Automation of Rapid Whole Genome Sequencing (rWGS) – The Need for Speed

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Rady Children's Institute
Genomic Medicine

No conflict of interest

- Informed consent was obtained for this research
- Patient and parent photos, videos, and names are used with their permission

RCIGM – Who are we?

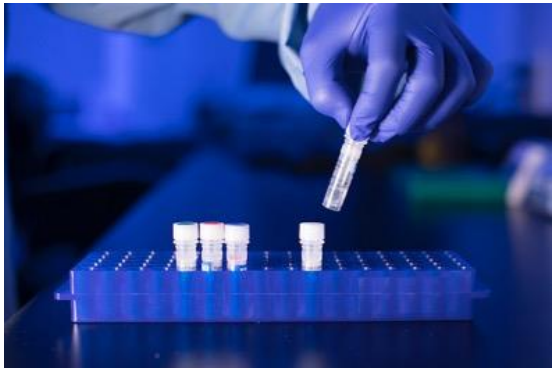
Clinical Genome Center

Rady Children's Institute Genomic Medicine

CLIA certification April 2017

CAP accreditation Sept. 2017

Mission: To prevent, diagnose, treat and cure childhood diseases through genomic and systems medicine research.



Rare diseases are predominantly caused by Genomic Variation and result in dire consequences for patients

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Rare disease: 1 in 2,000 affected

Ultra-rare disease: 1 in 50,000 affected

Rare diseases affect over 30 million patients in the US

6,517 Single gene diseases

22,928 Chromosomal diseases

>1,556 Genetic diseases of unknown cause

66% of rare diseases are serious and disabling – 50% are life-limiting

75% of rare diseases affect children

95% of rare diseases lack an FDA/EMA approved treatment

450 new medicines in development for rare diseases

27% of patients with the most common 350 rare diseases will not reach their 1st birthday

Sources: 1. The Personalized Medicine Report. Personalized Medicine Coalition. 2017. 2. Accelerating Rare Disorder Patient Identification to Drive Orphan Drug Adoption. PerkinElmer. 3. Orphan Drug Sales to Reach $262 Billion by 2024. RDMAG. 5. OMIM and dbVar. Accessed 10/19/2019

Initial Focus of Pediatric Genomic Medicine

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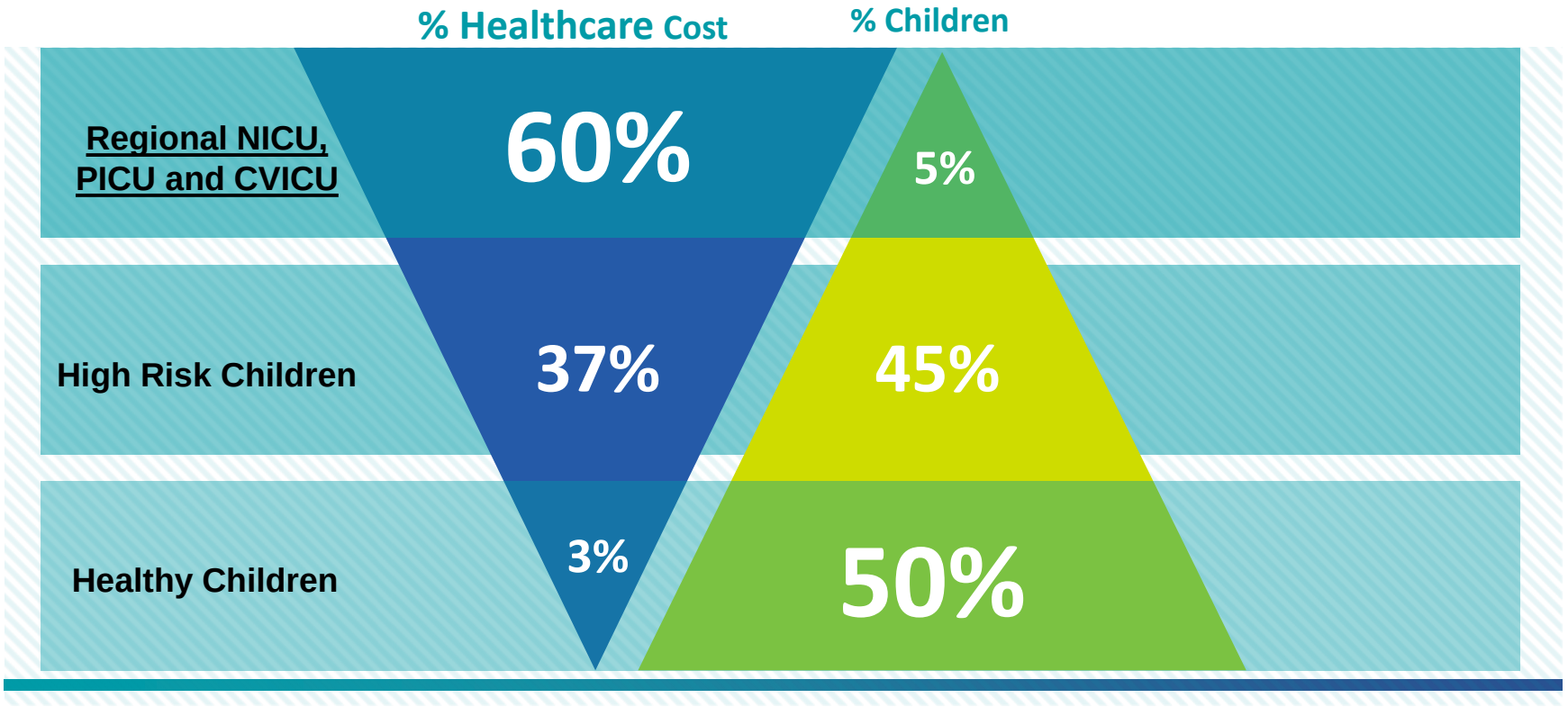

Rapid Whole Genome Sequencing

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NICU: Opportunity for Biggest Impact

Comprehensive genetic testing

↓

Timely, targeted treatment

↓

Better patient outcomes

The Rapid Precision Medicine Paradigm

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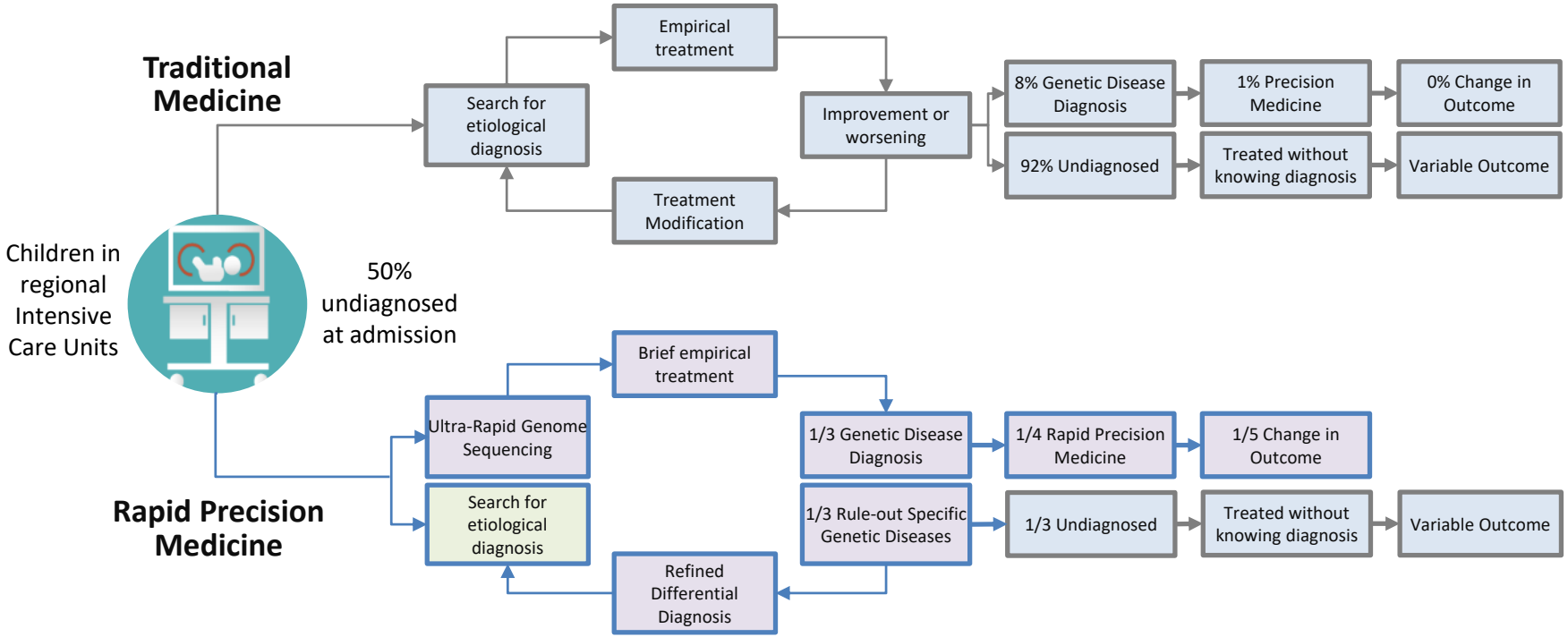

Focus: Infants in ICUs with diseases of unknown or incorrect aetiology

From: Willig et al 2015, Petrikin et al 2018, Farnaes et al 2018, Dimmock et al In Preparation

Evolution of rWGS

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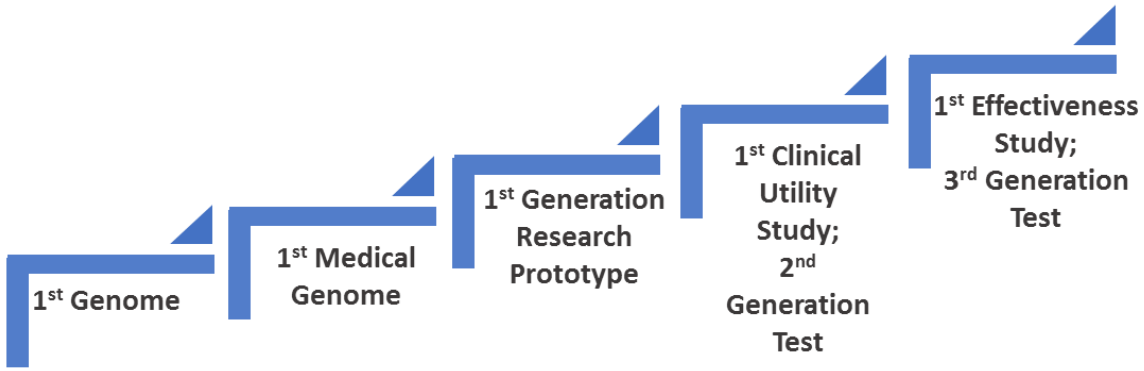
2003	2009	2012	2015	2018
$2.7 Billion 13 years	$2 Million 1.5 years	$13,000 50 hours	$13,000 26 hours	$8,500 19 hours



Medical literature consistently demonstrates clinical utility

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1st Author	Date	Study Type	Seq Type	NICU and PICU Enrollment Criteria	Size	Dx Rate	Clinical Utility	Change in Outcome	TAT (d)	Savings per patient tested
Saunders	2012	Cases	rWGS	NICU infants with suspected genetic disease	4	75%	n.d.	n.d.	2	
Willig	2015	Cohort	rWGS	<4 mo of age; Suspected actionable genetic disease	35	57%	31%	29%	23	
Meng	2017	Cohort	rWES	<100 days of life; Suspected genetic disease	63	51%	37%	19%	13	
van Diemen	2017	Cohort	rPanel	Infants; Suspected genetic disease	23	30%	22%	22%	12	
Petrikin	2018	RCT	rWGS	<4 mo of age; Suspected genetic disease	32	41%	22%	n.d.	13	
Farnaes	2018	Cohort	rWGS	infants; Suspected genetic disease	42	43%	31%	26%	23	$3,500
Stark	2018	Cohort	rWES	Acutely ill children with suspected genetic diseases	40	53%	30%	8%	16	$1,100
Mestek-Boukhibar	2018	Cohort	rWGS	Children; PICU and Cardiovascular ICU	24	42%	13%	n.d.	9	
Ceyhan-Birsoy	2019	RCT	rWES	NICU neonates	32	16%	n.d.	n.d.	n.d.	
Sanford	2019	Cohort	rWGS	4 months-18 years; PICU; Suspected genetic diseases	38	48%	39%	8%	14	
French	2019	Cohort	rWGS	Suspected genetic disease	195	21%	14%	n.d.	21	
Clark	2019	Cases	urWGS	Infants; Suspected genetic disease	7	43%	100%	n.d.	1	
Kingsmore/ Dimmock	2019	RCT	rWGS rWES urWGS	Infants; disease of unknown etiology; within 96 hours of admission	94 95 24	19% 20% 46%	24% 19% 54%	10%	11 11 5	In Progress
Baby Bear	2019	Cohort	rWGS	MediCal infants; within 1 week of admission; suspected genetic disease	132	43%	39%	In progress	3	$3,300
Average					**880**	**33%**	**26%**	**14%**		**$2,900**

NICU: neonatal ICU; PICU: pediatric ICU; RCT: randomized controlled trial; rWES: Rapid exome sequencing; *£ saved per QALY

Rapid Precision Medicine can decrease cost of care

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Effect of rWGS-based precision medicine on acute healthcare utilization in six infants and three matched controls

Subject ID	Presentation and modeled change in care	Gene	Time-to-diagnosis, days (method)	Hospital stay, Days	Decreased hospital stay, days (%)	Total cost	Cost avoided
6011	Cholestasis, 1st admission for etiologic Dx Cholestasis, 2nd admission for etiologic Dx	NPC1	7 (G)	8 15	15 (35%)	$ 25,278 $ 27,004	$ 27,004
6012	Palliative care started DOL 250 Palliative care started DOL 292	ARID1B	26 (G)	250 292	42 (17%)	$ 1,949,438 $ 2,276,944	$ 327,506
6014 Control 1	Hypotonia, Avoided EMG, GA, muscle biopsy Electromyogram, GA, muscle biopsy	NEB1	7 (G)	45	2 (6%)	$ 156,914 $ 9,900	$ 9,900
6026 Control 2 Avg cost	Cholestasis and congenital heart disease Avoided hepatoportoenterosomy Kasai hepatoportoenterosomy Cost of liver transplant x 43% occurrence	JAG1	3 (G)	11	3 (18%)	$ 50,327 $ 44,451 $ 87,344	$ 131,795
6041	Seizures. Diagnosis DOL 4 Seizures. Diagnosis DOL 42	KCNQ2	4 (G) 42 (S)	18 59	41 (69%)	$ 79,675 $ 261,156	$ 181,481
6053	Hypoglycemia. Diagnosis DOL 12 Hypoglycemia. Diagnosis DOL 32	ABCC8	7 (G) 28 (S)	10 31	21 (68%)	$ 59,769 $ 185,283	$ 125,514
Healthcare ngs				398			$ 803,199
Cost of rWGS in 42 families							$ 674,645
Net healt savings							$ 128,554

Ongoing studies will answer the questions of how much RPM saves and how much it is generalizable

Farnaes et al 2018

Physicians think RPM™ is useful

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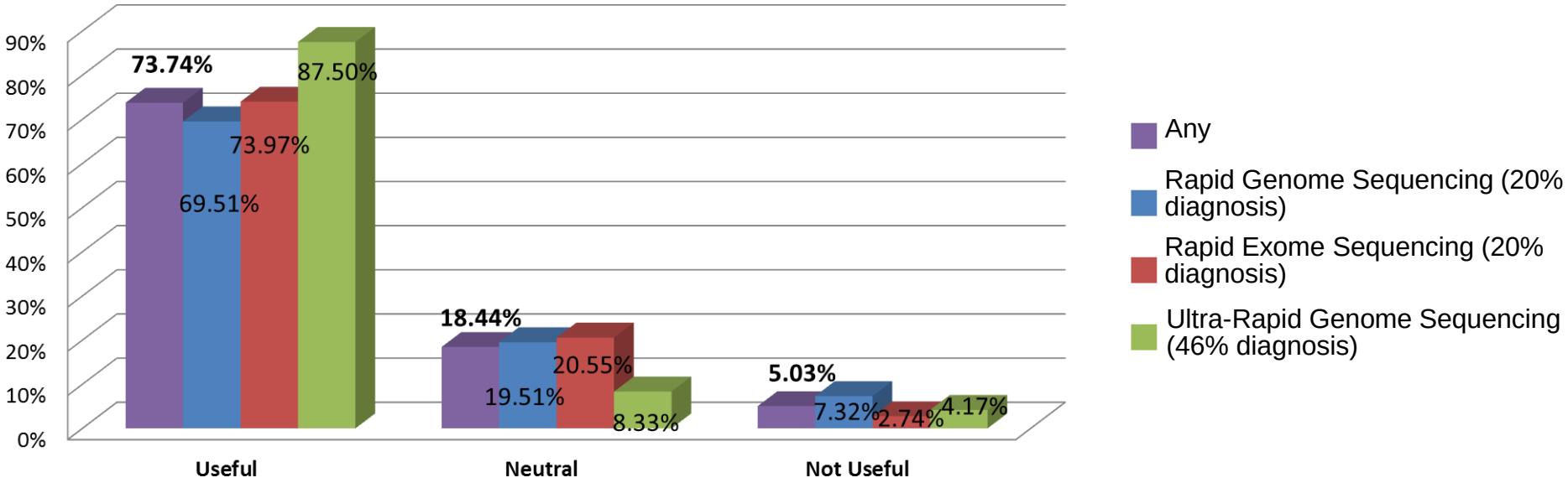
Was rapid genome sequencing useful?



ClinicalTrials.gov NCT03211039 Exome sequencing: 1% of genome Rapid: 12 days Ultra-rapid: 2 days

So do parents

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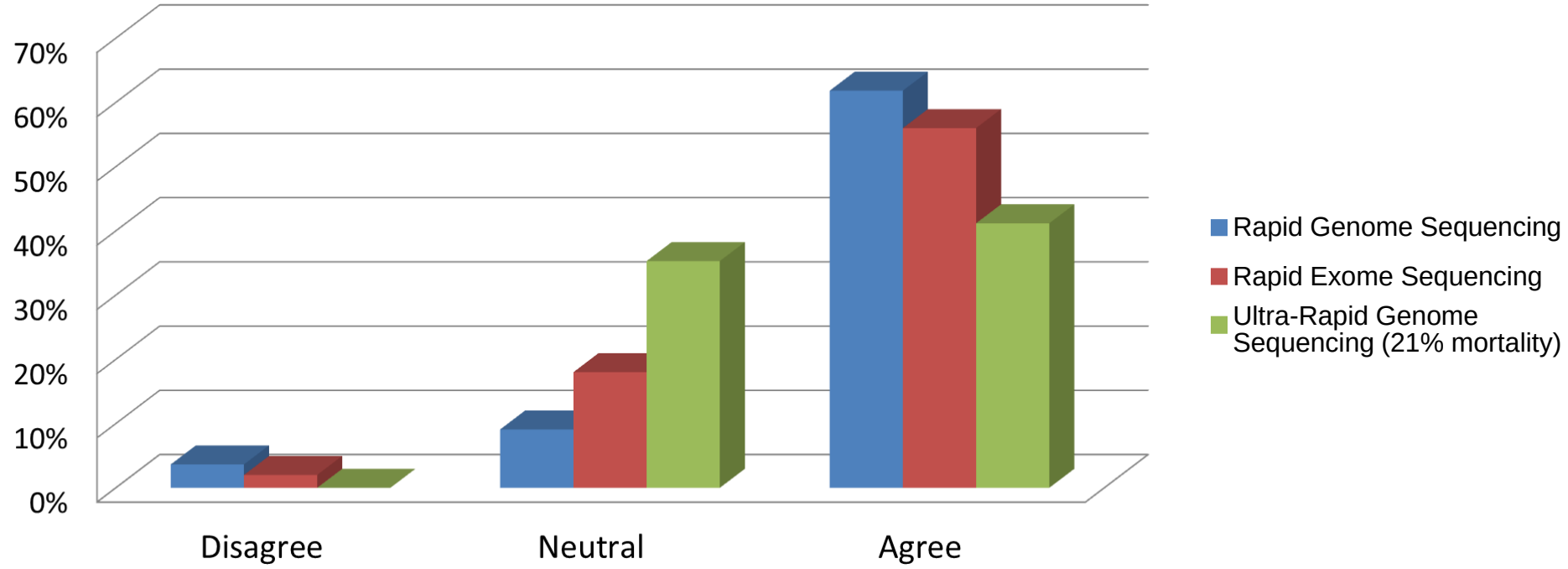
The choice to sequence did my child a lot of good:



ClinicalTrials.gov NCT03211039

28-day mortality: Rapid genome and exome sequencing 1.6%

3pm, October 24, 2017 – NICU family 243

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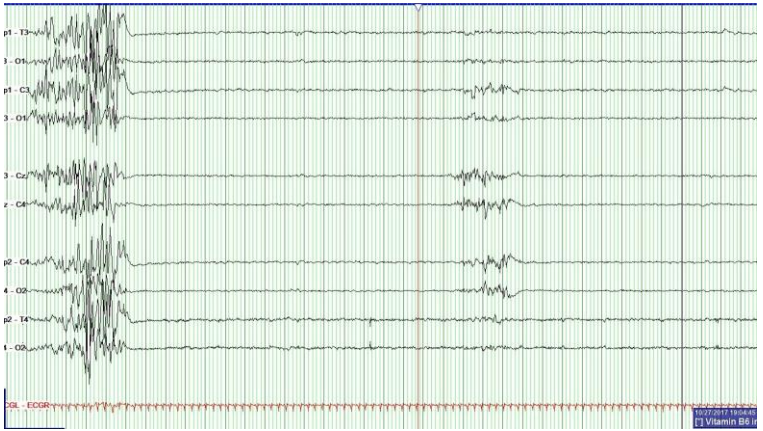
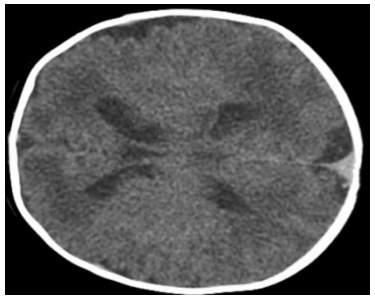
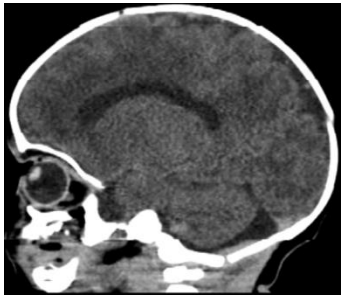
- 8-day-old ♂ admitted from ER with *Status Epilepticus*
- History:
    - 23-yo G2P1 healthy mother
    - Fetal ventriculomegaly detected by ultrasound during pregnancy
    - Delivery by uncomplicated C-section 39 1/7 weeks
    - Breast-feeding well, discharged home on day of life 3

NICU family 243: Initial NICU Workup

- **Electroencephalogram:** seizures & background burst suppression
- **Brain computed tomography:** mild hypoplasia of cerebellum; Borderline lateral ventriculomegaly
- **Infection workup:** negative
- Cerebrospinal fluid lactic acid 6.3 mmol/L (normal 1.1-2.8)
- Serum creatinine kinase 1,195 U/L (normal 13-80, not in acute renal failure range)







Disease Progressed Overnight

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- **"Last night was rough with ongoing…multifocal seizures that continued despite…levetiracetam or phenobarbital"**
    - Maximal anti-epileptic drugs
    - Worsening seizures
    - No response to phenytoin, carbamazepine
    - Midazolam drip increased until respiratory failure, emergent intubation
- "I discussed with his parents the range of outcomes I have seen with Neonatal Burst Suppression encephalopathy which usually entails limited life expectancy and at least moderate to severe developmental disabilities."

Diagnosis reported at 8pm October 27

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- **Disease:** Pyridoxine-Dependent Epilepsy
- **Gene:** Aldehyde dehydrogenase 7 family member A1
- **Inheritance Pattern:** Autosomal Recessive
- **Variants:** 2 pathogenic variants

Genome variant (g.)	Gene variant (c.)	Protein variant (p.)
Chr5 g.125,919,689C>T	*ALDH7A1* c.328C>T	p.Arg110Ter
Chr5 g.125887751C>G	*ALDH7A1* c.1279G>C	p.Glu427Gln

C=Cytosine; T=Thymidine; G=Guanine
Arg=Argenine; Ter=Termination Codon; Gln=glutamine; Glu=glutamic acid

Glossary: Gene – a sequence of nucleotides in a genome that codes for a protein

Recessive – A disease expressed in offspring only when inherited from both parents

Step 11: Rapid Precision Medicine Guidance

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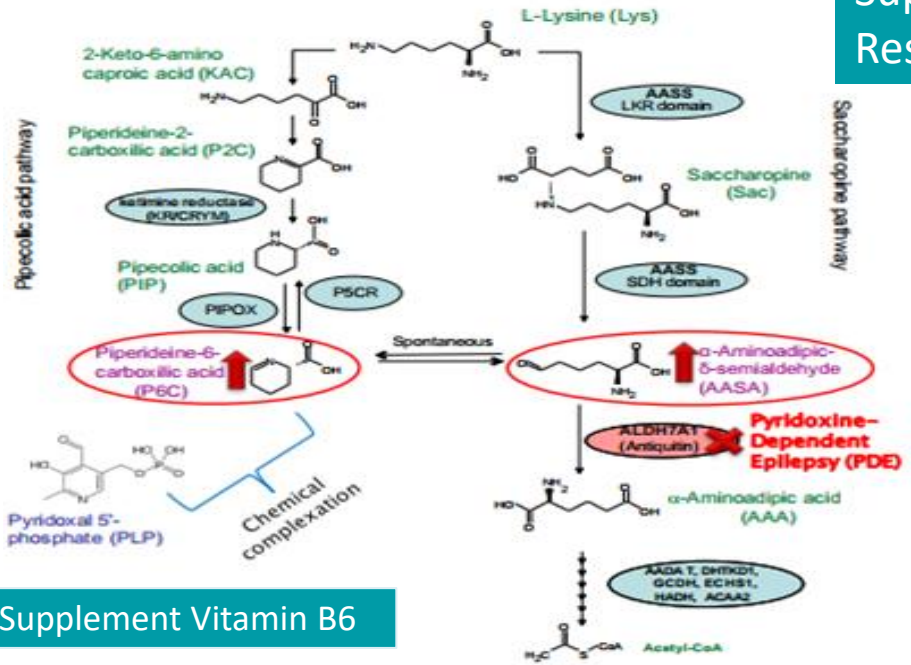
Supplement argenine
Restrict dietary lysine



Supplement Vitamin B6

Coughlin CR et al. Mol Genet Metab 2015 116:35

Impact of diagnosis 55 hours after consent

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Following triple therapy with pyridoxine, L-arginine supplementation and dietary lysine restriction

- Electroencaphalogram normalized
- Seizures stopped

Within 36 hours

- Extubated
- All anti-epileptic drugs stopped

Discharged Home

- Meeting milestones @ 22 months of age

The Rapid Precision Medicine Paradigm Applied to Neonatal Seizures

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Newborn 6 Months Before RPM

Search for etiological diagnosis

42 days empirical treatment

Treatment Modification

Unremitting seizures

Genetic Disease Diagnosis on DOL 43: Early Infantile Epileptic Encephalopathy Type 7

Natural history of disease

Profound neurologic damage

59 day hospitalization$ 211,484

DOL 1 Ultra-Rapid Genome Sequencing

3 days of empirical treatment

Unremitting seizures

Genetic Disease Diagnosis on DOL 43: Early Infantile Epileptic Encephalopathy Type 7

Rapid Precision Medicine: Carbamazepine

Minor developmental delay

17 day hospitalization$ 79,675

From: Farnaes et al 2018; *de novo KCNQ2* variant

Current Barriers

- **Is the expense justified?**
  - Is more needed?
- **Genomic analysis time**
  - Automated variant prioritization approaches
  - Artificial intelligence tools in development
- **Limitations of genomic sequencing approaches**
  - Polynucleotide repeats, regions with high homology, translocations
- **Responsible consent and return of results**
  - Education/outreach, collaboration with genetics services
- **Decision support for molecular diagnoses**
  - Education/outreach, medical fellow training

rWGS Interpretation Conundrum

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Sequencing

Genomes sequenced in 17 hours

Alignment/Variant Calling

Genomes processed in 45 min

Interpretation/Reporting

Evidence of pathogenicity	Category
Very strong	PVS1 null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease Caveats: • Beware of genes where LOF is not a known disease mechanism (e.g., GFAP, MYH7) • Use caution interpreting LOF variants at the extreme 3' end of a gene • Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact • Use caution in the presence of multiple transcripts
Strong	PS1 Same amino acid change as a previously established pathogenic variant regardless of nucleotide change Example: Val→Leu caused by either G>C or G>T in the same codon Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level PS2 De novo (both) maternity and paternity confirmed) in a patient with the disease and no family history Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, and so on, can contribute to nonmaternity. PS3 Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product Note: Functional studies that have been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting are considered the most well established. PS4 The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls Note 1: Relative risk or OR, as obtained from case–control studies, is >5.0, and the confidence interval around the estimate of relative risk or OR does not include 1.0. See the article for detailed guidance. Note 2: In instances of very rare variants where case–control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated patients with the same phenotype, and its absence in controls, may be used as moderate level of evidence.
Moderate	PM1 Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation PM2 Absent from controls (or at extremely low frequency if recessive) (Table 6) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium Caveat: Population data for insertions/deletions may be poorly called by next-generation sequencing. PM3 For recessive disorders, detected in trans with a pathogenic variant Note: This requires testing of parents (or offspring) to determine phase. PM4 Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants PM5 Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before Example: Arg156His is pathogenic; now you observe Arg156Cys Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level. PM6 Assumed de novo, but without confirmation of paternity and maternity
Supporting	PP1 Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease Note: May be used as stronger evidence with increasing segregation data PP2 Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease PP3 Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.) Caveat: Because many in silico algorithms use the same or very similar input for their predictions, each algorithm should not be counted as an independent criterion. PP3 can be used only once in any evaluation of a variant. PP4 Patient's phenotype or family history is highly specific for a disease with a single genetic etiology PP5 Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation

Still takes hours/days

Why is it Taking so Long? Manual Process!!!

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Steps in the Process
Initial Case Tracking - test type, family members
Phenotyping - HPO terms - gene lists
Case Creation
Variant selection/filtering
Curation of variants
Confirmation ordering
LD review/reporting
Report delivery
Verbal communication

A lot to Interpret!

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Variant Type	Details
Single Nucleotide Variants	>99.8% sensitivity
Small Insertions/Deletions	Reported up to 40 bp
Small Copy Number Variation	Down to 1 kb (187 bp finding this month!)
Large Copy Number Variation	Microdeletion/duplication syndromes
Aneuploidy	Whole chromosome (trisomy)
SMN1 and SMN2 Copy Number	0,1,2, and >3 copies
Mitochondrial Variants	Validated down to heteroplasmy levels of 1%

Variant Type	Plans for Validation
Balanced translocations	Feasible for WGS data
Repeat expansions	Myotonic dystrophy screening
Intronic variants	Assessing current tools
Mosaic Copy Number Variants	Validation planning underway
Robust/automated UPD calling	Gathering truth samples

Role of the Laboratory Director in the rWGS Era

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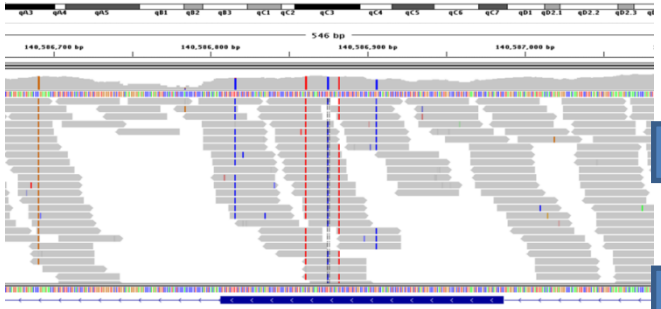

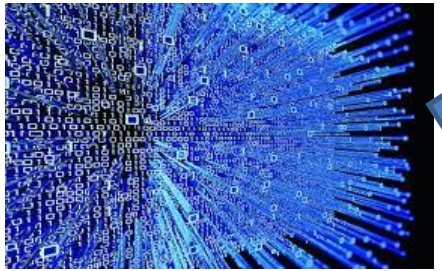

2nd FRONTIERS IN PEDIATRIC GENOMIC MEDICINE CONFERENCE

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]*

Phenome + Genome

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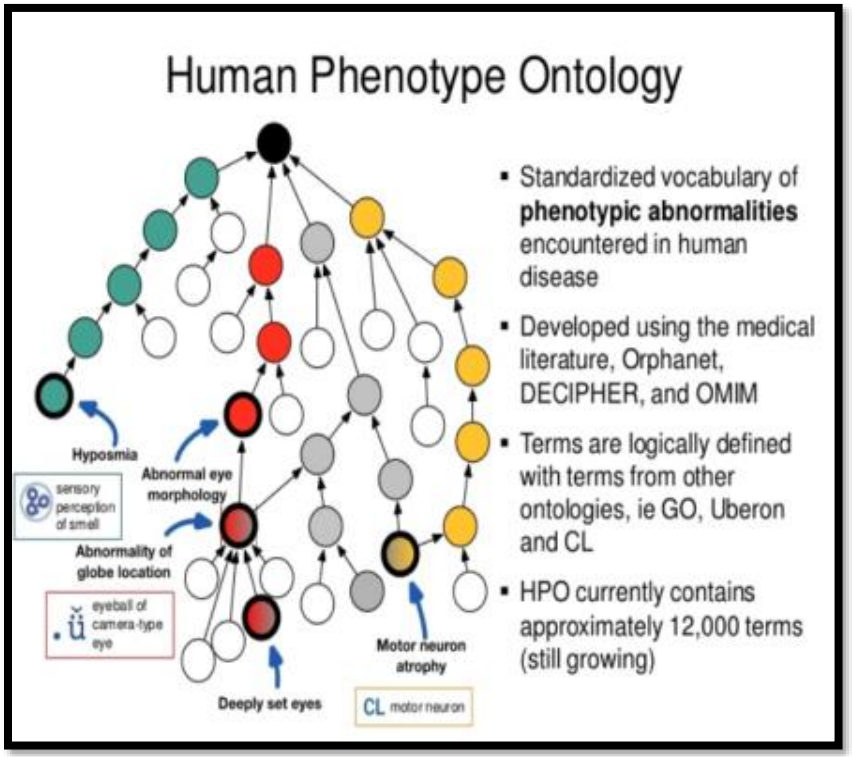

Deep Phenotyping by Natural Language Processing of Epic EMR: 20 sec

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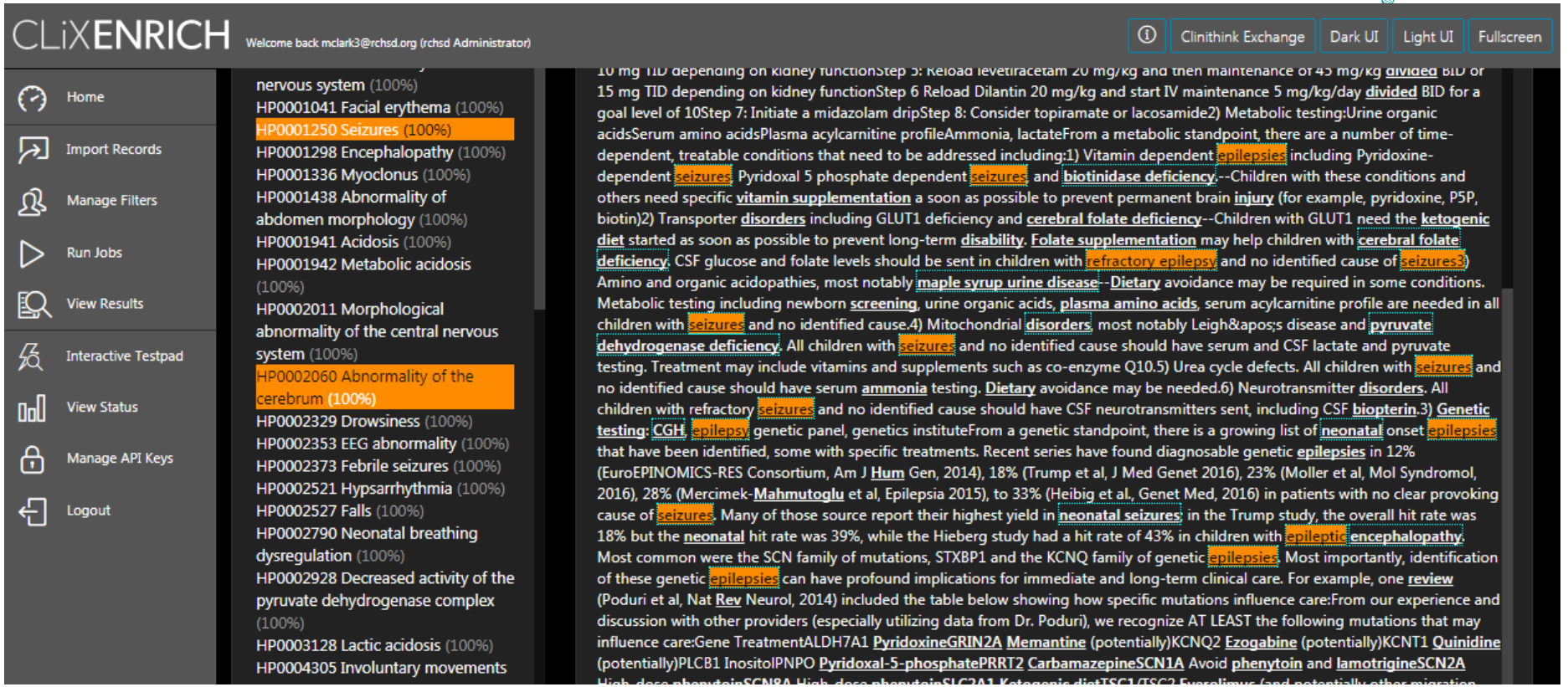
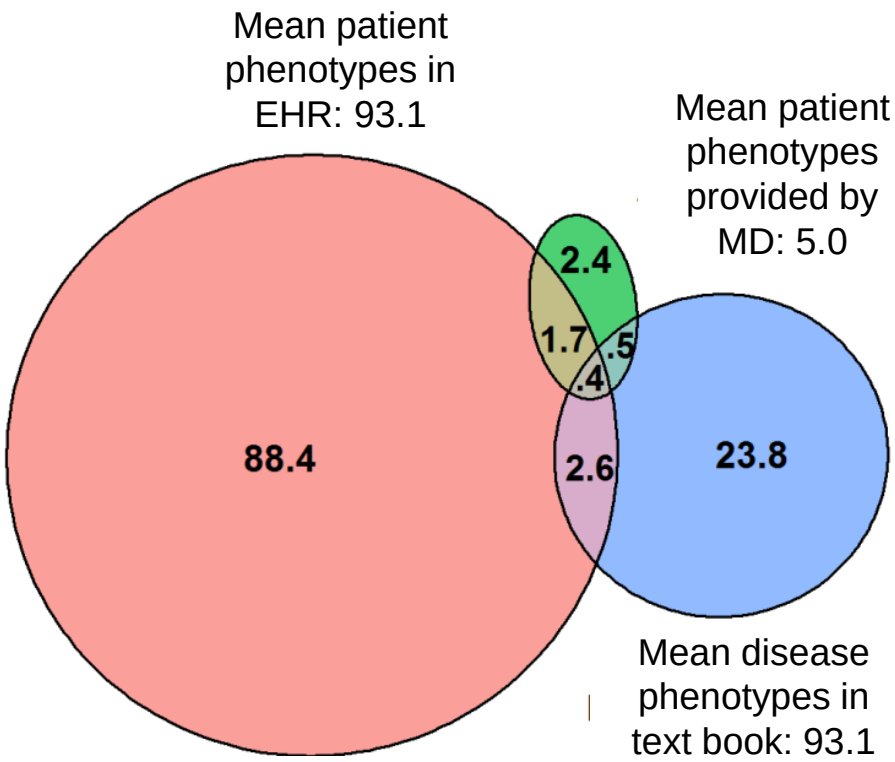


CliniThink CLiXENRICH natural language processing software

Why collect a deep phenotype

- 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



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

Automated provisional diagnosis:

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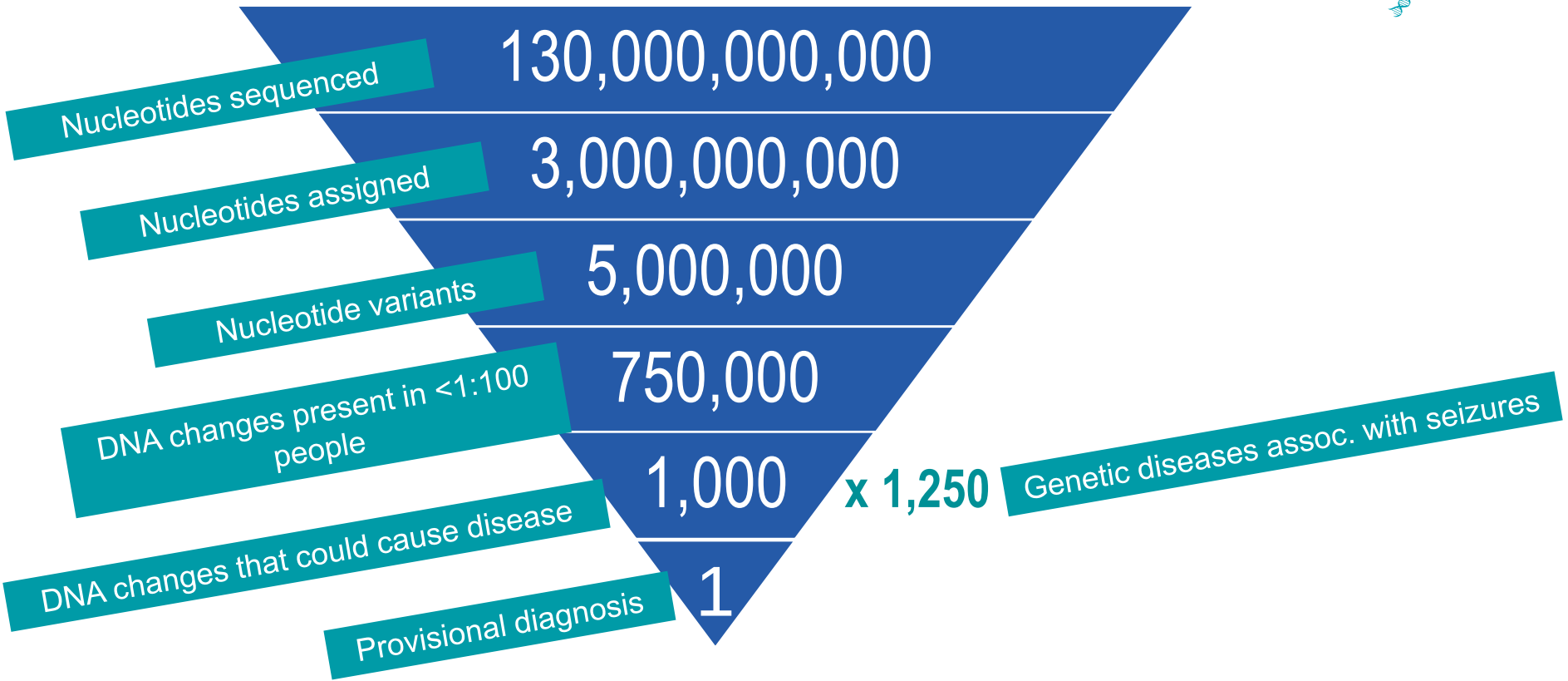

Next Steps and Where Does AI/NLP Come in?

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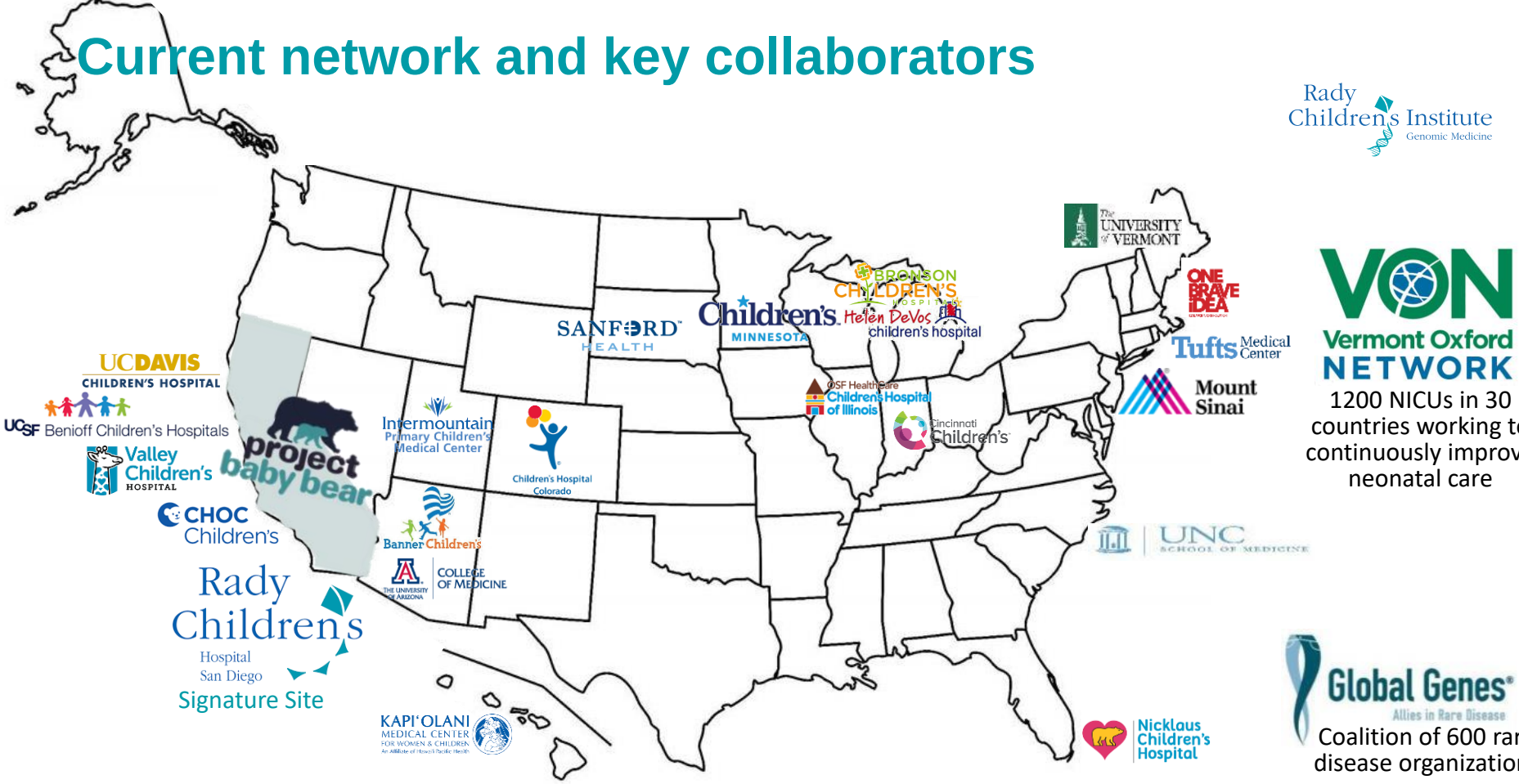
Steps in the Process	Automation/NLP
Initial Case Tracking - test type, family members	AUTOMATION
Phenotyping - HPO terms - gene lists	NLP/AI
Case Creation	AUTOMATION
Variant selection/filtering	AUTOMATION
Curation of variants	NLP/AI
Confirmation ordering	AUTOMATION
LD review/reporting	NLP
Report delivery	AUTOMATION
Verbal communication	NLP/AI – RPM GUIDANCE

Current network and key collaborators



Early, comprehensive diagnosis with therapy guidance will increase orphan drug markets

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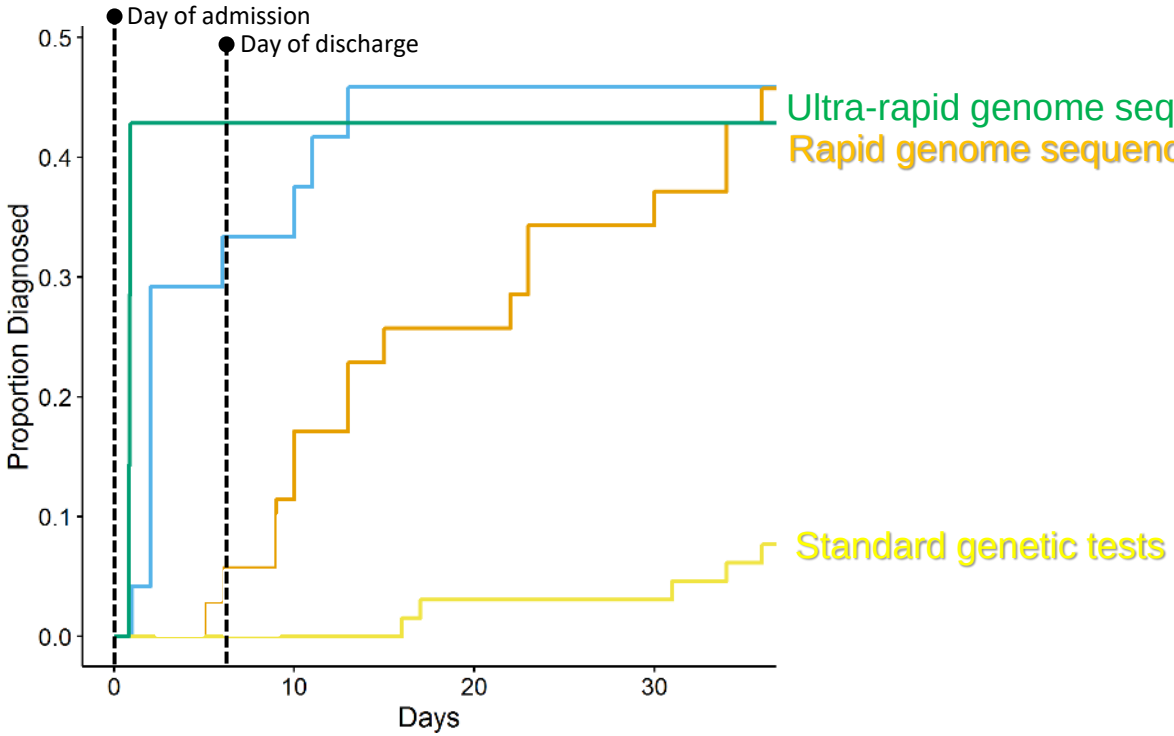

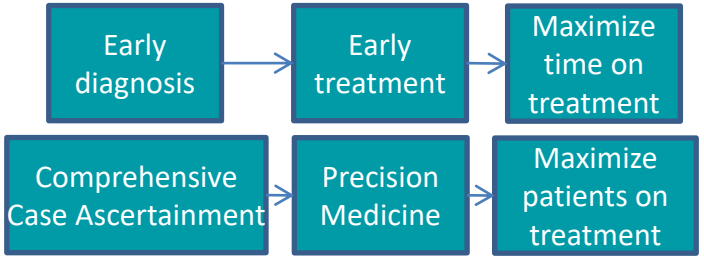

urWGS ordered on the day of admission with 1-2 day time to result is optimal

We are developing a healthcare delivery system for national implementation of Rapid Precision Medicine

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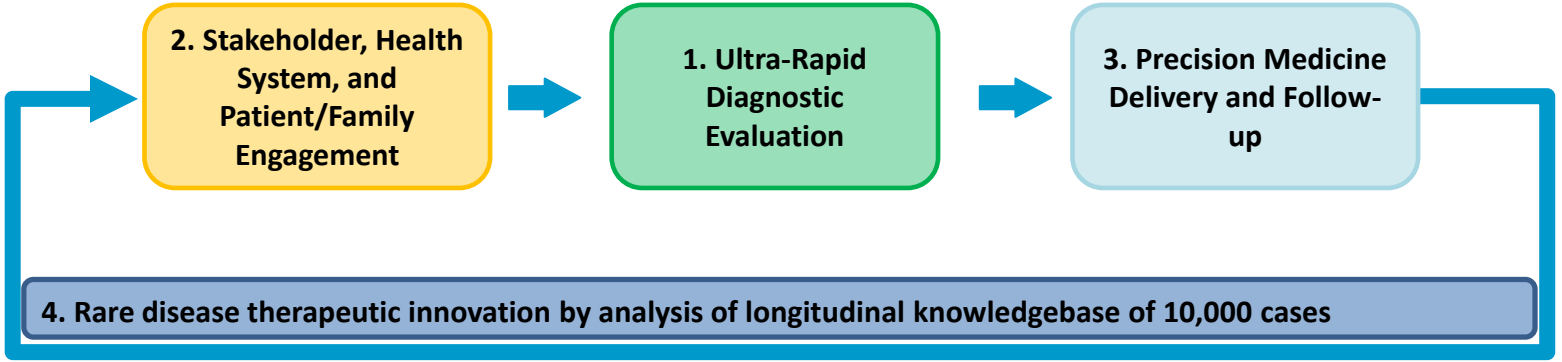
- Engages all stakeholders
- Genomic consult service
- Conext-specific implementation
- Simplified, non-expert ordering
- Automated deep phenotype extraction

- 1 day to result
- Semi-automated interpretation
- 3,000 cases / year
- State-of-the art diagnostic performance

- Results effectively communicated to non-expert ICU teams & parents
- Management guidance to change Rx before discharge
- Implications understood by parents
- Precision medicine follow-up clinic



Virtuous circle: Implementation identifies unmet needs that drive innovation

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