Clinical Applications of Whole Genome Sequencing

Hunter Best, PhD, FACMG
Professor of Pathology
University of Utah School of Medicine
Division Head, Molecular
Medical Director, Molecular Genetics & Genomics
ARUP Laboratories

Financial Disclosure

- The work presented from the Utah NeoSeq Project was supported, in part, by Illumina, Inc.

Learning Objectives

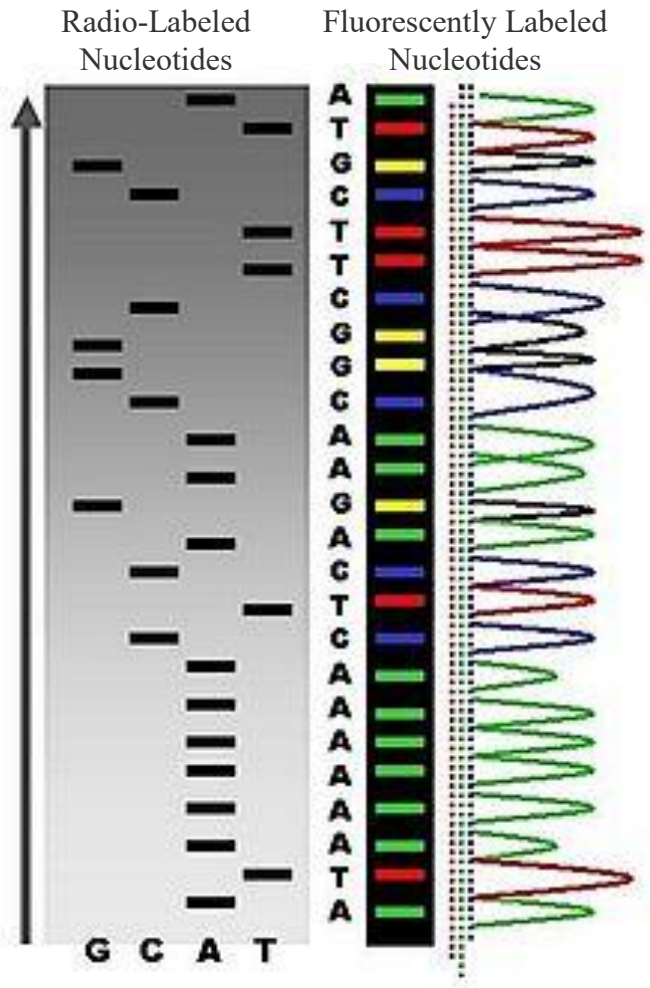
- Describe how massively parallel sequencing differs from traditional sequencing methods
- Explain the difference between rapid whole genome sequencing and standard whole genome sequencing
- Identify how whole genome sequencing is used clinically in suspected genetic disease diagnosis
- Recognize the benefits and limitations of whole genome sequencing

What We're Going to Cover

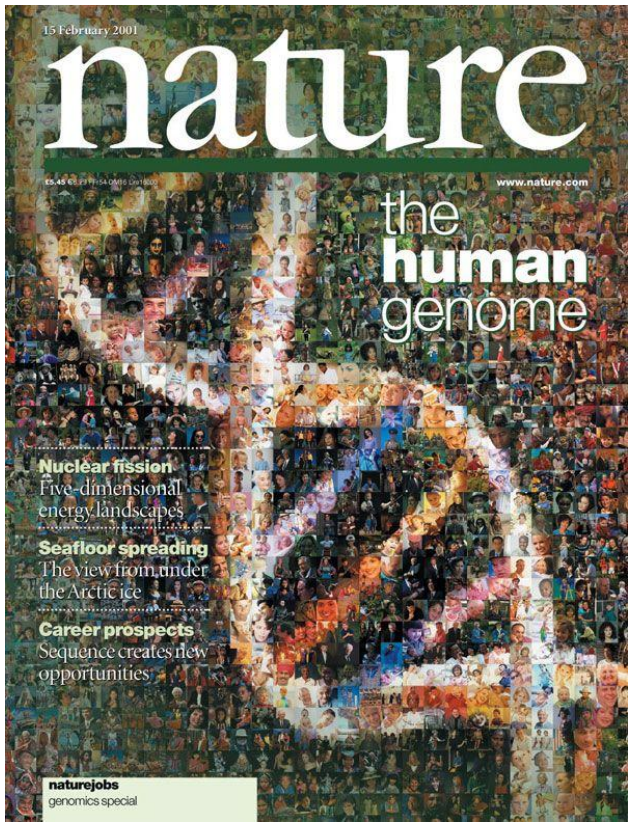
- Basics of DNA Sequencing
- Rapid Whole Genome Sequencing/Utah NeoSeq
- Standard Turnaround Time Whole Genome
- WGS benefits/limitations

Basics of DNA Sequencing

- **Sanger Sequencing**
  - Developed in the 1970's
  - Originally used radio-labeled chain-terminating dideoxynucleotides
    - Cytosine (C)
    - Guanine (G)
    - Adenine (A)
    - Thymine (T)
  - Sequencing gels
- Automated capillary electrophoresis
  - Fluorescently labeled dideoxynucleotides
  - Largely used for sequencing of fragments between 300-1000 bp
  - ABI 3730 capable of generating ~1 Mb of sequence per day



Human Genome Project



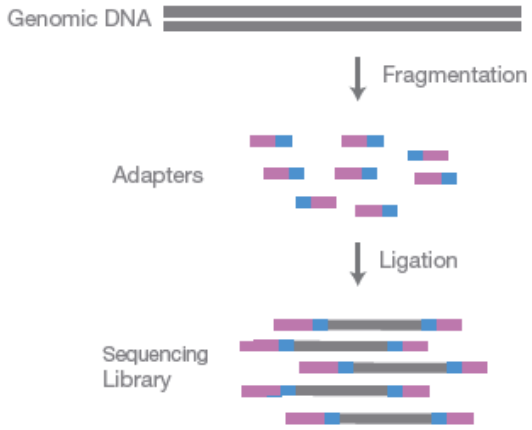
- **Started in 1990**
  - Goal to sequence the ~3,000,000,000 bp human genome
  - 20 institutions across 6 countries
  - Sanger sequencing methodology
  - Cost ~$3 billion
- **Completed in 2003**
  - 22,300 protein coding regions in the genome
- **Demonstrated that there was a need to develop high throughput, cheaper and faster DNA sequencing technologies**

Massively Parallel Sequencing (aka next-generation sequencing (NGS))

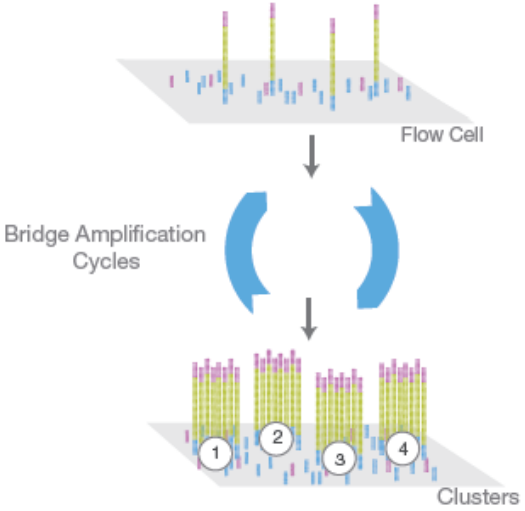
- Rapid and cost-effective method for determining the sequence of millions of DNA molecules simultaneously
- Instruments can fit on a desktop
- Requires complex and powerful computing processes for data analysis
  - Anyone can generate data; analysis and interpretation are typically the bottlenecks

MPS Sequencing Overview

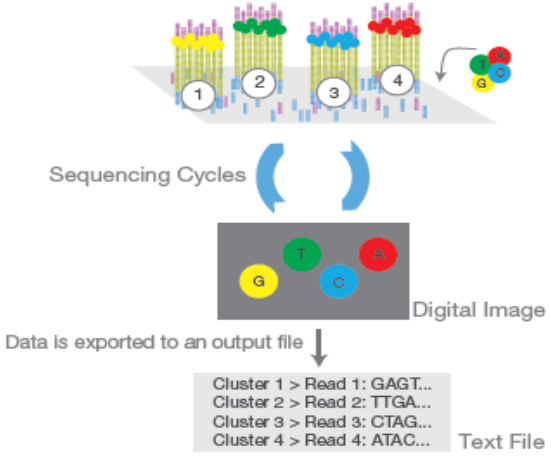
A. Library preparation

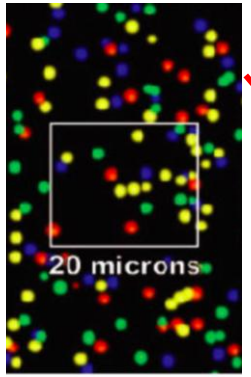


B. Cluster amplification



C. Sequencing





50-150 cycles (bases)

```
@JDQNNM1:207:c6ckfacxx:7:1101:1457:2221 1:N:0:TGACCA
AATTCCGCAAAAATTGATCATTTGCAAAGTCAAAACTATAGCCATATCCAAATCTTTTCCCCCTCCCAAGAGTTCTCAGTGTCTACATGTAGACTATTCCT
+
@@CDFFFFHHGHHJJIGJIIIIJIGIJHJEHIGJJJJJIJIIIJJEGHIEHJJIJJIIJJJJIIGJIJGEFHECDADDDDDFEEEEEEDDCCDEDDDDEEED
```

www.illumina.com

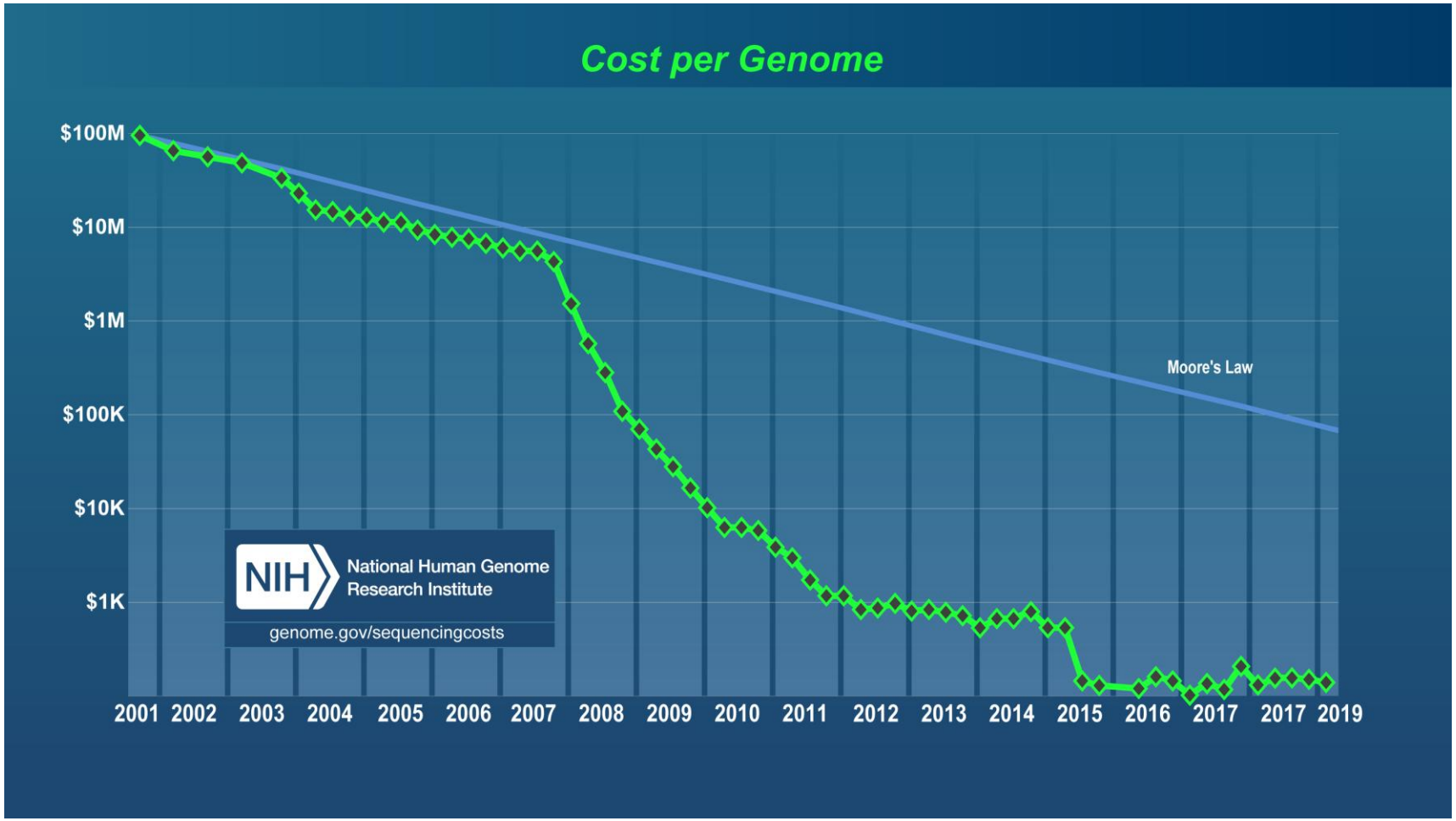
Massively Parallel Sequencing

millions of individual sequences

```
@JDQNNM1:207:c6ckfacxx:7:1101:1457:2221 1:N:0:TGACCA
AATTCCGCAAAAATTGATCATTTGCAAAGTCAAAACTATAGCCATATCCAAATCTTTTCCCCCTCCCAAGAGTTCTCAGTGTCTACATGTAGACTATTCCT
+
@@CDFFFFHHGHHJJIGJIIIIJIGIJHJEHIGJJJJJIJIIIJJEGHIEHJJIJJIIJJJJIIGJIJGEFHECDADDDDFEEEEEEDDCCDEDDDDEEED
@JDQNNM1:207:c6ckfacxx:7:1101:1517:2156 1:N:0:TGACCA
CCTGGTGAAAGTGGTGGGGCTGTATGGCTTCATGCTCAGCATATCGCCTCGACTCACCCTCCTTTCTCTGCTGCACATGCCCTTCACAATAGCAGCGGAGA
+
??@BDDD?FDF?DG2ABEHE<@?EFFHBABGFADGE9BBGGFDD4?DB:AAAE=ECCHH?DEC;@CCCCACC>AC@C>@CCCACCCCCCCCCC@ABB@B>B
@JDQNNM1:207:c6ckfacxx:7:1101:1893:2234 1:N:0:TGACCA
CATTTTGGTAAAGACAAATTATACAGACAGTAAAAAGTAAAAGGCAAAATTATAGAGACAGTAAAAAATCTGGAAAGGCTATATACTGTATGATTCCATAC
+
BBCFFFFFHHHHHIJJJJJJJJJJJJJJJJHIJJJJJGFHIJJIJJJJJJJJJJJJJJJJJGIJIJJJIHHHHHFFFFFEEEEEEEEEEEEEDEEFFFFCD
@JDQNNM1:207:c6ckfacxx:7:1101:3454:2163 1:N:0:TGACCA
AGGGGCGATGCATTCGAACGCGTTGGGGTTCTTCTCTCGGGAGTAGATGATGGTCTCCGTCTTCTGCCTTCTGAGGAACTCATCTCTTGTGACGATATCTG
+
8?@4B<?A1<CBF?FGGGE:FBH8@FHI;;DECAEECH8BE>A(5;CDCC>@@,:A@:=@??BC>CAC>C:@:>@?:<<CCCAC>4>:4>@>@<<BBB>::
@JDQNNM1:207:c6ckfacxx:7:1101:3554:2157 1:N:0:TGACCA
AGTTGGAGGTATTGATGCATGTGTTGGTTTGCCAACATTATCTCTATGATATTGATCTCTATTTGCTTGACGGCTGCCCTTCTTAAGATCATGAATTACAA
+
1=8BDA?BB+2<<A4AFBHFIBBCACCGEFC<D?ED;1:B>??DF>?D>?BBD<DFFEI<F8BFD===3(8;B8=BBD.;;>ACA@A5@;A>::>A;@:A>
@JDQNNM1:207:c6ckfacxx:7:1101:3932:2232 1:N:0:TGACCA
AGGTAGTGGCTTGACAGCTCAGGGATGTTGGTATGGGTCTCGTTAATCCAATTAACAAACCAGGCAATACTTTCACACGTGCAATCAAAGGGATTAAAGCG
+
@@CADEEFHHHHHHIGGGGHGIIJBFGEGEH?FCGIJDFFGEGHGHEBHGHIJIIGHHJCGGGIHFHFFFFFFFFCECDBBDDDDDCCDCDDDCCDDDDDB
@JDQNNM1:207:c6ckfacxx:7:1101:4020:2157 1:N:0:TGACCA
GTCTCACCCTCAGCACACTGGCAGACTTCAGCAGAACACAAGGTGGCCAAGAGTCTGCTCTTACTTGGTGCCCCGTAAAACACAGAACATCTGCGCTCTGG
+
@BBDFFFFFHHHHJJJJJJJIJJJGIJJJIIJJIJJIJJIJGIDFFCHHIIIJCHGIIIIGGIJIIHFHEHHFFDDCDDDDDDDDCCDCDDDDDDDDD@CC
@JDQNNM1:207:c6ckfacxx:7:1101:4229:2203 1:N:0:TGACCA
TATAGAGGAACCCTAAAGTGGGGTCCATTGTTCCGGGGGCTGGAGCTGTCCCCGGTGCTGGAAGACTCAGCAGATGGGGGGTGGCGGGGAAAGAGGGGAGC
+
CCCFFFFFGHHHHJIJJJAFHHID@FAHIJGHIIJJJIJHEDFBCEDEDDDDDDD@BDDDCACDDDCDDDDDDDCCCDDD@09<BDDDD@BDDCDBDBDDD
```

Millions of sequencers sequencing in parallel!

Sequencing Cost of Human Genome Keeps Going Down



What Limits Adoption of Whole Genome Sequencing (WGS)?

- Cost is still prohibitive for many clinical labs:
  - Sequencers are expensive
    - Illumina NovaSeq is >$1M
  - Data processing/analysis is difficult and costly
    - Literally millions of variants identified
    - Studies show everyone has between 3500-8500 unique variants
  - Insurance reimbursement for whole genome can vary between state and provider
- Still some advantages to targeted testing methods (exome, gene panels):

  - Generally cheaper capital costs

  - Well established data processing methods

  - More reliable reimbursement

Some Advantages of Clinical WGS

- No probes or enrichment = faster sequencing turnaround time
  - Sequencing can be completed in ~24 hours

- Provides more uniform coverage in difficult to sequence regions
  - Data shows that variant calling is more accurate in WGS

Exome vs. Genome

A. Whole Exome Sequencing (WES)

Selection (capture):

All exons of all known genes (1.5–2% of all human DNA)

- Variable read depth at boundaries
- Greater sequencing depth
- More cost effective

B. Whole Genome Sequencing (WGS)

No selection:

Entire human DNA analyzed including introns, RNA genes, etc.

- Moderate read depth
- Similar read depth across the genome
- Can identify copy number variants, repeat expansions
- Higher price

Image from https://www.idtdna.com

Why order WGS instead of Exome?

- WGS provides more data on coding and noncoding regions increasing diagnostic yield
  - WGS catches diagnostic variants not identified by whole exome sequencing (WES)
  - In one study, 18% of undiagnosed WES cases were found to have a causative variant by WGS*
  - Another study demonstrated that 34% of WES negative cases were diagnosed by WGS**
  - Detection of cytogenomic abnormalities is significantly better in WGS

- Healthcare economic analysis has demonstrated that when used as a first tier test it results in larger overall cost savings (compared to WES)***

***Shashi *et al*. (2019) *Genet Med*. 21(1): 161-172**
****Ewans *et al*. (2022) *Eur J Hum Genet*. 30: 1121-1131**
*****Nurchis et al. (2024) *JAMA Netw*. 7(1):e2353514**

When should you order WGS?

- WGS should be considered a first line diagnostic test when an inherited disorder is suspected but the patient phenotype does not suggest any single disorder:
  - Patient has a constellation of findings
  - Findings potentially suggest multiple disorders

- WGS is also an excellent test in patients that have experienced a diagnostic odyssey

Clinical Application of WGS

Rapid Whole Genome Sequencing (rWGS)

What is rWGS?

- Performing WGS and data analysis in 1 week or less
  - » Depending on the lab, a standard TAT genome can take a month or longer to report
- Requires that the entire testing pipeline be optimized for speed while maintaining quality and accuracy
- Usually only a positive or negative result is released
  - » Reporting of variants of unknown significance (VUS) limited to those suspected to be causative

The Utah NeoSeq Project

The Utah NeoSeq Project

- A research collaboration between several departments at the University of Utah
  - » Neonatology/Pediatrics, Pathology (ARUP), Human Genetics
- The purpose of Utah NeoSeq project was to evaluate the utility of rWGS in critically ill infants in the NICU at the University of Utah
  - » Enrollment of a total of 65 patients into the study over the course of 18-24 months
  - » Expected that WGS would reduce time to diagnosis
  - » Goal was to provide final results in seven days or less

NeoSeq Enrollment Criteria

- Patient phenotype must appear to be genetic in nature with no obvious diagnosis
- Testing limited to patients where trio (proband + parents) was available
- Enrollment could be pre- or post-natal
- Extensive consent process required
  - » Patients are carefully informed of the research nature of testing offered
- Standard of care testing was also ordered

Why is rWGS ideal for NICU Patients?

- Genetic disorders are a leading cause of morbidity/mortality in this patient population
  - Standard TAT WGS and exome clinical tests take ~1 month to report
  - NGS targeted panel testing usually has 2-3 week turnaround time

- Rapid diagnosis can have a high clinical utility:
  - Improved outcomes
  - Change in management
  - Reduction in time in the hospital
  - Help with end-of-life decision making

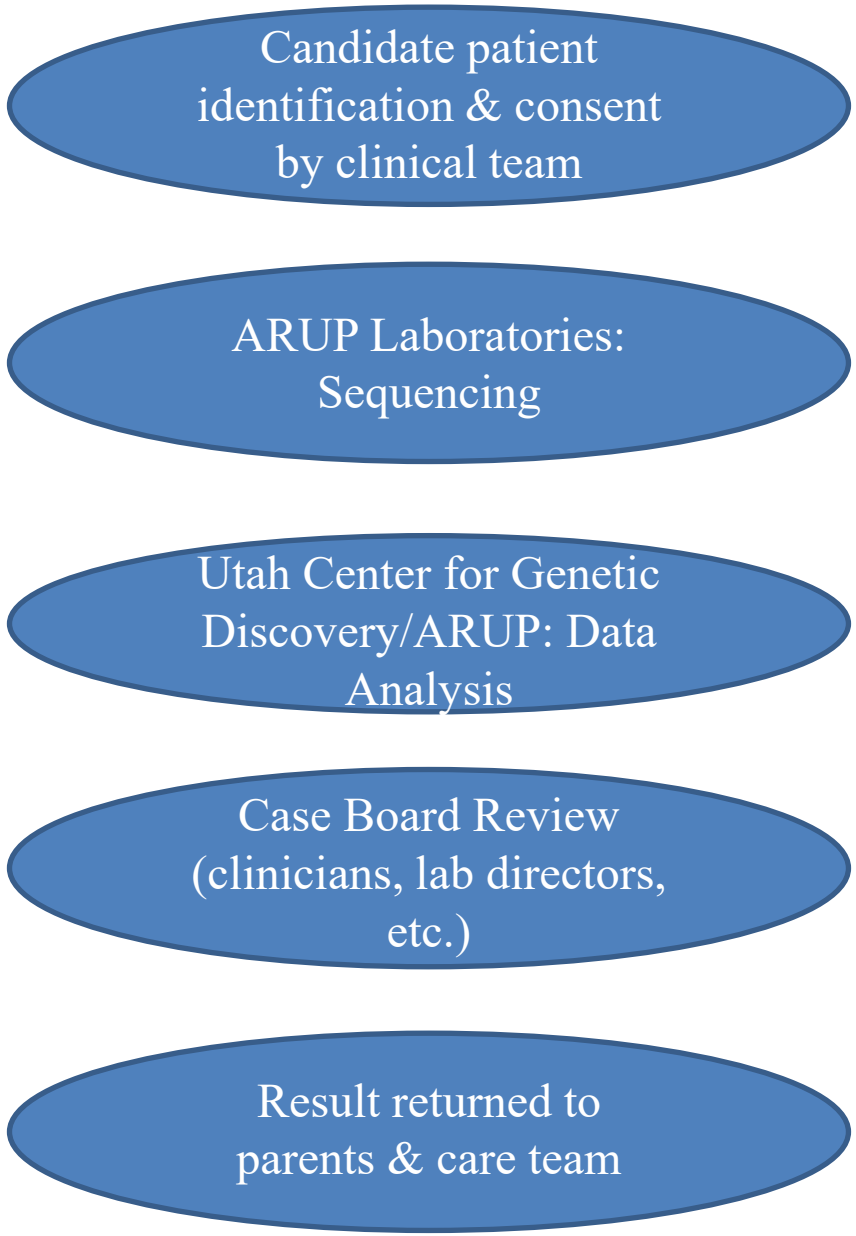
SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

GENETIC DIAGNOSIS

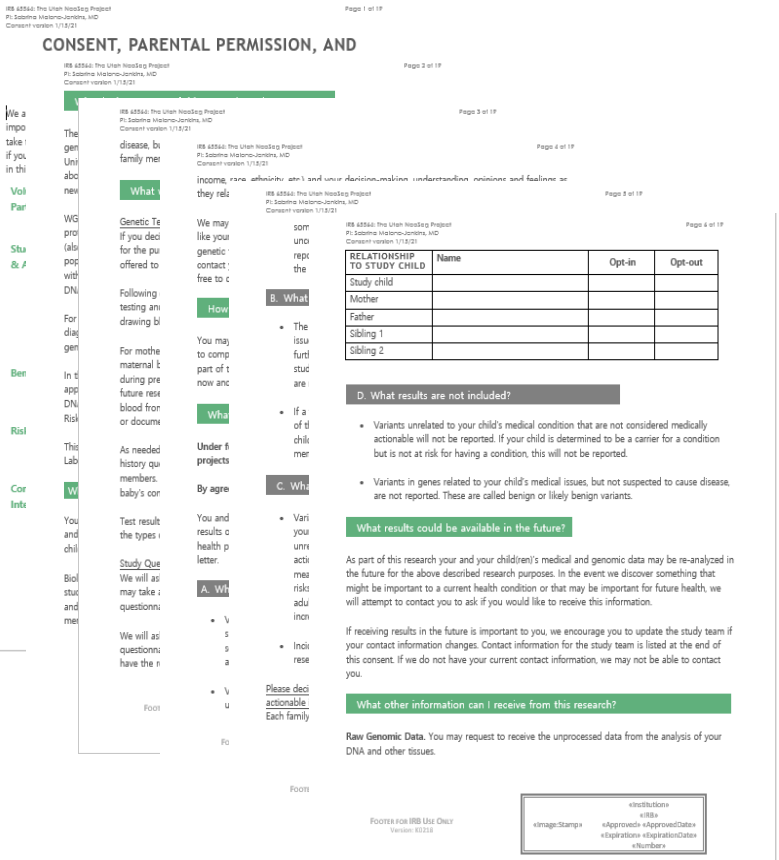
Diagnosis of genetic diseases in seriously ill children by rapid whole-genome sequencing and automated phenotyping and interpretation

Michelle M. Clark[1], Amber Hildreth[1,2,3], Sergey Batalov[1], Yan Ding[1], Shimul Chowdhury[1], Kelly Watkins[1], Katarzyna Ellsworth[1], Brandon Camp[1], Cyrielle I. Kint[4], Calum Yacoubian[5], Lauge Farnaes[1,2], Matthew N. Bainbridge[1,6], Curtis Beebe[7], Joshua J. A. Braun[1], Margaret Bray[8], Jeanne Carroll[1,2], Julie A. Cakici[1], Sara A. Caylor[1], Christina Clarke[1], Mitchell P. Creed[9], Jennifer Friedman[1,10], Alison Frith[5], Richard Gain[5], Mary Gaughran[1], Shauna George[7], Sheldon Gilmer[7], Joseph Gleeson[1,10], Jeremy Gore[11], Haiying Grunenwald[12], Raymond L. Hovey[1], Marie L. Janes[1], Kejia Lin[7], Paul D. McDonagh[8], Kyle McBride[7], Patrick Mulrooney[1], Shareef Nahas[1], Daeheon Oh[1], Albert Oriol[7], Laura Puckett[1], Zia Rady[1], Martin G. Reese[13], Julie Ryu[1,2], Lisa Salz[1], Erica Sanford[1,2], Lawrence Stewart[7], Nathaly Sweeney[1,2], Mari Tokita[1], Luca Van Der Kraan[1], Sarah White[1], Kristen Wigby[1,2], Brett Williams[5], Terence Wong[1], Meredith S. Wright[1], Catherine Yamada[1], Peter Schols[4], John Reynders[8], Kevin Hall[12], David Dimmock[1], Narayanan Veeraraghavan[1], Thomas Defay[8], Stephen F. Kingsmore[1]*

Clark *et al*. (2019) *Sci Transl Med*. 11(489)


Candidate patient identification & consent by clinical team
ARUP Laboratories: Sequencing
Utah Center for Genetic Discovery/ARUP: Data Analysis
Case Board Review (clinicians, lab directors, etc.)
Result returned to parents & care team

WGS Consent is a Complex Process



CONSENT, PARENTAL PERMISSION, AND

RELATIONSHIP TO STUDY CHILD	Name	Opt-in	Opt-out
Study child			
Mother			
Father			
Sibling 1			
Sibling 2			

D. What results are not included?

- Variants unrelated to your child's medical condition that are not considered medically actionable will not be reported. If your child is determined to be a carrier for a condition but is not at risk for having a condition, this will not be reported.
- Variants in genes related to your child's medical issues, but not suspected to cause disease, are not reported. These are called benign or likely benign variants.

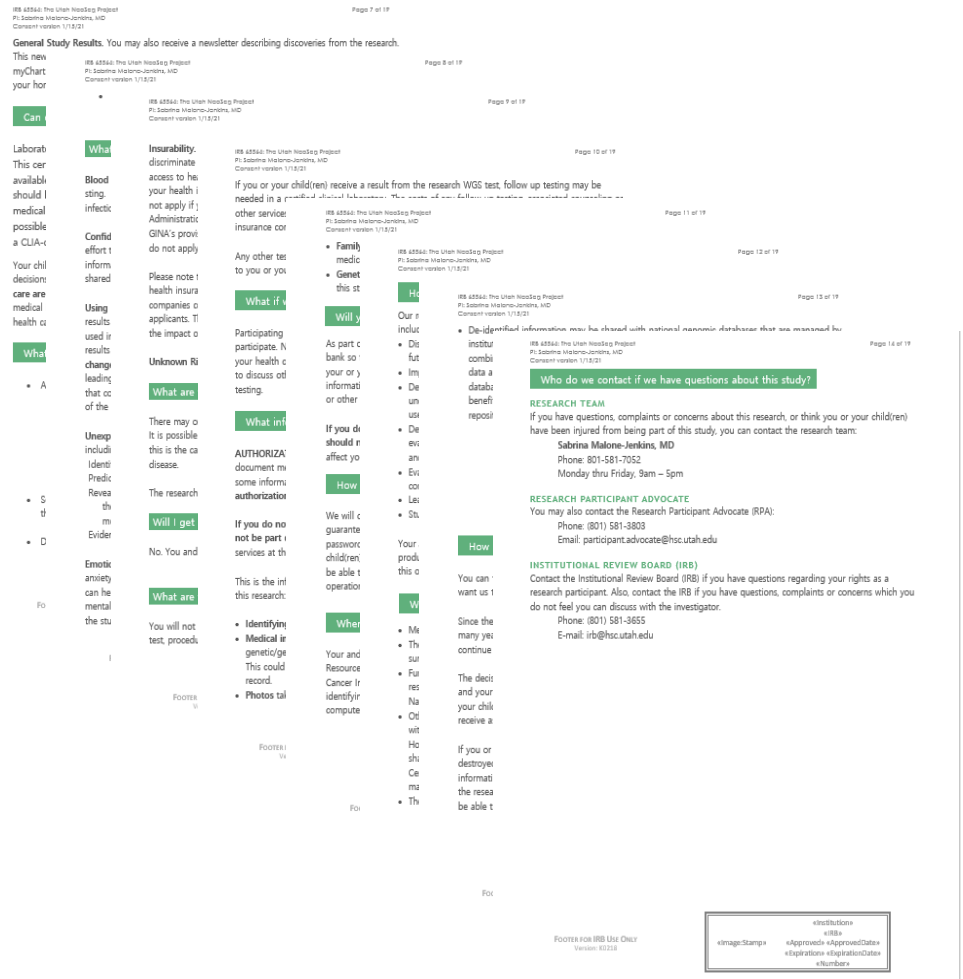
What results could be available in the future?

As part of this research your and your child(ren)'s medical and genomic data may be re-analyzed in the future for the above described research purposes. In the event we discover something that might be important to a current health condition or that may be important for future health, we will attempt to contact you to ask if you would like to receive this information.

If receiving results in the future is important to you, we encourage you to update the study team if your contact information changes. Contact information for the study team is listed at the end of this consent. If we do not have your current contact information, we may not be able to contact you.

What other information can I receive from this research?

Raw Genomic Data. You may request to receive the unprocessed data from the analysis of your DNA and other tissues.



General Study Results. You may also receive a newsletter describing discoveries from the research.

Who do we contact if we have questions about this study?

RESEARCH TEAM

If you have questions, complaints or concerns about this research, or think you or your child(ren) have been injured from being part of this study, you can contact the research team:

Sabrina Malone-Jenkins, MD
Phone: 801-581-7052
Monday thru Friday, 9am – 5pm

RESEARCH PARTICIPANT ADVOCATE

You may also contact the Research Participant Advocate (RPA):
Phone: (801) 581-3803
Email: participant.advocate@hsc.utah.edu

INSTITUTIONAL REVIEW BOARD (IRB)

Contact the Institutional Review Board (IRB) if you have questions regarding your rights as a research participant. Also, contact the IRB if you have questions, complaints or concerns which you do not feel you can discuss with the investigator.
Phone: (801) 581-3655
E-mail: irb@hsc.utah.edu

Common rWGS Lab Workflow

Sample Received

↓

DNA Extraction

↓

Tagmentation
(Library Prep)

↓

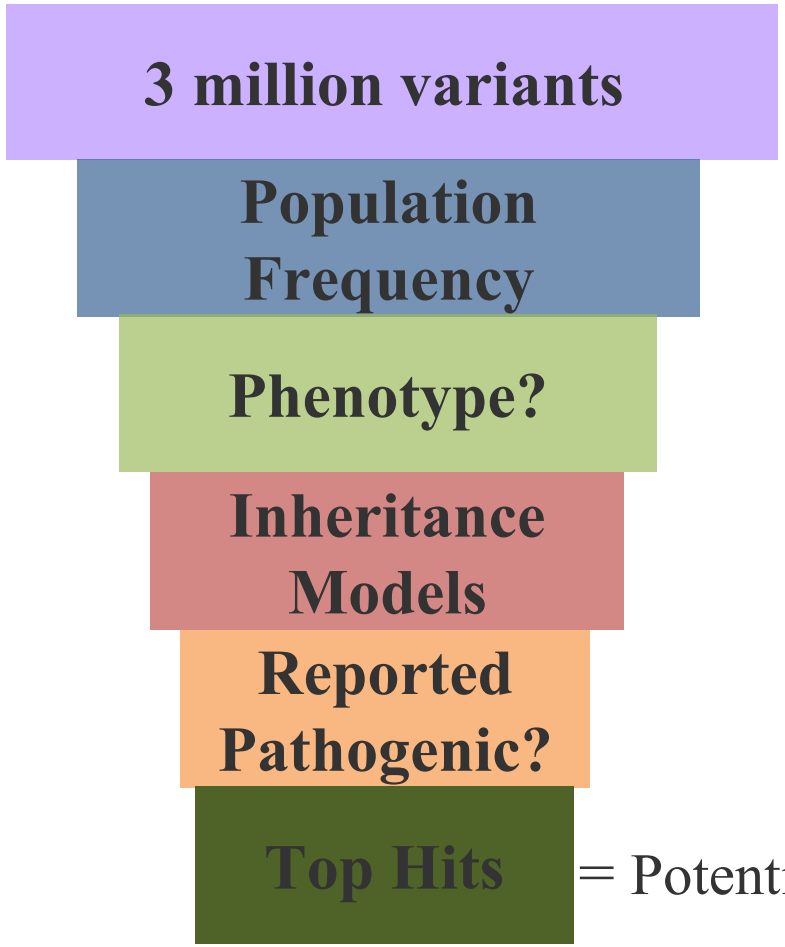
Sequencing

↘

Data Transfer &
Analysis

S1 Flowcell per Trio
= ~50X coverage/sample
(500-550Gb Total)

Applying Data Filters is Critical!

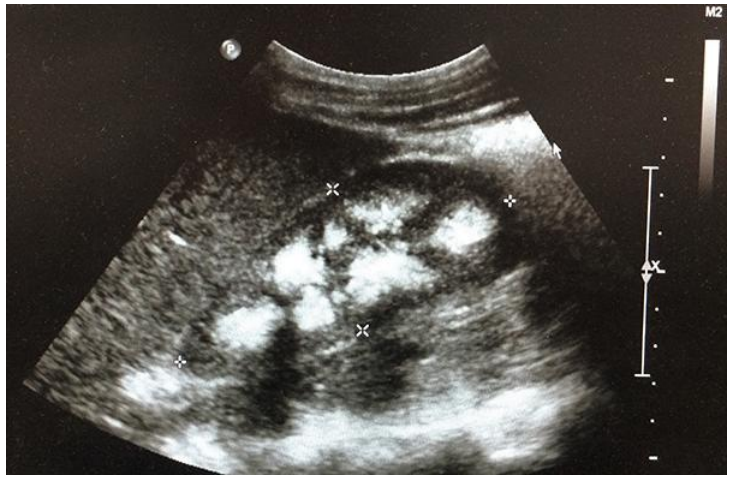


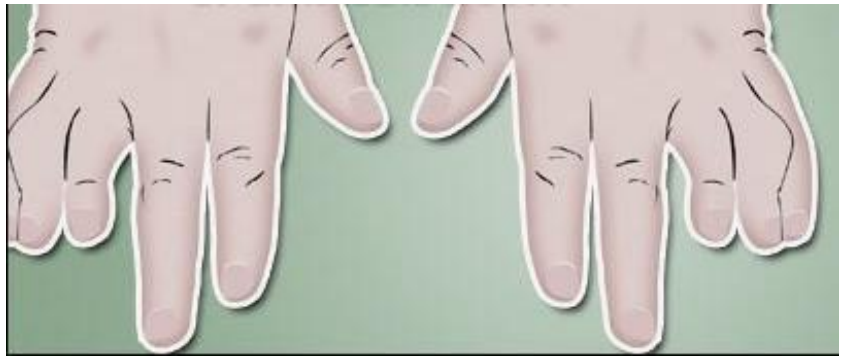
= Potentially Causative Variants

Case Example #1

Case History

- Prenatal ultrasound at 32 weeks gestation reveals:
  - » Enlarged echogenic kidneys
  - » Postaxial polydactyly of hands
  - » Club feet
  - » Patient enrolled in NeoSeq on DOL 2



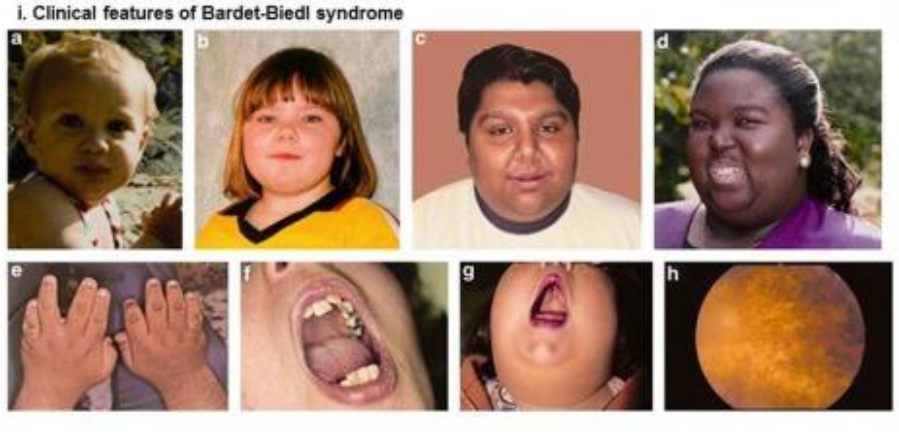


Sequencing Results

- Standard variant filters applied
- HPO keyterms used to further target variant list
  - » polydactyly, club feet, enlarged echogenic kidneys
- Two candidate variants identified in the *BBS10* gene
  - » c.909_912del (p.Ser303Argfs*3) and c.271dup (p.Cys91fs*5)
  - » Both are rare alleles in the general population in gnomAD
  - » Both are frameshift variants reported as pathogenic in ClinVar
  - » Both are predicted to truncate mRNA in *BBS10*
  - » Based on parental results, variants are in trans
  - » Gene is an autosomal recessive cause of Bardet-Biedl Syndrome

Bardet-Biedl Syndrome

- A multisystem ciliopathy
- Characterized by cone-rod dystrophy, obesity, postaxial polydactyly, cognitive impairment, renal malformations, genitourinary malformation, musculoskeletal abnormalities, etc.
- Clinical features are progressive and present from infancy through young adulthood making clinical diagnosis difficult



ii. Diagnostic features

	Frequency
Primary features	
Rod-cone dystrophy	93%
Polydactyly	63–81%
All four limbs:	21%
Upper limbs only:	9%
Lower limbs only:	21%
Obesity	72–92%
Genital anomalies	59–98%
Renal anomalies	53%
Learning difficulties	61%
Secondary features	
Speech delay	54–81%
Developmental delay	50–91%
Diabetes mellitus	6–48%
Dental anomalies	51%
Congenital heart disease	7%
Brachydactyly/ syndactyly	46–100%/8–95%
Ataxia/ poor coordination	40–86%

Forsythe *et al*. *Front Pediatr*. (2018)

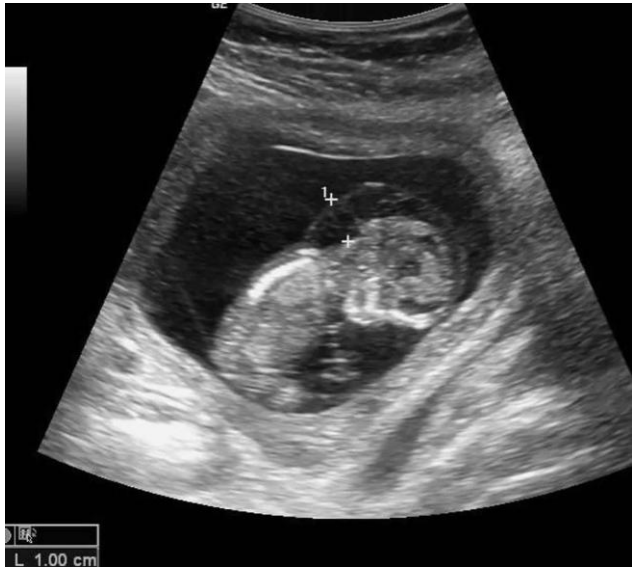
Case Outcome

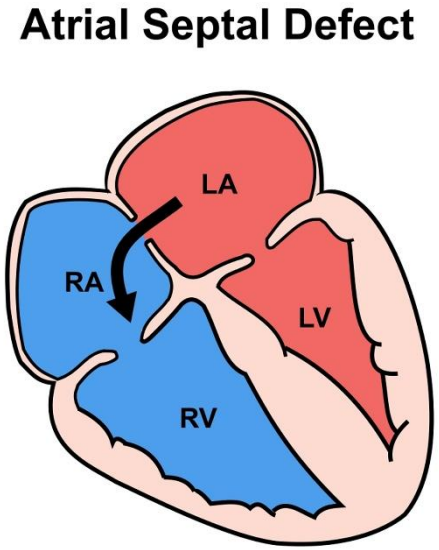
- Diagnosis of Bardet-Biedl Syndrome delivered on DOL 6
  - » "Standard of care" genetic tests results returned two weeks later
- Reported changes in management:
  - » Referral to specialists and additional medical screening recommended
- Parents reported a high degree of satisfaction with the speed of the diagnosis and genetic counseling received

Case Example #2

Case History

- 3-day-old male with hydrops fetalis, abnormal positioning of feet, patent foramen ovale, atrial septal defect, ascites, hydrocele and skin edema
- Patient enrolled in NeoSeq on DOL 4



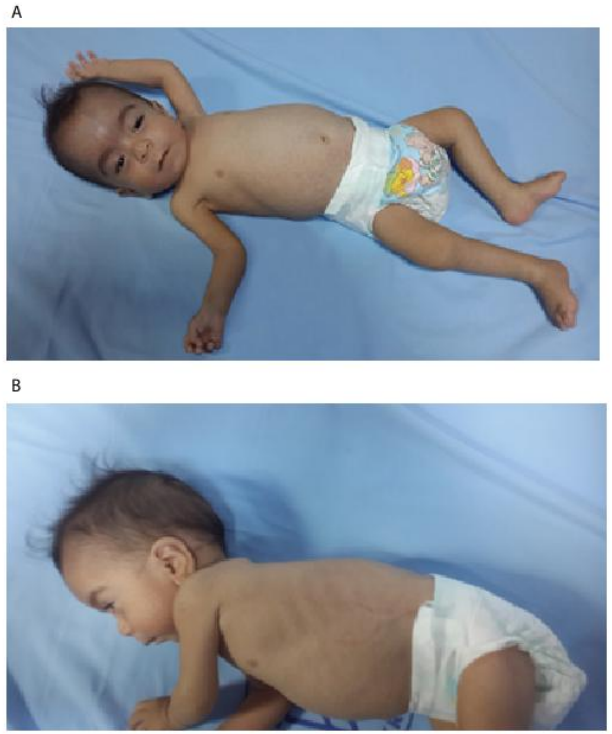


Sequencing Results

- Standard variant filters applied
- HPO keyterms used to further target variant list
  - » hydrops fetalis, ascites, positional foot deformity, cholestasis, hydrocele testis, etc.
- Two candidate variants identified in the *CTSA* gene
  - » c.184C>T (p.Gln62X) and c.1372T>G (p.Phe458Val)
  - » Both are rare alleles in the general population in gnomAD
  - » Based on parental results, variants are in trans
  - » Nonsense variant assumed pathogenic. The missense variant is in a well conserved region and previously characterized as pathogenic in ClinVar and that interpretation is supported by functional studies
  - » Gene is an autosomal recessive cause of galactosialidosis

Galactosialidosis (aka Neuramidase Deficiency)

- A lysosomal storage disorder with a combined deficiency of beta-galactosidase and neuraminidase
- Three forms of the disease exist: early infantile, late infantile and juvenile/adult
- Early infantile form characterized by fetal hydrops, edema, visceromegaly, skeletal dysplasia and death in infancy
- Disease is exceedingly rare with fewer than 150 cases reported in the literature



re 1: Frontal view (A) and lateral view. (B) of the patient's appear

Nur *et al*. (2014) *J Genet Syndr Gen Ther*.

Outcome

- Diagnosis of Galactosialidosis provided on DOL 9
- Reinforced the family's decision to move to compassionate care
- Patient passed away a few weeks after receiving diagnosis
- Clinical standard of care test results were not received until 2 days after patient passed away

Are negative rWGS results helpful?

Negative rWGS Cases are Still Clinically Useful

- Post-result surveys have been conducted by Utah NeoSeq and Rady Children's Hospital
- Clinicians and family members are almost as satisfied with negative results as they are with a positive
- Negative results provide additional information for management decisions

Utah NeoSeq Summary

- 65 infants enrolled
  - » Causative variants found in 26 (40%) patients
  - » Strong candidates found in an additional 7 (11%)
- 52 provider surveys were conducted and 41/52 (79%) characterized the results as ‘very useful’ and associated with management changes

Malone-Jenkins *et al*. (2025) *NPJ Genom Med*. 10(1):26

Standard Turnaround Time
Whole Genome Sequencing (WGS)

What is Standard TAT WGS?

- WGS performed without a clinical need for rapid results
  - » Reduced cost compared to rWGS
  - » Report may include larger number of VUSs
  - » Typically reported in about a month but depends on the lab
    - ARUP TAT is between 2-3 weeks

Case Example #1

Case History

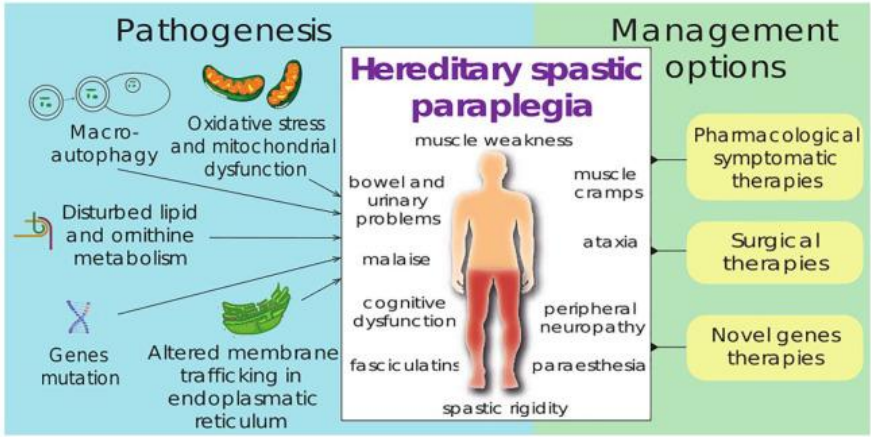
- 26-year-old male with psychosis, auditory hallucinations, weight gain, hair loss, and marked mood shifts
  - » Brain MRI revealed microadenoma
  - » Lumbar puncture revealed anomalies in neurotransmitter levels
- Clinician suspects that patient has a CNS inflammatory process issue or a lysosomal storage disorder
- WGS ordered on proband only

Sequencing Results

- Standard variant filters applied
- HPO keyterms used to further target variant list
  - » Psychosis, auditory hallucination, increased body weight, alopecia, emotional liability, abnormality of the nervous system
- Two candidate variants identified in the *SPG7* gene
  - » c.1529C>T (p.Ala510Val) and c.759-2A>G
  - » Both are rare alleles in the general population in gnomAD
  - » c.1529C>T is the most commonly reported *SPG7* variant and has been seen in both the compound heterozygous and homozygous state
  - » C.759-2A>G is a canonical splice site variant and assumed pathogenic
  - » Gene is an autosomal recessive cause of Hereditary Spastic Paraplegia (HSP)

Hereditary Spastic Paraplegia

- Characterized by ataxia, multisystem atrophy and peripheral neuropathy
- Some individuals also present with schizophrenia-like features such as cognitive and emotional dysfunction, psychosis, auditory hallucinations and language impairment
- Schizophrenia-like features are a rarely reported feature of this disease in the literature. This is thought to be due to less rigorous cognitive evaluations being performed in patients with the physical findings
- Treatment is symptom specific and no overall cure is available



Awuah *et al*. (2023) *Sage Open Med*. 12: 20503121231221941

Case Summary

- WGS results provide diagnosis of Hereditary Spastic Paraplegia
  - » Disorder wasn't on differential provided by referring clinician
  - » Case illustrates the power of using whole genome sequencing in older patients with nonspecific symptoms

Case Example #2

Case History

- 5-month-old Arabic male with necrotizing enterocolitis totalis, bowel resection, chronic respiratory failure, pulmonary hypertension, cholestasis, anemia, cardiomegaly, hepatosplenomegaly, congenital hypothyroidism, DVT and seizures
- Abnormal echocardiogram showing increased right ventricular pressure, right ventricular fluid overload and ventricular septal defect
- EEG was abnormal
- Brain MRI was unremarkable
- Family history is noncontributory
- WGS ordered on proband and parents

Sequencing Results

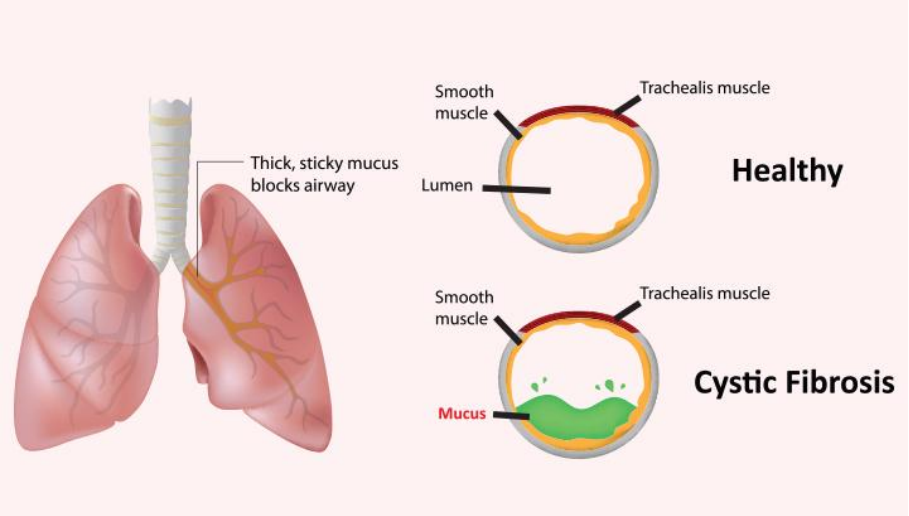
- Standard variant filters applied
- HPO keyterms used to further target variant list
  - » Necrotizing enterocolitis, respiratory failure, pulmonary arterial hypertension, pulmonic stenosis, chronic lung disease, tracheobronchomalacia, cholestasis, congenital hypothyroidism, anemia, adrenal insufficiency, cardiomegaly, hepatosplenomegaly, deep venous thrombosis, seizure, cardiorespiratory arrest, recurrent infections, conjunctival icterus, abnormal anterior fontanelle morphology, polyhydramnios, hypertonia, hyperreflexia, global developmental delay, ventricular septal defect
- Homozygous pathogenic variants identified in both the *CFTR* and *TPO* genes
- Homozygous likely pathogenic variant identified in the *SLC12A6* gene

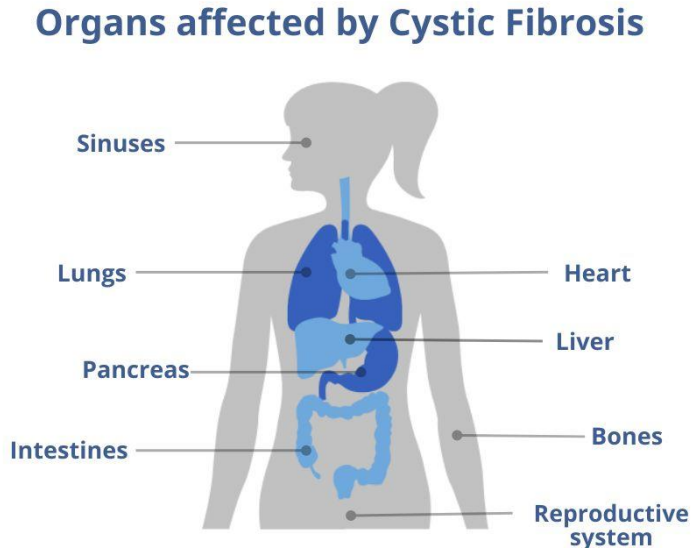
Sequencing Results

- Homozygous *TPO* Variant
  - » c.2422del (p.Cys808AlafsTer24). Frameshift variant known to be pathogenic
  - » Autosomal recessive gene variants in *TPO* result in Thyroid dyshormonogenesis type 2A
    - Heritable form of congenital hypothyroidism
  - » Identified variant is reported in the literature in numerous individuals with congenital hypothyroidism

Sequencing Results

- *CFTR* Variant
  - » c.1521_1523del (p. Phe508del) homozygous. Well known pathogenic variant
  - » Autosomal recessive gene variants result in Cystic Fibrosis
  - » Disease is characterized by pulmonary dysfunction, recurrent bronchitis, pancreatic insufficiency, malnutrition, myriad GI issues, liver disease, bronchiectasis and male infertility





Sequencing Results

- *SLC12A6* Homozygous Variant
  - » c.106_124dup (p.Arg42GlnfsTer5)
  - » Variant not reported in literature but causes a frameshift in a gene where loss of function is a known mechanism of disease
  - » Variant not reported in gnomAD
  - » Autosomal recessive *SLC12A6* variants result in Agenesis of the Corpus Callosum with Peripheral Neuropathy

Agenesis of the Corpus Callosum with Peripheral Neuropathy

- Also known as Andermann Syndrome or Charlevoix Disease
- Characterized by hypotonia, areflexia, developmental delay, intellectual disability, dysmorphic features, variable dysgenesis of the corpus callosum, contractures, tremors and seizures
- Restrictive lung disease leading to respiratory distress may be present in some patients
- Agenesis of the corpus collosum can be absent with only polyneuropathy or sensorimotor issues present
- Death is usually in third or fourth decade of life usually due to respiratory infection

Case Summary

- WGS results provide diagnosis of multiple genetic disorders:
  - » Cystic Fibrosis
  - » Thyroid Dyshormonogenesis 2A
  - » Agenesis of the Corpus Callosum w/ Peripheral Neuropathy
- Given the broad clinical spectrum of the phenotype, a genome wide testing method was the ideal genetic test for this patient

WGS Benefits/Limitations

WGS Benefits

- WGS allows for screening of the entire human genome for genetic variants that may be contributing to the phenotype in patients
  - » Most appropriate for testing of patients where the clinical findings do not suggest any one specific genetic disorder
- From a technical perspective, WGS is a better testing method than Exome
  - » As mentioned earlier, WGS detects some variants that are missed by exome sequencing resulting in increased diagnostic yield
  - » Exome should still be considered in circumstances where insurance providers are unwilling to authorize use of WGS
- Some variant classes (i.e., copy number variants) are more easily detectable by genome that exome

WGS Limitations

- Rapid or not, WGS is still an expensive test
  - » Should not be used in the place of targeted gene panels or targeted molecular testing when a specific diagnosis is suspected
- WGS is not currently a replacement for chromosomal microarray testing
  - » WGS still misses some structural rearrangements and other cytogenetic abnormalities
    - Current WGS detection rates for these abnormalities is about 85% while array is usually reported to be well over 90%. WGS is missing variants in this category
    - Eventually, WGS will be as good as (or better than) array but we're not quite there yet
- Detection of triplet repeat disorders is hit and miss but improving
- We are still unable to detect some variants in high homology regions of the genome using WGS. This too is improving and will eventually be possible

Summary

- Whole Genome Sequencing is a powerful diagnostic tool that is applicable in a number of clinical scenarios including:
  - » Rapid disease diagnosis in newborns and in adults where a quick diagnosis may impact clinical outcome
  - » Genetic diagnosis in patients with many findings that do not point to one specific disorder
  - » Diagnosis in patients that have undergone a long diagnostic odyssey without a clear answer
- WGS is technically superior to exome but insurance coverage may necessitate the continued use of exome sequencing
- There are still limitations to WGS but constantly improving bioinformatic tools are likely to eliminate most of these in the coming years

Utah NeoSeq Acknowledgements

Mudsar Ahmad
Najla Al-Sweel
Katherine Anderson
Meagan Barrows
Pinar Bayrak-Toydemir
Dawn Bentley
Hunter Best
Anne Blaschke
Josh Bonkowsky
Lorenzo Botto
Steven Boyden
Luca Brunelli
Ashley Bunker
Russell Butterfield
Janice Byrne
John Carey
Samuel Cheshier
Devin Close
Richard Coleman
Michael Cormier
Jacob Durtschi
John Farrell
Josue Flores-Daboub
Eric Fredrickson
Makenzie Fulmer
Bushra Gorsi
Stephen Guthery
Emily Hardy
Alexander Henrie
Edgar Hernandez
Carson Holt
Teresa Janecki
Yuan Ji
Ashley Joseph
Ashley Kapron
Mary Anne Karren
Gordon Lemmon
Tracey Lewis
Manndi Loertscher
Nicola Longo
Sabrina Malone-Jenkins
Luke Maese
Rong Mao
Kandace McGrath
Christine Miller
Marvin Moore
Tara Newcomb
Thomas Nicholas
Katherine Noble
Brendan O'Fallon
Betsy Ostrander
Rachel Palmquist
Brent Pedersen
Aaron Quinlan
Carrie Rau
Hayley Reynolds
Paul Rindler
Erin Rothwell
Shawn Rynearson
Joshua Schiffman
Kathryn Szczotka
Carrie Torr
Martin Tristani-Firouzi
Matt Velinder
David Viskochil
William Watkins
Sergiusz Wesolowski
Kathryn Woodbury
Joseph Worden
Mark Yandell
Jian Zhao

HEALTH
UNIVERSITY OF UTAH

ARUP LABORATORIES

Utah Center for Genetic Discovery

ARUP LABORATORIES

HEALTH
UNIVERSITY OF UTAH

ARUP is a nonprofit enterprise of the University of Utah and its Department of Pathology.