Updates on Genetic Technologies: It is Time for the Miracles!

Owen Phillips, MD, MPH

Disclosures

- None

Objectives

1. Learn where we are by following a little boy born with a rare genetic disorder: from dying to a cure

2. Technologies and disorders
    A. Gene sequencing
    B. Crispr Cas 9/Base editing
    C. In utero treatments


A little boy is born
KJ Muldoon
June, 2024
in Philadelphia, PA

Mom G3P2

No family history of genetic disease

Low risk obstetrical care

KJ born at 35 weeks

In first 48 hours

- Lethargy
- Respiratory Distress

- Blood ammonia level 1703 micrograms/dl (9-33)
- Elevated glutamine
- Thought to a urea cycle defect, but all common ones tested for were negative

- Blood drawn for whole genome sequencing.

Genetic disease

- More than 30 million people in the United States have one of more than 7,000 genetic diseases.
- Most are so rare that no company is willing to spend years ($$$) developing a gene therapy so few people would need.
- Genetic disorders in general are most common cause of pediatric hospitalizations
- Less than 5% have FDA-approved treatments

How we diagnose genetic disease has changed

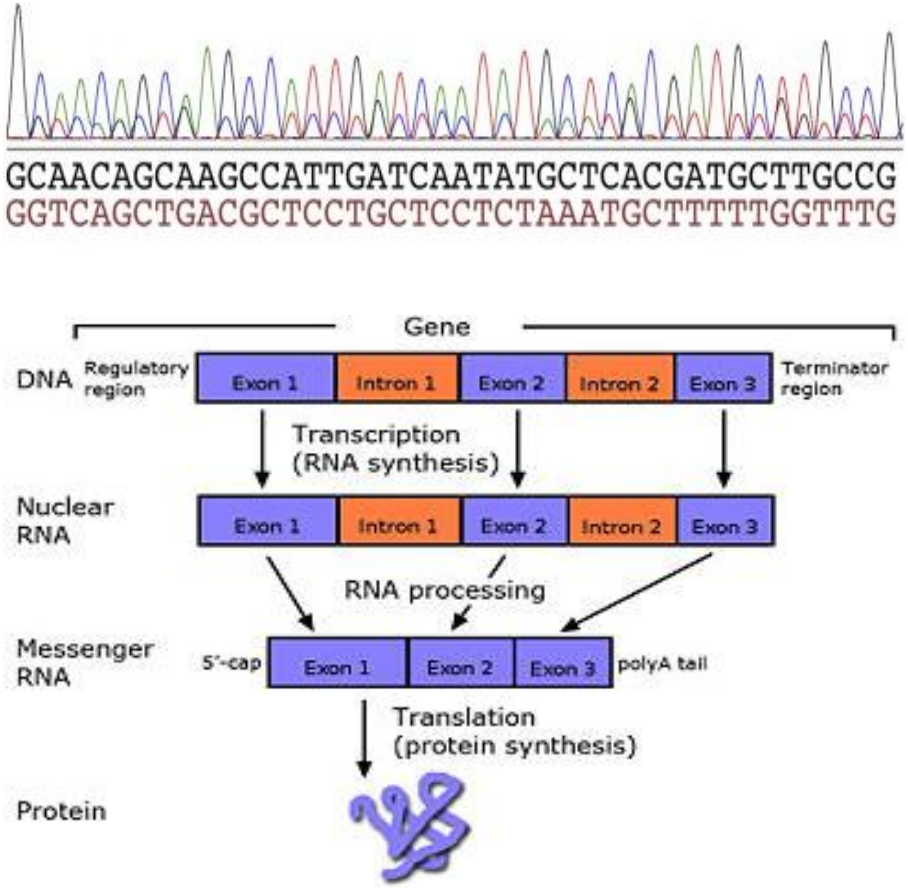
- Historically, physical exam of a child, detailed family history, determining pattern of inheritance and guess at a diagnosis.

- Next- testing for a gene that might be responsible for that disease (if that disease had been linked to a gene)
- Prenatal testing would be possible only when the mutation and the gene had been determined in the family.

- The Diagnostic Odyssey:
  - In pediatrics: trial and error
  - In Ob-Gyn: takes time

The Genome

- 3. 2 billion base pairs
- 40,000+ genes
- Coding for our vital proteins



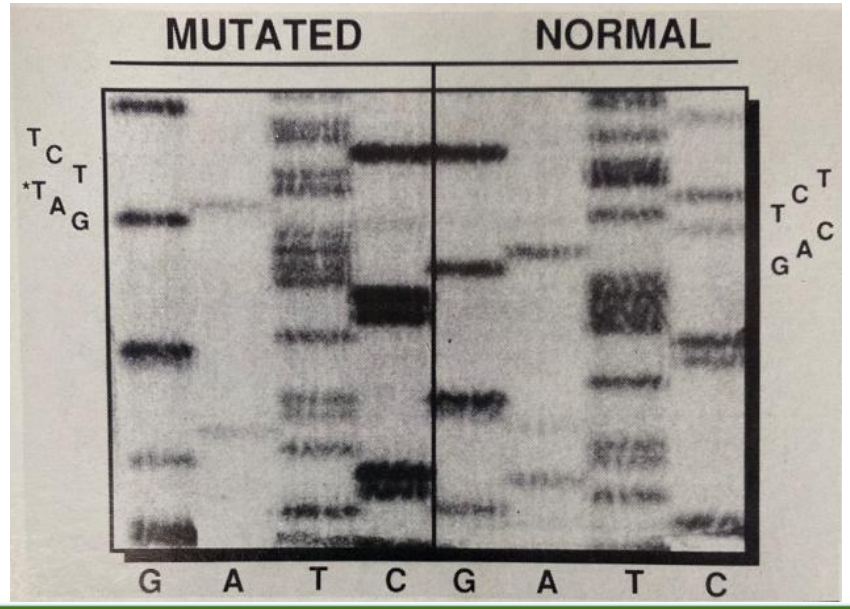
Sanger sequencing: computer print out of the order of the A's, T's, G's, and C's

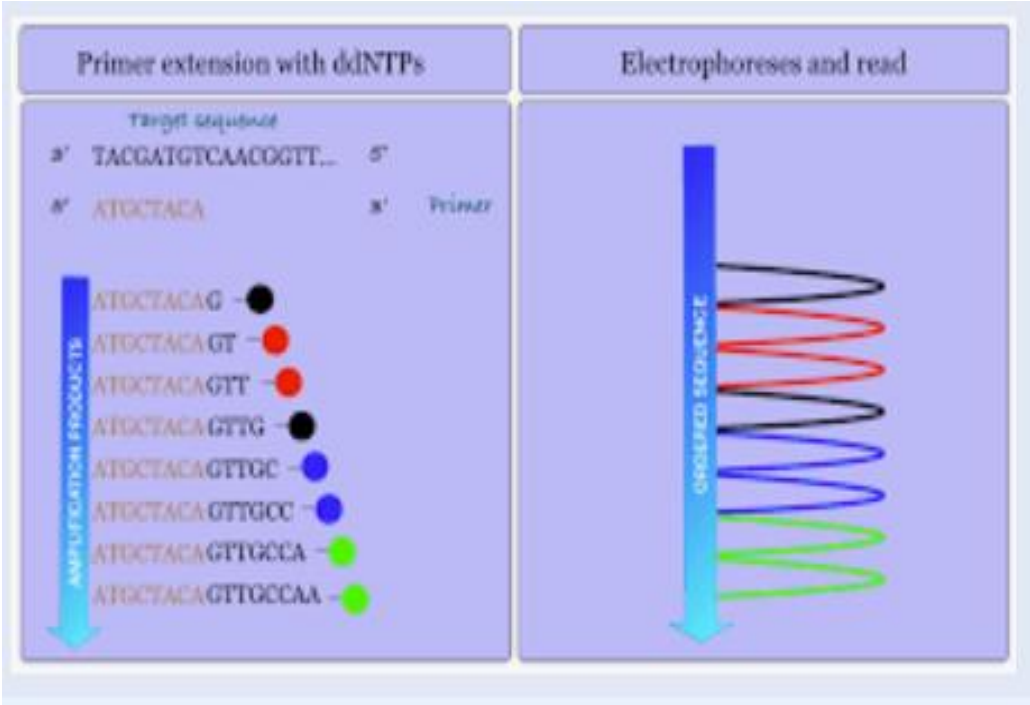
Chain termination sequencing

Good for short strands of DNA

Time consuming

Still in use



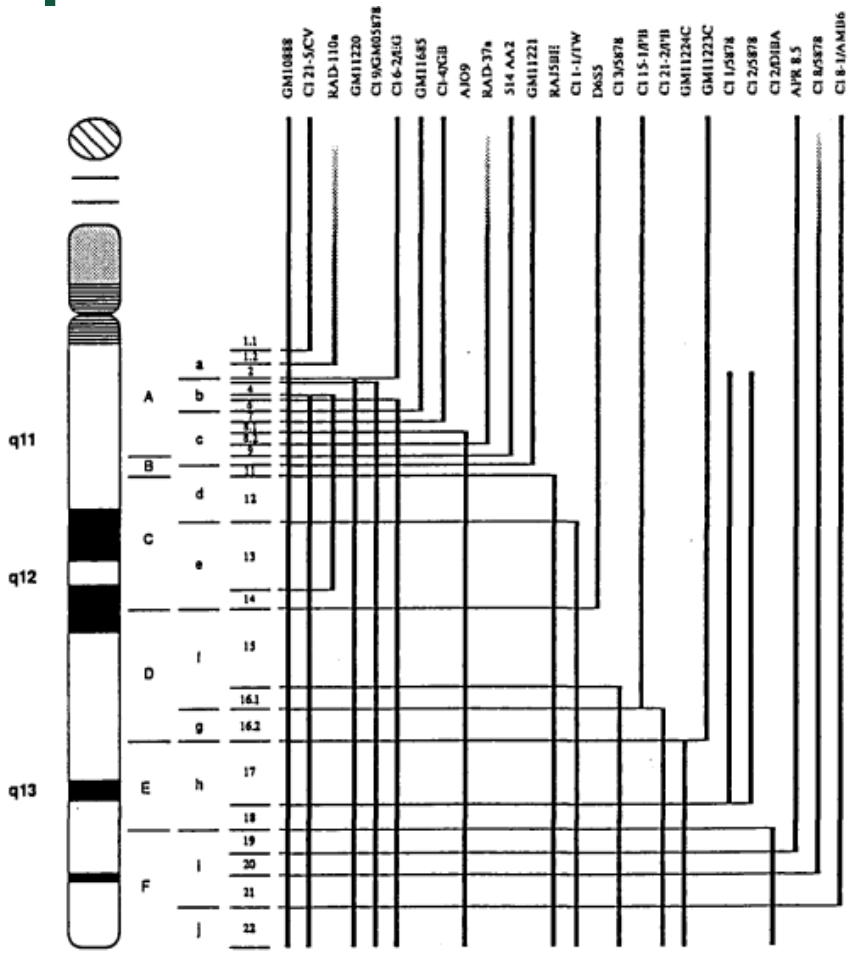


Human Genome Project

1988- NIH assembles scientists, administrators, science policy experts

- DOE and NIH sign a MOU for a 15-year project to map and sequence all 3.2 billion bases. A scientific ‘moon shot’
- 1990- NIH allocates first funds: funds went to institutions with infrastructure. Assigned a chromosome
- 1994- Genetic linkage map produced (a year ahead of schedule). The relative order of genes along chromosomes

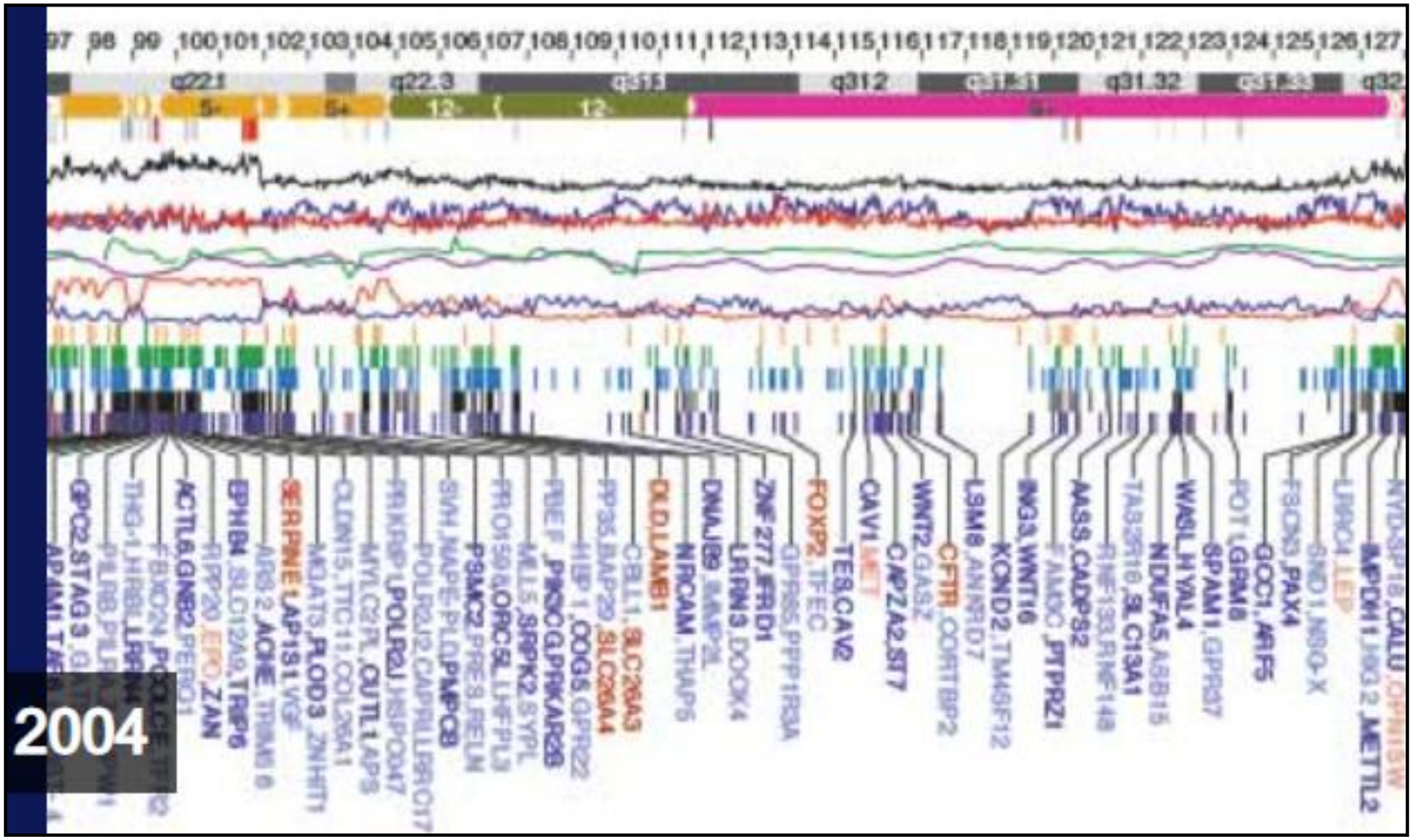
1995: HGP (Penn; MIT) published map of chromosome 22



Draft of Human Genome published in 2001

- 90% genome sequenced
- 20,000 genes and seem to be clustered
- Humans have only 2X the number of genes as plants and insects
- More than 90% genome stretches are DNA ‘junk’ (non-coding regions). But gives us the ability to define lineage

Humans are >99.9% identical genetically



Completion: Oct.20, 2004

Next Generation (NextGen) Sequencing

- Testing for genes from blood, hair, saliva, buccal swab, etc.
- Extract DNA

- Commercial labs, universities
- Whole genome sequencing (WGS or GWS)
- Whole exome (exons in genes of interest) sequencing (WES)

Whole Genome Sequencing (WSG or GWAS)

- Sequence everything
- The entire genome:

  Fishing

  EX: A baby born with multiple birth defects, no recognizable syndrome

  Shortens the diagnostic odyssey for families

  Does not detect 100% of variants that cause disease (wide but not deep): about 95%

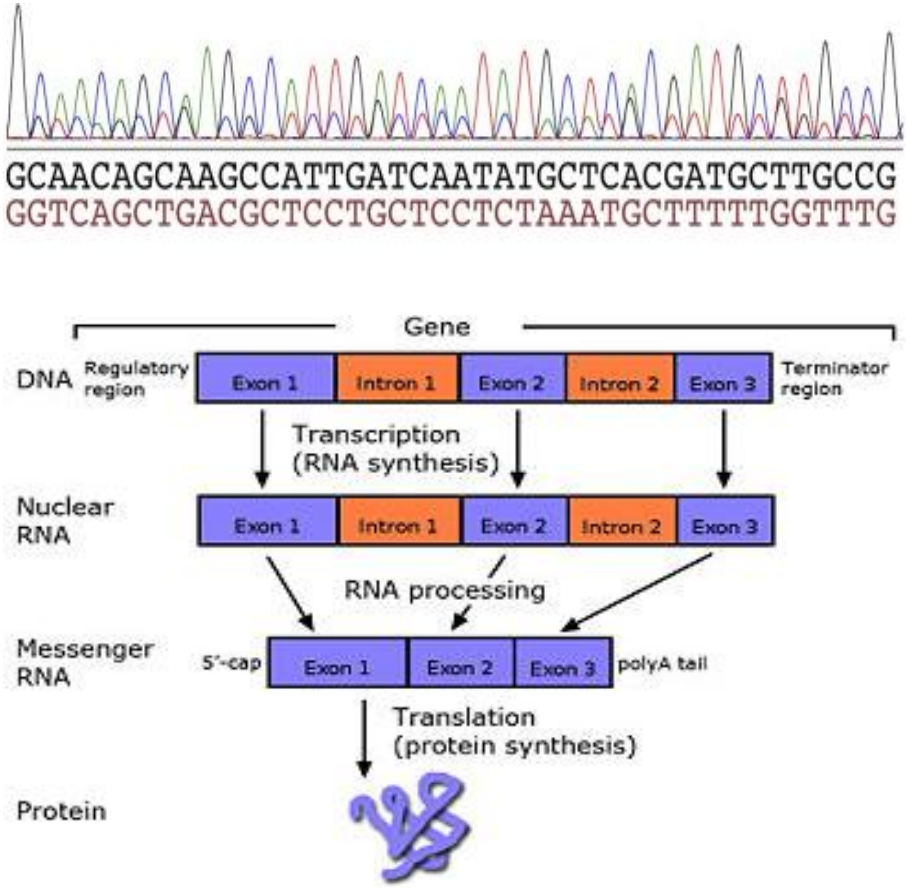
  Some surprises (consanguinity, risk for other disease)

Whole Exome Sequencing (WES)

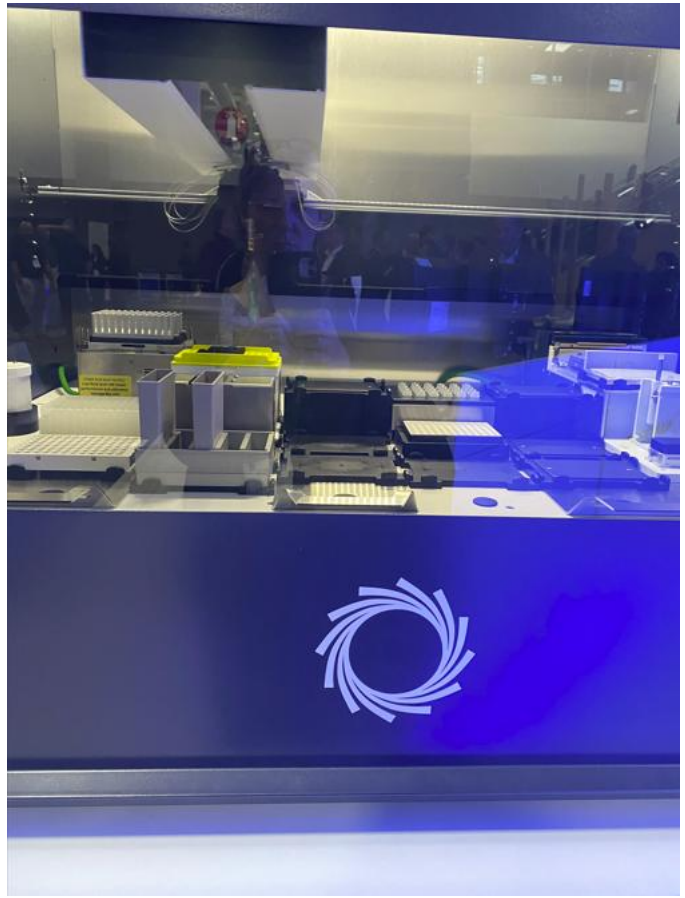
- Sequence just the exons (coding regions) of a known gene
    - Have a presumptive diagnosis
    - WES: a panel
    - Turn-around-time down to a few days
    - Detects 99%+ variants
- Cheaper
- Targeted

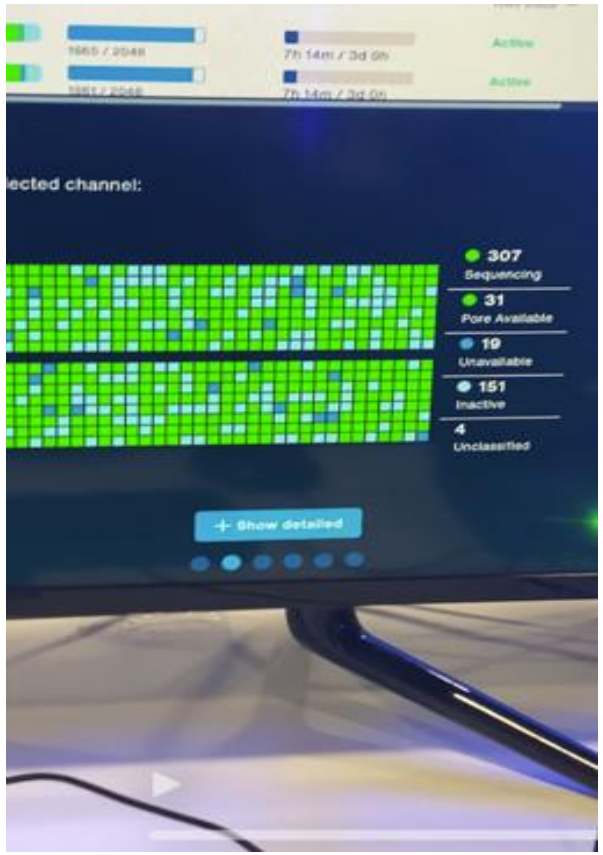
The Genome

- 3 billion base pairs
- 40,000 + genes
- Coding for our vital proteins



DNA extracted
From blood/saliva





Sequencing the entire genome may be first line in Peds cases

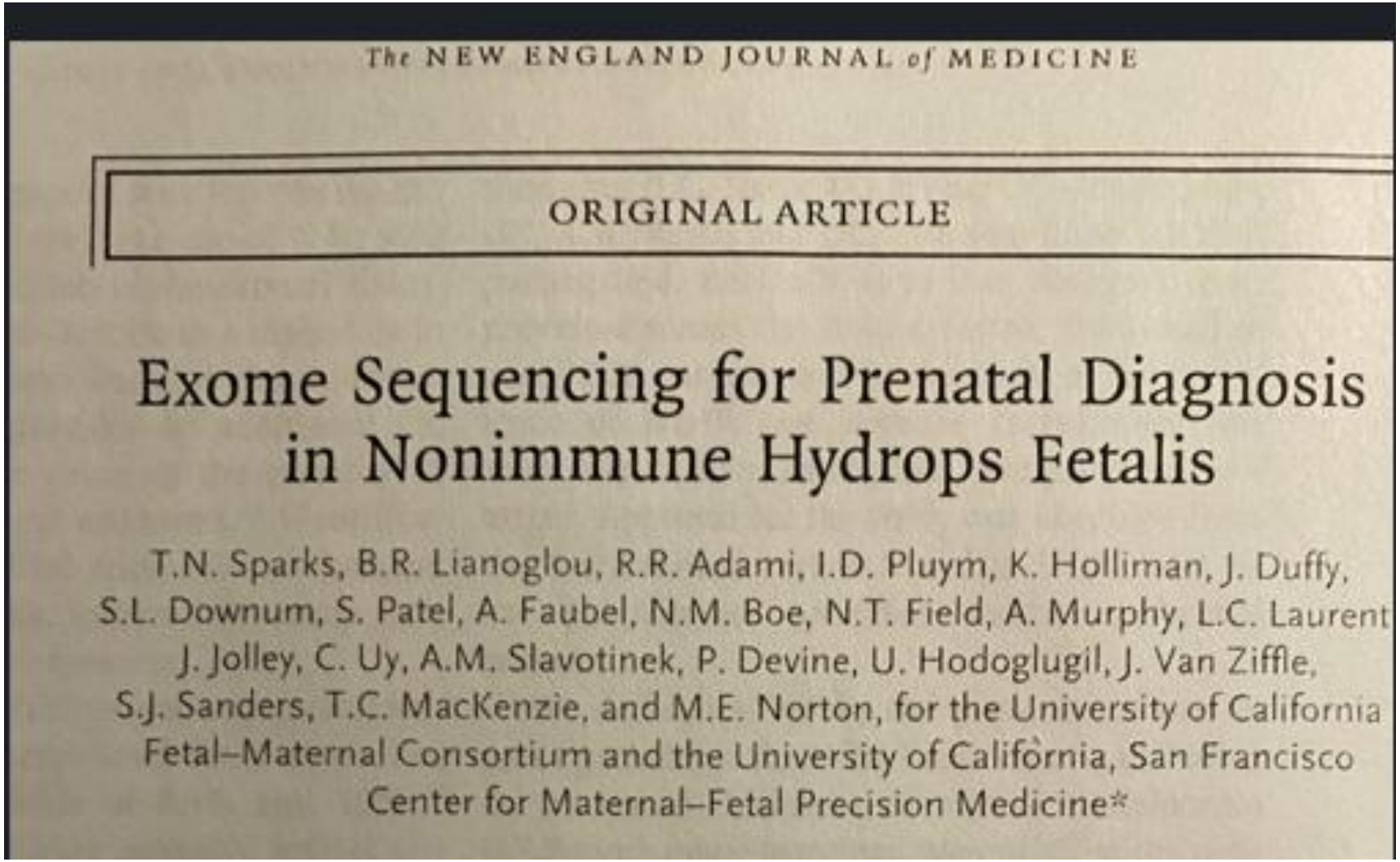
2001: >$1 million/genome to sequence

2019: $2-3000/exome panel
$1000/genome

Little fall in price since 2015

For the OBGYN

- WES-
- Panels for prenatal findings on ultrasound
- Expanded carrier screening (4 genes up to 129 genes) uses WES



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Exome Sequencing for Prenatal Diagnosis in Nonimmune Hydrops Fetalis

T.N. Sparks, B.R. Lianoglou, R.R. Adami, I.D. Pluym, K. Holliman, J. Duffy, S.L. Downum, S. Patel, A. Faubel, N.M. Boe, N.T. Field, A. Murphy, L.C. Laurent, J. Jolley, C. Uy, A.M. Slavotinek, P. Devine, U. Hodoglugil, J. Van Ziffle, S.J. Sanders, T.C. MacKenzie, and M.E. Norton, for the University of California Fetal–Maternal Consortium and the University of California, San Francisco Center for Maternal–Fetal Precision Medicine*

Received: 21 September 2019 | Revised: 2 January 2020 | Accepted: 6 January 2020

DOI: 10.1002/pd.5653

ORIGINAL ARTICLE

PRENATAL DIAGNOSIS WILEY

Rapid prenatal diagnosis of skeletal dysplasia using medical trio exome sequencing: Benefit for prenatal counseling and pregnancy management

Jin Han[1] | Yan-Dong Yang[2] | Yi He[3] | Wen-Jie Liu[4] | Li Zhen[1] | Min Pan[1] | Xin Yang[1] | Victor Wei Zhang[4,5] | Can Liao[1] | Dong-Zhi Li[1]

The Now Future

- 2012 National Institutes of Child Health and Development (NICHD) director Dr. Alan Guttmacher: “One can imagine the day that 99% of newborns will have their genomes sequenced immediately at birth”

- 2025: The BabySeq project: implementing genomic sequencing in **all** newborns

The BabySeq Project is a research study exploring the use of genome sequencing in newborns.

KJ

- A few days old, getting sicker
- Protein-free diet, treated with glycerol for hyperammonemia
- Palliative care discussed
- Discussed possible liver transplant, but too sick and small

KJ

- Getting sicker

- **Then in one week, the lab called, with a diagnosis derived from WGS.**

Carbamoyl-phosphate synthetase 1 deficiency

Autosomal recessive: both parents carriers

1 in 1.3 million births: 50% mortality rate as neonate. If survived, 100% neurological damage

Lab found two variants in the CPS1 gene: Q335X on the paternal allele and E714X on the maternal allele.

The Q335X variant has been reported in only one other case of CPS1 deficiency.

A single base pair substitution was found in Q335X

With the diagnosis of CPS1 deficiency

- Supplementation and diet changes
- His biochemical status worsened
- With each hyperammonemic episode, death and neurological damage was nearing.
- At 5 months of age, he was placed on the list for a liver transplant

But in the meantime

- Work was being done to create a genetic cure.
- Laboratories with expertise in CRISPR- CAS9 were prepared for this challenge through past research
- In month 7 of life, an injection was ready for administration
- KJ became the first patient of any age to have a custom gene-edited treatment

University of PA CHOP, Penn, University of CA, Berkley, 2 private biotech companies, FDA fast-tracked

Kiran Musunuru, MD, PhD, MPH, ML, MRA

Rebecca Ahrens-Nicklas, MD, PhD

How?

- Two gene editing tools
- CRISPR Cas9
- Base editing

CRISPR CAS9

1987 Atsuo Nakata in Osaka, Japan

CRISPR: “Clustered regularly interspaced palindromic repeats”, sites along DNA

Discovered in E.Coli

Natural way for bacteria to disable viral invasions

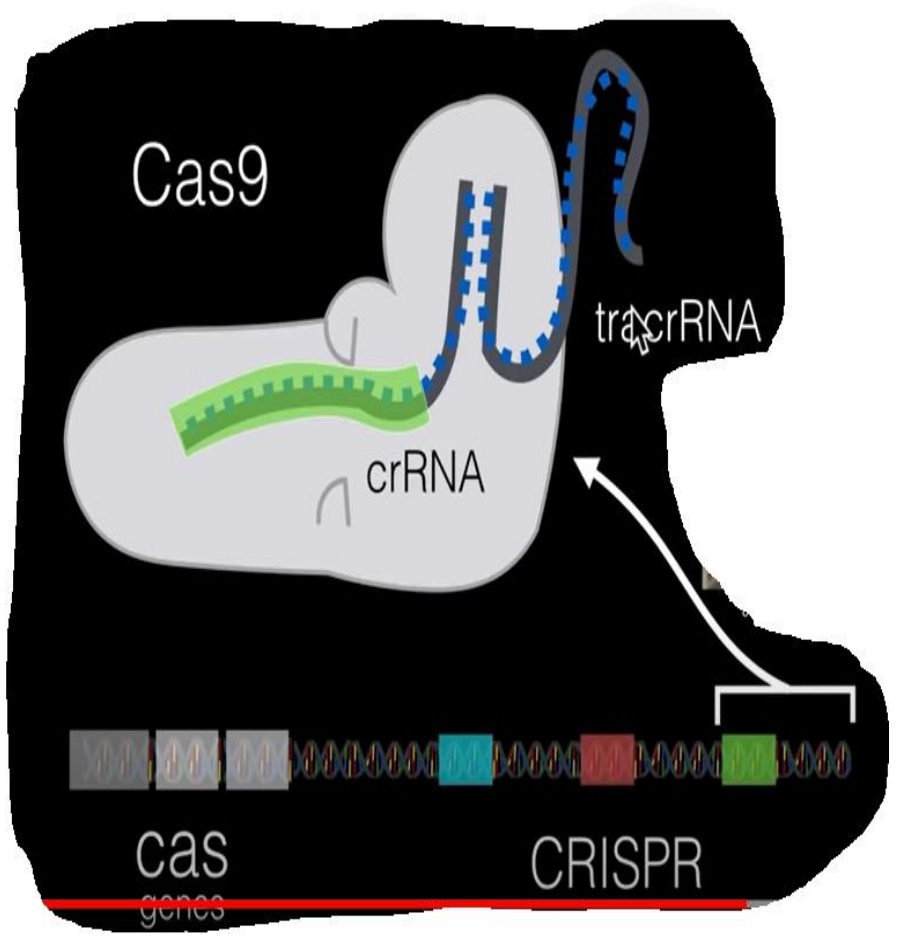
When bacteria are exposed to a virus, it creates a piece of RNA that matches the viral sequences. This ‘guide or cr’ RNA links with CAS 9 protein to search for virus in the cell- cut it and disable the virus

In the bacteria

CAS 9 protein has cutting properties (endonuclease)

crRNA- the viral DNA the system is looking for to chop up

Tracer RNA holds it all together



C (clustered) **R** (regularly) **I** (interspaced)
S (short) **P** (palindromic) **R** (Repeats)

- In bacteria, all that happens is DNA or RNA is chopped up in a specific location by matching a tracer RNA to a viral DNA segment and cutting with the endonuclease (CAS9)- destroying a virus

- What if it were possible in humans to target a specific piece of DNA and disable it ?!?

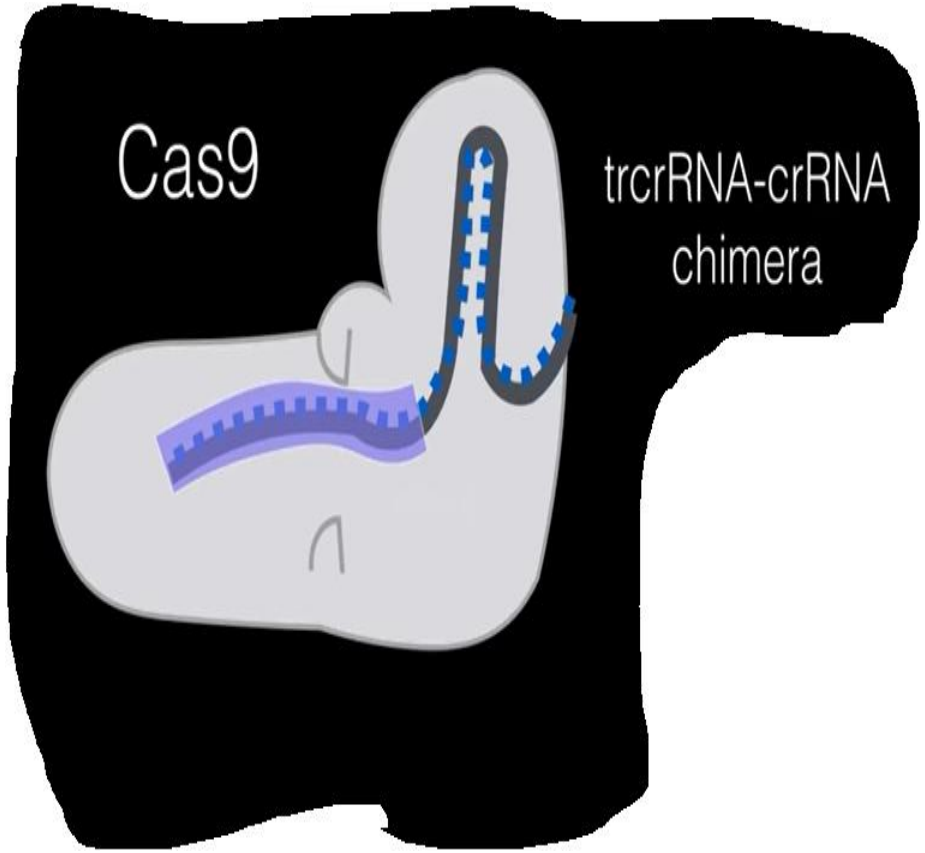
- Or what if it were possible to cut at a specific site and introduce a corrected or normal piece of DNA

Emmanuelle Charpentier and Jennifer Doudna
Work published in 2012
Accepting Nobel Prize for Chemistry, 2020

- https://www.youtube.com/watch?v=UKbrwPL3wXE

System for introducing a new piece of DNA

- CAS9 endonuclease
- Purple now is new piece of DNA
- trcrRNA- tracer (guide) RNA looking for the right spot in the DNA sequence to cut and insert
- And ‘snip’



“First Molecular Disease”: Sickle Cell Disease

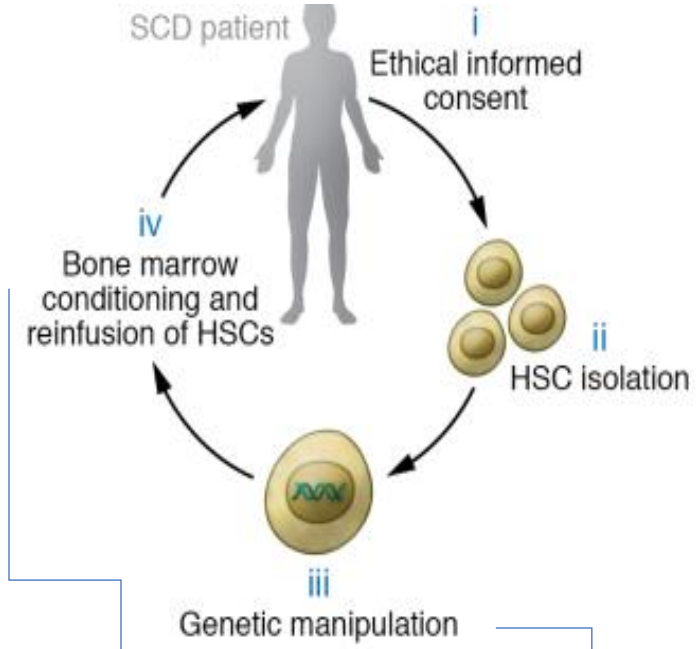
SCD affects 100,000 Americans

Pain, low quality of life, shortened life spans

Recent improvements in treatments

- Hydroxyurea
- Therapies aimed at preventing sickling
- Bone marrow transplant (many obstacles and complications)

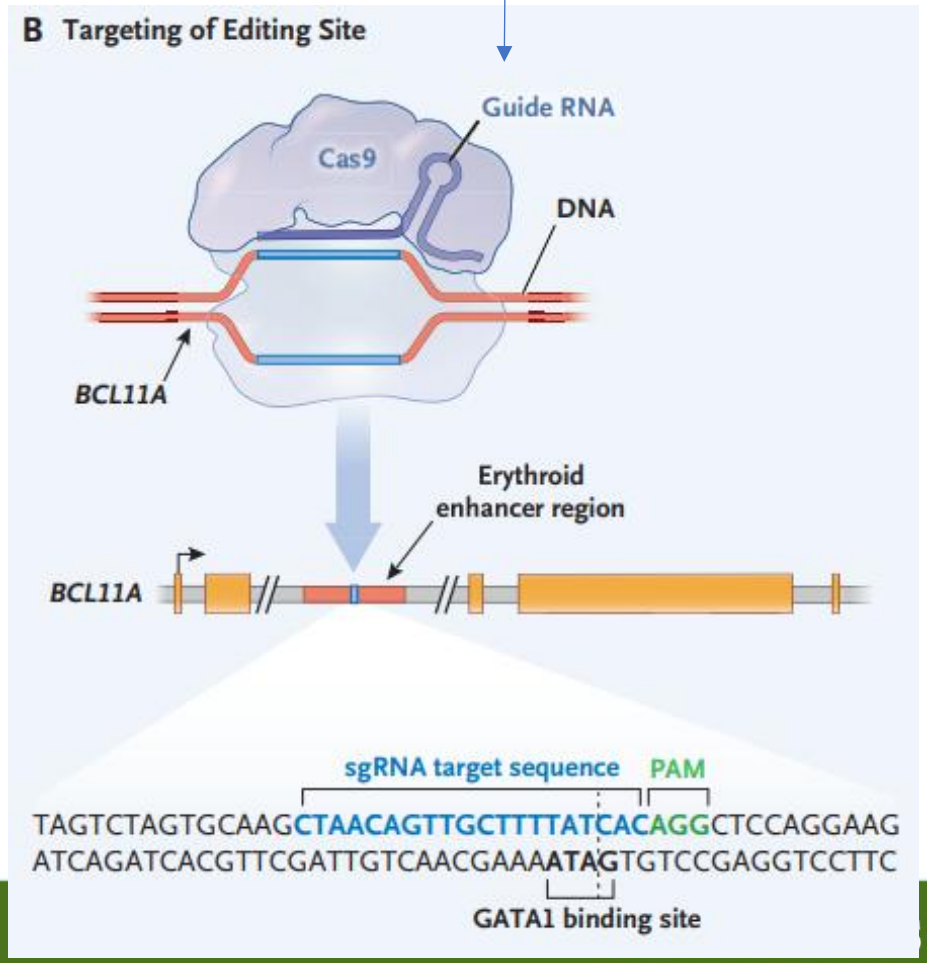
Now, CRISPR



Use of Busulfan (or other high-dose chemo) to ablate the bone marrow

- Prolonged hospitalization
- Immunosuppression
- Infections

AAV



Sharon Gray

- First patient to be treated with gene therapy for SCD
- She says ‘cured’

FDA approval 12/8/2023: First gene therapy to be approved

- Nakata discovered the CRISPR CAS9 system in E.Coli 1987
- BCL11A discovered 2008 by Vijay Sankaran
- Doudna and Charpentier first published 2012
- 2022: SCD cure

Concerns

- Complications of the myeloablative process: infections, death
- Off target effects
- Warning of increased risks of leukemia
- Cost- $2.2 million

CRISPR CAS9

- Thalassemia: like SCD
- SMA: children are walking and alive
- Cystic fibrosis: some genetic variant carriers now have a cure
- Duchenne’s muscular dystrophy:

Duchenne's muscular dystrophy

NYT editorial: (2/17/2024)

"Before Eliot received his treatment, he had difficulty going up stairs. Hopping on one foot, a milestone for a 4-year-old, was impossible.

On Aug. 29, he finally received the one-time infusion. Three weeks later, he was marching upstairs and able to jump over and over. After four weeks, he could hop on one foot. Six weeks after treatment, Eliot's neurologist decided to re-administer the North Star Ambulatory Assessment, used to test boys with D.M.D. on skills like balance, jumping and getting up from the floor unassisted.

In June, Eliot's score was 22 out of 34. In the second week of October, it was a perfect 34 — that of a typically developing, healthy 4-year-old boy. "

Congenital Deafness

- Aissam Dam; age 11

- Mutations in gene ‘Otoferin’

Treated with CRISPR-CAS9

“there’s no sound I don’t like”

Base editing: Not inserting a corrected copy of a gene. But editing one base.

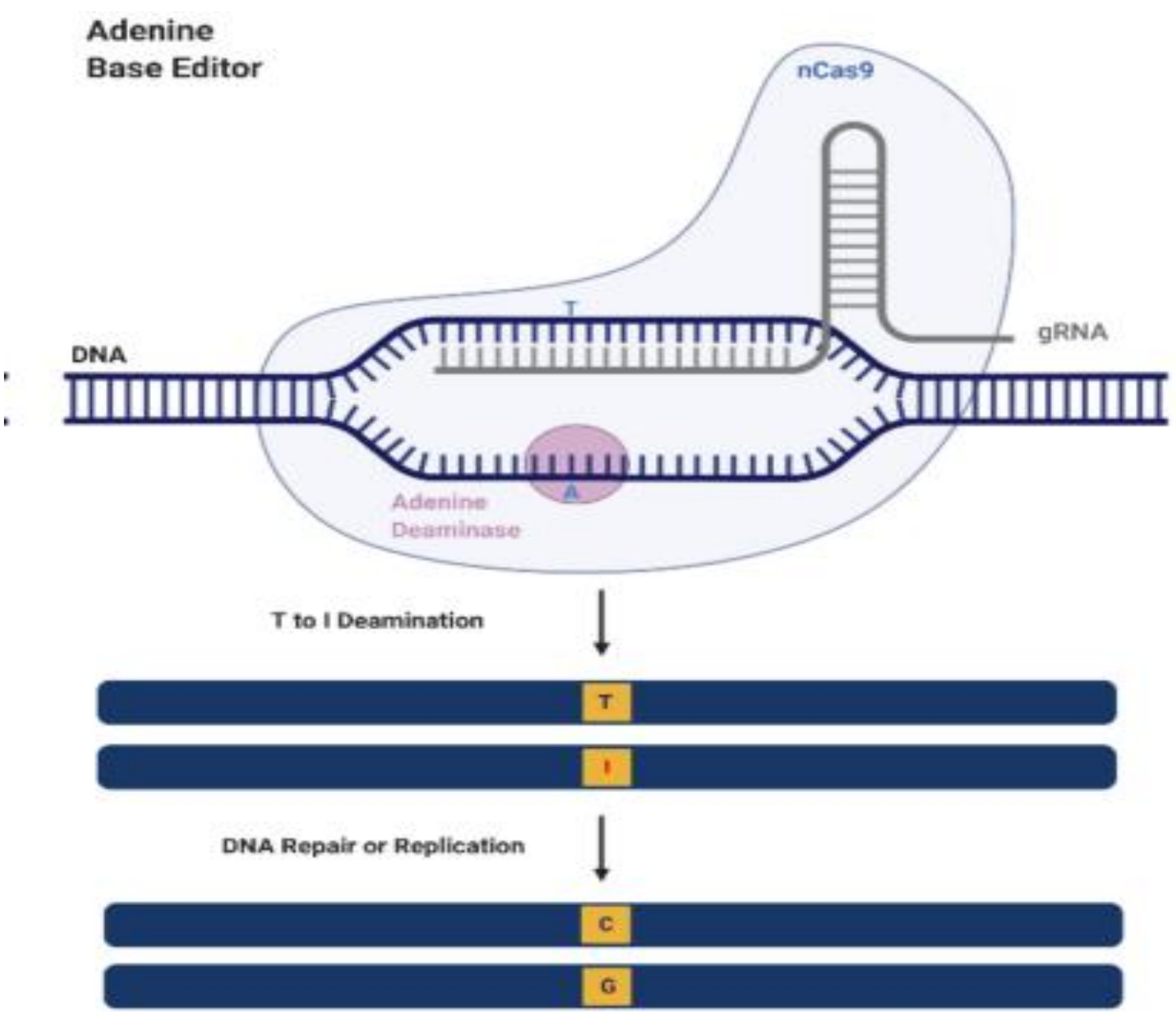
Base editors combine three core components into a single molecular complex:

- **A modified CRISPR protein:** It acts as a guide to find and bind to the exact target location on the genome.

- **A guide RNA (gRNA):** Directs the Cas9 protein to the exact sequence of DNA that requires an edit.

- **A DNA deaminase enzyme:** Fused to the Cas9, this enzyme chemically alters the targeted base—converting it into a different base. From a C to T, e.g.

- The cell's natural DNA repair pathways stabilize the edit.


Adenine
Base Editor
nCas9
gRNA
DNA
T
A
Adenine
Deaminase
T to I Deamination
T
I
DNA Repair or Replication
C
G

For KJ: Gene editing- one base pair substitution in a gene that functions in the liver

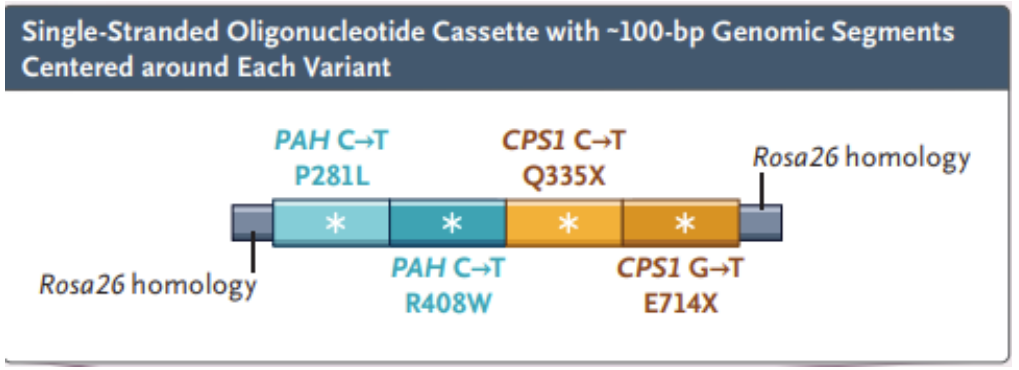
NEJM June 12, 2025

Q335X variant was inherited from dad.

a 100-bp human genomic segment spanning the CPS1 Q335X variant

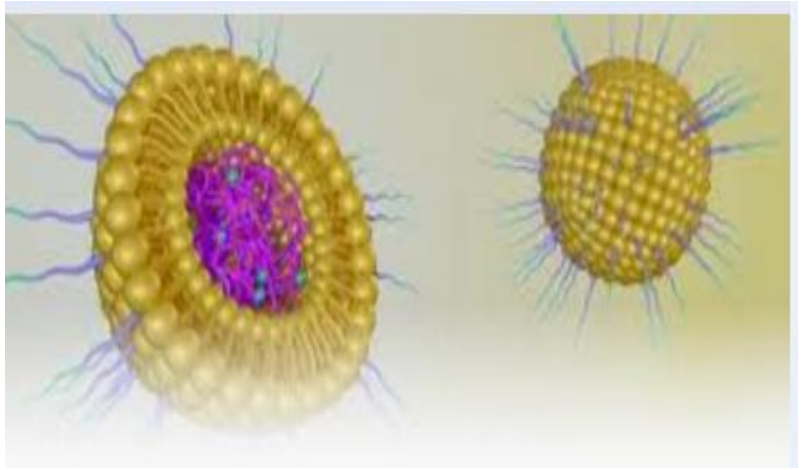
A lipid nanoparticle therapy (cassette) gRNA with the target Q335X *in the eighth position of its sequence* to correct his CPS1 variant in the liver (From a C to T)

Mouse trials successful



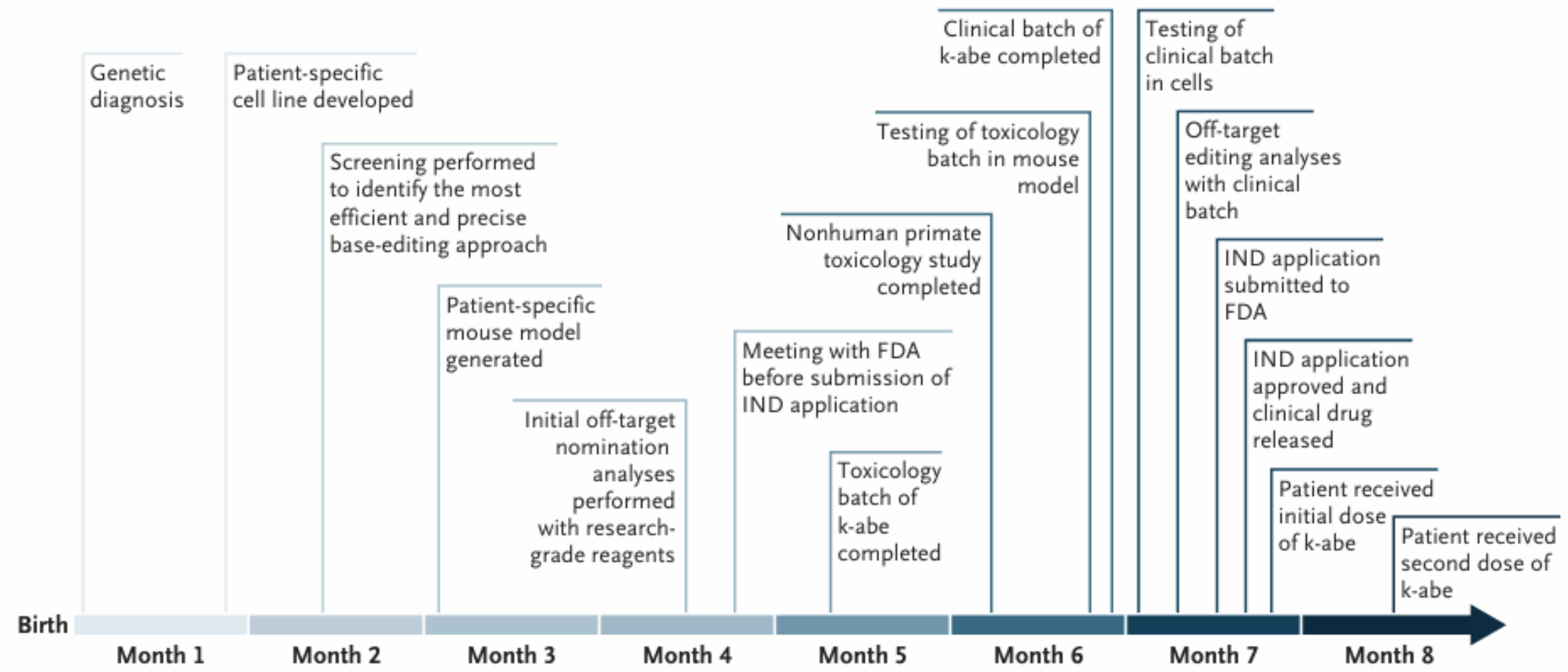
Used a lipid rich packaging of corrected gene and CAS9 base editing system

- KJ underwent an immunosuppression
- IV injection of new drug (tRNA) at day 208 (about 7 months) of life
- After 2 weeks received another dose
- Ammonia levels fell to 13
- Neurological condition stabilized



KJ

- Update from CHOP 2/24/26
- Has been given 3 doses- “not a cure”
- May still need a liver transplant
- “walking and talking, as he continues to grow and thrive”


Genetic diagnosis
Patient-specific cell line developed
Screening performed to identify the most efficient and precise base-editing approach
Patient-specific mouse model generated
Initial off-target nomination analyses performed with research-grade reagents
Meeting with FDA before submission of IND application
Toxicology batch of k-abe completed
Nonhuman primate toxicology study completed
Testing of toxicology batch in mouse model
Clinical batch of k-abe completed
Testing of clinical batch in cells
Off-target editing analyses with clinical batch
IND application submitted to FDA
IND application approved and clinical drug released
Patient received initial dose of k-abe
Patient received second dose of k-abe
Birth
Month 1
Month 2
Month 3
Month 4
Month 5
Month 6
Month 7
Month 8

Now we know we can….

- 7000 genetic diseases: Most rare
- Cost of this process?
- Private companies?
- Universities?

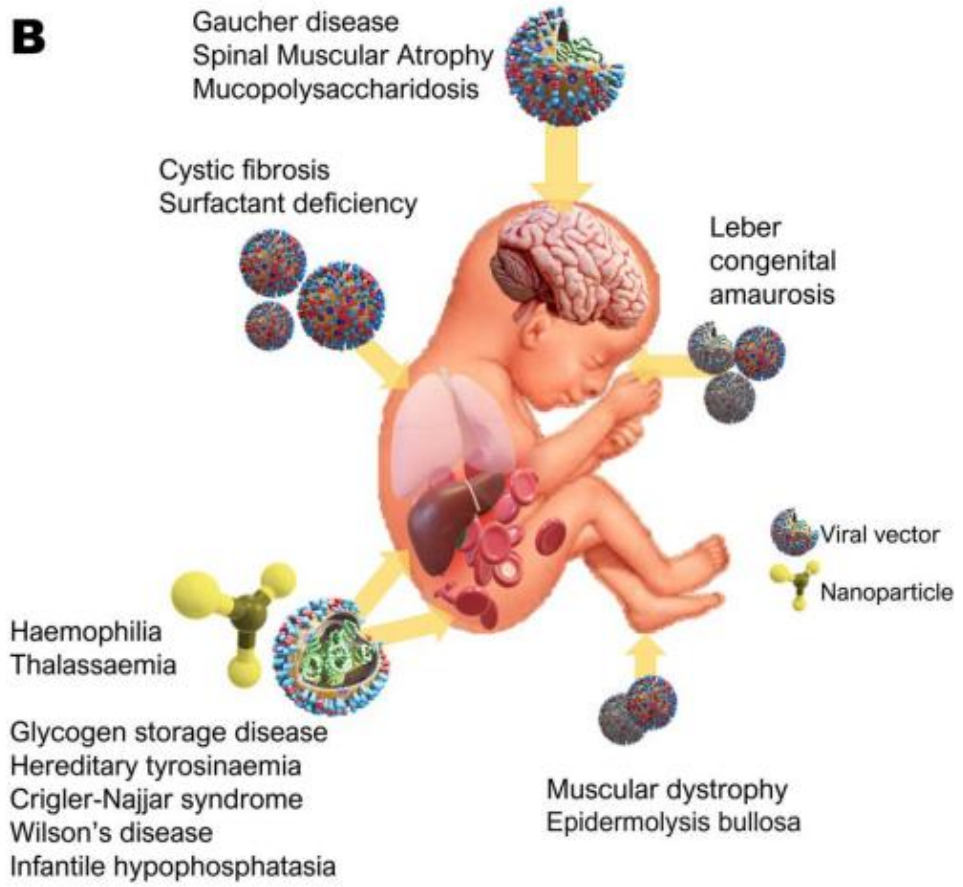
In utero gene editing: experiments in mice

- Many diseases have their detrimental effects during the critical developmental period
- The drug to mass ratio higher (lower doses/cost)
- Fetal immune system more tolerant of viral vector with CRISPR
- Blood brain barrier easier to overcome

In utero treatments

Mattar, et al; Prenatal diagnosis, 2022

- Studies on going
  - CF- success in mice by venous injection or amnio
  - SMA: oral medication modulator to mom (Risdiplam). Fetus affected. Born and normal development



Cure of familial hypercholesterolemia

NEJM, May, 2026

- Gene inherited: PCSK9 (increases LDL; associated with early CVD)

- VERVE-102 consists of a messenger RNA encoding an adenine base-editor protein and a guide RNA targeting PCSK9. The editing takes place in the liver

- “One IV dose led to dose-dependent, substantial, and sustained reductions in LDL cholesterol levels and PCSK9 levels”
- Results lasted beyond hepatocyte turnover (300 days)

Two potential target cell types

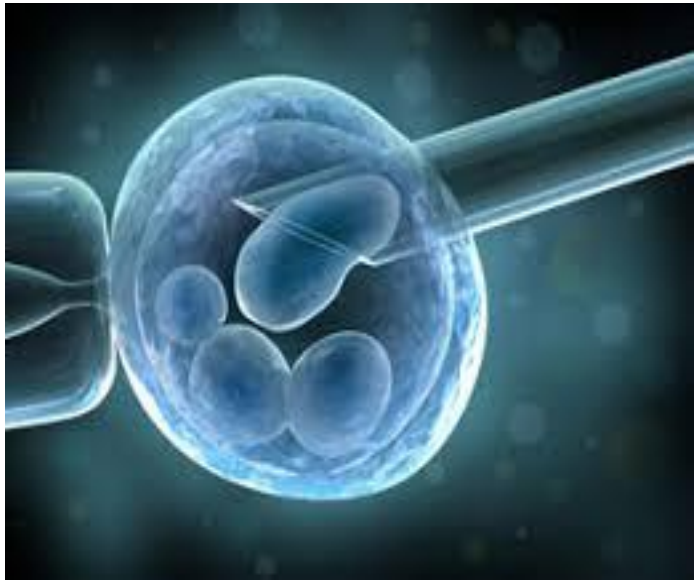
1. Somatic cells- all we have been discussing.
   - Brain cells
   - Muscle cells
   - Blood cells
   - Cochlear cells

Even in utero are somatic cells

2. Germ line (embryonic or eggs/sperm)

Altering these cells would affect every cell in body

Would be passed onto every subsequent generation



NYT: June 5, 2026: ***Scientists Edit Human Embryo Genes With Startling Precision***

- “Scientists have edited the DNA of early human embryos with unprecedented accuracy, an achievement that could open the way to babies engineered with particular characteristics.”

- “Prospect has long alarmed bioethicists”

- Dr. Dieter Egli (Columbia; now of Nucleus Genomics): “As a scientist, you can provide the data for discussion, but then essentially there you stop and let others take over.”

And yes, we have lots of questions

- Questions?