DAVID DIMMOCK M.B.B.S.

RAPID WHOLE GENOME SEQUENCING IMPROVES THE CARE OF HOSPITALIZED INFANTS

Rady Children's Institute
Genomic Medicine

Acknowledgements

- Stephen Kingsmore
- Jennifer Azares
- Wendy Benson
- Matt Bainbrindge, PhD
- Sergei Batalov, PhD
- Julie Cakici
- Shimul Chowdhury PhD
- Michelle Clark, PhD
- Yan Ding, MD
- Todd Laird
- Patrick Mulrooney
- Shareef Nahas Ph.D
- Daeheon "Danny" Oh
- Albert Oriol
- Julie Reinke
- Lisa Salz
- Jennifer Silhavy,
- Olivia Simonides
- Ray Veeraraghavan, PhD
- Vanessa Wertheim, PhD
- Catherine Yamada

Rady Children's Hospital San Diego

UC San Diego

- Lauge Farnaes, MD, PhD
- Amber Hildreth, DO
- Nathaly Sweeney, MD
- Jenni Friedman, MD
- Joe Gleeson, MD, PhD
- Julie Ryu, MD
- George Chiang, MD
- Lance Prince, MD, PhD
- Farhad Imam, MD, PhD
- Nicole Coufman, MD, PhD
- James Perry, MD
- Tina Chambers, PhD
- Jeff Neul, MD, PhD
- Gail Knight, MD
- Mallory Boutin
- Cynthia Kuelbs, MD
- Lynn Byrd, MD
- Jonathan Sebat, PhD
- Bruce Barshop, MD

Support:

- Rady Children's Institute for Genomic Medicine
- NICHD
- NHGRI
- NIDDK
- Illumina

Initial Focus For Precision Medicine- Infants

8,000 known genetic diseases

These affect 3% of US children

- Leading cause of death in infants,
- Leading cause of death in PICUs and NICU

Presentation less confounded by environment

Biggest timespan for benefit

San Diego Synergy
Industry Collaboration for Rapid WGS

Newsweek

Europe Edition

HOME POLITICS BUSINESS CULTURE TECHNOLOGY SCIENCE HEALTH THIS WEEK'S EDITION

SCIENCE

Whole Genome Sequencing Time Cut to Just 26 Hours

By Conor Gaffey 10/1/15 at 9:31 PM

Whole genome sequencing can be the difference between life and death for newborns suffering from genetic diseases. A new sequencing procedure cuts down the time involved from 50 hours to just 26. Michael Dalder/Reuters

RECORD GUINNESS WORLD RECORDS™ HOLDER

How:

1. Same work-day blood sample to HiSeq 2500
2. 2 x 101 nt WGS in RRM
3. DRAGEN alignment & variant calling
4. Deep phenotyping and differential diagnosis
5. Sophisticated bioinformatics Pipeline
6. **FDA permission to report WGS results before confirmation in life-threatening situations in not life threatening confirm via sanger or array**

Earlier rapid comprehensive genetic testing

Timely specific care

Better patient outcomes

Birth History

Product of 40wk 2 days Gestation

- Birth Weight: 40%
- Birth Length: 95%
- Head Circumference: 79%

Born via repeat C-section to a G6P2

Prenatal labs: Normal

Pregnancy complicated by polyhydramnios

Delivery complicated by vacuum extraction

APGARS were 4 at 1 minute, 9 at 5 minutes

Transferred to NICU due to Apneic episode and bradycardia during delayed cord clamping

Transferred to RCHSD on DOL 9 for concern of PPHN

Family History

Father, Schizophrenia

Mother, healthy

1 year old brother, full sibling, healthy

8 year old maternal half-sister, healthy

Course at Rady Children's Hospital

Cardiovascular:

- Echocardiogram:
  - severe pulmonary hypertension, small PDA, small PFO with bi-directional flow, and moderate RV dilatation
  - Partial Anomalous Pulmonary Venous Return (PAPVR) of Right Upper Vein (RUV) ->SVC
- Patient on Tadalafil, Bosentan, iNO 20ppm via NCPAP and Remodulin initially
- iNO discontinued due to no benefit

Course at Rady Children's Hospital

Respiratory:

- Apnea and floppiness during delayed cord clamping, required PPV and deep suctioning
- Started Low Flow Nasal Cannula (LFNC) due to failed CCHD screen
- Increased work of breathing and oxygen requirements → NCPAP7
- Chest CT:
  - diffuse ground glass opacities, suggestive of edema, interstitial disease or airspace disease
- Weaned to LFNC

Course at Rady Children's Hospital

FEN/GI:

- Abdominal Ultrasound 4/22:
  - Small or contracted gallbladder
  - Otherwise within normal limits

Neurology

- Normal head ultrasound scan
- Getting Physical Therapy for increased tone

Metabolic/Genetics:

- **NBS:** normal except for sickle cell trait

HPO terms

- **Persistent Fetal Circulation of the newborn**

“Persistent fetal circulation is a condition caused by a failure in the systemic **circulation** and pulmonary **circulation** to convert from the antenatal **circulation** pattern to the "normal" pattern. ... Because of this, the condition is also known as "**persistent** pulmonary hypertension of the **newborn**"

- ***pulmonary_artery_hypertension***
- ***respiratory_failure***
- ***right_ventricular_hypertrophy***


-96:00
Baby with respiratory distress
Rady
Children's
Institute

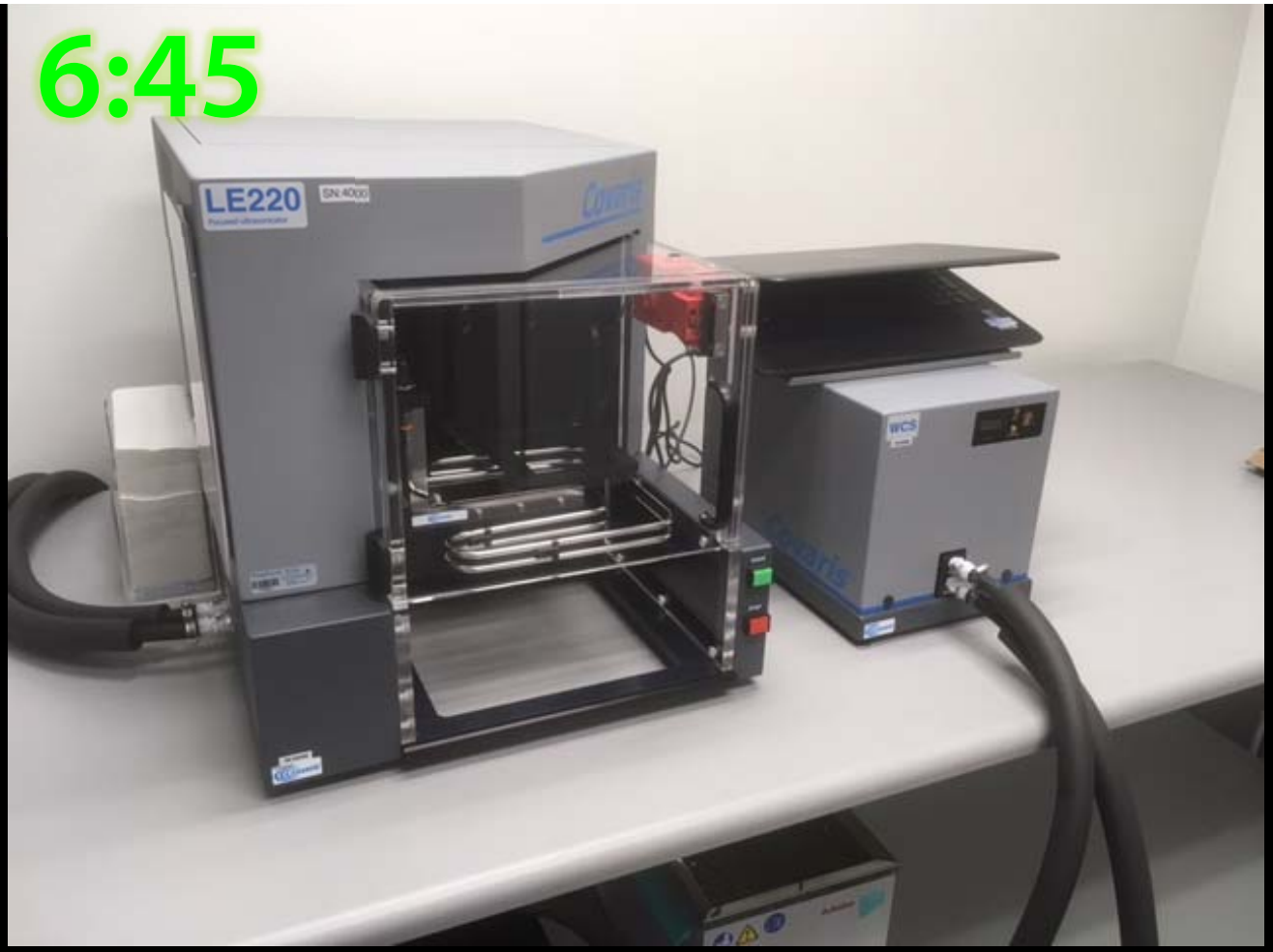
-16:30

Blood drawn


-16:00
Rady
Children's
Institute
Genomic Medicine
Transport to Institute
Rady
Children's
Institute


01:20
Isolate DNA
Rady
Children's
Institute

6:45



Prepare DNA for sequencing

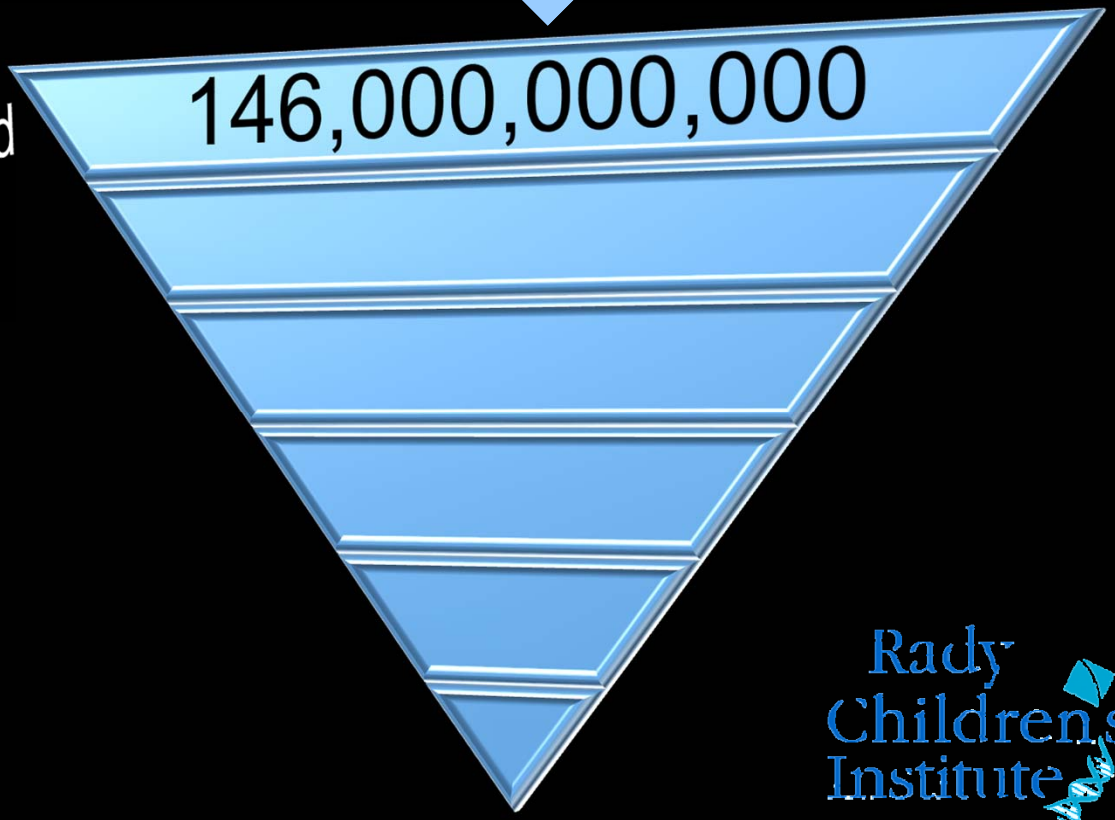
Rady Children's Institute
Genomic Medicine

Rapid genome sequencing

35:31

Infant with respiratory distress

Total DNA letters detected

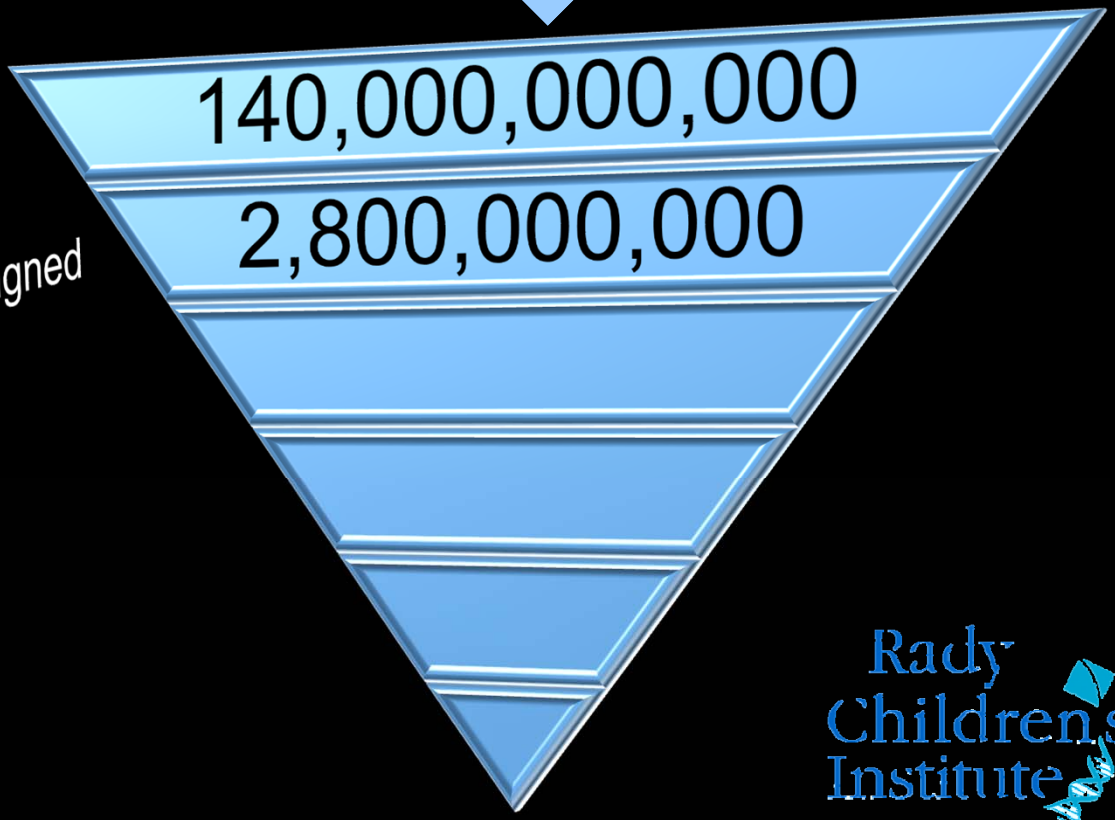


Rady Children's Institute
Genomic Medicine


36:30
DRAGEN
by edico genome
DRGN5500-001
4225EXSQLJCA
V0.9-000
DRAGEN™
Bio-IT Processor
Rady
Children's
Institute

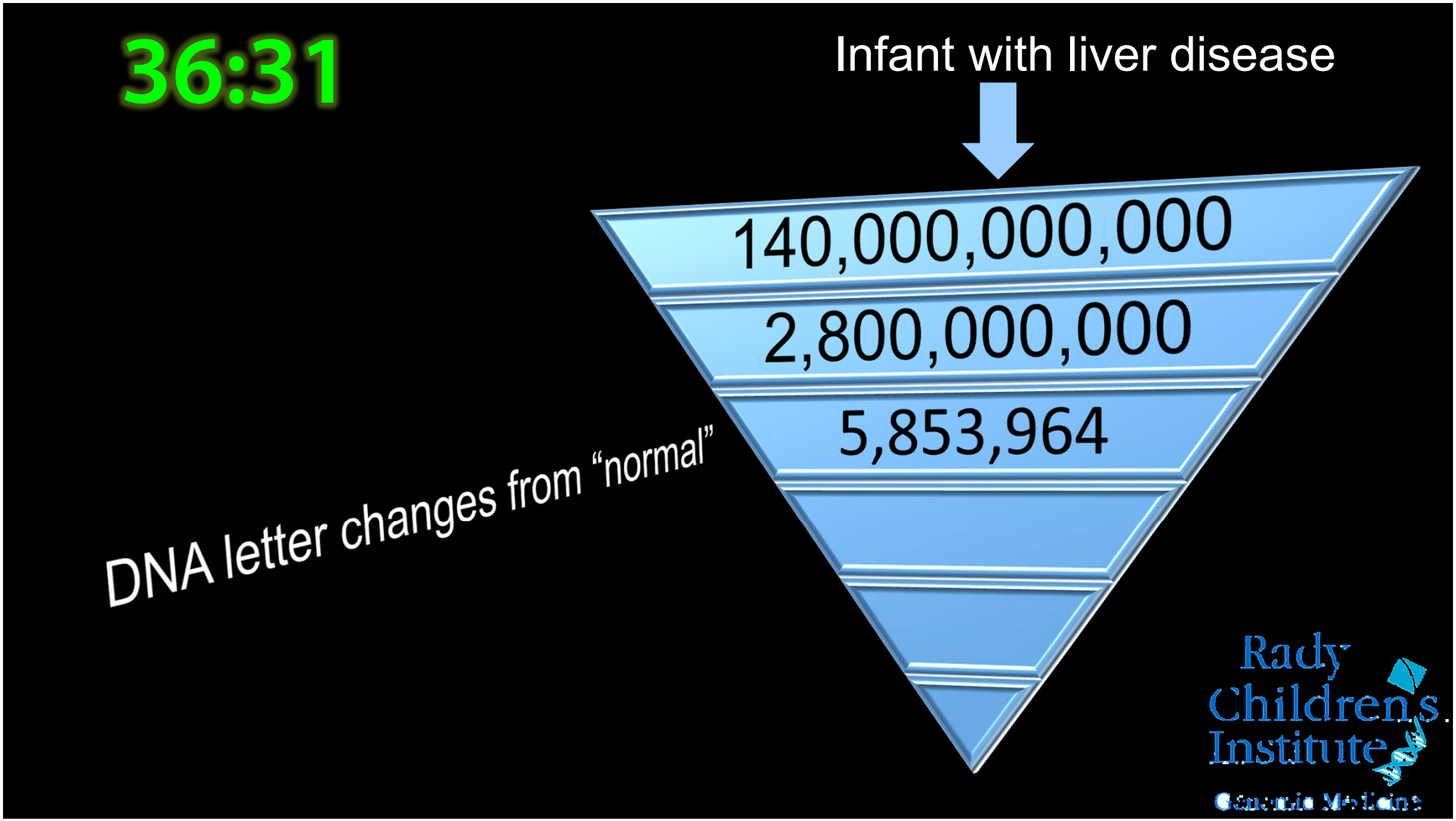
36:30

Infant with respiratory distress

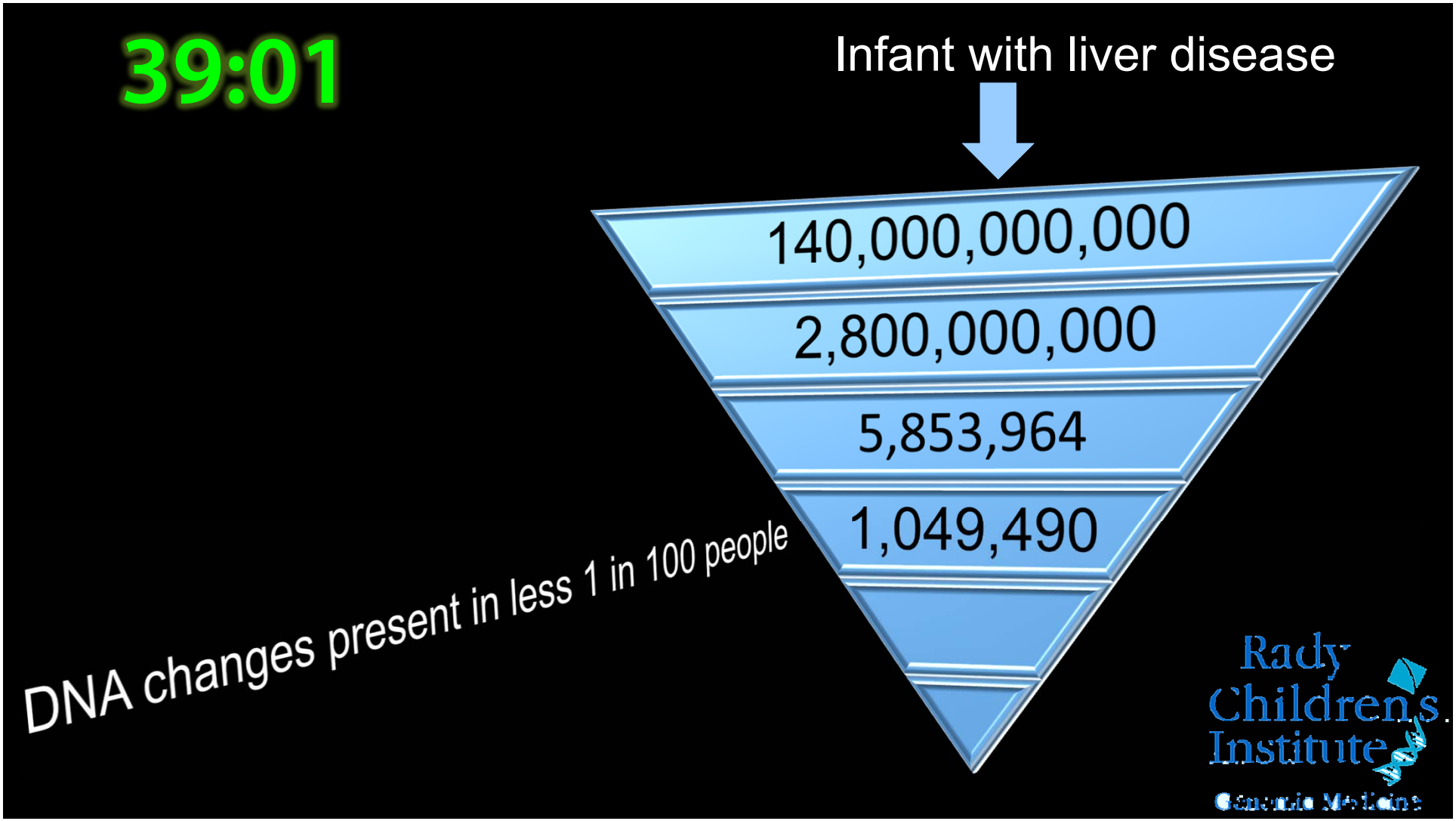


DNA letters of genome code assigned

Rady Children's Institute
Genomic Medicine

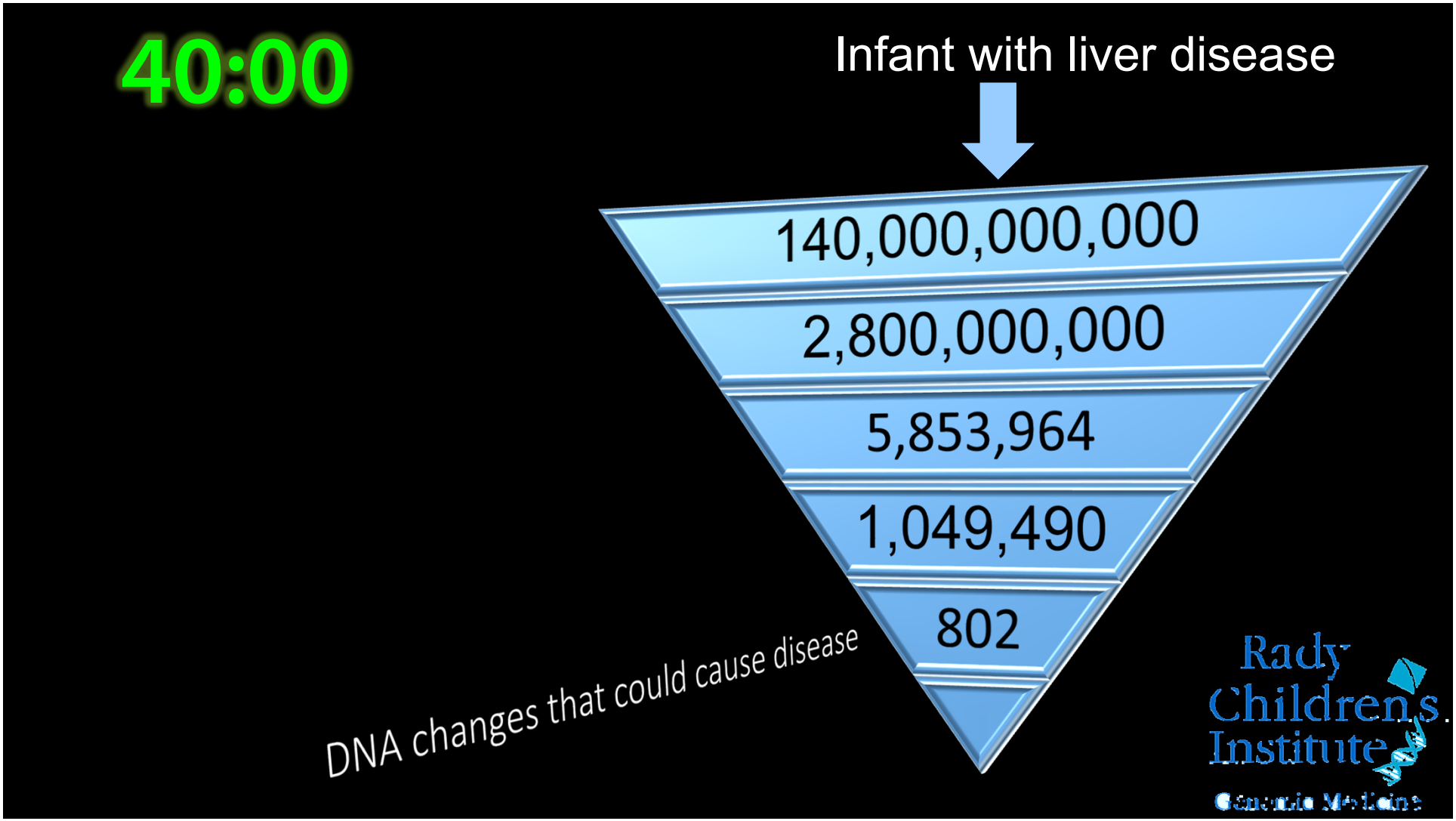

36:31
Infant with liver disease
140,000,000,000
2,800,000,000
5,853,964
DNA letter changes from "normal"
Rady Children's Institute

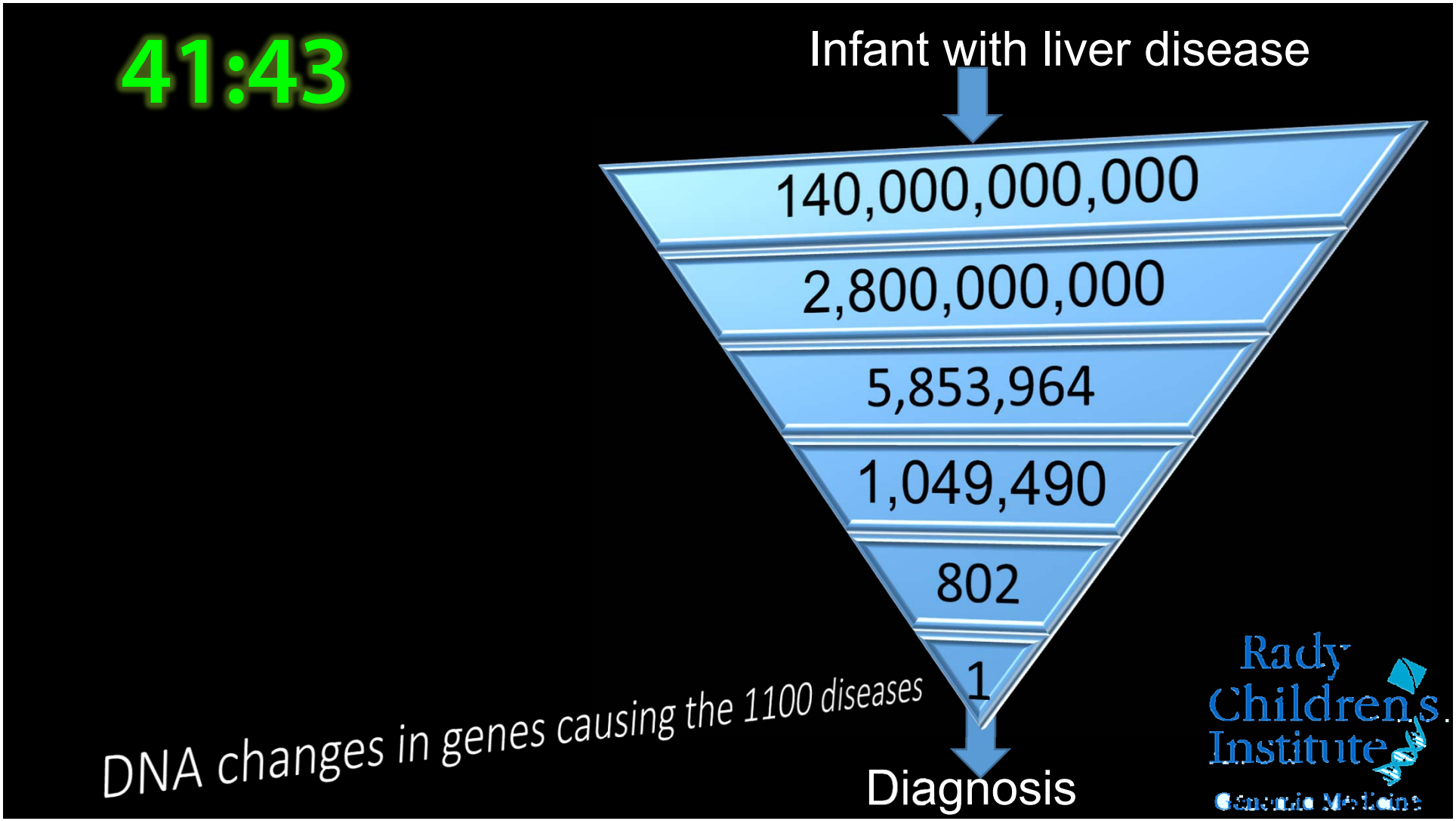
FAMILY 6096	100257-proband	UNITS	REFERENCE RANGE
Sex / maternity / paternity / Mendelian model	**M verified**		
Yield: raw/bulk	**146.0**		
% mapped	**98.60%**	pct	98-100
% duplicates	**2.60%**	pct	< 15%
Yield	**144.1**	Gbp	> 130
Insert size: Mean +/- std.dev	**403.3**	bp	300-480
Average and median coverage across genome	**41.8**	x	> 40
Average coverage over OMIM genes	**40.0**	x	> 40
# of OMIM genes with coverage at <10X (and list)	588	ENST	< 2% (282)
# of OMIM genes with 100% coverage at >=10X	95.8%	pct	> 98%
# of OMIM genes with 100% coverage at >=20X	81.3%	pct	> 94%
# of OMIM genes with 100% coverage at >=30X	20.2%	pct	> 80%
# of genes with 100% coverage at >=40X	**2.4%**		
Variation (VCF) metrics			
# of calls Total	**5853946**		2.5-6.0M
# of PASS calls	**5753090**		2.5-6.0M
# of calls Total coding	**31736**		25000-30000
Total # of SNVs	**4719925**		
Total # of Indels	**1033165**		
Hom/Het ratio (in coding regions)	0.47	ratio	0.5-0.61
Ti/Tv ratio (in coding regions)	1.96	ratio	2-2.2 (2.8-3)
# of het calls (# of hom call)	**3961190 (1892756)**	units	
In-silico sample swap check	**N/A**		Mendelian test
Automated upload of VCF to Omicia	**PASS**		
Inform sign-out of analysis-ready state	**PASS**		
Detect sample analysis completion state on Omicia	**PASS**		
Update LIMS	**TBD**		
Download annotated VCF to RCI	**TBD**		


39:01
Infant with liver disease
140,000,000,000
2,800,000,000
5,853,964
1,049,490
DNA changes present in less 1 in 100 people
Rady Children's Institute

Gene List: 664 Genes associated with HPO terms

Rank	Gene	ID	Score
1	FOXF1	2294	1
2	STAMBP	10617	0.2288
3	PM20D1	148811	0.09365
4	SGCG	6445	0.08241
5	CBLN2	147381	0.08016
6	VPS33B	26276	0.06181
7	BMPR2	659	0.05173
8	FGFR3	2261	0.03816
9	SMAD9	4093	0.0299
10	PTH1R	5745	0.02902
11	SLC35D1	23169	0.02731
12	FLNB	2317	0.02719
13	ALMS1	7840	0.0266
14	DHCR7	1717	0.02311
15	CHD7	55636	0.02177
16	MGP	4256	0.01927
17	NFIX	4784	0.0191
18	TGFA	7039	0.01896


40:00
Infant with liver disease
140,000,000,000
2,800,000,000
5,853,964
1,049,490
802
DNA changes that could cause disease
Rady Children's Institute


41:43
Infant with liver disease
140,000,000,000
2,800,000,000
5,853,964
1,049,490
802
1
DNA changes in genes causing the 1100 diseases
Diagnosis
Rady Children's Institute

Variant Identified

Review Priority	Gene	Position dbSNP	Change	Effect	Zygosity	Quality GQ Coverage	1KG AF EVS AF ExAC AF	Omicia Score	Evidence
●●●	FOXF1	chr16:86544363	G → T c.188G>T p.Ser63Ile	missense splice site impact	●○	808 99 39 : 17 : 22		0.846	CV HGMD

FOXF1

chr16:86544363

Het c.188G>T; p.Ser63Ile

Molecular Analysis

FOXF1 c.188G>T; p.Ser63Ile

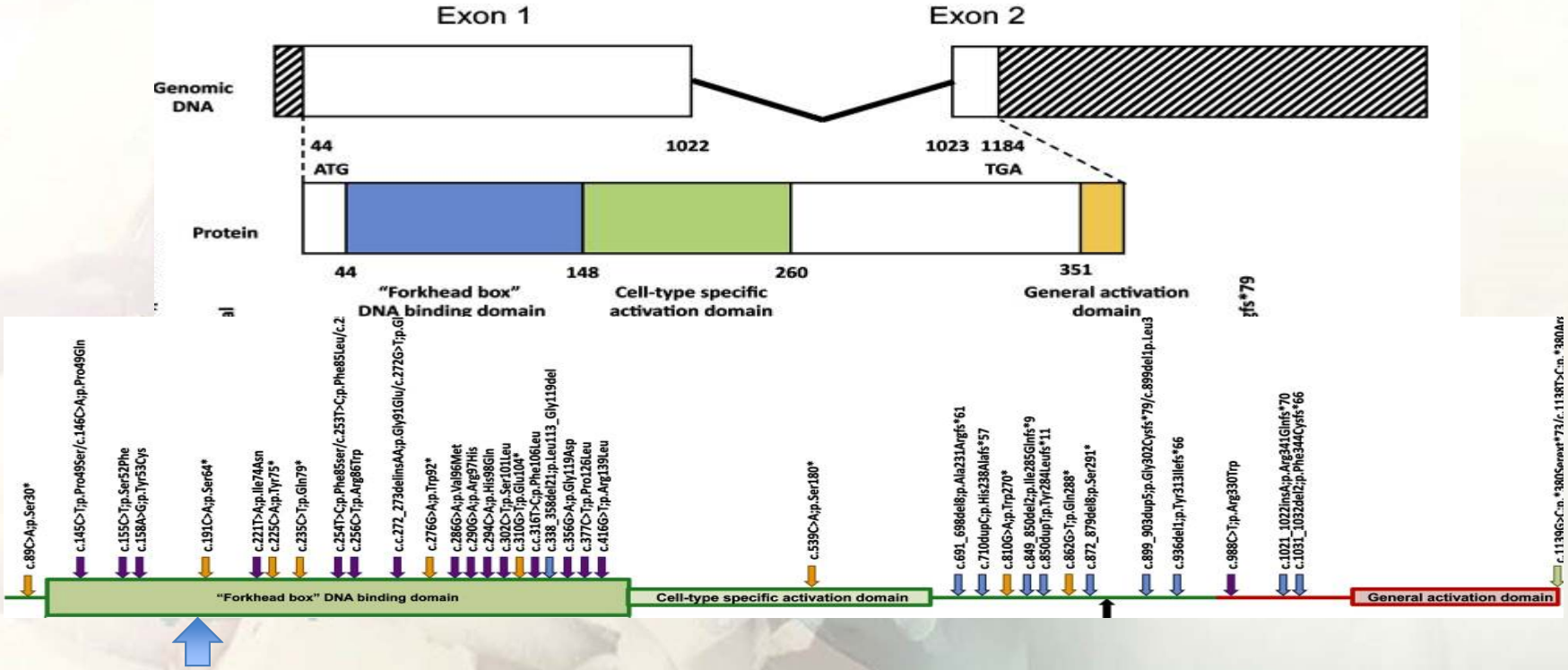
Functional Scores for Position 86544363

Omicia Score	0.846	Omicia Score assesses whether a variant is likely to be deleterious. It is a meta classifier that combines scores from the variant scoring algorithms below. The output is a 0 to 1 score, with higher scores indicating damaging variants.
MutationTaster	Disease Causing - D Bayes Classifier Probability: 1	MutationTaster employs a Bayes classifier to predict the disease potential of an alteration. The prediction of disease causing (D) or polymorphism (P) is either from the Bayesian model or based on external data, which is noted in the classification. Prediction confidence (range 0 to 1) with values closer to 1 indicating higher confidence.
Polyphen-2 - HDIV	0.999	Polyphen-2 predicts possible impact of an amino acid substitution on the structure and function of a human protein using straightforward physical and comparative considerations. The output is a 0 to 1 score.
SIFT	0	SIFT predicts whether an amino acid substitution affects protein function, by aligning homologous protein sequences using PSI-BLAST. The output is a 0 to 1 score, with lower scores indicating damaging variants.
phyloP - Vertebrate	3.53	phyloP computes conservation or evolutionary acceleration p-values based on an alignment and model of neutral evolution. Values between -11.764 and +6.424. Positive scores indicate conservation; negative scores fast-evolution.
phyloP - Placental	1.97	Values between -12.709 and +2.941. Positive scores indicate conservation; negative scores fast-evolution.

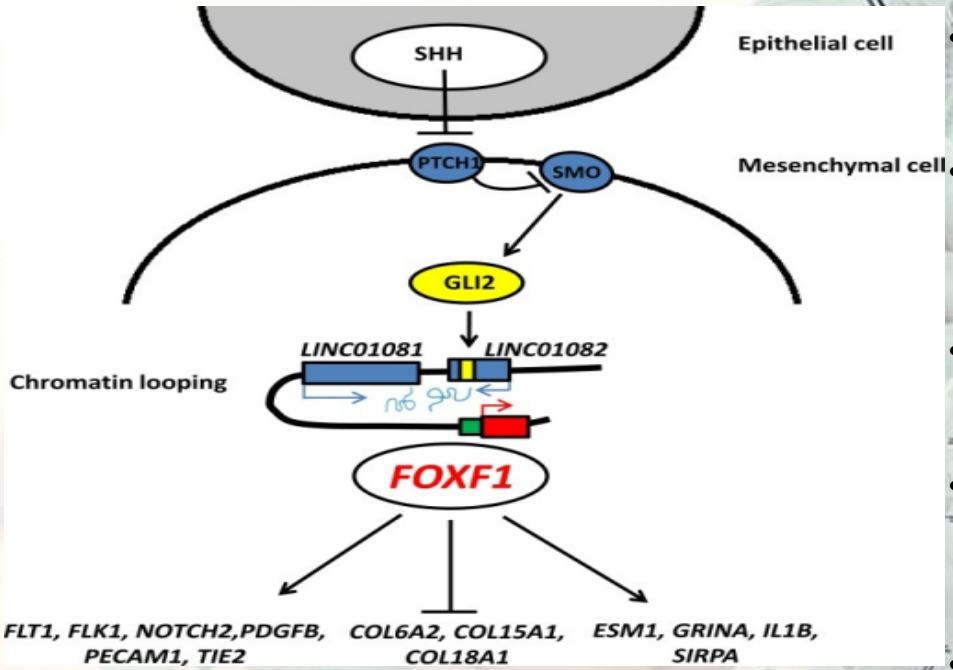
Molecular Analysis

FOXF1 c.188G>T; p.Ser63Ile:

Novel located in highly conserved DNA binding domain



FOXF1 Gene Function



- The FOXF1 gene provides instructions for making the forkhead box F1 (FOXF1) protein.
- This protein is a transcription factor, which means that it attaches (binds) to specific regions of DNA and helps control the activity of many other genes.
- **characterized by a distinct forkhead DNA binding domain**
- The FOXF1 protein is important in the development of pulmonary mesenchyme, the embryonic tissue from which blood vessels of the lung arise.
- FOXF1 downstream target of Sonic hedgehog pathway.
- It is also involved in the development of the gastrointestinal tract.

FOXF1 OMIM

INHERITANCE
- Autosomal dominant

CARDIOVASCULAR
Heart
- Right-to-left shunt via the foramen ovale or ductus arteriosus or both
- Atrial septal defect (in some patients)

Vascular
- Alveolar capillary dysplasia (ACD)
- Malposition of pulmonary vein branches adjacent to pulmonary artery branches (MPV)
- Deficient capillarization of airspace walls
- Increased muscularization of arterioles
- Neonatal pulmonary hypertension

RESPIRATORY
- Pulmonary insufficiency

Lung
- Abnormal lung lobation

ABDOMEN
Pancreas
- Annular pancreas

Biliary Tract
- Gallbladder agenesis

Gastrointestinal
- Intestinal malrotation
- Duodenal atresia
- Meckel diverticulum

ENITOURINARY
Kidneys
- Hydronephrosis

Ureters
- Hydroureter

Bladder
- Bladder dilatation

PRENATAL MANIFESTATIONS
Amniotic Fluid
- Polyhydramnios

MISCELLANEOUS
- Lethal in the neonatal period

Rady Children's Institute
Genomic Medicine

Alveolar Capillary Dysplasia

- Very rare congenital malformation
- There is abnormal development of the capillary vascular system around the alveoli of the lungs
- Rare cause of persistent pulmonary hypertension in the newborn (PPHN)
- Does not respond to medical management with pulmonary vasodilators
- Caused by heterozygous mutation in the FOXF1 gene on chromosome 16q24
- Diagnosis is frequently postmortem via histology
- **Mortality thought to be 100%**
- **Possible treatment is lung transplant**


RADY CHILDREN'S INSTITUTE
GENOMIC MEDICINE
GOING BIG
FOR THE LITTLE ONES


RADY CHILDREN'S INSTITUTE
GENOMIC MEDICINE
GOING BIG
FOR THE LITTLE ONES