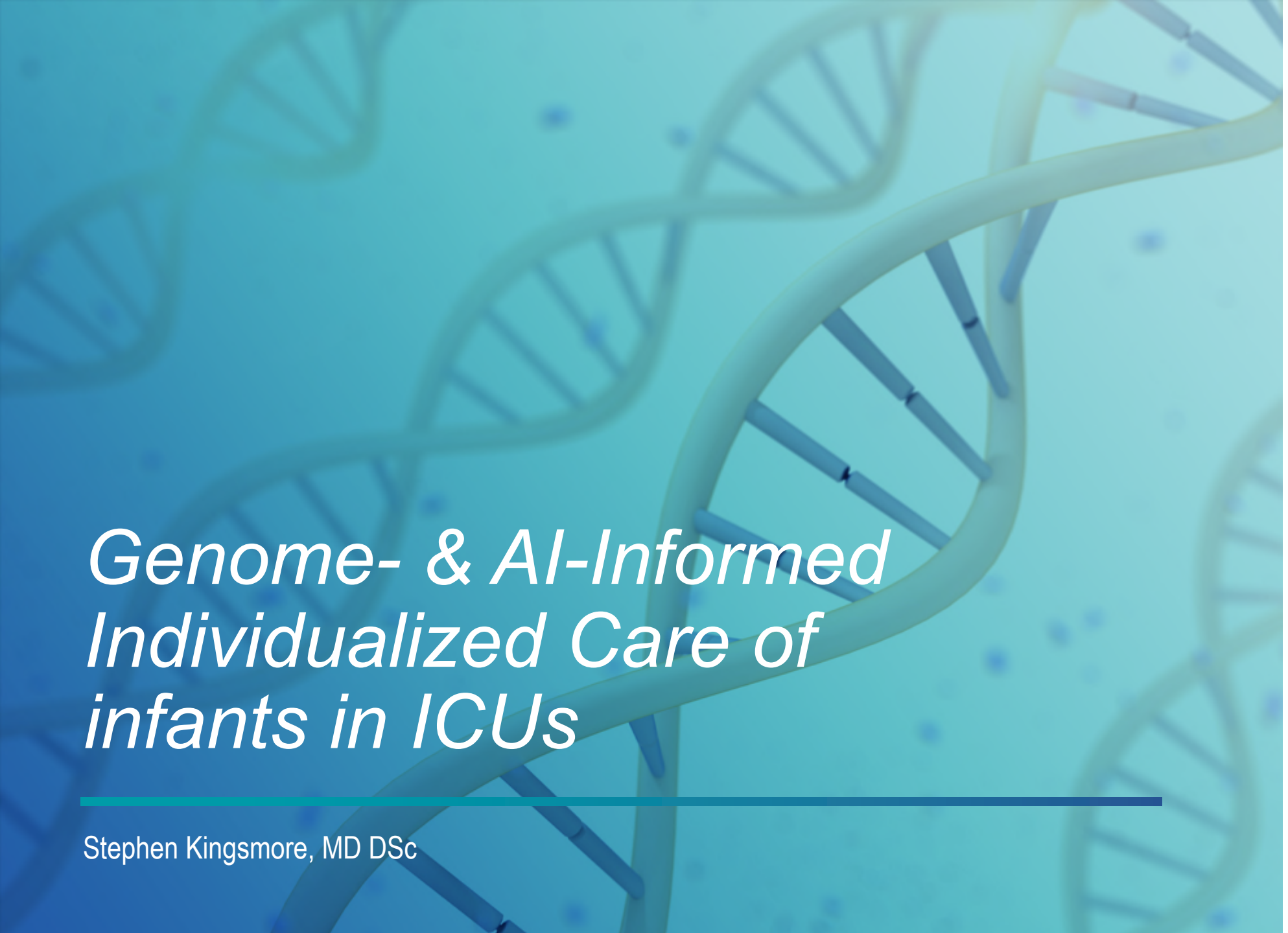


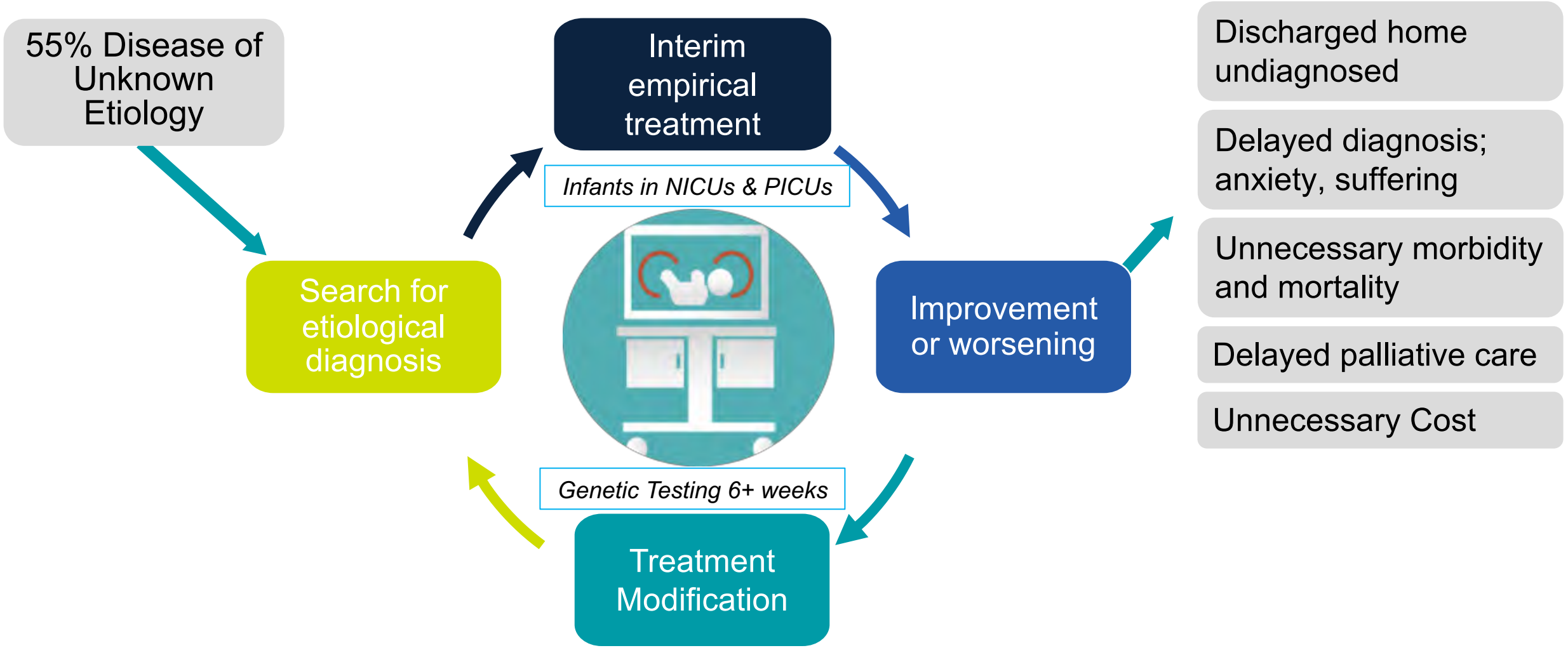
Genome- & AI-Informed Individualized Care of infants in ICUs

Stephen Kingsmore, MD DSc

Rady Children's Institute
Genomic Medicine

I do not have a conflict of interest with regard to this presentation

Rady Children's Institute
Genomic Medicine



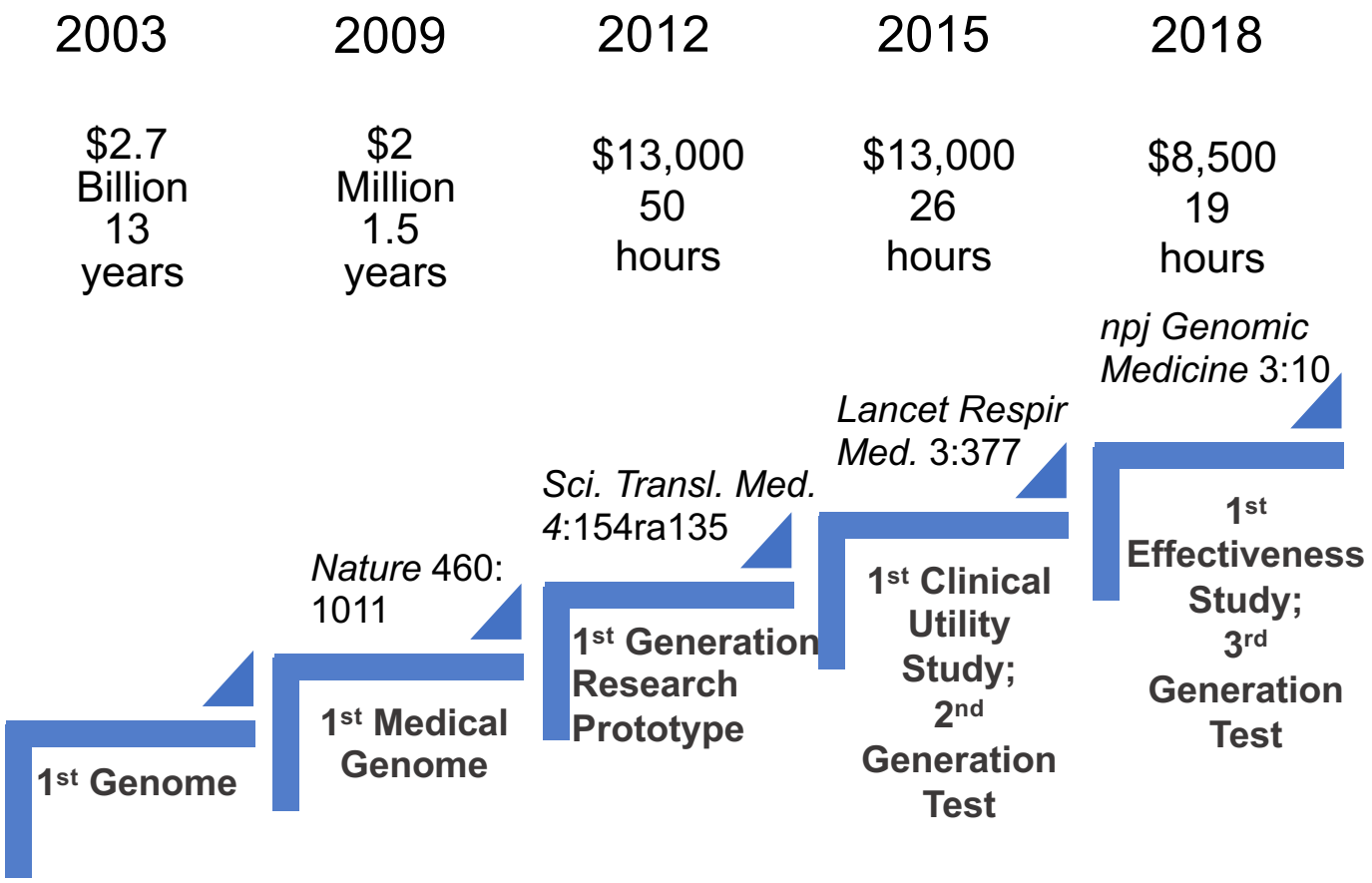
- **15% of 250,000 US NICU and PICU infants have 1 of 14,000 genetic diseases**
- **60% go undiagnosed and ∴ are mistreated; leading cause of death**

NSIGHT2, ClinicalTrials.gov ID NCT03211039, 213 enrollees, 47% of NICU, 15% incidence


DMC

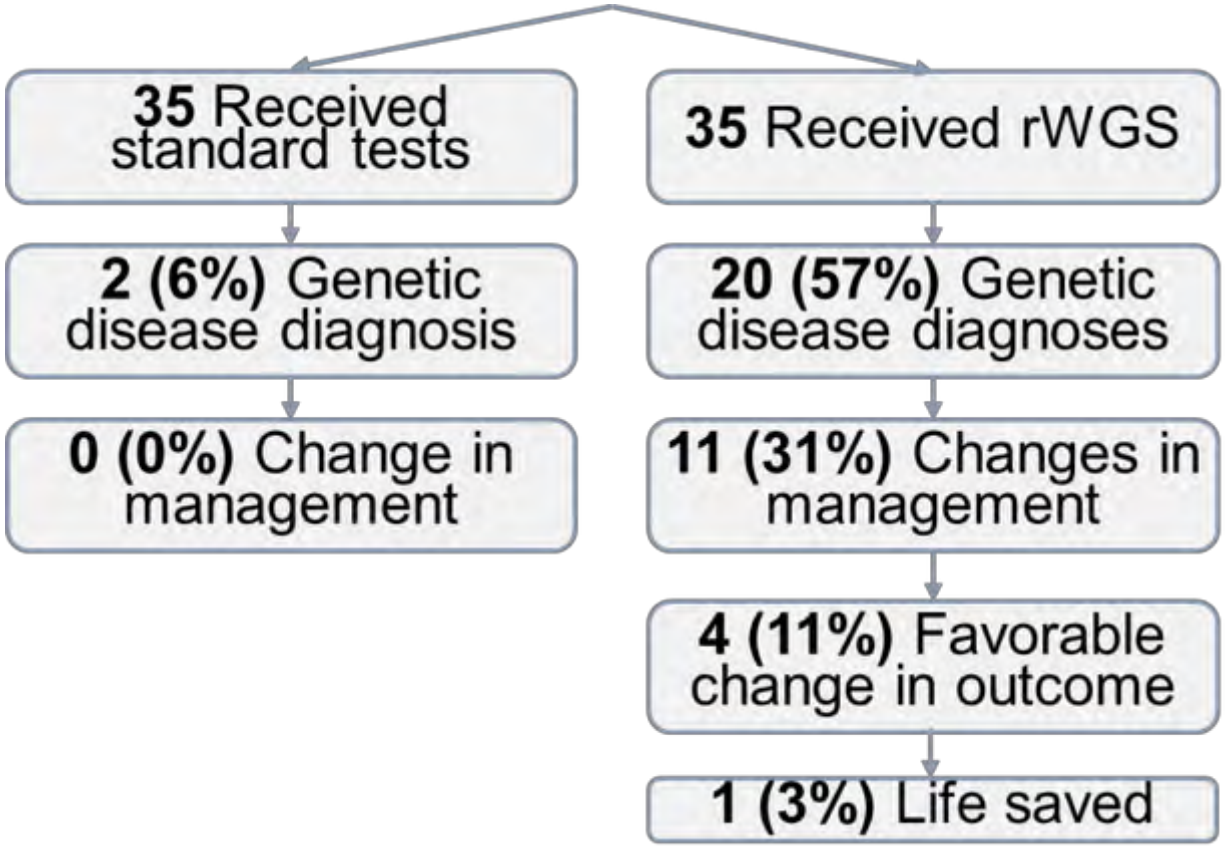
Evolution of Individualized Medicine in Children's ICUs

Rady Children's Institute Genomic Medicine

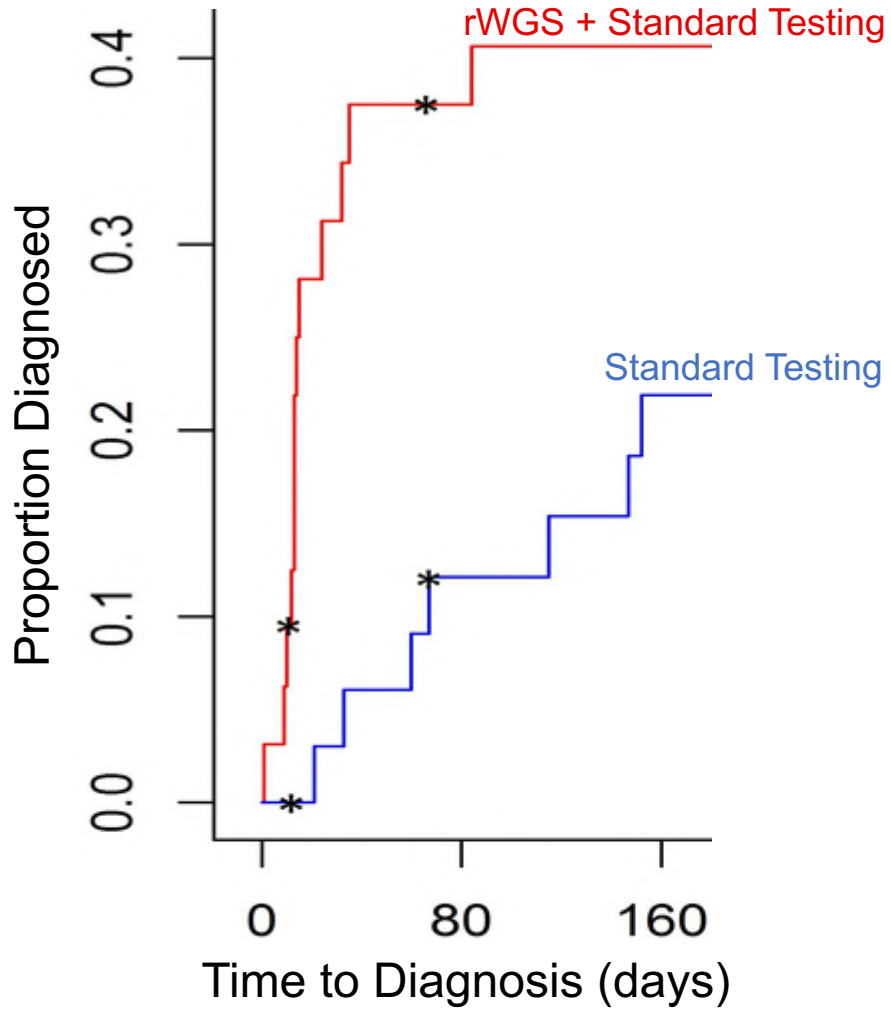


Two Early Studies of Clinical Utility of rWGS-based Individualized Medicine

Rady Children's Institute Genomic Medicine



(before individualized medicine mentoring)

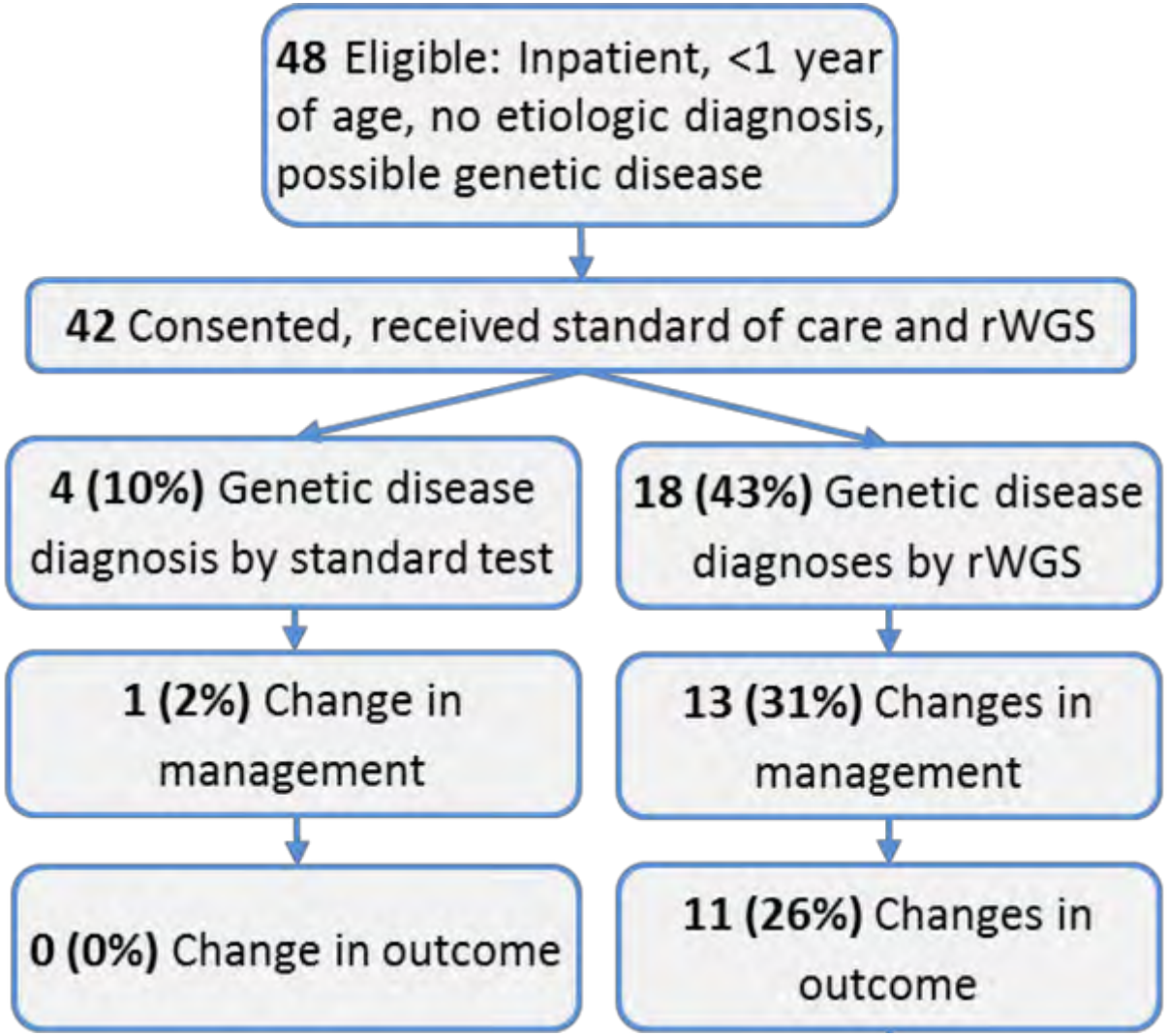


Lancet Respir Med (2015) 3:377

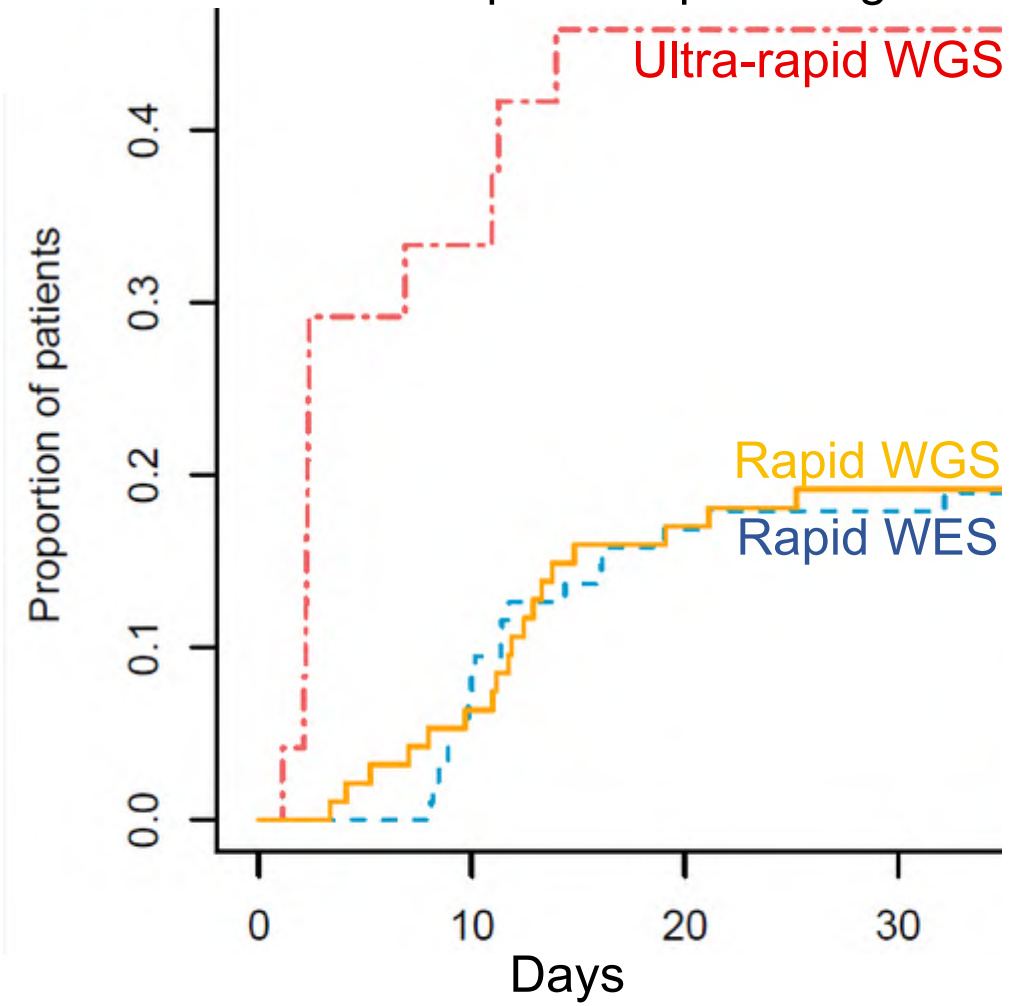
npj Genomic Med (2018 3:6)

Two Recent Studies of Effectiveness of rWGS-based Individualized Medicine

Rady Children's Institute Genomic Medicine



Time from blood sample receipt to diagnosis



npj Genomic Medicine (2018) 3:10

NSIGHT2, ClinicalTrials.gov ID NCT03211039

2019 status of rWGS-based individualized medicine for NICU and PICU Infants

Rady Children's Institute Genomic Medicine

55% Disease of Unknown Etiology

Search for etiological diagnosis inc. rWGS

Interim empirical treatment

rWGS 1.5-10 days

35% Genetic Disease Diagnosis

Infants in NICUs & PICUs

25% Individualized Medicine

20% improved outcomes
Less cost & suffering

Barriers to Adoption of rWGS-based individualized medicine for NICU and PICU infants

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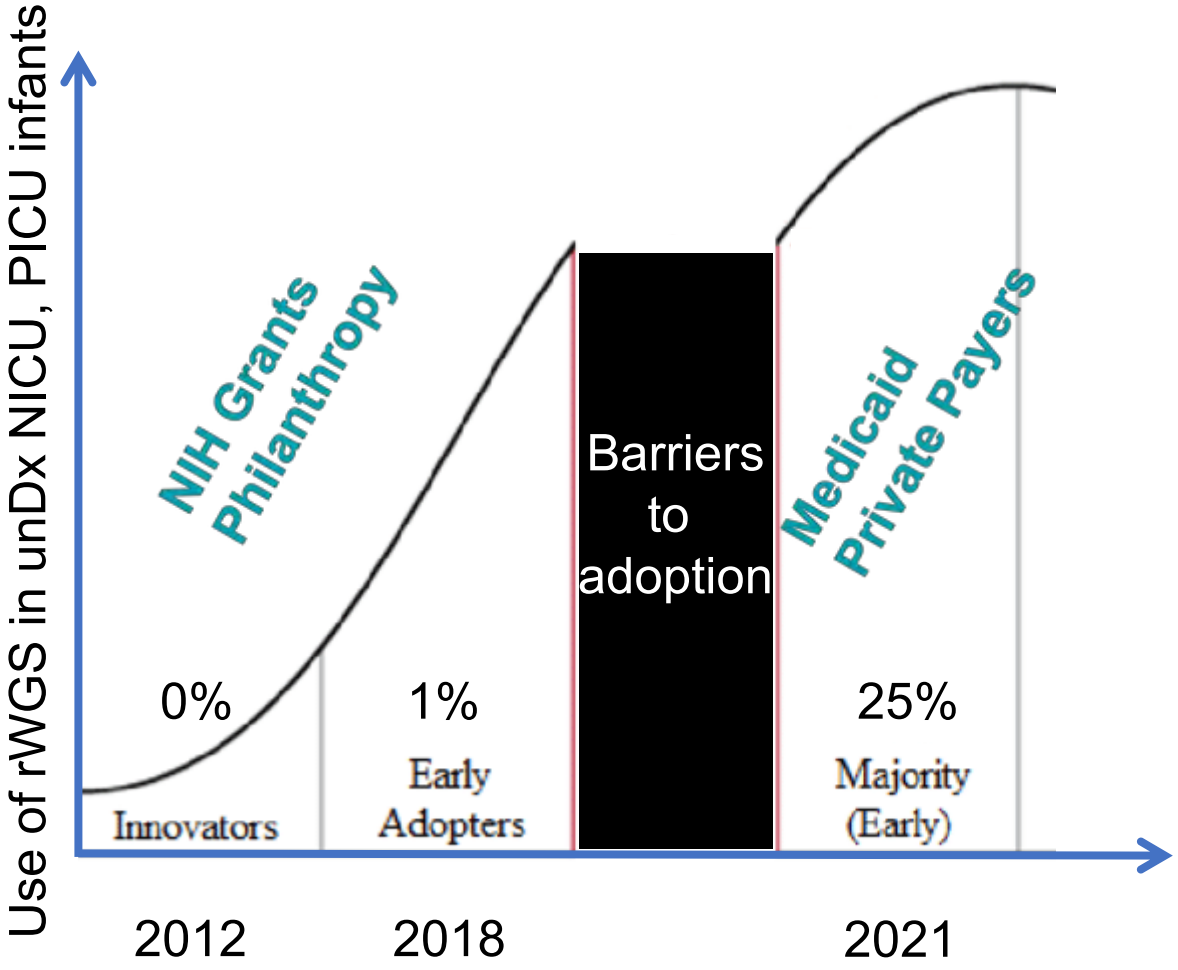
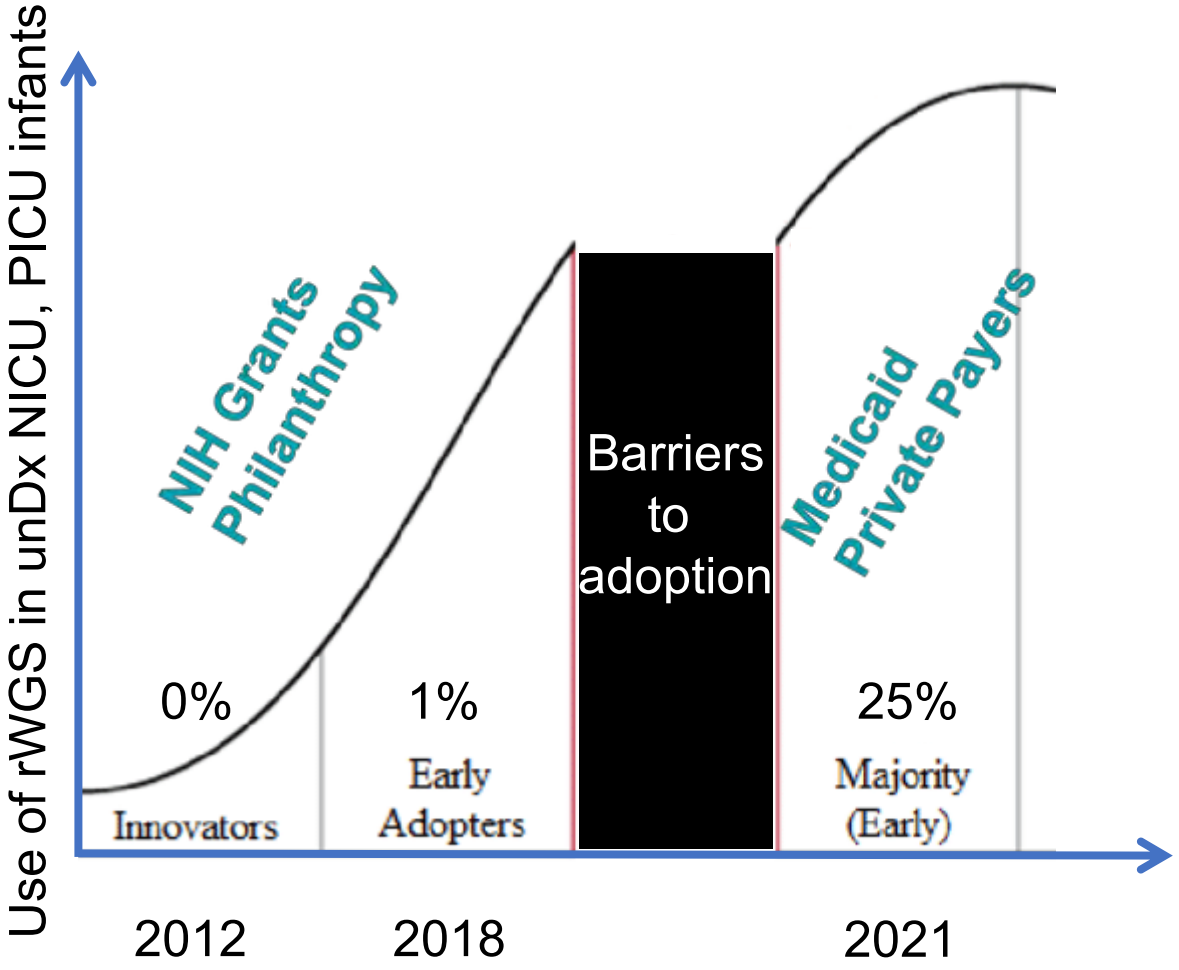


- Capital & labor intensity of individualized medicine
  - rWGS and rWES methods are too slow to guide NICU and PICU management
  - rWGS and rWES methods not scalable
  - Shortage of expert medical geneticists, laboratory directors, genetic counselors
- Lack of clinician familiarity with individualized medicine
  - Shortage of expert medical geneticists, genetic counselors
- Insufficient evidence of efficacy
  - Absence of reimbursement
- Many genetic diseases lack effective Rx
  - Most treatments lack adequate evidence

Barriers to Adoption of rWGS-based individualized medicine for NICU and PICU infants

Rady Children's Institute Genomic Medicine



- ~~Capital & labor intensity of individualized medicine~~
  - ~~rWGS and rWES methods are too slow to guide NICU and PICU management~~
  - ~~rWGS and rWES methods not scalable~~
  - ~~Shortage of expert medical geneticists, laboratory directors, genetic counselors~~
- Lack of clinician familiarity with individualized medicine
  - Shortage of expert medical geneticists, genetic counselors
- Insufficient evidence of efficacy
  - Absence of reimbursement
- Many genetic diseases lack effective Rx
  - Most treatments lack adequate evidence

3rd generation whole genome sequencing with automated genetic disease diagnosis

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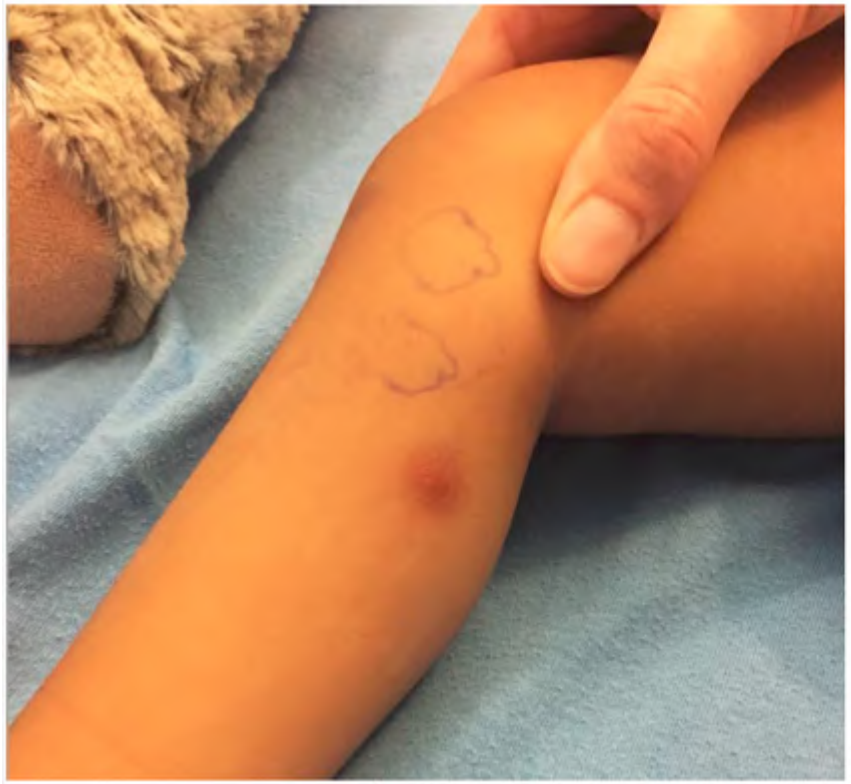
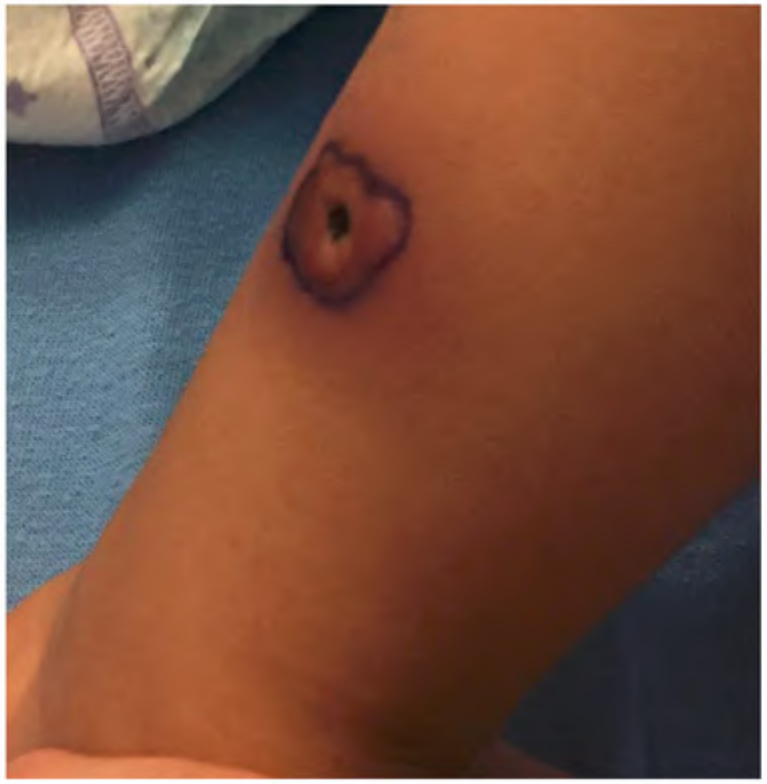
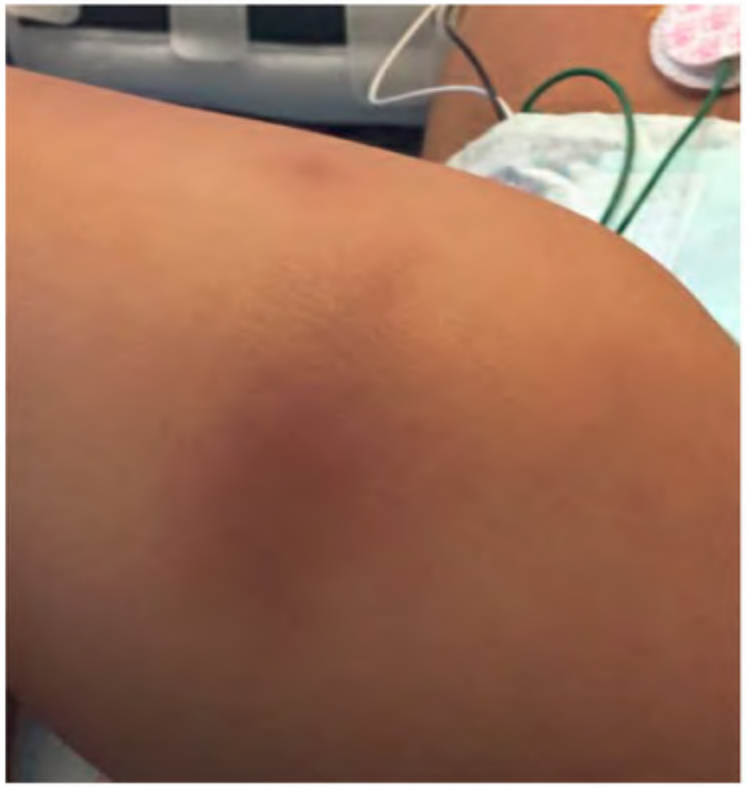
Median time to result 20 hours; In Press

17 month old boy with fever (T_{max} 103^0F) x 3 days, vomiting, diarrhea, abdominal pain, labored breathing, skin lesions x 1 day

Rady Children's Institute Genomic Medicine







Rady Emergency Department Work Up

Rady Children's Institute Genomic Medicine

- Blood tests:
  - Metabolic panel: metabolic acidosis
  - C-reactive protein: markedly elevated
  - Complete blood count: low white cell count
- Abdominal ultrasound & computed tomography: No intussusception, possible mild colitis
- Lumbar puncture
  - Cardiovascular decompensation = hypovolemic shock → intravenous fluids
  - SiO_2 88% on $Fi0_2$ 21% → Continuous Positive Airway Pressure ventilation
  - Sepsis suspected → intravenous vancomycin + ceftriaxone
  - Admitted to PICU → switched to intravenous meropenem

Hospital Day 2

Rady Children's Institute Genomic Medicine

- Blood culture: Pseudomonas aeruginosa
- Skin rash diagnosed as echthyma gangrenosum
- Rapid genome sequencing ordered

Step 1: Order in Epic Electronic Health Record

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LAB000796 RCIGM Rapid Whole Genome Test - Proband

Counseling Provided and Consent Obtained? **Yes** No

Select Test Type **Proband** **Trio** Other

Opt Out of Additional Use? **No** Yes

Include Incidental Findings? **Yes** No

Briefly Provide Clinical Description: Pseudomonal sepsis, leukopenia

Are Family Member Samples Expected? **Yes** No

Name and DOB of Expected Family Member Sample

Mother **Father** **Sibling** Other

Ultra-Rapid Processing? No **Yes**

Step 2: Genome library preparation (2.5 hours)

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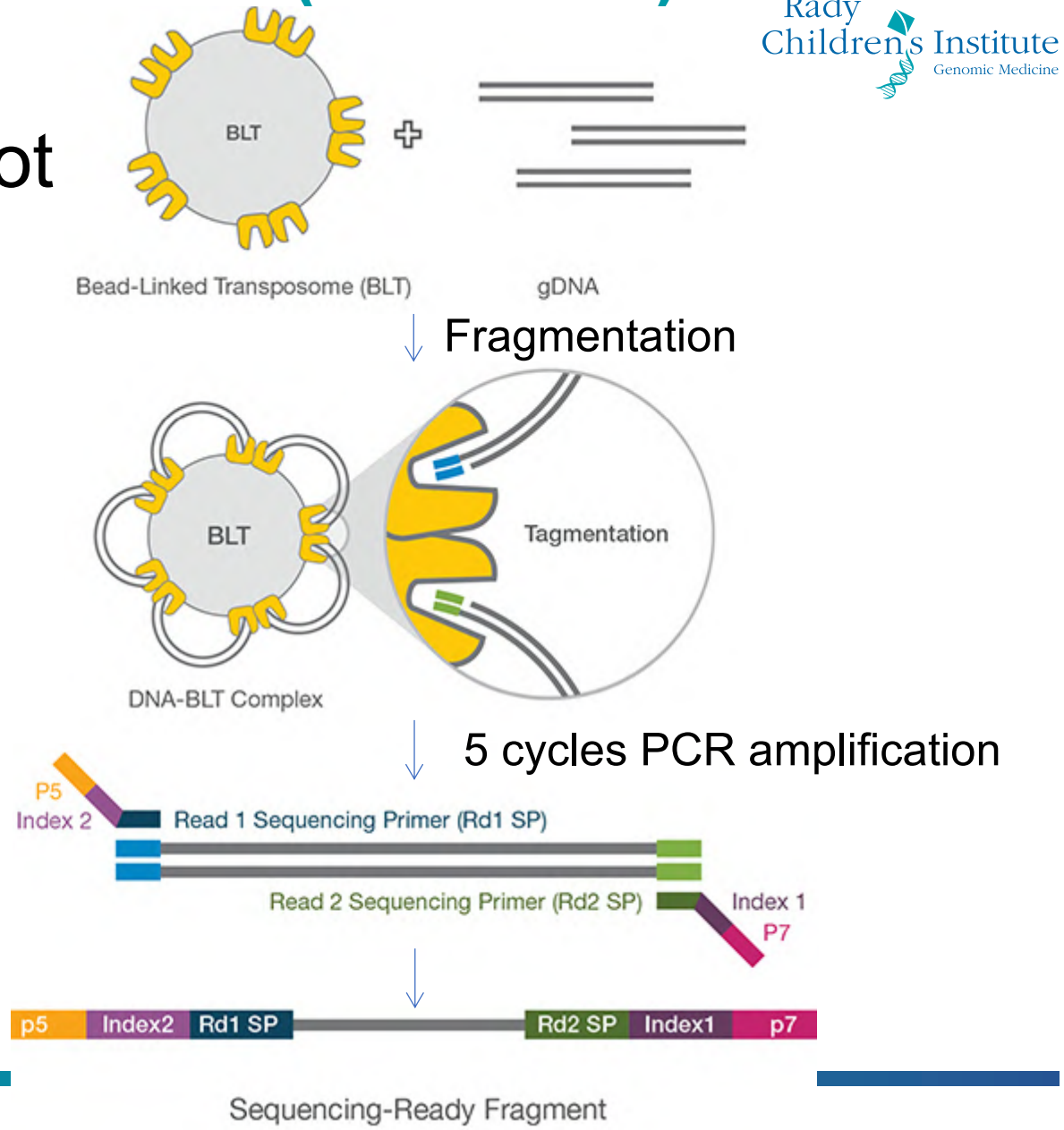
- 0.5 ml blood, dried blood spot
- Illumina Nextera Flex



Step 3: Genome Sequencing (15.5 hours)

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- 2 x 100 nucleotide paired sequences
- Illumina NovaSeq 6000 instrument
- S1 flowcell
- Trio or 2 Probands per flowcell
- 40X proband; 30X parents

RADY CHILDREN'S INSTITUTE SETS DNA SEQUENCE WORLD RECORD

Step toward faster diagnoses of puzzling, threatening illness

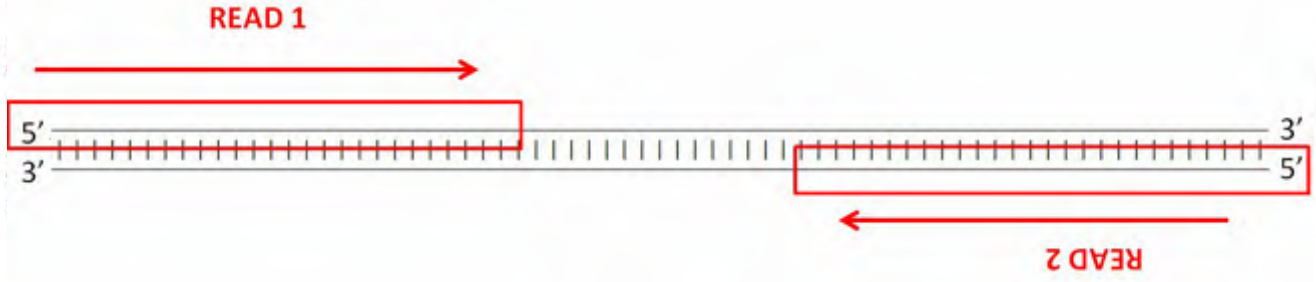
BY PAUL SISSON

It took 13 years to build the first full set of genetic blueprints for the human race by sequencing the DNA inside our cells that governs everything from eye color to risk of debilitating disease.

But a team at Rady Children's Institute for Genomic Medicine, working closely with homegrown sequencing sensation Illumina Inc., just proved it's possible to get the job done

EDUARDO CONTRERAS U-T

Clinical laboratory scientist Sarah White pulls a flow cell from a new genetic sequencer at Rady Children's Institute for Genomic Medicine.



Step 4: Identify all disease-causing variants in child's genome: 45 min

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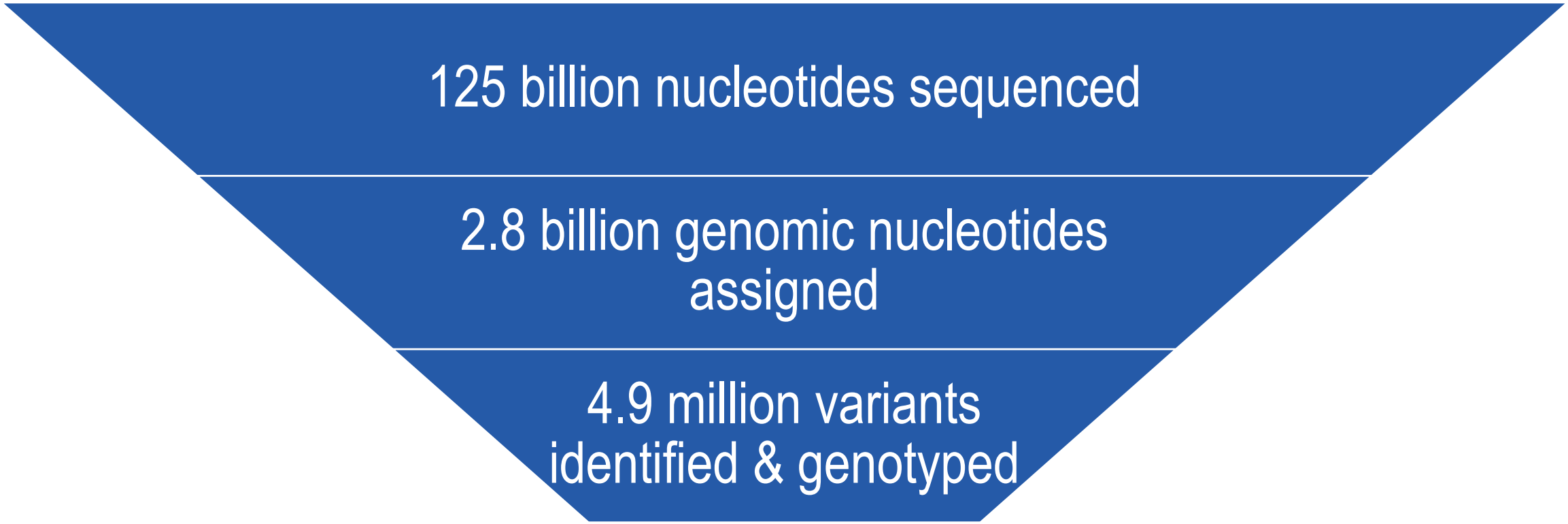

Glossary: Nucleotide – a single DNA letter (base); Adenine, Cytosine, Guanine or Thymidine

Variant – a DNA change from the (normal) reference genome sequence

Illumina DRAGEN 2.0

Step 5: Variant pathogenicity scoring

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Variant Category	Criteria
Pathogenic (P): 99% disease causing	1 VS + (1S or 2M/Sup) 2S 1S + (3M or 2M+2Supp)
Likely Pathogenic (LP): 90% disease causing	1 VS/S + 1 M 1 S + (1 M or 2 Supp) 3 M 2 M + 2 Supp 1 M + 4 Supp
Variant of Uncertain Significance (VUS): 10% disease causing	

Very Strong (VS)	Null variant (nonsense, frameshift, ±1 or 2 splice site position, initiation codon, exon deletion) in gene where LOF known to cause disease
Strong (S)	• Same amino acid change as previously established pathogenic variant • De novo in a patient with the disease and no family history • Functional studies show damaging effect on the gene • Prevalence in affected individuals significantly greater than controls
Moderate (M)	• Located in mutational hot spot/functional domain without benign variation • Extremely low frequency in Gnomad • Recessive disorders, detected in trans with a pathogenic variant • Protein length changed by in-frame indel in nonrepeat region or stop-loss • Novel missense at amino acid where different missense known to be pathogenic • Assumed de novo, but without confirmation of paternity and maternity
Supporting (Supp)	• Cosegregation with disease in multiple affected family members in gene known to cause disease • Missense variant in gene with low rate of benign missense variants and where missense variants commonly cause disease • Multiple computational tools call deleterious • Phenotype highly specific for disease with single genetic etiology • Reputable source reports as pathogenic, but unpublished

Richards S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of ACMG and AMP. Genet Med. 2015 Mar 5.

Step 5: Variant Pathogenicity Scoring: 2 mins

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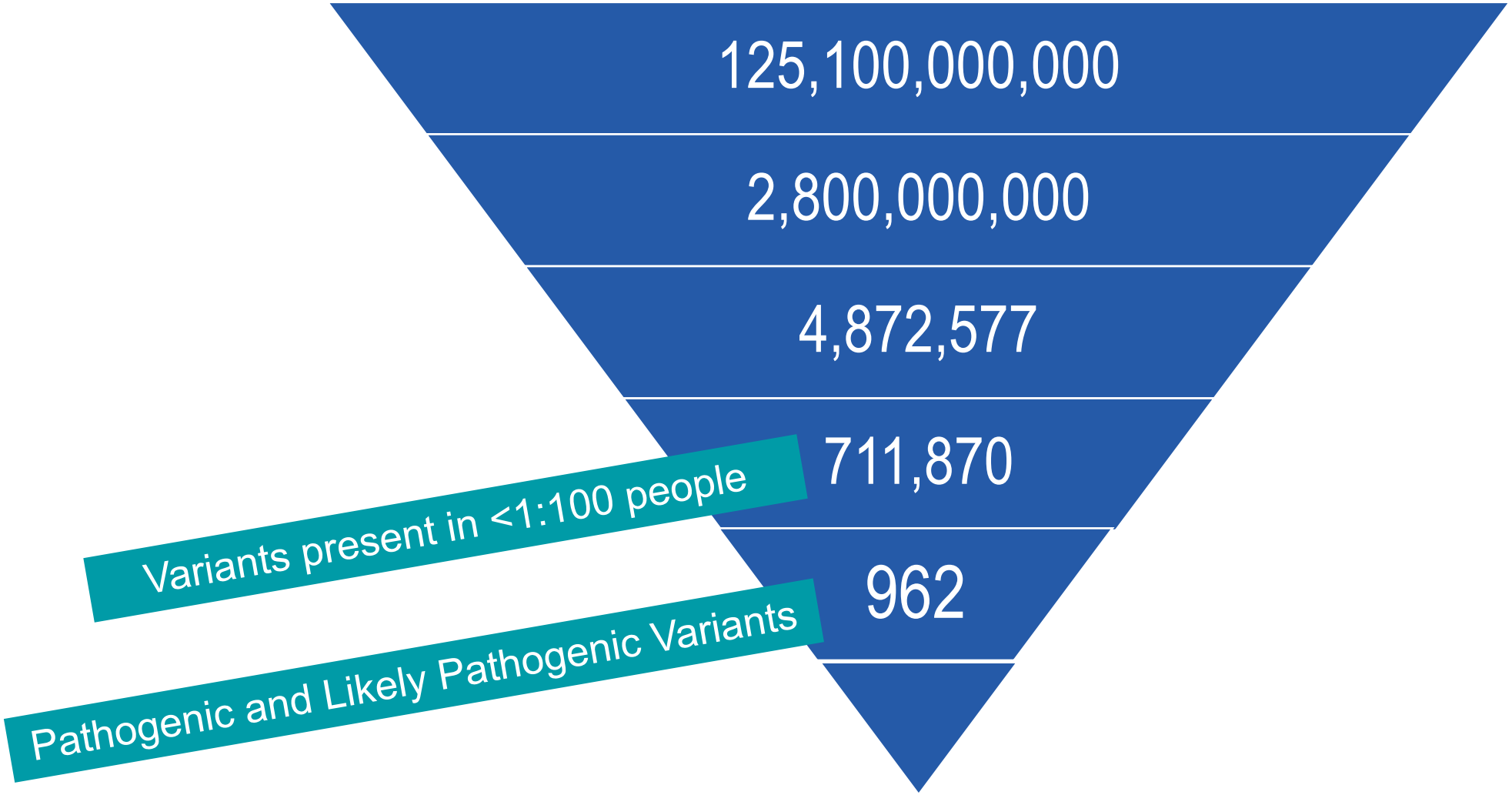
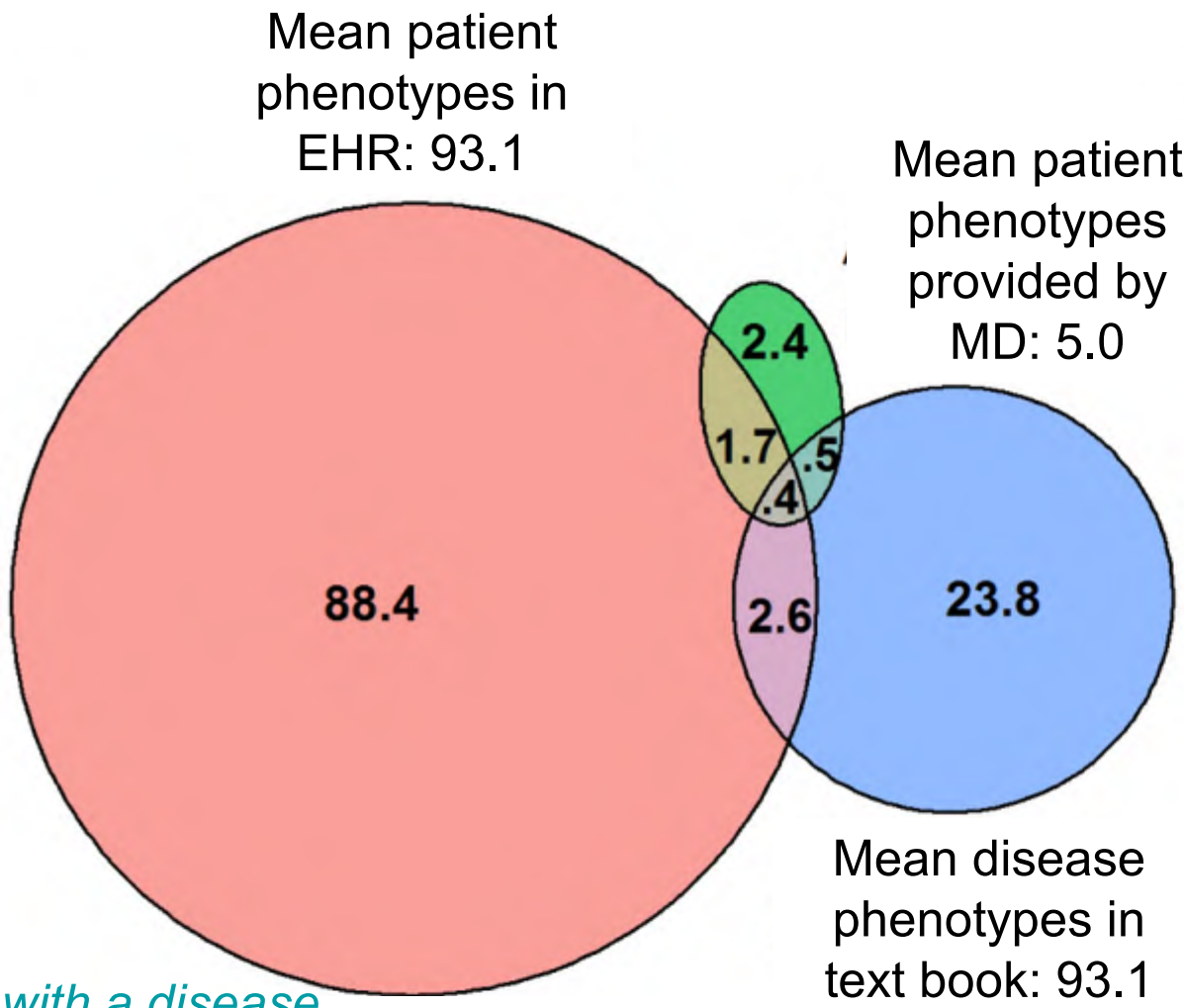


Diploid MOON software with InterVar post-processing

Why collect a deep phenotype

Rady Children's Institute Genomic Medicine

- The clinical features of NICU infants do NOT correspond well with classical descriptions of their disease
- The ability to make a diagnosis is critically dependent on a full clinical description



Glossary: Phenotype – the clinical features of a patient with a disease

76 children with genetic diseases; natural language processing of EHR; Text book: Mendelian Inheritance in Man

Step 6: Deep Phenotyping by Natural Language Processing of Epic EMR: 20 sec

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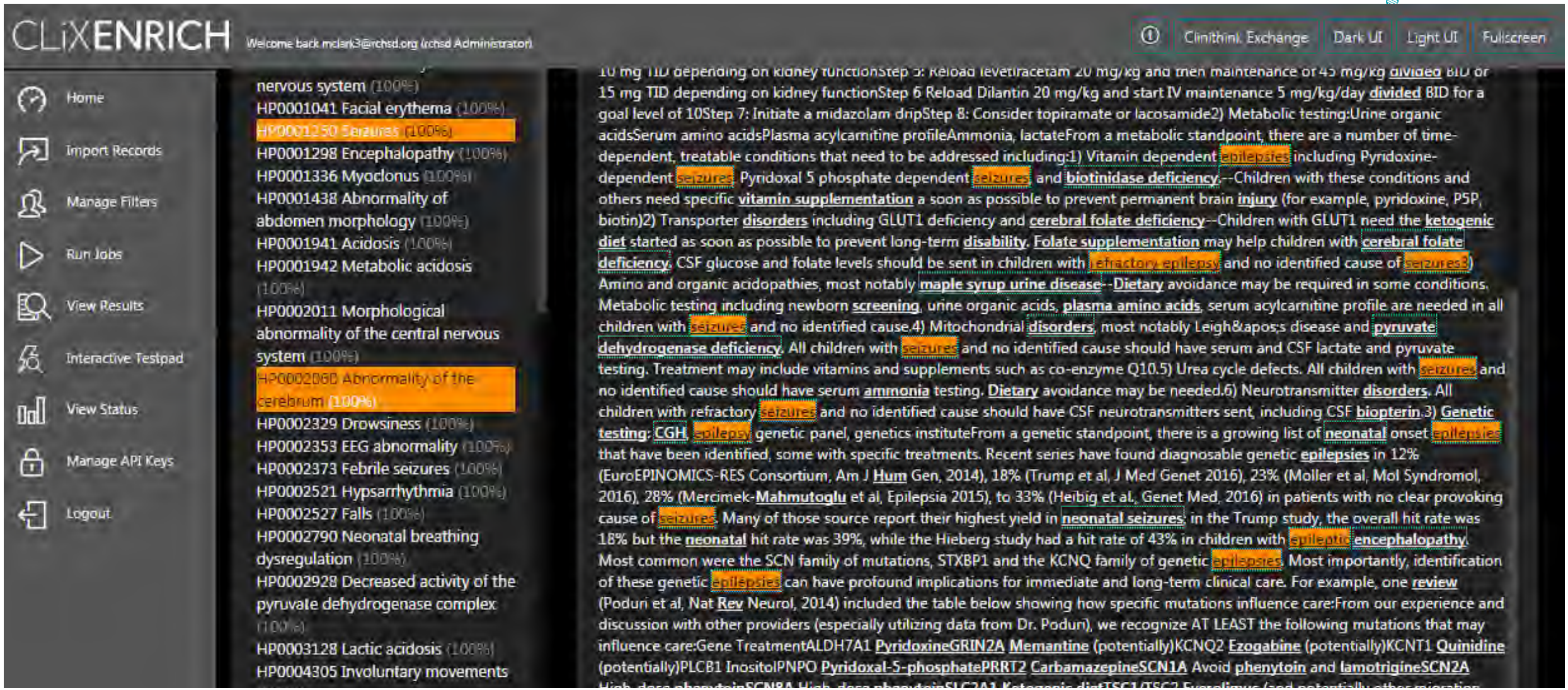

CliniThink CLiXENRICH natural language processing software

Step 7: Translate phenotypes to a hierarchical standardized vocabulary

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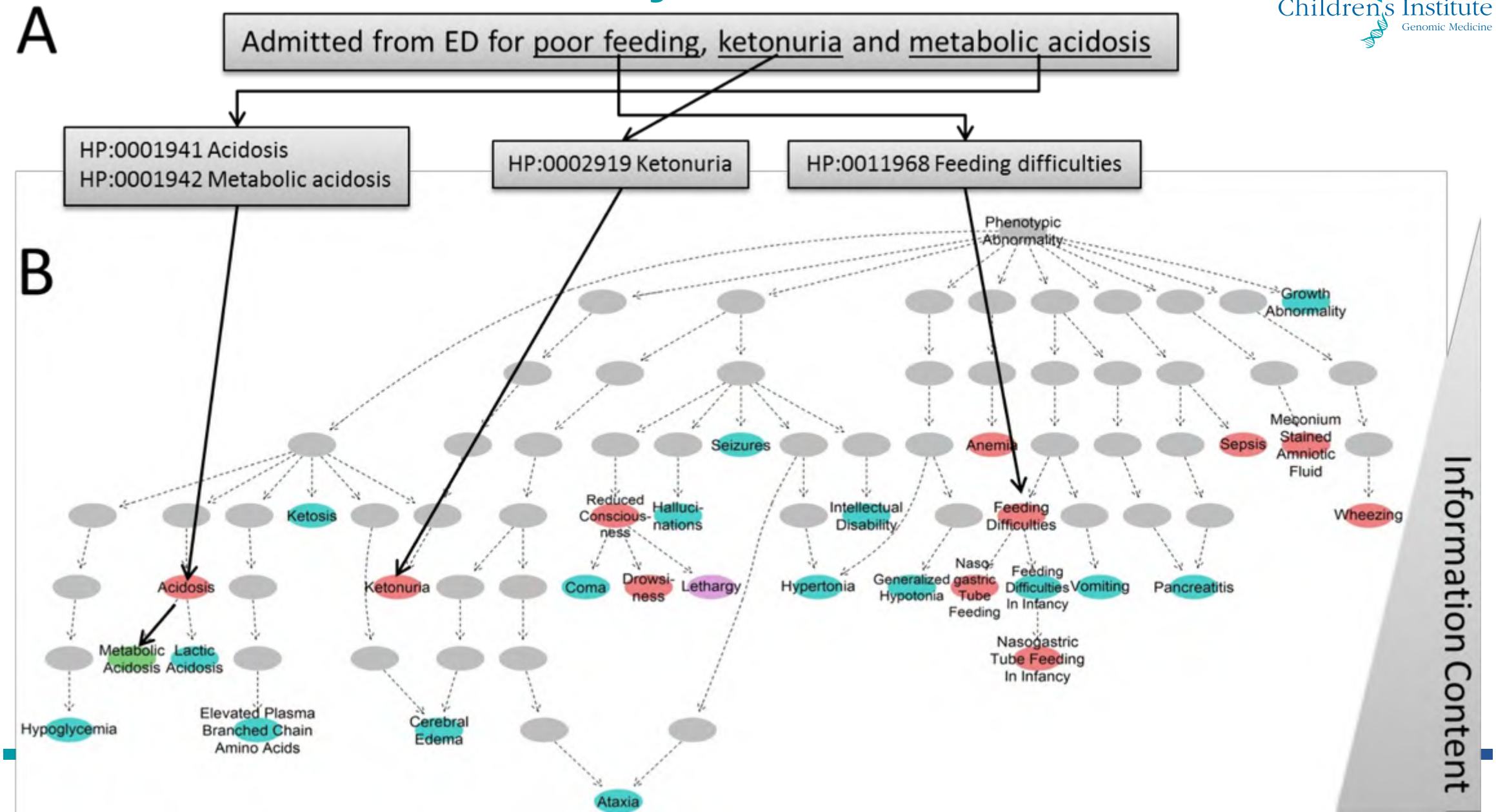

Step 8: Pattern Recognition creates a comprehensive differential diagnosis

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Patient's Features.

HPO.	Feature.
category.: Abnormality of blood and blood-forming tissues (1 Item)	
HP:0001882	Leukopenia
category.: Abnormality of metabolism/homeostasis (3 Items)	
HP:0011227	Elevated C-reactive protein level
HP:0001945	Fever
HP:0001942	Metabolic acidosis
category.: Abnormality of the abdomen (2 Items)	
HP:0002014	Diarrhea
HP:0002013	Vomiting
category.: Abnormality of the cardiovascular system (1 Item)	
HP:0011106	Hypovolemia
category.: Abnormality of the immune system (4 Items)	
HP:0001882	Leukopenia
HP:0100806	Sepsis
HP:0100806	Sepsis
HP:0000988	Skin rash
category.: Abnormality of the integument (1 Item)	
HP:0000988	Skin rash
category.: Abnormality of the respiratory system (1 Item)	
HP:0012418	Hypoxemia

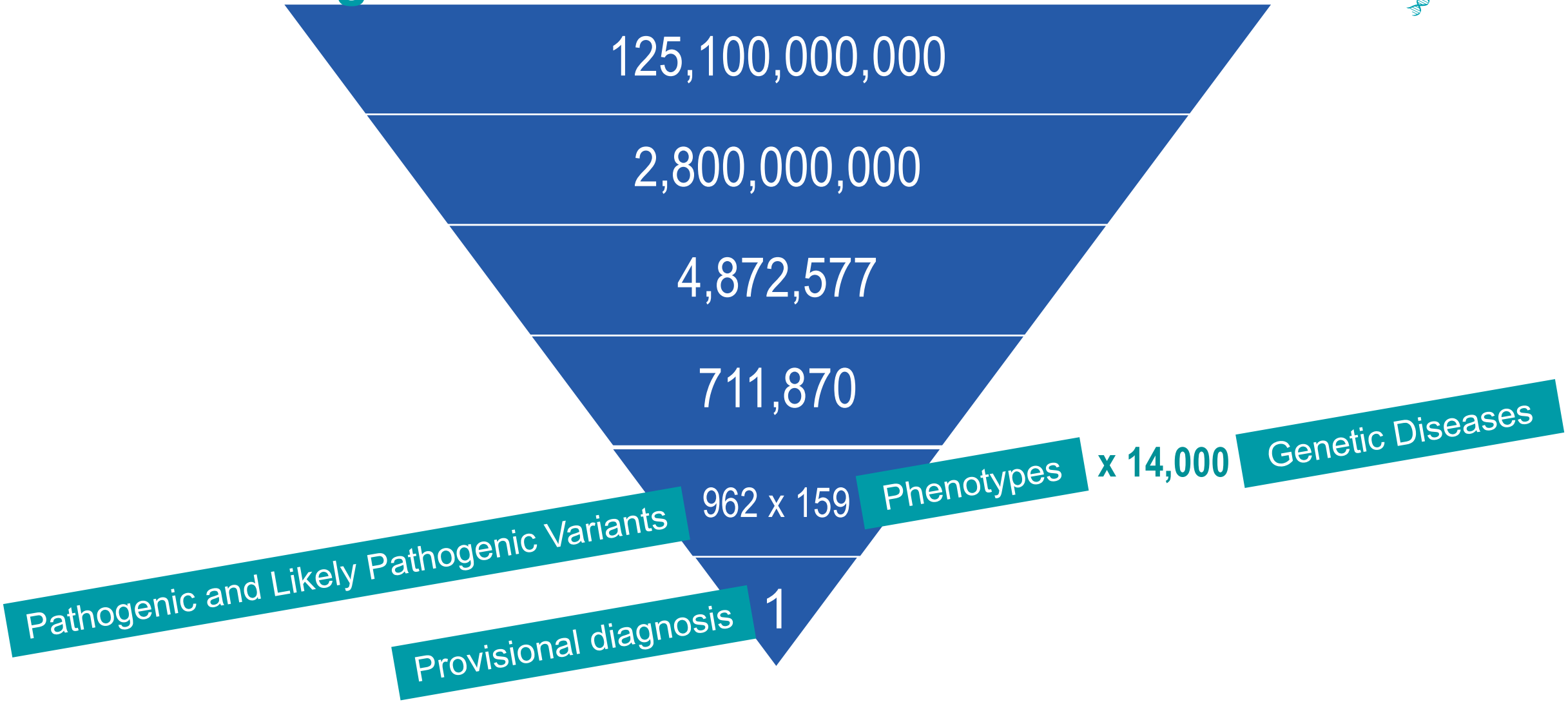
Patient's Features. | **Diagnosis.**

Algorithm: resnik (Unsymmetric). | 10 Features.

p-value.	Disease Id.	Disease name.	Genes.
0.0010	OMIM:26...	#260920 HYPER-IGD SYNDROME; HIDS;;HYPERIMMUNOGLOBULINEMIA D AND PERIODIC FEVER SY...	MVK (4598)
0.0010	ORPHAN...	LEGIONELLOSIS	
0.0010	OMIM:61...	DENGUE FEVER	
0.0010	ORPHAN...	LASSA FEVER	
0.0010	ORPHAN...	EBOLA HEMORRHAGIC FEVER	
0.0010	ORPHAN...	MARBURG HEMORRHAGIC FEVER	
0.0010	ORPHAN...	CRIMEAN-CONGO HEMORRHAGIC FEVER	
0.0010	OMIM:60...	STEVENS-JOHNSON SYNDROME	IKZF1 (10320...
0.0010	OMIM:26...	RETICULAR DYSGENESIA	AK2 (204)
0.0010	ORPHAN...	LUJO HEMORRHAGIC FEVER	
0.0010	OMIM:61...	#611762 FAMILIAL COLD AUTOINFLAMMATORY SYNDROME 2; FCAS2	NLRP12 (916...
0.0010	ORPHAN...	SPONTANEOUS PERIODIC HYPOTHERMIA	
0.0010	OMIM:13...	GASTRITIS, FAMILIAL GIANT HYPERTROPHIC	
0.0010	OMIM:16...	CYCLIC HEMATOPOIESIS	ELANE (1991)
0.0010	OMIM:60...	ENTEROPATHY, FAMILIAL, WITH VILLOUS EDEMA AND IMMUNOGLOBULIN G2 DEFICIENCY	
0.0010	OMIM:14...	HYPERTHERMIA, CUTANEOUS, WITH HEADACHES AND NAUSEA	
0.0018	ORPHAN...	SCRUB TYPHUS	
0.0018	OMIM:60...	600643 CAROLI DISEASE, ISOLATED	
0.0023	OMIM:25...	#251000 METHYLMALONIC ACIDURIA DUE TO METHYLMALONYL-COA MUTASE DEFICIENCY;;METHY...	MUT (4594)
0.0023	OMIM:24...	#243500 ISOVALERIC ACIDEMIA, IVA;;ISOVALERIC ACID COA DEHYDROGENASE DEFICIENCY;;IVD D...	IVD (3712)
0.0023	OMIM:61...	#615592 IMMUNODEFICIENCY 15; IMD15	IKBKB (3551)
0.0026	ORPHAN...	TYPHOID	
0.0026	ORPHAN...	RABIES	
0.0026	OMIM:61...	#614102 IMMUNOGLOBULIN KAPPA LIGHT CHAIN DEFICIENCY; IGKCD;;KAPPA CHAIN DEFICIENCY	IGKC (3514), ...
0.0026	OMIM:26...	260570 PELGER-HUET-LIKE ANOMALY AND EPISODIC FEVER WITH ABDOMINAL PAIN	
0.0030	ORPHAN...	LYELL SYNDROME	
0.0030	OMIM:61...	#614699 IMMUNODEFICIENCY, COMMON VARIABLE, 7; CVID7	CD19 (930), ...

Step 9: Automated Diagnosis: 2 mins
Manual Diagnosis: 1 – 10 hours

Rady Children's Institute Genomic Medicine



Diploid MOON software with InterVar post-processing

Step 9: Automated Diagnosis: 4 mins
Manual Diagnosis: 10 hours

Rady Children's Institute Genomic Medicine

The patient's phenotype might be caused by:

BTK	G	splice donor	Agammaglobulinemia, x-linked	
X:100,613,603	ref: A	c.974+2T>C	XR	

- **X-linked recessive**
- **Inherited from mother**
- **Loss of splice donor site of intron 11**
- **Classified as pathogenic**
- **Confirmed by functional studies**

		Ref Range & Units
Total IgG	<135 ↓	413-1,112 mg/dL
IgM	16 ↓	30 - 146 mg/dL
IgA	<5 ↓	21 - 117 mg/dL

	2wk ago	Ref Range & Units
LYMPHOCYTES ABSOLUTE	2962 ↓	4000 - 10500 cells/uL
CD19 ABSOLUTE	<20 ↓	830 - 1880 cells/uL
CD19 PERCENTAGE	1 ↓	20 - 38 %

Hospital Course

Rady Children's Institute Genomic Medicine

- Diagnosis after 22 hours
- Individualized medicine
  - Double coverage, double duration antibiotics
  - Intravenous immunoglobulin to maintain IgG level >600mg/dL
  - Magnetic resonance imaging: no additional septic emboli
- Prognosis
  - Normal life
  - 10% have significant infections despite treatment
- Genetic counseling
  - Mother is a carrier
  - Maternal relatives at-risk
- Discharged home on day 13

Smith and Berglof, Gene Reviews, 2016

Diagnostic performance of 3rd generation rWGS-based individualized medicine

Rady Children's Institute Genomic Medicine

- Retrospective, n=84 children with 86 diagnoses
  - Expert manual interpretation: Precision 98%, Recall 98%
  - Automated interpretation: Precision 99%, Recall 95%

	Retrospective Patients				Prospective Patients											
Subject ID Age	**263** 8 days		**6124** 14 years	**3003** 1 year	**6194** 5 days		**290** 3 days		**352** 7 weeks		**362** 4 weeks		**374** 2 days		**7052** 17 months		**412** 3 days	
Abbreviated Presentation	Neonatal seizures		Rhabdo-myolysis	Dystonia, Dev. delay	Hypoglycemia seizures		Pulmonary hemorrhage		Diabetic ketoacidosis		Neonatal seizures		HIE, anemia		Pseudomonal septic shock		Neonatal seizures	
Method	**Auto.**	**Auto.**	**Auto.**	**Auto.**	**Auto.**	**Std.**	**Auto.**	**Std.**	**Auto.**	**Std.**	**Auto.**	**Std.**	**Auto.**	**Std.**	**Auto.**	**Std.**	**Auto.**	**Std.**
Total (hours)	**20:25**	**19:56**	**19:20**	**19:14**	**20:42***	**56:03**	**19:29**	**48:46**	**19:11**	**42:04**	**19:10**	**57:21**	**31:02†**	**34:38**	**22:04**	**38:37**	**20:53**	**48:23**
Molecular Diagnosis	Early Infantile Epileptic Encephalo-		Glycogen Storage Disease V	Dopa-Responsive Dystonia	None		None		Permanent neonatal diabetes		None		None		X-linked agamma-globulinemia		Benign familial neonatal seizures 1	
Gene and Causative Variant(s)	*KCNQ2* c.727C>G		*PYGM* c.2262delA c.1726C>T	*TH* c.785C>G c.541C>T	n.a.		n.a.		*INS c.26C>G*		n.a.		n.a.		*BTK* c.974+2T>C		*KCNQ2* c.1051C>G	

Summary: Strategies are being implemented to address barriers to rWGS-based individualized medicine for NICU and PICU infants

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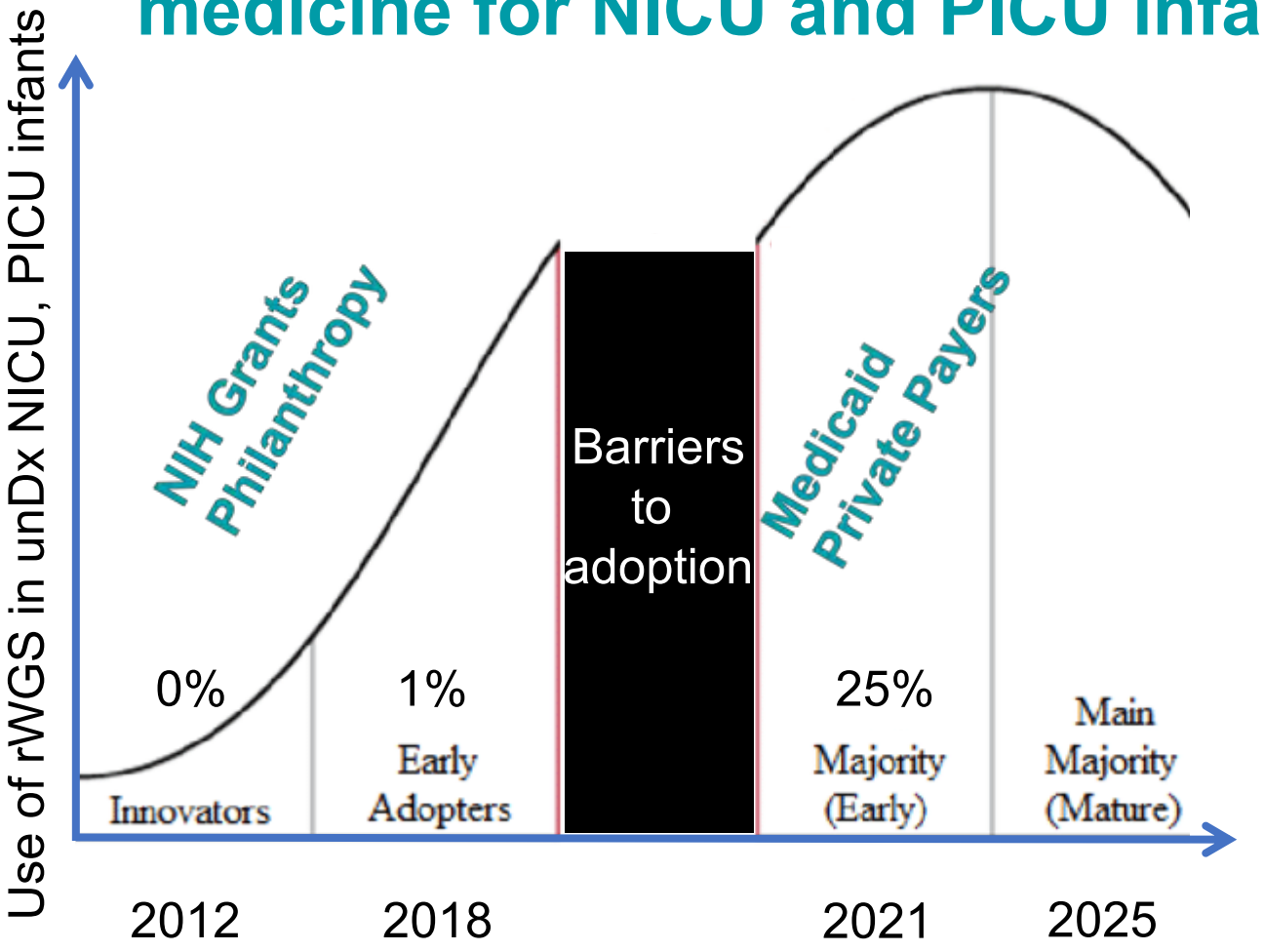

- Automated 20-hour diagnosis by rWGS
- Lack of clinician familiarity with individualized medicine
  - APIs for FAQs, counseling
  - Case-based learning via podcast/webinar
- Insufficient evidence of efficacy
  - Cost effectiveness studies
  - Payor collaborations
- Many genetic diseases lack effective treatments
  - APIs for reference individualized management guidance

Summary: By 2021 we predict the early majority of children's ICUs to implement individualized medicine

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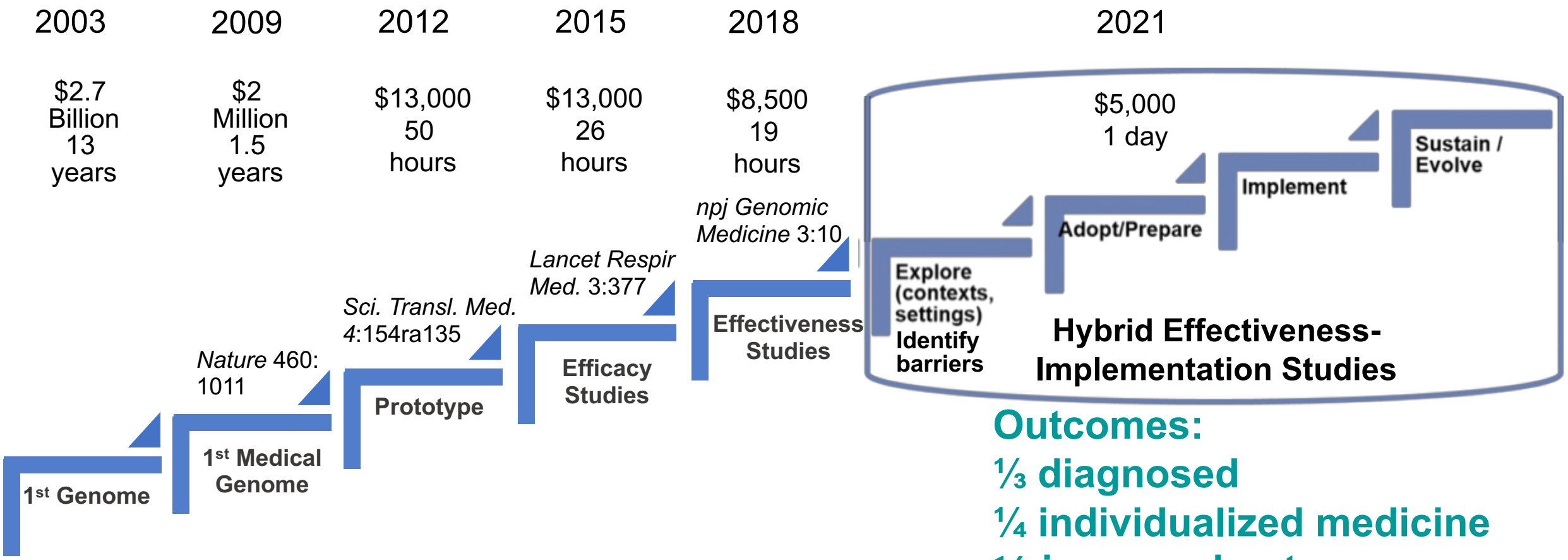

Outcomes:

⅓ diagnosed

¼ individualized medicine

⅕ improved outcomes

Acknowledgments

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- John Reynders
- Margaret Bray
- Paul McDonagh
- Brett Williams

CliniThink
- Calum Yacoubian
- Alison Frith
- Richard Gain

Diploid
- Peter Schols
- Cyrielle Kint