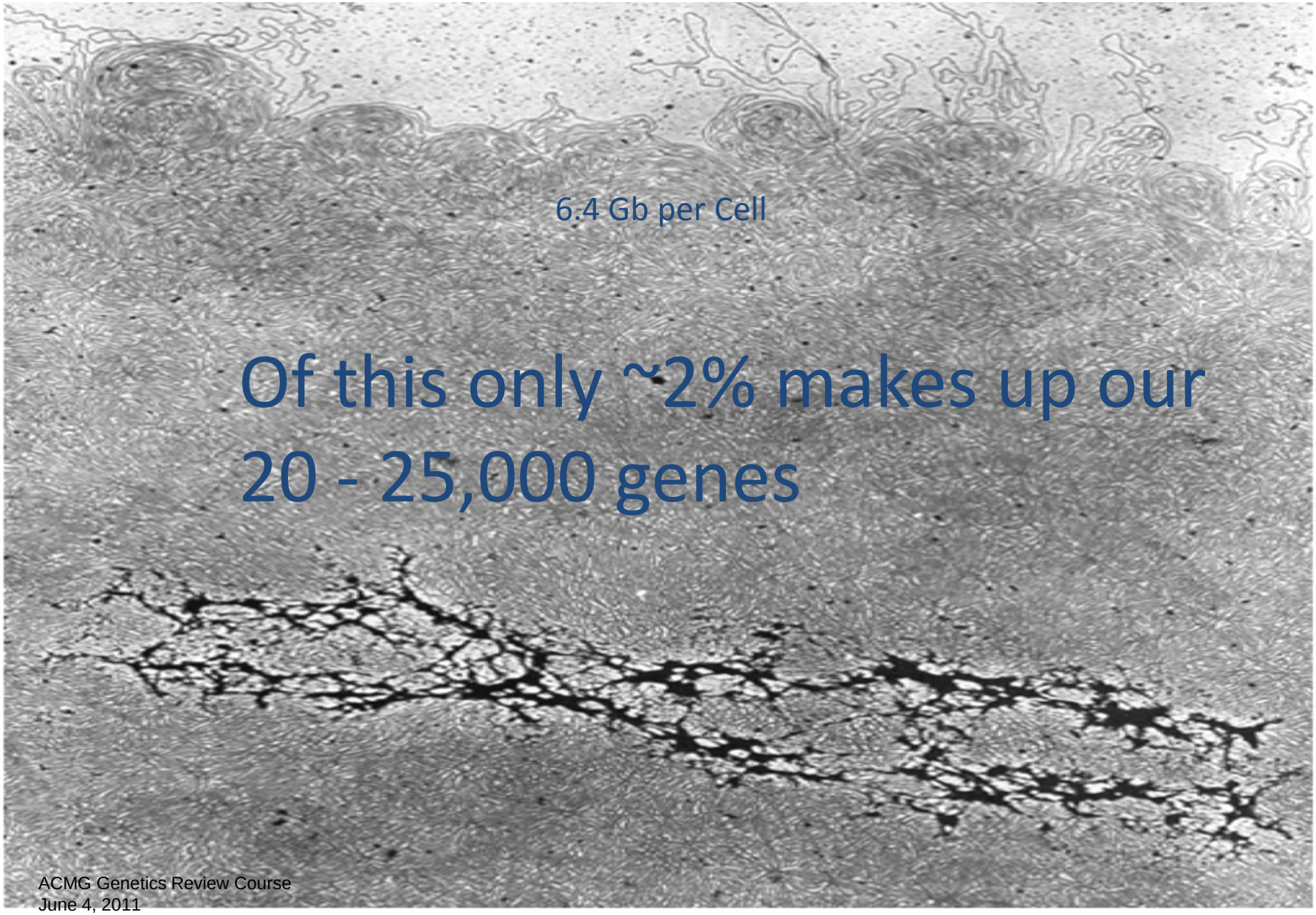
- Genomes
- Basics of sequencing (more clinical than analytical)
- Uses of genome and exome sequencing (GS/ES)
- Research in Newborn Screening and Sequencing

“DNA is Important!” OT Avery 1944





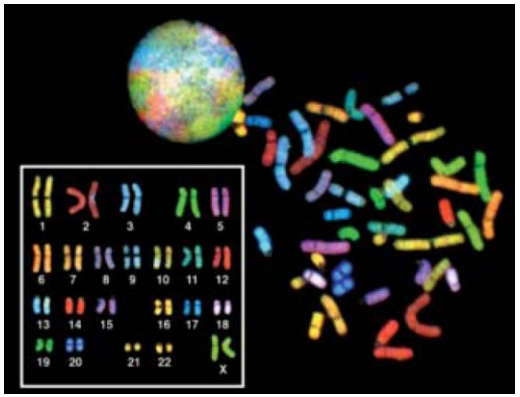
How Much DNA Do we Have?



The Human Genome

"It's a history book – a narrative of the journey of our species through time. It's a shop manual, with an incredibly detailed blueprint for building every human cell. And it's a transformative textbook of medicine, with insights that will give health care providers immense new powers to treat, prevent, and cure disease."

----Francis Collins, Director National Institutes of Health (NIH)



Human Genome

Human Haploid Genome 3.2GB

Diploid somatic cells require sequencing of 6.4Gb

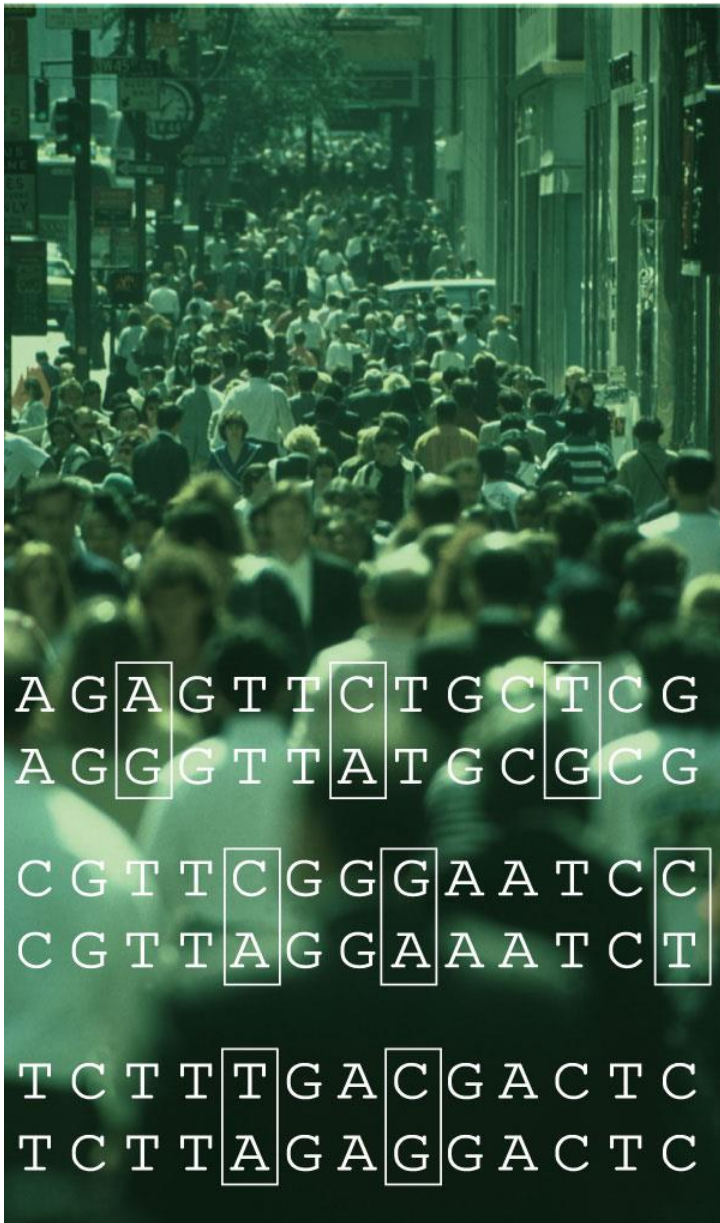
Gigabyte = 10^9 = billion

Megabyte = 10^6 = million

Kilobyte = 10^3 = thousand

Genomic Variation

- No “normal” genome sequence
- Everyone carries millions of differences in DNA sequence
- Requires informatic filtering to find variants of clinical significance
- Differences contribute to risk for disease and drug response



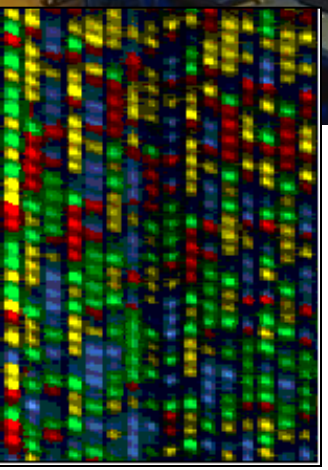
Genomic Variation

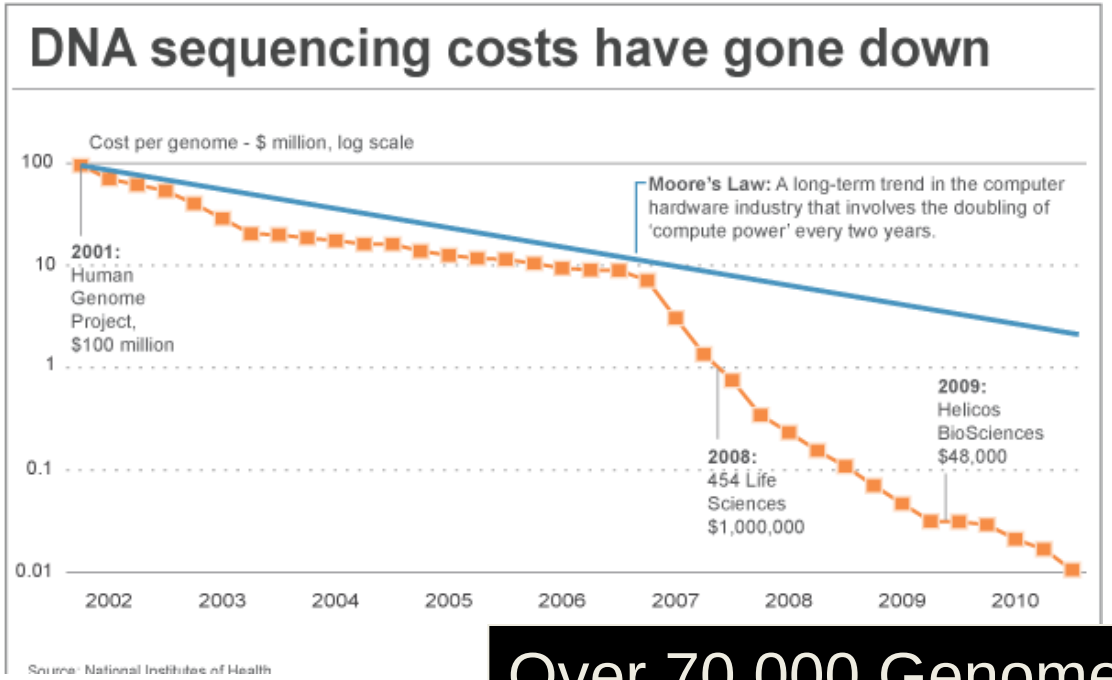
- ~3.8 million variants per person
  - 3.1 million are known common variants
  - 0.6 million are rare or novel variants
  - 400 genes per person have rare or novel nonsynonymous variants at conserv ed loci
    - 136 predicted to be deleterious
  - Huge level of inaccuracy in genome variation annotation databases

Technology Advancements

1000 Genomes

A Deep Catalog of Human Genetic Variation

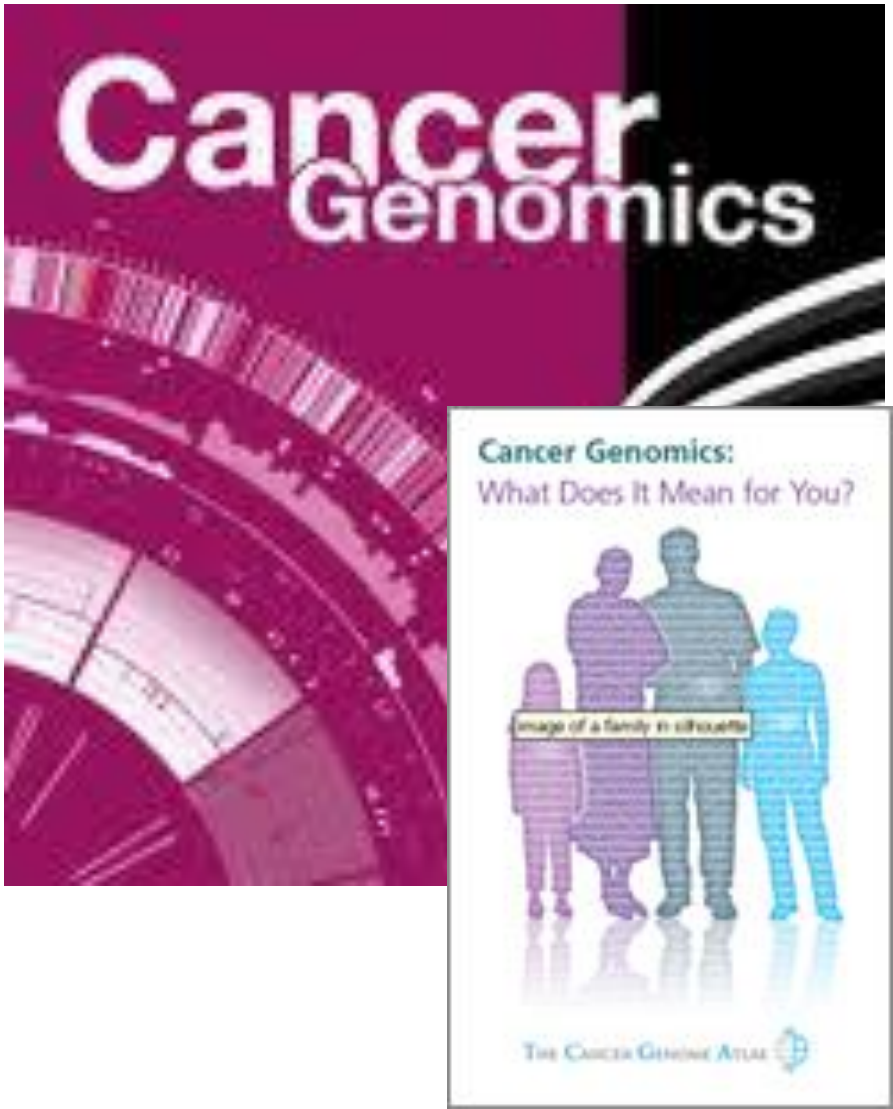




Over 70,000 Genomes

Cancer Genome

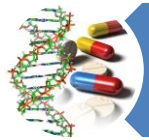
- The DNA in cancer cells differs from DNA in non-cancer cells
- Influences cancer onset, progression, response to therapy, prognosis
- Understanding these changes is important



Genetic Testing Across the Lifespan

Newborn Screening

Diagnostic



Treatment Choice

Presymptomatic

Predispositional

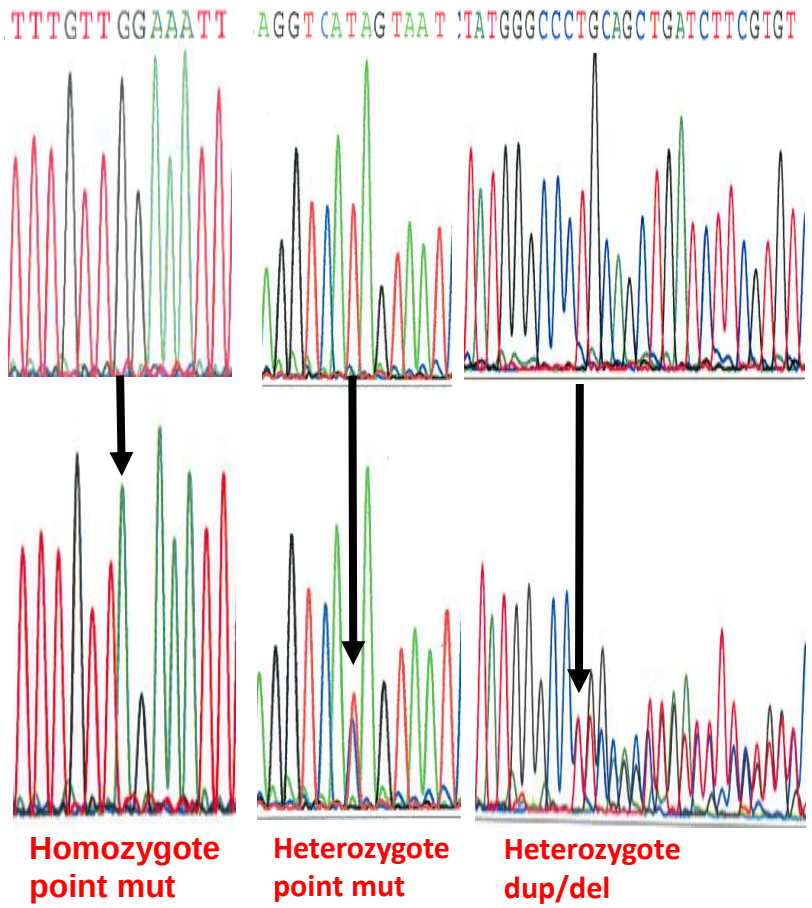
Sequencing

- First Generation
  - Classical sequencing (e.g, Sanger, Maxam Gilbert)
- Next Generation
  - Massively parallel sequencing that blends two disruptive forces, DNA sequencing and Information Technology
- Third Generation coming
  - Single strand sequencing (e.g., nanopore

Classical Sequencing

- Low throughput
- Relatively high cost
  - 1985 – 1 base for $10
  - 2005 – 10,000 bases for $10
- Amplify one amplicon from one sample and sequence it
- 1000 bp per run (parallel can improve reads over time)
- Difficult to detect mosaicism

Dideoxy Sequencing



Next Generation Sequencing (NGS)

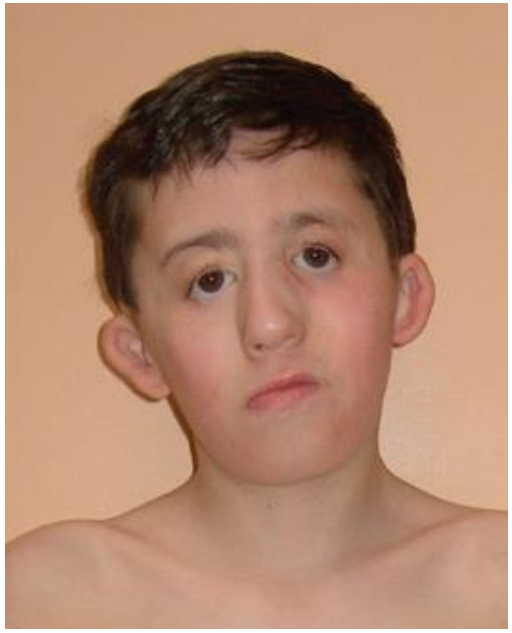
- <$1,000 per exome; $5,000 per genome
- Diverse chemistries that can amplify everything, sequence it many times in parallel, and integrate the data
  - Requires PCR
- NGS Bioinformatics
  - Sequence alignment against a reference sequence
  - Base calling and variant detection
  - Filtering to identify important variants

Whole Exome Sequencing (WES)

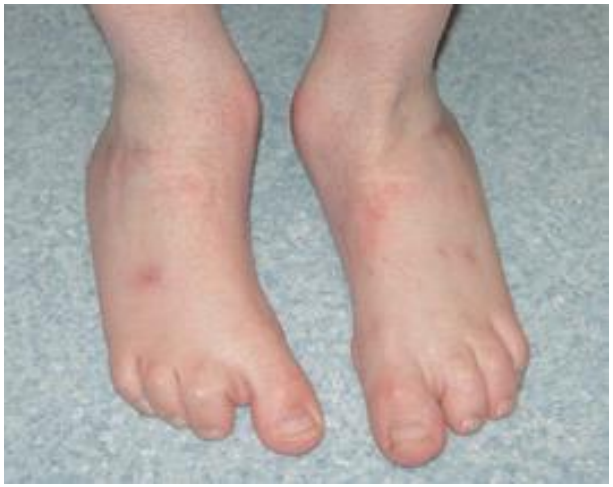
- ~1% of genome is in protein encoding exons that contain ~85% of known mutations
- WES detects SNPs, synonymous, nonsynonymous, nonsense; some splicing & in/del variations
- Since detect many variations from WES must use informatics ie conservation & SIFT, co-segregation & function to prioritize candidates

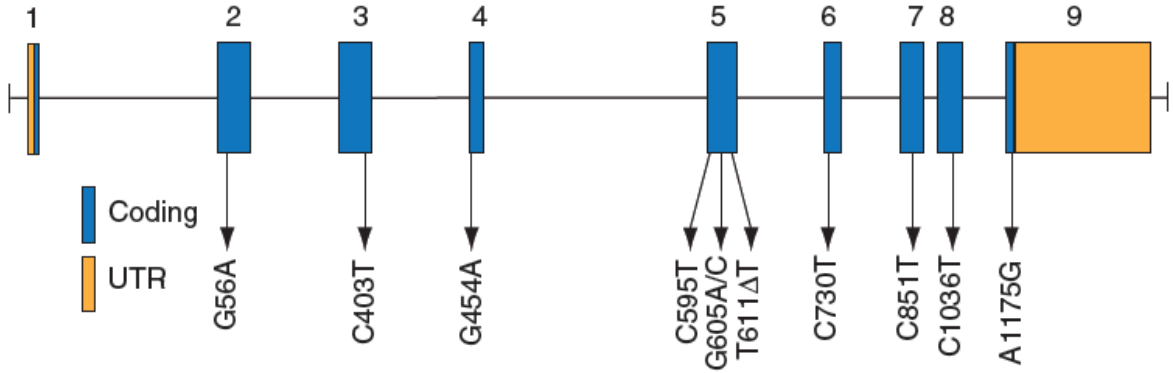
Whole Exome Sequencing (WES)

Ng et al Nat Genet 42: 30-36, 2010



Filter	Fam 1	Fam 1+2	Fam 1+2+3
NS/SS/I	2,362	1,810	1,525
Not dbSNP129	53	25	21
Not HapMap8	46	7	4
Neither	9	1	1
Predict Damaging	1	0	0





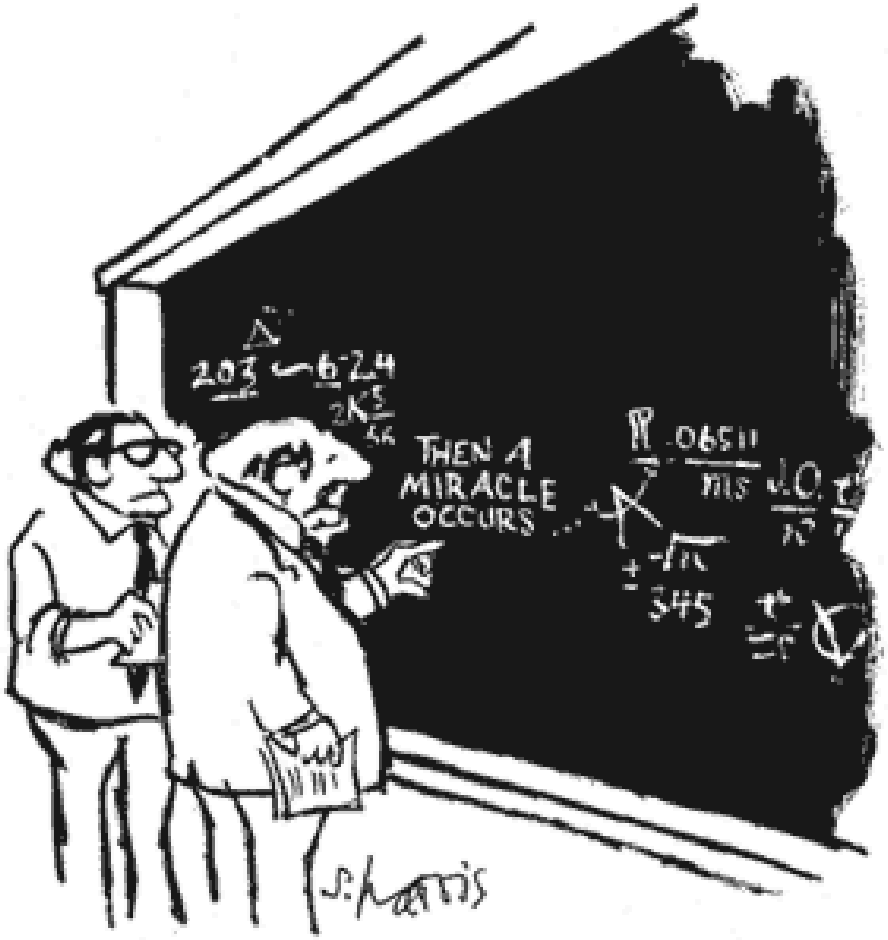
DHODH: 10 missense & 1 bp del in 6 Kindreds

Third Generation Sequencing

- Coming quickly
- Single strand sequencing; no amplification
- Several chemistries being used

- Still limited by interpretation

$1000 Personal Genome



"I THINK YOU SHOULD BE MORE EXPLICIT HERE IN STEP TWO."

Applications of Next-Generation Sequencing

Type of Sequencing	Application
De novo	New Species/Strains
Emergency, public health	SARS Virus, Bioterrorism
Ancient DNA – Extinct Species, Pathogens	Evolutionary Biology, Epidemiology
Resequencing/Genotyping	Disease Susceptibility,/Diagnosis Carrier Status, Pathogens, Cancer
Deep Sequencing	HIV Mutations in Single Patient, Scan for Mutations in Tumor
Chip-Seq	Epigenetics
RNA Sequencing	Gene Expression

Current Clinical Uses of GS/ES

- Exome is the part of the genome we understand whether targeted chemically or informatically
- Diagnostic
  - Multigene panels (cardiomyopathy, hearing loss)
  - Nonspecific phenotypes in undiagnosed patients
- Screening
  - Carrier
  - Noninvasive prenatal (cell free fetal DNA or fetal cell based)
  - Preimplantation genetic screening

Current Uses of Sequencing in NBS

- Multigene diagnostic panels for screen positives for physiological or phenotype based screens (i.e., hearing, T-cell lymphopenia, CCHD)
- Research to identify genetic modifiers of penetrance and expressivity
- Research into if and/or where GS/ES fits into NBS

Human Genome Project Guilt

- $3 billion+ Human Genome Project and a $1 million dollar interpretation

- Research and clinical investigation to understand and improve clinical utility of sequence data is rapidly expanding

New Project

Genomic Sequencing and Newborn Screening Disorders

NIH *Eunice Kennedy Shriver* National Institute of Child Health and Human Development

DEPARTMENT OF HEALTH & HUMAN SERVICES · USA

- The *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), the National Human Genome Research Institute (NHGRI), and the NIH Office of Rare Diseases Research (ORDR) sponsored a workshop, *Newborn Screening in the Genomic Era: Setting a Research Agenda*.

- The purpose of the meeting was to identify elements of a trans-NIH research agenda that would lead to the application of new genomics concepts and technologies to newborn screening and child health.

- The meeting was attended by experts from academia, industry, and federal agencies in the fields of newborn screening (NBS) and genomics

- Chaired by Drs. David Valle (Johns Hopkins, University) and Piero Rinaldo (Mayo Clinic).

https://www.genome.gov/Pages/PolicyEthics/StaffArticles/Newborn_Screening_Meeting_Summary.pdf

NBSTRN
Newborn Screening Translational Research Network

NBSTRN and Sequencing

- Supporting and adapting NBSTRN tools to grantees work
  - ELSI Workgroup
  - VRDBS as source of true positives or new clinical groups for research
  - Longitudinal Pediatric Data Resource (LPDR) to aggregate clinical data
  - Finalizing development of ‘Harvest’ integration engine that allows clinical data in LPDR to be integrated with GS/ES data to identify genomic modifers

The Interpretive Challenge

- Associating sequence variation with disease vs. predicting implications of sequence variation in asymptomatic people
- Penetrance and ascertainment
  - Current data is biased towards sick people
  - Moving from clinically biased data to general population data is next major hurdle

Can we depend on *in silico* analysis?

- Short-read sequence alignment algorithms
  - Only 55! (e.g. Bowtie, BWA, Novoalign, MOSAIK)
- Evaluation of disease-causing potential of sequence alterations:
  - PolyPhen2, SIFT, Mutation Taster, SNAP, Panther, Pmut, Phlyo P, BDGP, GeneSplicer, SoftBerry, ASPic, GERP
- Do they all agree with each other?
- Can we rely on a composite score?

Interpreting Genomic Sequence

- What is “normal”?
- Current mutation/variation databases are a mess
- New efforts
  - Clinically Relevant Variant Resource (CRVR)
    - develop a resource for the identification and dissemination of consensus information on genetic variants relevant for clinical care
  - 2013 Interpretation of Sequence Variant Workshop
    - ACMG, AMP, CAP
    - propose standard terms and an evidence-based strategy for classifying sequence variants

Deciding what to report: The issue of "secondary" findings

- AKA "incidental findings" - reporting is currently being debated. Could include:
  - Mutations related to late-onset disorders where medical intervention/life-style intervention is available
  - Mutations related to late-onset disorders where no intervention is available
  - Identification of carrier status for recessive disorders
  - Variants related to drug metabolism

NICHD/NHGRI NBS and Sequencing Grant Program

The Trade-offs for use of ES/GS in NBS

- Can look at many more disease associated markers
  - Many don't fit into the current paradigm for why we do NBS
- Not all variants are well enough understood to be used as NBS targets
  - Won't find everyone with a particular disease or risk of developing disease
- Could shift functional screens to second tier to identify those most likely to cause disease

NICHD/NHGRI Newborn Screening and Genome Sequencing Grantees

- Three major components of program
  - Genomic sequencing and anaysis
  - Research related to patient care
  - ELSI issues in use of genomic information in newborn period
- Funding is $25 million over five years to four awardees

Grantee 1: Brigham and Women's Hospital, Boston, MA

- P.I.s: Robert Green, MD and Adam Beggs, PhD
- 450 consented newborns sequenced with data available as a resource for parents and doctors throughout infancy and childhood
  - Genetic counselor returns sequencing and NBS results to families
  - Parents asked about impact of receiving the results and if they respond differently to receiving results as compared to current NBS results
  - Develop a process for reporting results to the newborns' physician and investigate how they act on it.

Grantee 2: Children's Mercy Hospital – Kansas City, MO

- P.I.: Stephen Kingsmore, MD
- Will use GS/ES to test newborns in the NICU to provide rapid diagnosis. Studies will assess:
  - Risks and benefits of using sequencing in newborns in the NICU
  - The ability to reduce turn-around-time to results to 50 hrs.
  - Whether sequencing increases the number of diagnoses or decreases the the time it takes to reach a diagnosis in NICU newborns
  - Parents' perception of the benefits and risks associated with the results changes over time

Grantee 3: University of California, San Francisco

- P.I.: Robert Nussbaum, MD
- To explore the potential for exome sequencing as a method of NBS of disorders currently screened for and others not currently screened for, but with potential benefit. They will:
  - partner with California NBS Program to assess 1400 children previously screened by classical NBS to see what this contributes
  - offer genetic testing to patients in a UCSF immune system disorders clinic to determine if it adds to SCID screening or allows other immune diseases to be screened
  - Provide pharmacogenetic testing to reduce adverse drug effects
  - develop a participant protection framework for conducting genomic sequencing during infancy and will explore legal issues related to using genome analysis in newborn screening programs.

Grantee 4: University of North Carolina at Chapel Hill

- Cynthia Powell, MD and Jonathan Berg, MD, PhD
- Will sequence the entire genome of 400 infants to determine what useful clinical data can be acquired through the tests. In conjunction with the testing, the UNC team has partnered with Research Triangle Park-based RTI International to develop educational and consent tools to determine how best to educate parents and physicians about the test and its results. Studies will:
  - include infants with health infants and infants with conditions such as PKU, CF, or other disorders of metabolism
  - assess best ways (e.g., ELSI issues) of returning results to doctors and patients
  - develop tools to help parents understand meaning of results and additional challenges physicians may face.
  - include special focus on multicultural families and diverse populations.

Related NIH Research

- Clinical Genome Project that includes
  - Clinically Relevant Variant Resource (CRVR) (U01 grantees)
  - U41 group (ICCG related) to capture patients' variant and clinical data for analytical and clinical curation by CRVR
  - ClinVar (NCBI)
- Clinical Sequencing Exploratory Research (CSER) program to assess integration of sequencing into clinical care
- Return of Results Consortium assessing issues associated with return of results of different types

Critics will even question whether we should learn how best to use new technologies

- “There’s great danger in sequencing newborns who have no say in the matter and whose parents may really have no clue what a Pandora’s Box they’re opening for themselves, their child, their future and their relationship,” says Twila Brase, president and co-founder of Citizens’ Council for Health Freedom in St Paul, Minnesota.
  - Points to the importance of this research program

The Promises

- Improve ability to make molecular diagnoses
- Understand the range of phenotypes associated with individual genes
- Guide individual patient care
- Provide options for family planning
- Open doors to new avenues of research
- Increase our understanding of human genetic variability