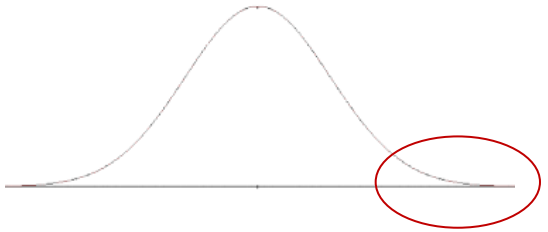
Mystery Illnesses:
Developing a Path to Caring and Discovery

Penelope: University of Utah's
Undiagnosed and Rare Disease Program

Presenters:
Lorenzo Botto, MD
Rong Mao, MD



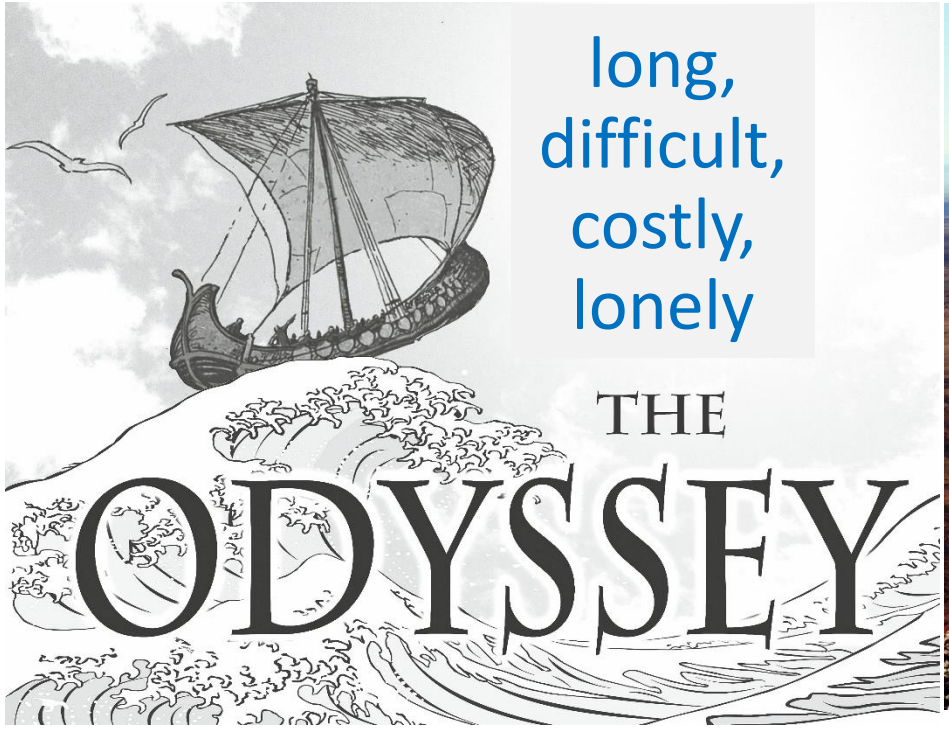
21 December 2017

T 12 year old boy

Clinical:

- Progressive, neurodegenerative
- Many tests, several invasive
- Seen at multiple institutions
- Started at age 5 years, now 12

Diagnostic Odyssey: 7 years



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Clinical:

- Progressive, neurodegenerative
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Diagnostic Odyssey: 7 years

Value of diagnosis: answering the family's key questions

• **What is this ?**	End the odyssey
• **What will happen now?**	Outcomes
• **How do we treat it?**	Care (*cure*)
• **Why did it happen?**	Cause
• **What will happen to my family?**	Risk

ORIGINAL RESEARCH ARTICLE | Genetics inMedicin

The National Institutes of Health Undiagnosed Diseases Program: insights into rare diseases

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Lynne Wo
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Sandra Yang, MS[1,2], A
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omparative Sequencing
vid Adams, MD, PhD[1,3]

Key Lesson learned:

- **Value of careful phenotyping**
- **Evaluation as iterative process**
- **Expect new/very rare conditions, variant presentations**
- **Advance care, advance knowledge**

Purpose: This report describes the National Institutes of Health Undiagnosed Diseases Program, details the Program's application of genomic technology to establish diagnoses, and details the Program's success rate during its first 2 years.

Methods: Each accepted study participant was extensively phenotyped. A subset of participants and selected family members (29 patients and 78 unaffected family members) was subjected to an integrated set of genomic analyses including high-density single-nucleotide polymorphism arrays and whole exome or genome analysis.

Results: Of 1,191 medical records reviewed, 326 patients were accepted and 160 were admitted directly to the National Institutes of Health Clinical Center on the Undiagnosed Diseases Program service. Of those, 47% were children, 55% were females, and 53% had neurologic disorders. Diagnoses were reached on 39 participants (24%) on clinical, biochemical, pathologic, or molecular grounds; 21 diagnoses involved rare or ultra-rare diseases. Three disorders were diagnosed based on single-nucleotide polymorphism array analysis and three others using whole exome sequencing and filtering of variants. Two new disorders were discovered. Analysis of the single-nucleotide polymorphism array study cohort revealed that large stretches of homozygosity were more common in affected participants relative to controls.

Conclusion: The National Institutes of Health Undiagnosed Diseases Program addresses an unmet need, i.e., the diagnosis of patients with complex, multisystem disorders. It may serve as a model for the clinical application of emerging genomic technologies and is providing insights into the characteristics of diseases that remain undiagnosed after extensive clinical workup.

Genet Med 2012:14(1):51–59

Key Words: neurological disorders; rare disease; SNP arrays; undiagnosed disease; whole exome sequencing

Understanding what is important: begin from the end

Parents

Care Coordinators

Physicians, Attendings

Valued Outcomes

Coordination

- Plan and deliver efficient path to diagnosis and care
- Avoid duplications, leverage synergies, be timely
- Have a single point of contact with program

Communication

- Integrated medical Information: families, providers
- Visual, clear summary for family and PCP

Team, Process & Tools

Diagnosis

- low throughput with high demand: screen
- Review and respond to all: accept or refer

Discovery and Training

- Aligned with clinical mission
- Opportunity for next generation of clinicians & scientists

Process (simplified)

Assess, Plan, See

Team evaluation

- Referral: engage and get data
- Review: discuss and score
- Decide: accept vs. refer
- Design evaluation plan: clinical team, testing, HPO & gene lists, prelim tests (SNP array)
- See Family, finalize testing

Team Members

Top Level Support

Executive Sponsor

E Clark, Chair, Department of Pediatrics

Core Team

Front Line

Team lead (L Botto)
Cardiogenetics (S Bleyl)
Dysmorphology (J Carey, D Viskochil)
Biochemical Genet (N Longo)
Neurology (J Bale)
Comprehensive Care (J Alvey, C Hagedorn)
Gastroenterology (S Guthery)
Rheumatology/Immunology (J Bohnsack, K Chen)
Molecular Genetics (R Mao, P Bayrak-Toydemir)
Fellows in Medical Genetics, Molecular Genetics

NP Coordinator (A Andrews)
Admin Assistant (M Smith)
(parent partner)

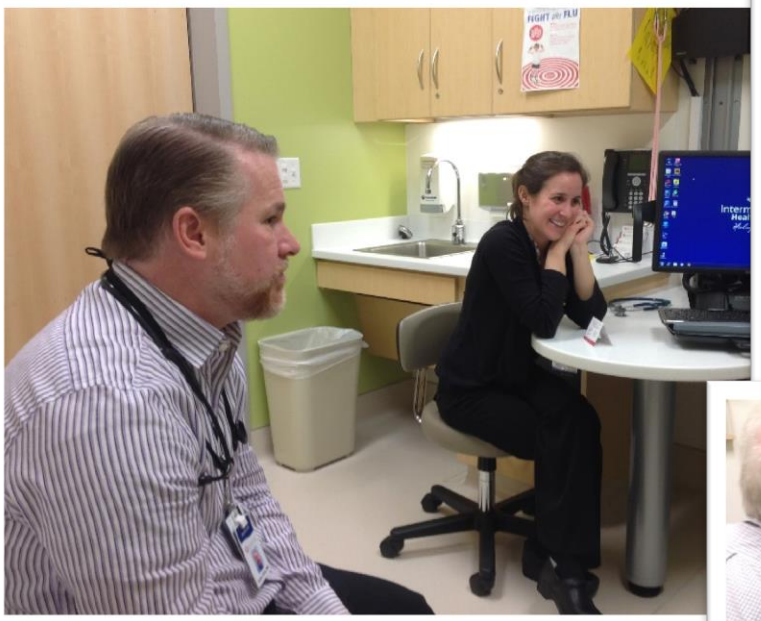
Front Line

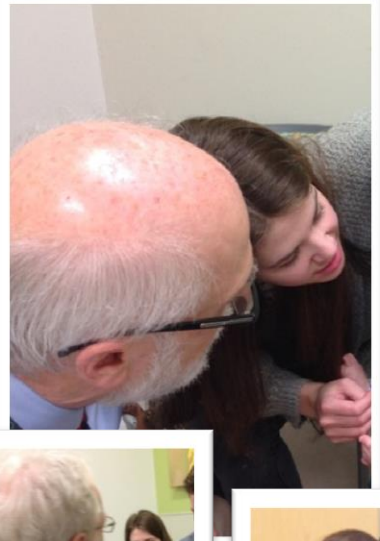
Consultants

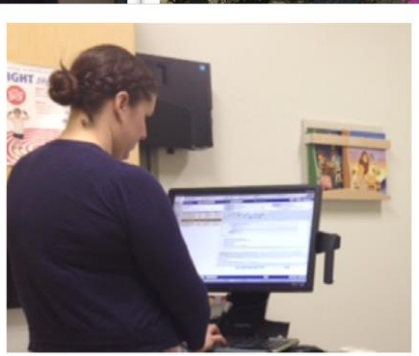
Neuromuscular
Hematology-oncology
Behavioral health
Endocrinology
Social work

Engagement at step 0:
Voice of Customer Workshops with key stakeholders

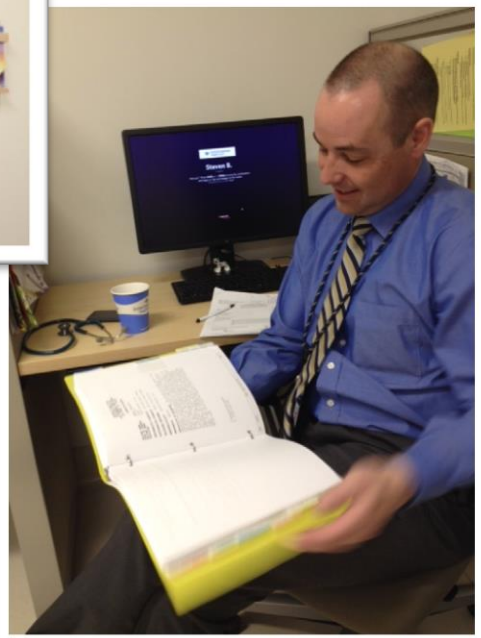
Parents
Clinical coordinators
Clinicians







think,
see,
think again



Process (simplified)

Assess, Plan, See

Team evaluation

- Referral: engage and get data
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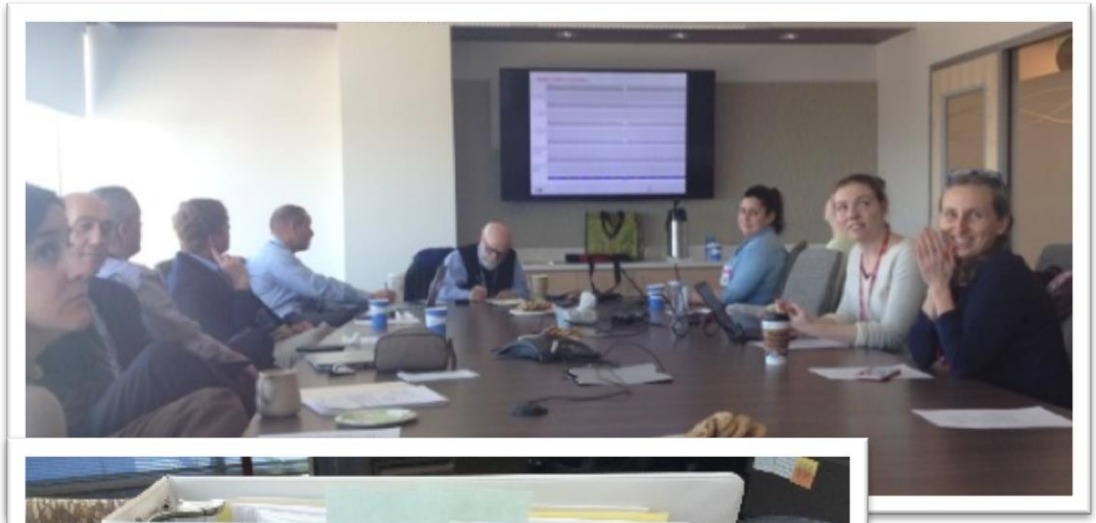
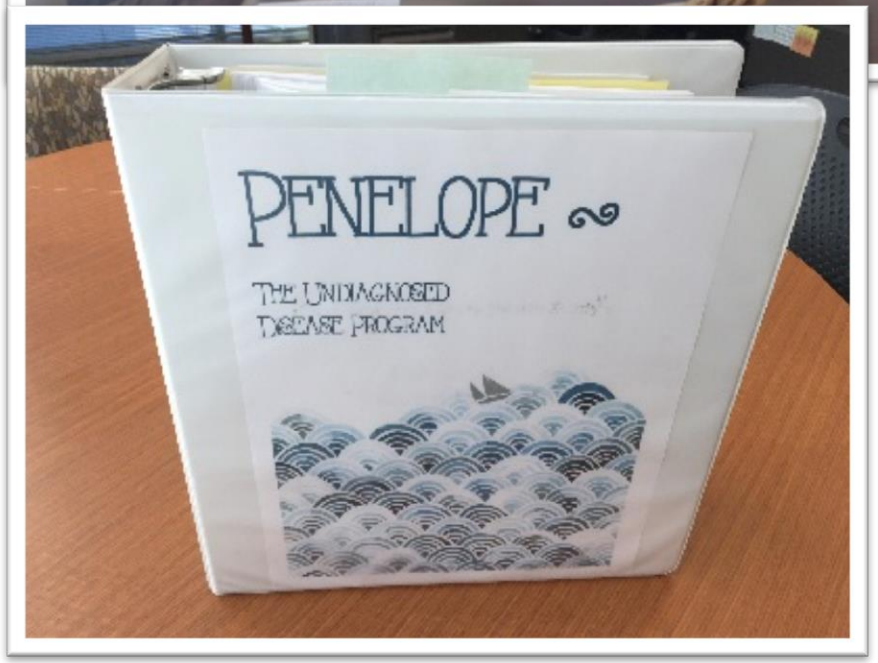
- See Family, finalize testing

Analyze

- Exome
- Bioinformatics
- Expand testing, RNA

Process (simplified)





Diagnose & Care

Team evaluation

- Diagnosis made?
- Care Plan
- Family binder
- Family Result Visit
- Referrals
- Follow up

- (12 year old boy)

Page 2 of 2 - Genomic details

agnostic data and gaps:

- Exome: one diagnostic finding = a single PRPS1 (OMIM 311850) missense mutation
  - on X chromosome, also present in mother (who compensated with her second copy)
- **Single gene testing: FSHD allele contraction (haplotype unknown), modifying role unclear**
- **Expansion repeats**: not done, but not needed given diagnosis
- **Mitochondrial genome**: not done, not needed given diagnosis
- **Genomic imbalances**: normal microarray
- **Environmental exposures**: significant regression after febrile episode, a possible contributor

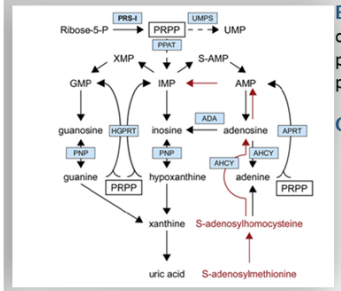
A. Genes with protein changing variants

Variants with EVS/ExAc frequency <1%

Internal database frequency <3%

OMIM hits

Predicted Class	Gene	Chrom	Position	rsId	Nucleotide Change	Protein Change	Zygosity
Disease Associated	PRPS1	chrX	chrX:106884159	none	c.344G>C	p.Val112Leu	hemizygou
Uncertain Significance	CFH	chr1	chr1:196711013	none	c.2965T>G	p.Cys989Gly	heterozygo



B. Biochemical / mechanistic pathways : the enzyme generated by the PRPS1 gene (PF
crucially involved in a biochemical pathway that makes and recycles purine and pyrimidine
pyrimidines are key compone nts of important substances (DNA, RNA, energy molecules su
principle, blocks in the pathway could be helped with certain supplements (SAM, nicotinar

C. Clinical presentations described with PRPS1: note the wide range and variabilit

optic atrophy
deafness
polyneuropathy (atrophy, areflexia)
development
infections, survival

Variable compone
the PRPS1-associated
conditions

Arts syndrome

Shorted lifespan (pre-teen)
Developmental regression
Marked neuropathy
Vision loss (including blindness)
Infections (often respiratory)

CMTX5

Long survival
Can have normal intellect
Slowly progressive neuropathy
Vision loss and deafness variable

Deafness XL

X linked deafness (SN)

Visual Summary (1)

Genetic Findings and Relation to Clinical Findings

N

Page 1 of 2 - Summary

Clinical:

- Global delays, brain anomalies (bilateral polymicrogyria/cortical dysplasia), macrocephaly
- Tetralogy of Fallot
- Morgagni (diaphragmatic) hernia
- Post axial polysyndactyly of hand and feet

Course: stable, growing well

Key evaluations so far : Normal SNP microarray, overgrowth panel (27 genes
KT1, AKT2, AKT3, GNAQ, and PIK3R2 in blood and atrial
anel (39 genes seq and del/dup), PIK3CA sequencing (blood).
Neurology, Cardiology, Ophthalmology
luation
PH/MCAP, ciliopathies
ions and testing:
s, Neurology, Comprehensive Care
, CBC, vit D (normal CK and CBC, vit D mildly low)
naging (normal)
xome Sequencing

ry of integrated clinical and genetic assessment

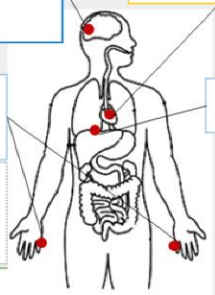
bilateral polymicrogyria
cortical dysplasia;
macrocephaly (>99%)
Global delays
Normal VEP and ABR

Tetralogy of Fallot
(complete repair)

Post axial polysyndactyly
hands and feet (tags, in hands and right foot, more fully formed digit on left foot).

Morgagni
hernia

Physical exam:
medial mobile masses on edge of the soles of both feet.
Reduced tone. DTR 0-1+ and symmetric.



Clinical Course:

regress plateau improve

Family History: healthy parents, no sibs

Visual Summary (2)

Clinical Evaluation and Plan

Process (simplified)

Assess, Plan, See

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Analyze

- Exome
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- Research
- Matching
- Follow up

Diagnose & Care

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- Diagnosis made?
- Care Plan
- Family binder
- Family Result Visit
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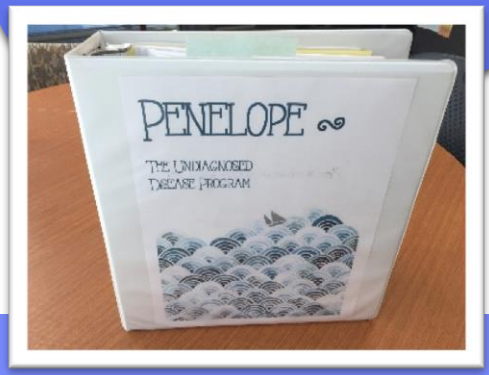
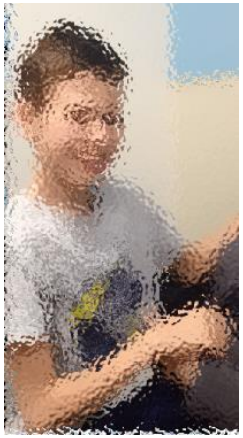


Clinical:

- Progressive neurodegenerative condition
- Many genetic tests, imaging, invasive tests
- Seen at NIH and multiple institutions

Diagnostic Odyssey: 7 years



Vision loss (optic atrophy)
Hearing loss (SN)

Regression
Neuropathy
Weakness

dysphagia

Predicted Class	Gene	Chrom	Nucleotide Change	Protein Change	Zygosity	Effect
Disease Associated	**PRPS1**	**chrX**	**c.344G>C**	**p.Val112Leu**	**hemizygous**	**Missense**
Uncertain Significance	CFH	chr1	c.2965T>G	p.Cys989Gly	heterozygous	Missense

optic atrophy
deafness
polyneuropathy (atrophy, areflexia)
development
infections, survival

Variable components in the PRPS1-associated conditions

Arts syndrome

Shorted lifespan (pre-teen)
Developmental regression
Marked neuropathy
Vision loss (including blindness)
Infections (often respiratory)

CMTX5

Long survival
Can have normal intellect
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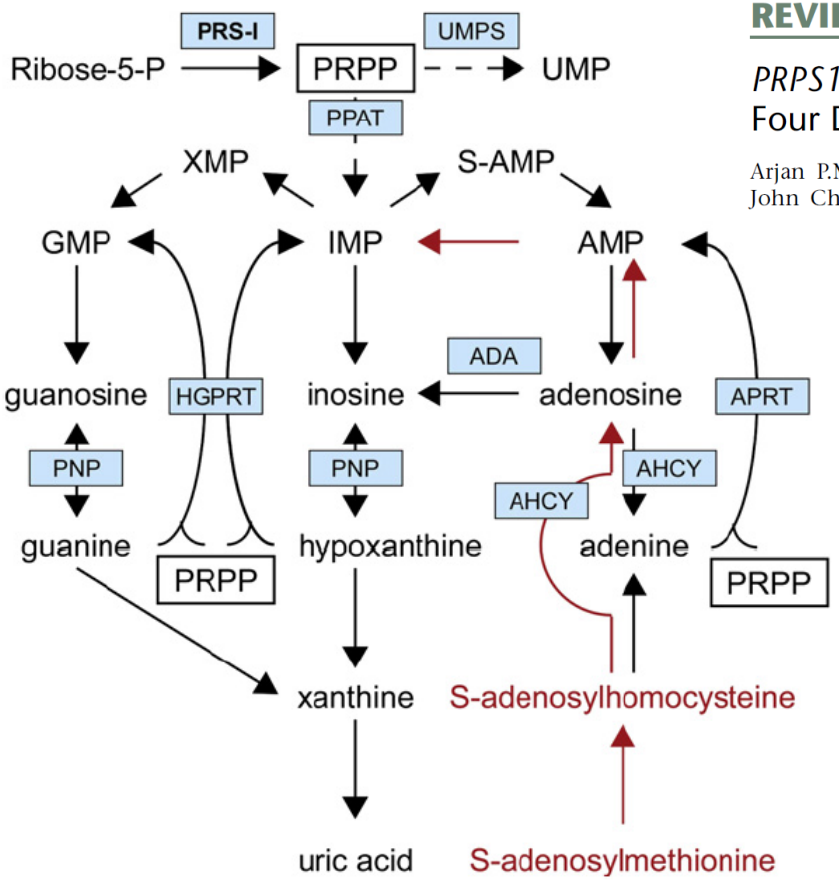


PRPS1 Mutations:
Four Distinct Syndromes and Potential Treatment

Arjan P.M. de Brouwer,[1,*] Hans van Bokhoven,[1,2] Sander B. Nabuurs,[3] Willem Frans Arts,[4] John Christodoulou,[5,6] and John Duley[7,8]

Figure 2. Simplified Overview of the Purine Metabolism Pathway

The scheme is derived from KEGG Pathways hsa00230 (purine metabolism) and hsa00240 (pyrimidine metabolism).[12] Indicated in red is the alternative pathway to replenish purine nucleotides via SAM. Enzymes central in this review are highlighted by blue boxes. PRS-I is printed in bold. Boxes show the essential role of PRPP in the purine metabolism. Dashed arrows indicate multiple intermediate steps that are not shown. PRPP is also used for the de novo synthesis of pyrimidines, as indicated by the arrow to UMP, the central intermediate in pyrimidine pathway. In addition, PRPP is essential for pyridine nucleotide (NAD/NADP) synthesis

Parental update (Dec 2017)

- **started walking with walker, per mom vision is improving, much happier kid "quality of life increased 100%"**

Comprehensive Care Update
- objective assessment vs. baseline

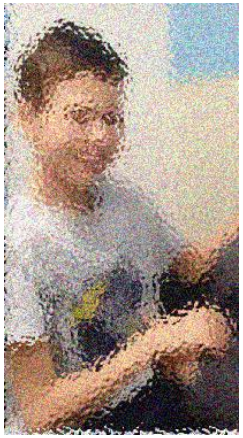
SAM supplementation in the diet may alleviate some of the symptoms of the patients with *PRPS1* loss-of-function mutations by replenishing purine nucleotides independent of PRPP production. An open-label clinical trial in the two affected Australian brothers is currently under way and appears to have improved the health of the patients, although it is too early to draw significant conclusions. Patients with DFN2 and CMTX5 and mildly affected carrier females from the original Arts syndrome may also benefit from SAM supplementation in their diet.

FTP - 12 year old boy

Report date: 9 May 2016

Clinical:

- Progressive neurodegenerative condition
- Many genetic tests, imaging, invasive tests
- Seen at multiple institutions



Hearing loss (SN)
Vision loss (optic atrophy)

Regression
Neuropathy
Weakness

dysphagia

Clinical Course:

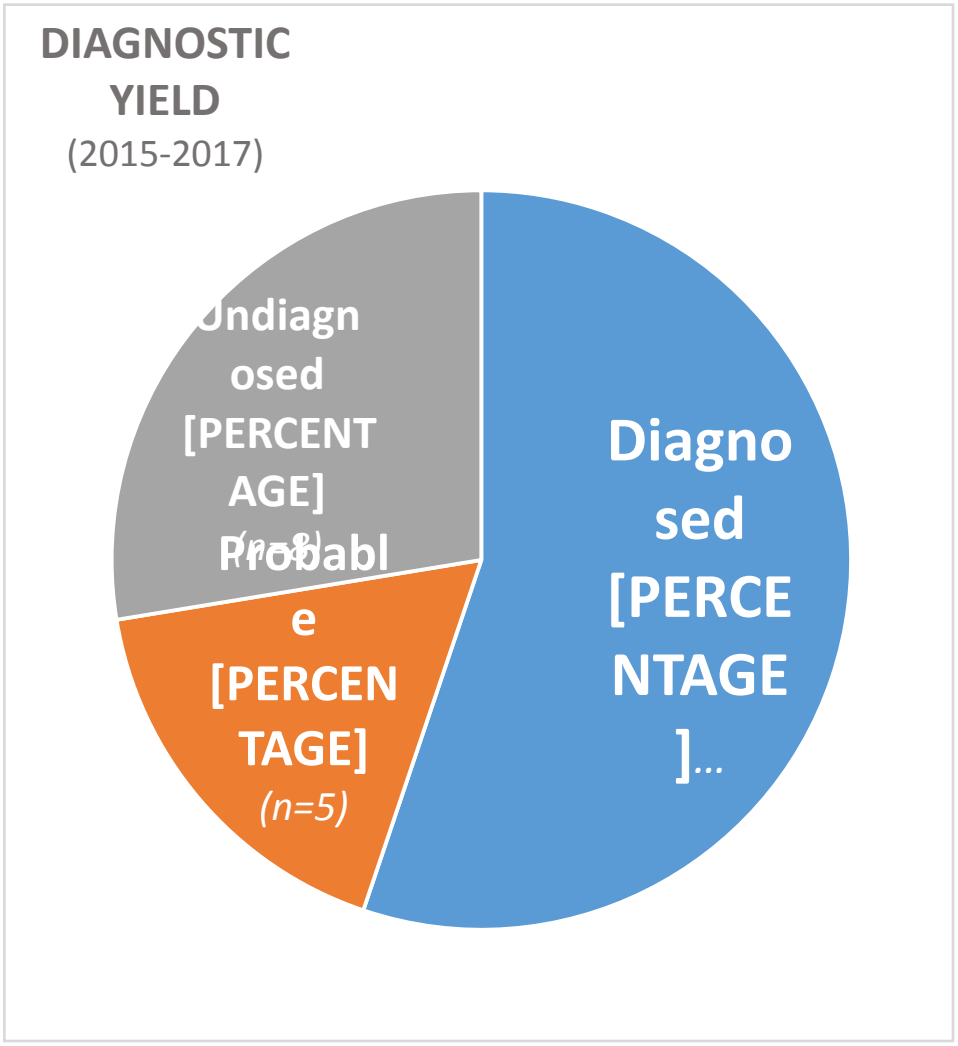
Diagnostic Odyssey: 7 years, now over

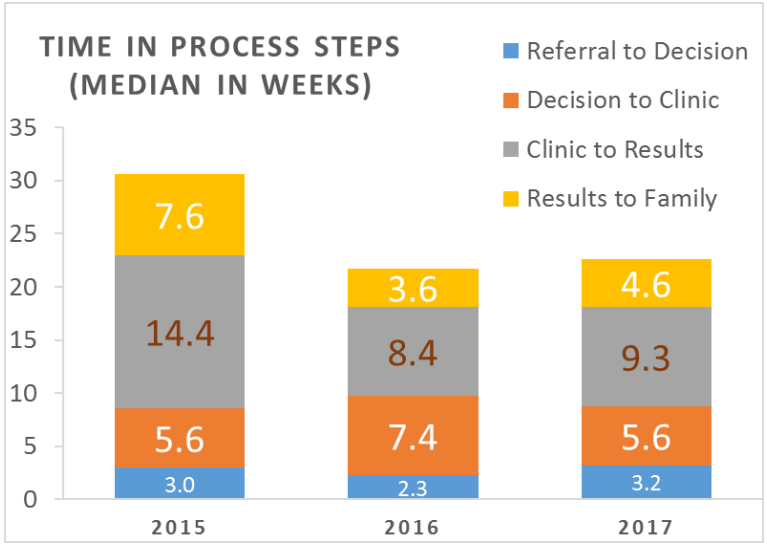
Value provided by Penelope Program

- **End of diagnostic odyssey:** **PRPS1 mutation (c.334G>C, p.V112L), associated with X-linked Charcot-Marie-Tooth disease-5 (CMTX5; OMIM 311070)**
- **Diagnosis-driven new treatment: identified pathway, connected with other clinicians (Australia), started supplementation with S-adenosyl methionine**
- **Actionable family information: X-linked, test mother for carrier status, can test other boys and treat early if affected (also, avoid diagnostic odyssey in sibs)**

Lesson 1: Good Processes and Teams Give Good Results

Favorable Diagnostic Yield (55 to 72%)
Improving Timeliness





Source: Penelope Program data updated 12/2017

Lesson 2: expect a high proportion of new or variant conditions

New condition
New gene

Atypical presentation

Ultra-rare diagnosis

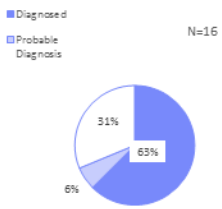
Diagnoses

- Charcot Marie Tooth type 5 (CMTX5) – Arts syndrome overlap: *PRPS1*
  - *Diagnosis-driven treatment (SAM +/- riboside), connected with other center*
- UDP Galactose transporter deficiency (CDG IIm): *SLC35A2-CDG*
  - *attempting diagnosis-driven treatment (galactose), connected to consortium*
- Progressive Osseous Heteroplasia: *GNAS*
  - *Treatment with topical thiosulfate*

- ARID1B-related intellectual disability: *avoided tumor surveillance*
- Torg-Winchester syndrome / multisystem nodular osteolysis: *MMP-2, connected to MMP-2 research (Alberta, Dr Fernandez Patron) for potential tx*

- KCND3-related early onset intellectual disability: *connected with consortium*
- HUWE1-related intellectual disability
- NONO-related syndrome (left ventricular non-compaction)
- Megalencephaly-Polymicrogyria-Polydactyly-Hydrocephalus type 3: *CCND2*
- Aicardi Goutieres type 2 (RNASEHB)

Lesson 3: clinical utility is real



Family

Family members at risk

★ Recurrence risk (de novo, Mendelian)

Management

Investigations stopped

Surveillance started stopped

New treatments ★ started

Child

Precision Diagnosis, Prognosis ★

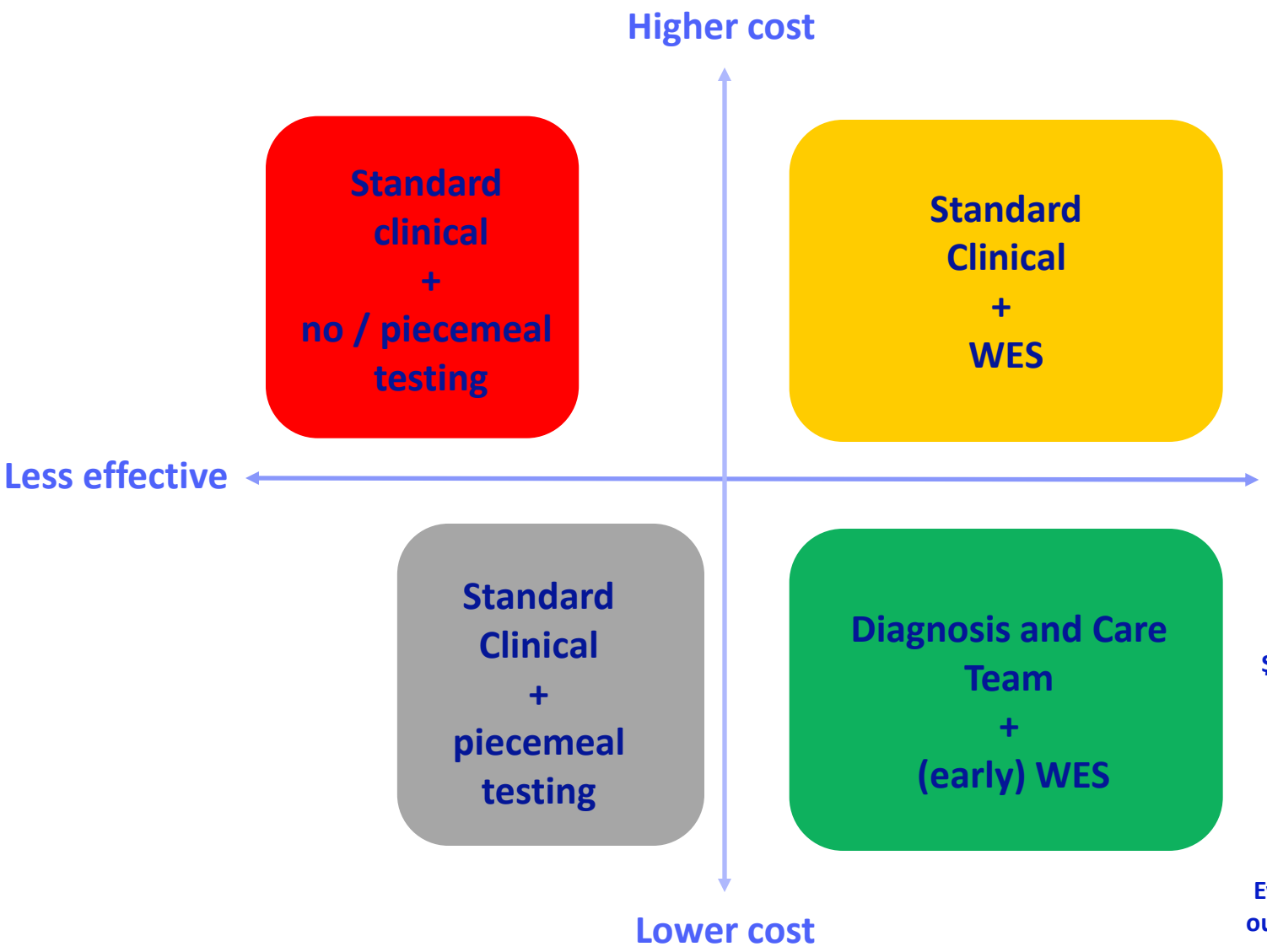
- SETD5-related intellectual disability
- STARD9-related epilepsy and developmental disability

- NOTCH1-related brain calcifications with Hirschsprung d.
- PIK3C3-related neurodevelopmental regression

- Multiple vascular hypoplasia (AR)
- Possible new osteoporosis/fractures condition: novel gene
  - connected with two other centers, working on functional studies

New vs. variant

Lesson 4: journey to better value continues



8 in-state children

cost prior to Penelope
$ 342,475
$ 82,000 for diagnostics

Penelope:
12 hrs consultant time /patient

Time: 20 to 15 weeks
Evaluation: Follow up for outcome and resource use

Who is making this happen

University of Utah
Justin Alvey
Ashley Andrews
James Bale
Carlos Barbagelata
Steven Bleyl
John Bohnsack
Lorenzo Botto
John C Carey
Stephen Guthery
Caroline Hagedorn
Nicola Longo
Melissa Joy Smith
Dave Viskochil

Department of Pediatrics
EC Clark

ARUP
Rong Mao, Pinar Bayrak-Toydemir
Colleen Carlston, Wei Shen, Tanya Tvrdik
Chris Miller, Patti Krautscheid, Sara Brown

Planning workshops
Parents: Gina Poley Money and Utah Family Voices families
Clinical coordinators: Athena Carola, Christa Jennings, Kim Orton, Clint Gibson, Melissa Smith, Ashley Andrews

Utah Genome Project

MOAB: Model Organism Advisory Board

Personalized Health Program
Will Dere, Emily Coonrod

Human Genetics - USTAR
Gabor Marth, Matt Velinder

Sorenson Foundation

Intermountain Healthcare

RUN – Utah Rare community

NIH Undiagnosed Disease Program
William Gahl, Cynthia Tifft, Lynne Wolfe

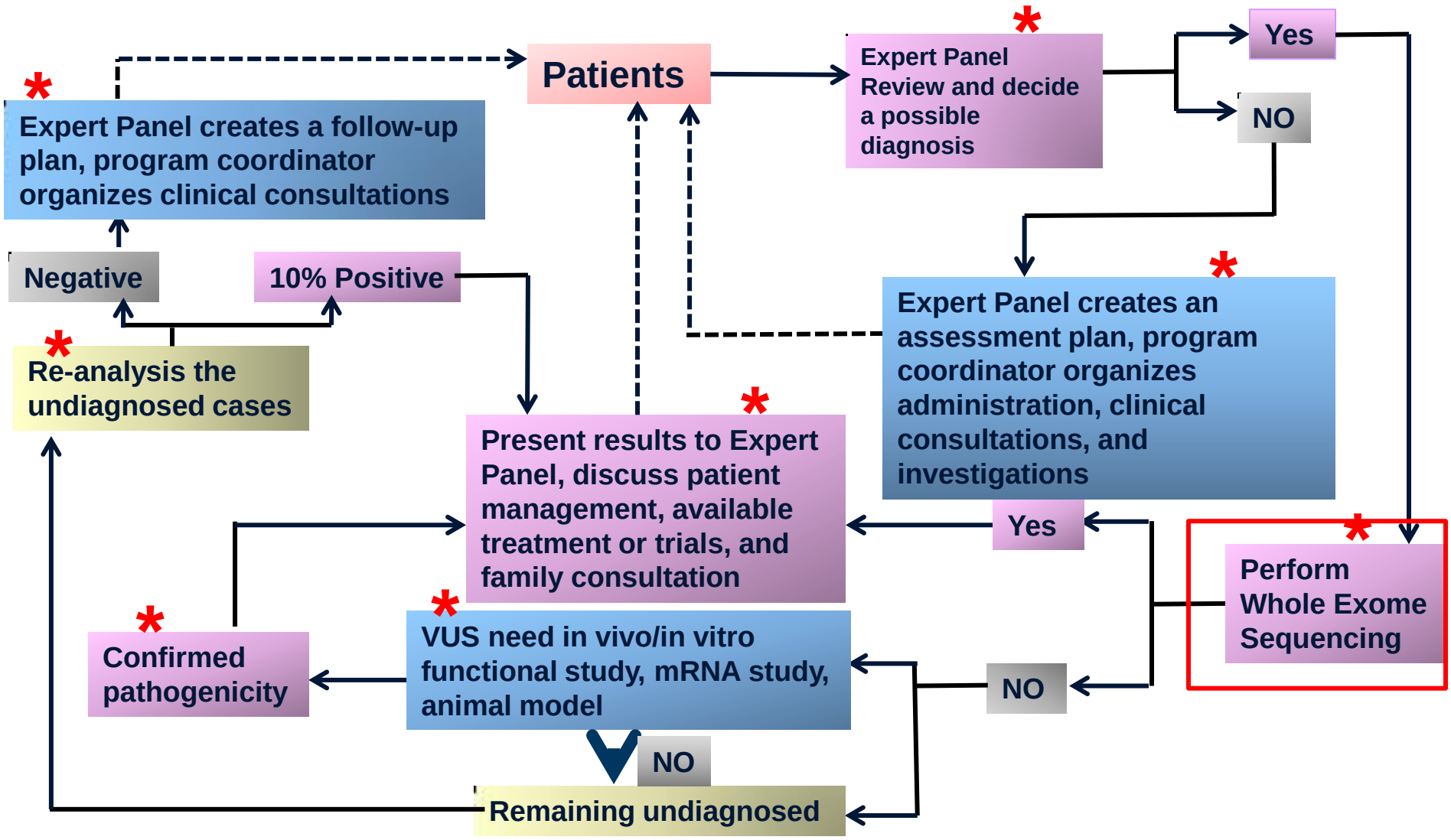
Keio University Global Center of Excellence
Kenjiro Kosaki

Part 2 Collaboration with Penelope UDP

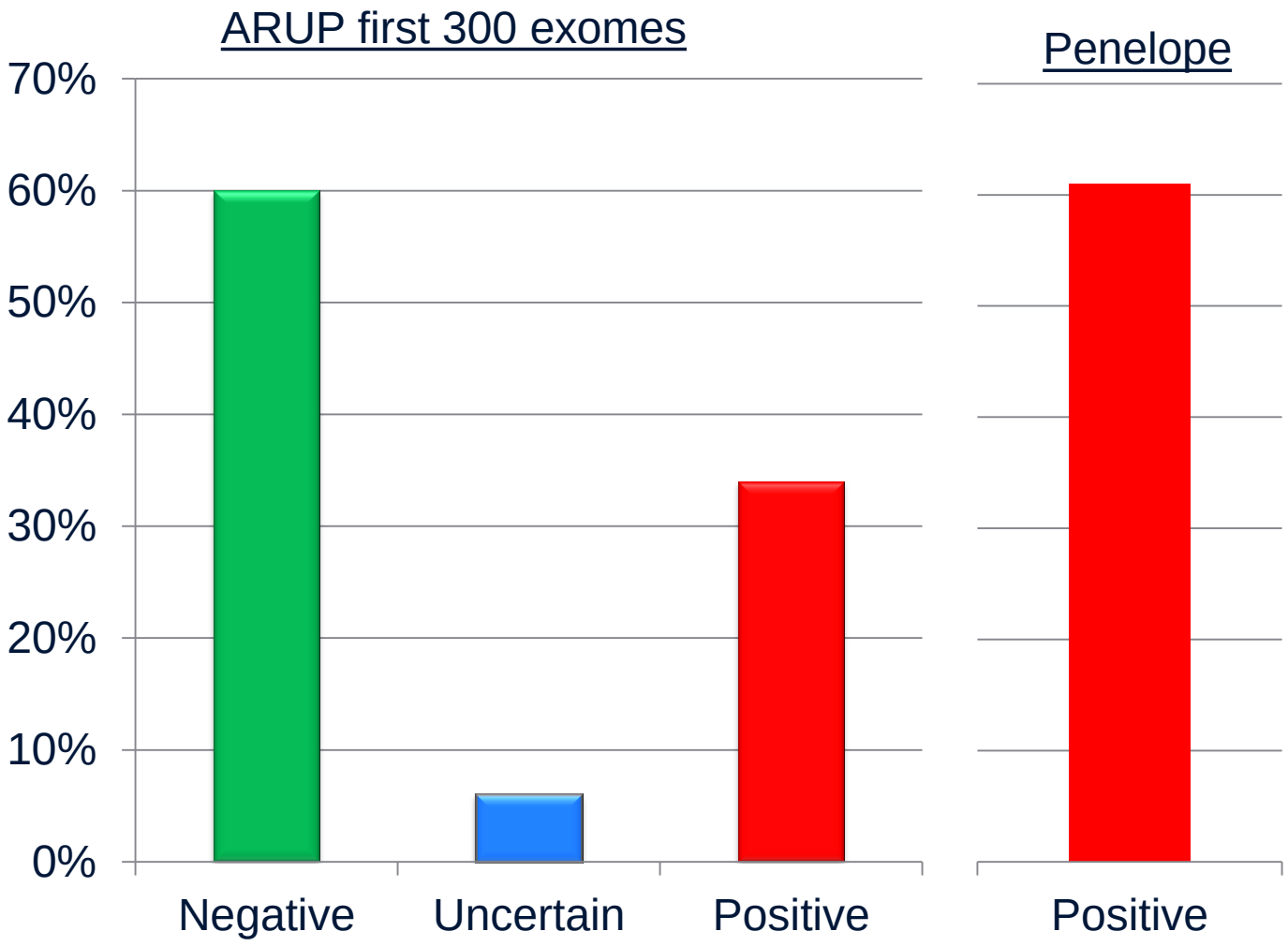
ARUP Participation in Penelope Program

- Medical directors are members of UDP steering committee
- UDP meetings twice a month
- Exome sequencing at ARUP Genomics Lab with TAT for 6-8 weeks.
- Learn every case prior to WES and present back to UDP
- Education

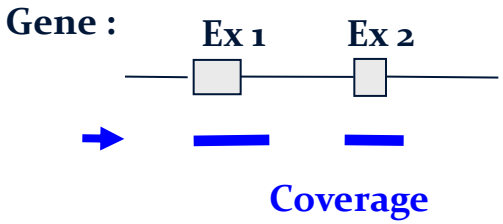
Penelope Program



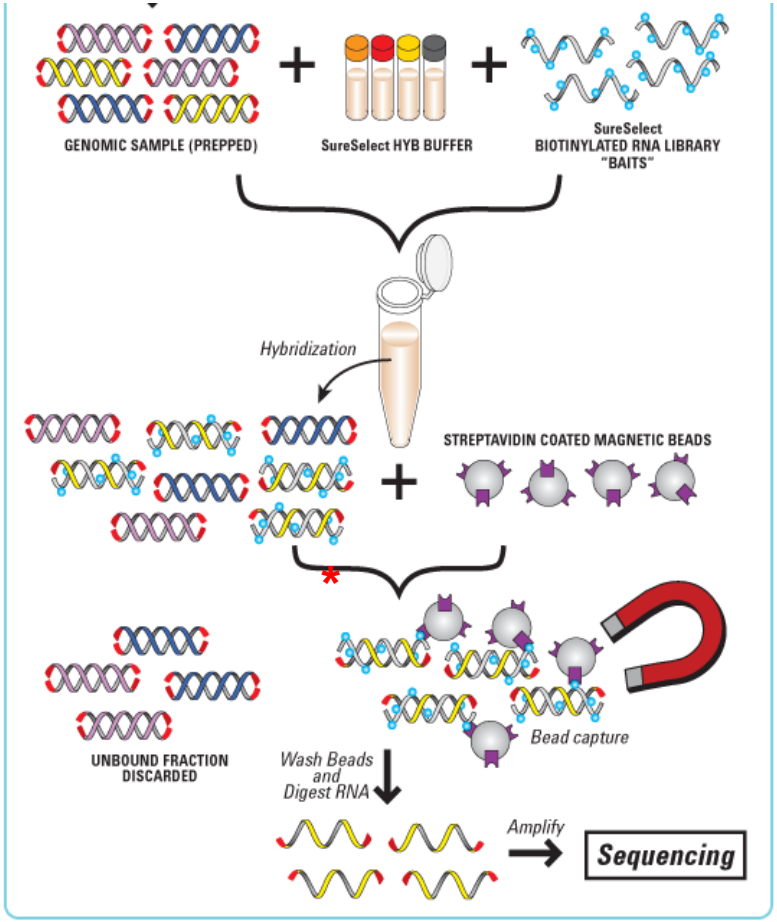
61% Positive Yield



“Clinical Research” Exome Capture

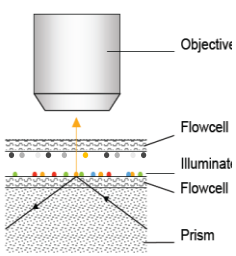


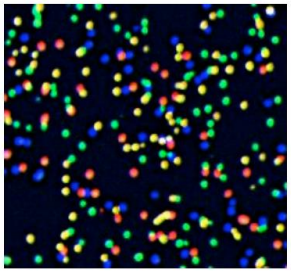
Biotinylated RNA library baits cover all exons annotated in the consensus CDS database, as well as flanking sequence for each targeted region and small non-coding RNAs



The capture probes boosted in difficulty regions and 4500 HGMD/OMIM genes, capture and sequencing efficiency is >99%

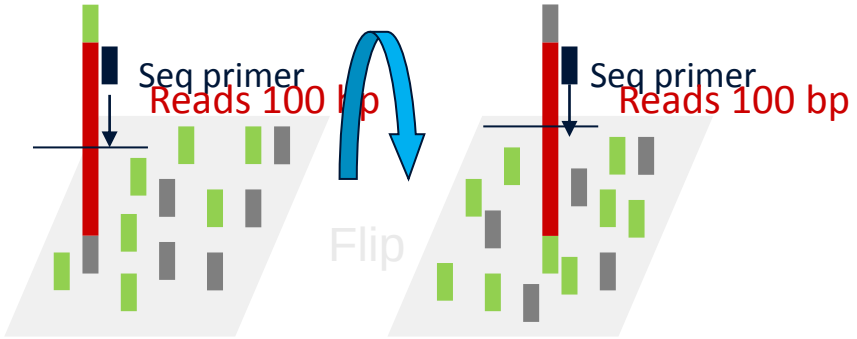
Sequencing on HighSeq2500





20 microns

Paired-End Reading (2X100 bp)



QA matrix:
>100 mean
10X coverage, >95%

Paired end reads

- **Increase read coverage per cluster**
- **More accurate reading and alignment**
- **Detect small and large insertions, deletions and other rearrangements**

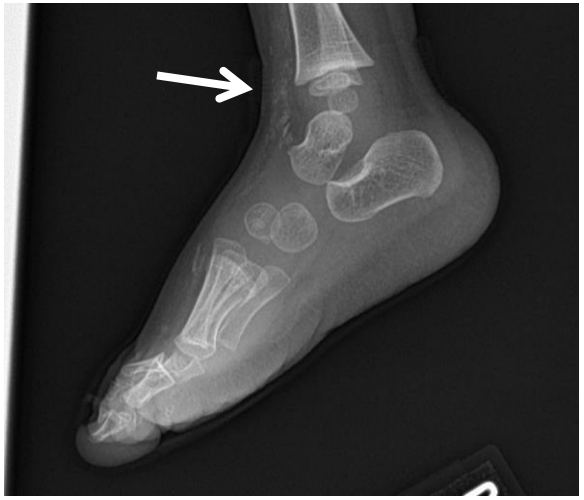
Case 1: *Imprinting Gene*

Slides courtesy of Colleen Carlston, PhD

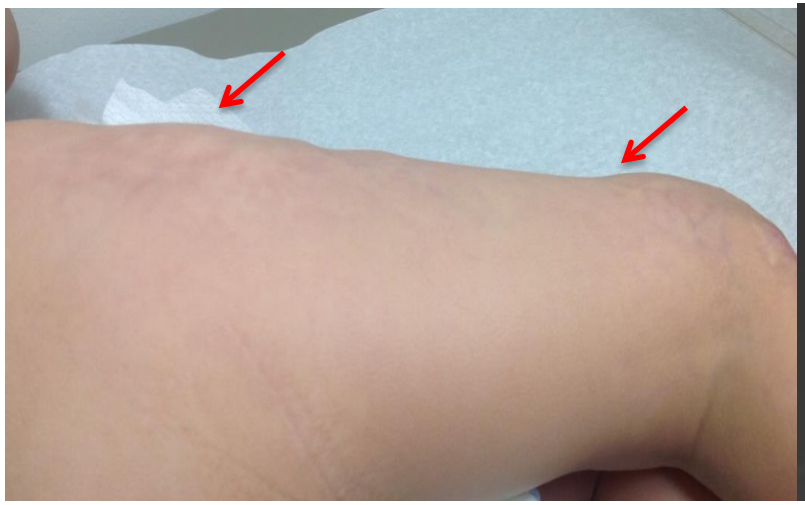
Clinical Information

- 2 y/o Hispanic male
- History of intrauterine growth restriction (IUGR)
- Short Stature, Microcephaly, Scoliosis
- Progressive ectopic sheet-like calcifications along the anterior ankle and foot (see IA, X-Ray)
- Subcutaneous masses over right patella, right thigh, right femoral head and near the lumbar spinal region (see IB and IC, X-Ray)

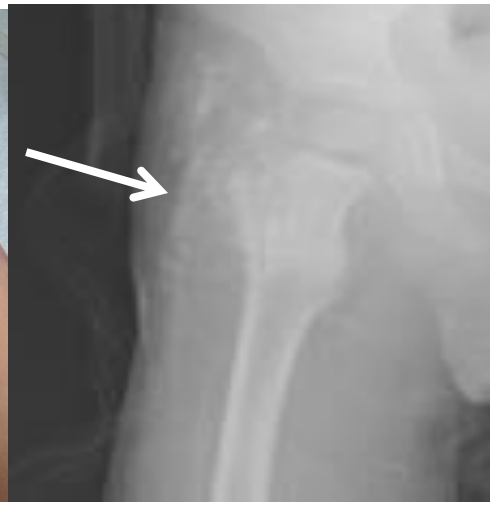
IA



IB



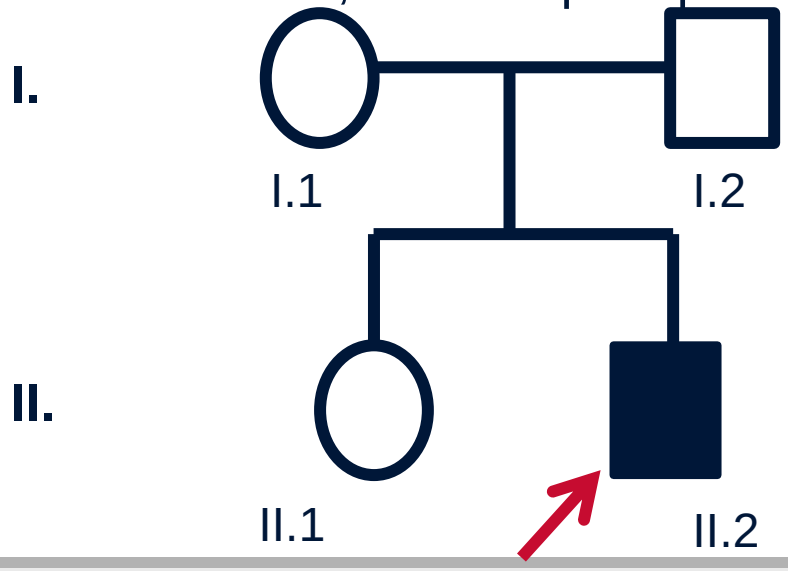
IC



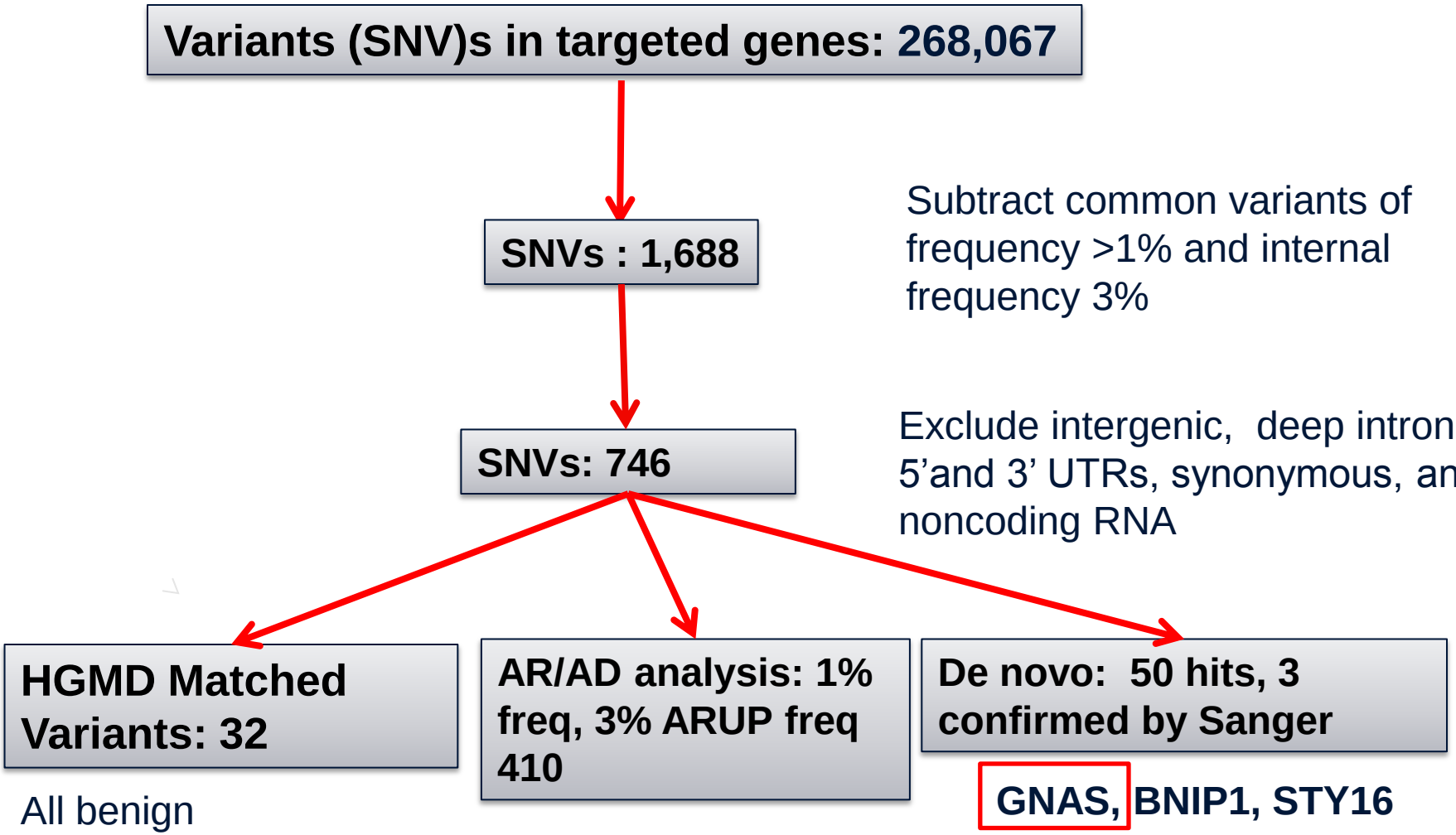
Clinical Information Cont

- Family: Not remarkable, no consanguinity, a heathy sister

- Biopsy from right knee showed bone trabeculae with osteoblastic rimming and scattered osteoclastic cells in fibromyxoid stroma
- Normal parathyroid hormone, thyroid-stimulating hormone, T4 and T3 uptake
- Normal comprehensive metabolic panel: lipid profile, urine analysis, complete blood count, alkaline phosphatase, urine calcium,

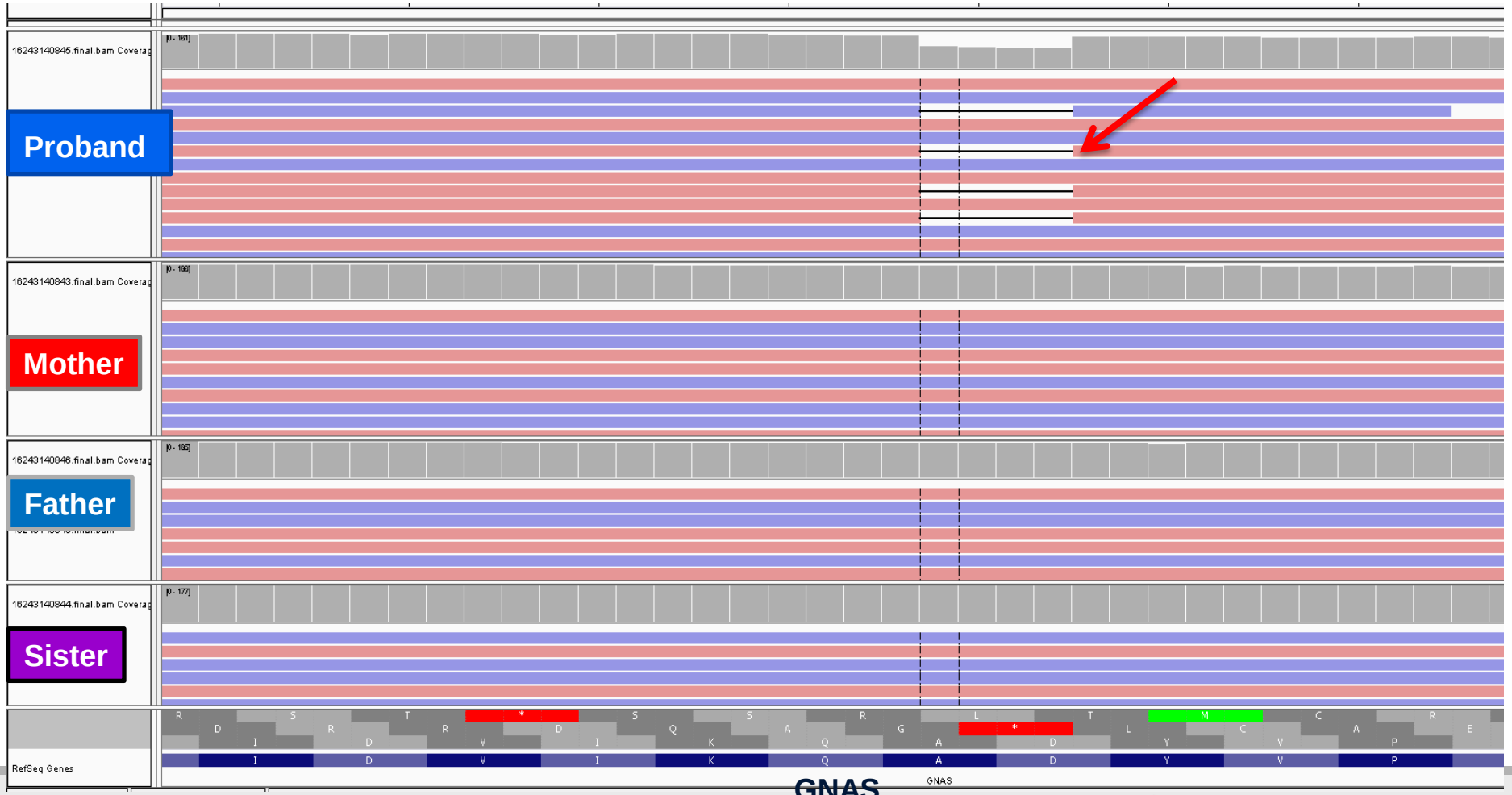


Exome Data


Variants (SNV)s in targeted genes: 268,067
SNVs : 1,688
Subtract common variants of frequency >1% and internal frequency 3%
SNVs: 746
Exclude intergenic, deep intronic, 5'and 3' UTRs, synonymous, and noncoding RNA
HGMD Matched Variants: 32
All benign
AR/AD analysis: 1% freq, 3% ARUP freq 410
De novo: 50 hits, 3 confirmed by Sanger
GNAS, BNIP1, STY16

GNAS De Novo

- Chr20(GRCh37): g.57484255_57484258del; NM_000516.4
  c.565_568del; p.Asp189fs



Mutational Hotspot

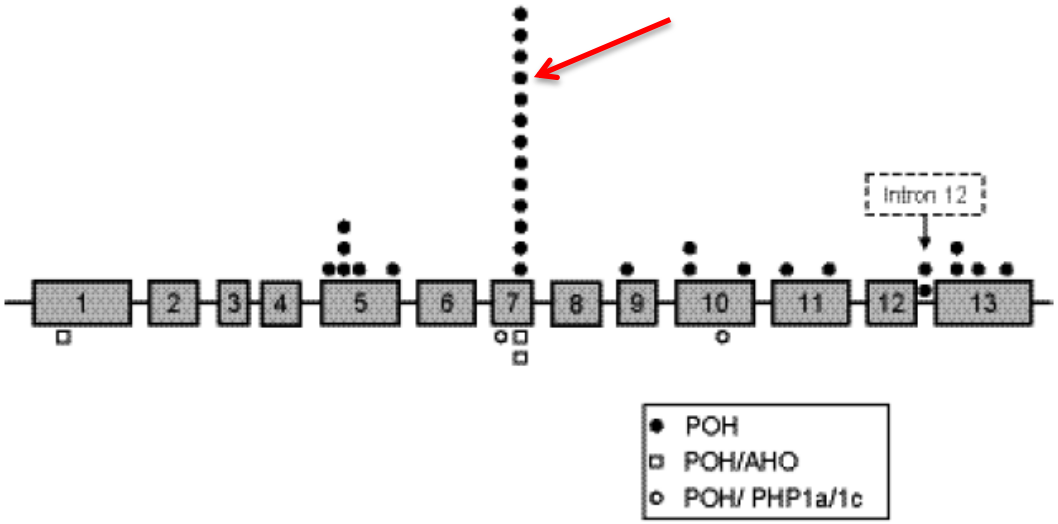


Fig. 1.
Distribution of *GNAS* mutations in POH and other conditions of progressive HO. Exons for the *GNAS* gene (Gsα mRNA) are identified by numbers and are approximately drawn to scale. Intronic sequences are represented by straight solid lines between exons. Information presented is from this study; recent reports of *GNAS* mutations in AHO and PHP1a/1c also show a wide distribution throughout the *GNAS* exons (e.g., Jan De Beur et al. [2003]; Linglart et al. [2002]; Aldred et al. [2000]). Symbols represent patients within each diagnostic group whose *GNAS* sequence analysis revealed specific mutations at the indicated approximate locations. A "hot spot" (4 base pair deletion) causing a frameshift mutation at c.565-568 occurs in exon 7.

Adegbite 2008 Am J Med Genet A

GNAS c.565_568del Reported

- This variant has been reported in patients:
  - with progressive osseous heteroplasia (**POH**) (Shore 2002, Adegbite 2008, Lebrun 2010, Schrander 2014)
  - pseudohypoparathyroidism type 1A (**PHP Ia**) (Linglart 2002, Nakamoto 1998, Yokoyama 1996, Walden 1999, Lebrun 2010, Inta 2014)
  - pseudohypoparathyroidism type 1A (PHP Ia) / pseudopseudohypoparathyroidism (PPHP) (Ahmed 1998)
  - Pseudopseudohypoparathyroidism (PPHP) (Walden 1999)
  - Albright hereditary osteodystrophy (AHO) (Weinstein 1992, Nakamoto 1998, Linglart 2002, Joseph 2011)
  - as well as in an unaffected carriers (Shore 2002, Adegbite 2008).

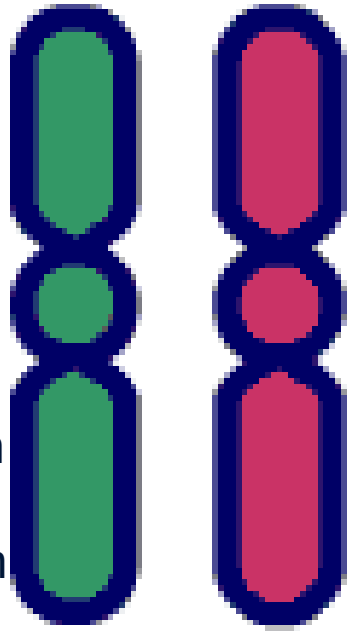
GNAS: Imprinting Gene

Paternal inactivating GNAS mutation:
Progressive osseous heteroplasia (POH) (OMIM:166350) Dominant

Phenotype:

- Onset in infancy or childhood
- Dermal ossification beginning in infancy, followed by increasing and extensive bone formation in deep muscle and fascia
- Growth retardation of limbs, short status

Paternal Maternal



Chromosome 20

Maternal GNAS mutation:
Pseudohypoparathyroidism IA (PHP Ia) (OMIM 103580) Dominant

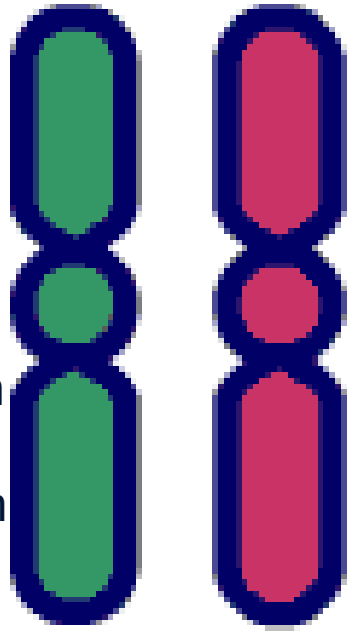
Variable phenotype:

- Resembled parathyroid hormone deficiency
- Short stature, round face, short neck, obesity, subcutaneous calcifications
- Hypocalcemia and hyperphosphatemia

Case 1: *GNAS* Paternal Mutation

Paternal inactivating GNAS mutation:
Progressive osseous heteroplasia (POH) (OMIM:166350) Dominant
Phenotype:

- Onset in infancy or childhood
- Dermal ossification beginning in infancy, followed by increasing and extensive bone formation in deep muscle and fascia
- Growth retardation of limbs, short status, scoliosis



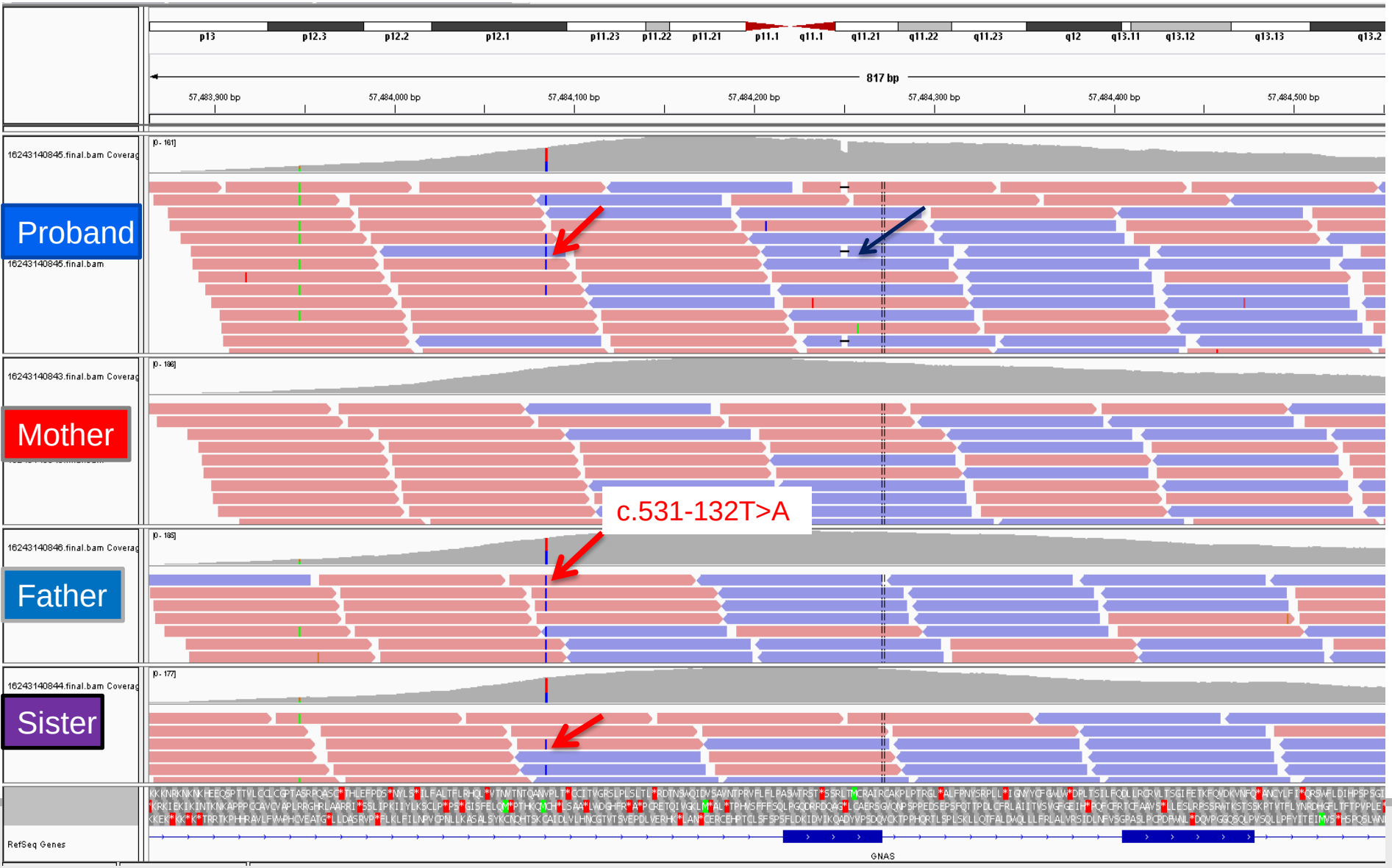
Chromosome 20

Maternal GNAS mutation:
Pseudohypoparathyroidism IA (PHP Ia) (OMIM 103580)
Dominant
Variable phenotype:

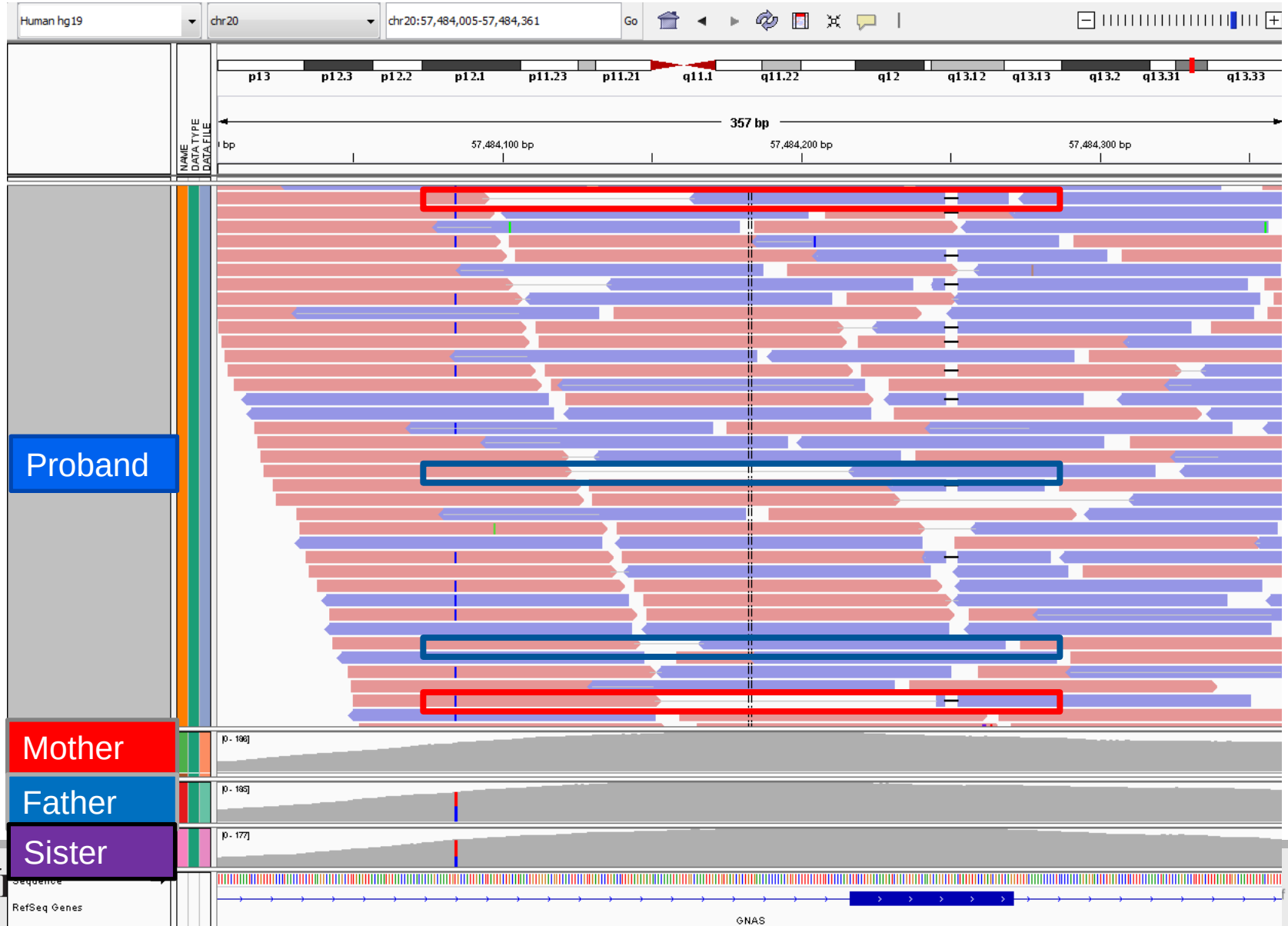
- Resembled parathyroid hormone deficiency
- Short stature, round face, short neck, obesity, subcutaneous calcifications
- Hypocalcemia and hyperphosphatemia

Case 1: *GNAS* Mutation Paternal Original?

Paternal polymorphism Chr20(GRCh37): g.57,484,085; NM_000516.4 c.531-132T>A, 170 bp away



GNAS mutation paternal original? Confirmed by Pair-end read



Case 1 *GNAS*: Paternal Inactive Mutation

- De novo c.565_568del mutation was confirmed on paternal allele.
- Consistent with dx of Progressive Osseous Heterotopia (POH)
- A very rare genetic disorder of abnormal bone formation

Therapeutic Assessment

- Physical therapy to preserve movement (unlike fibroplasia ossificans progressiva)
- Surgery not recommended
- New treatment (ongoing): topical thiosulfate (to improve solubility of Ca)
- Potential treatment: Hh inhibitors, Retinoic acid receptor γ agonists

Case 2: Unknown of Known Disease

Slides courtesy of Wei Shen, PhD

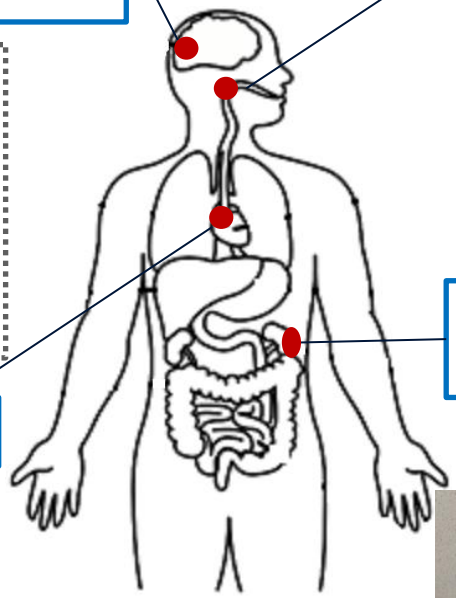
Clinical Information:

- 21-month girl

Global Developmental Delays, Hypotonia, leukoencephalopathy, brain calcifications

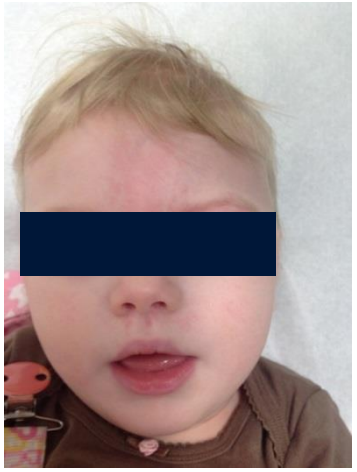
Dysphagia, poor feeding

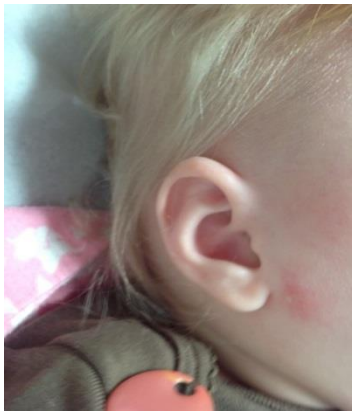
Physical exam:
- Short Stature
- Dysmorphic Facial Features – Mild brachycephaly, glabellar hemangioma

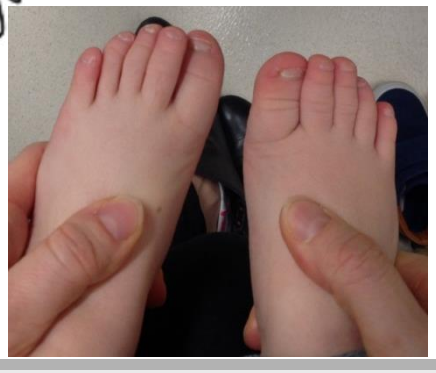


Hirschsprung's Disease

Atrial septal defect







- Family history: unremarkable, healthy parents

Exome Data:

Variants (SNV)s in targeted genes: 249,851

SNVs : 3,761

Subtract common variants of frequency >1% and internal frequency 3%

SNVs: 749

Exclude intergenic, deep intronic, 5'and 3' UTRs, synonymous, and noncoding RNA

HGMD Matched Variants: 37 hits

AD: 0.1%, 0.3% ARUP: 425 hits

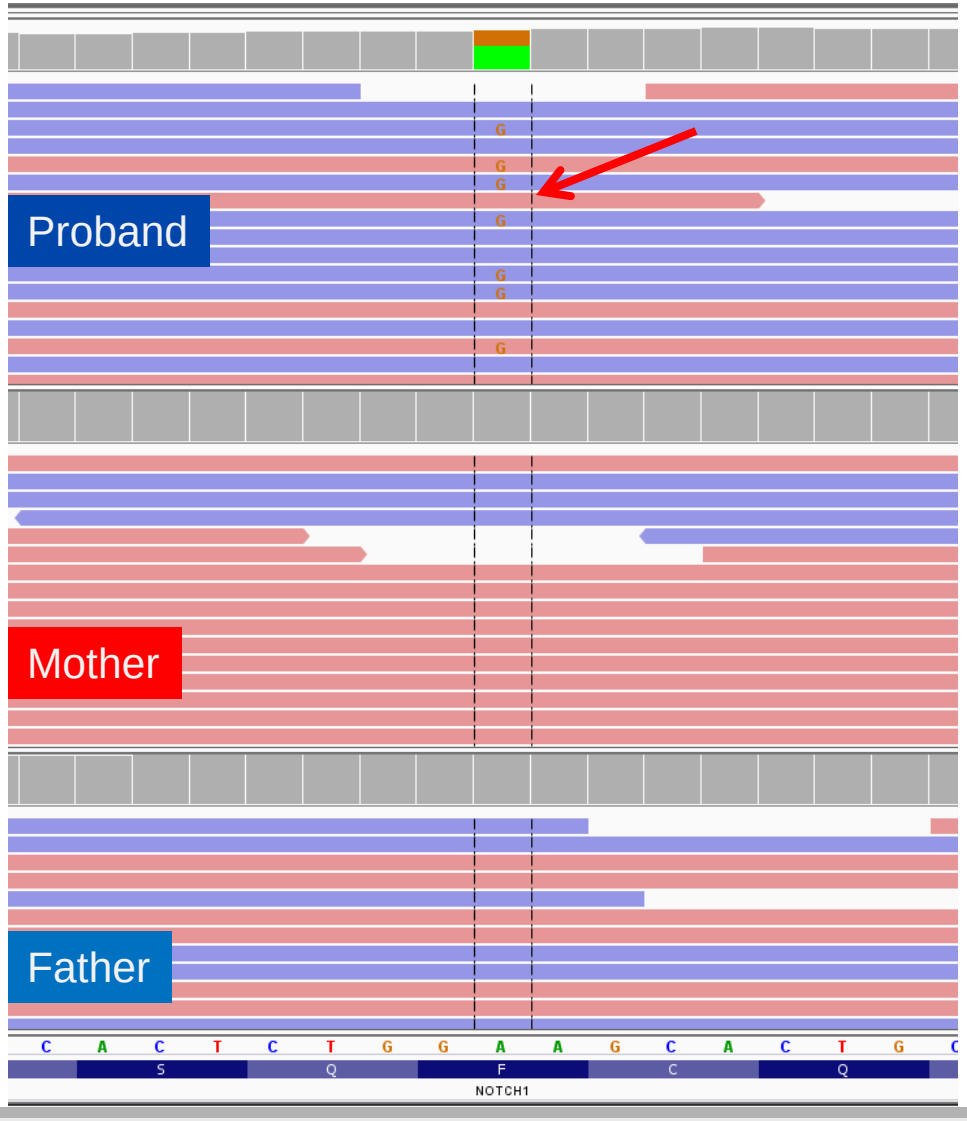
De novo: 87 hits, 1 real in IGV

NOTCH1	het	c.5078T>C	p.F1693S

AR analysis: 1% freq, 3% ARUP freq : 1 gene

NOTCH1 De Novo

Chr9(GRCh37):g.139397723 NM_017617.4 **c.5078T>C; p.Phe1693Ser**



- Very rare: not reported in ExAC, gnomAD
- Highly conserved amino acid in the HD domain
- Computational predictions (**PP3**):
  - SIFT: deleterious
  - PolyPhen-2: probably damaging
- Reported in one patient with acute leukemia (presumably somatic) (PMID 18281529)
- Variant of uncertain significance

NOTCH1 Mutations

- Adams-Oliver syndrome (MIM: 616028), autosomal dominant (loss-of-function mutations)
  - Aplasia cutis congenita of the scalp (80%)
  - Terminal transverse limb defect (85%)
  - Cutis marmorata telangiectatica congenita (20%)
  - Cardiovascular malformations/dysfunction (23%): left-sided obstructive lesions, septal defects, conotruncal defects
  - Brain anomalies (uncommon): microcephaly, cortical dysplasia, polymicrogyria, pachygyria, dysgenetic corpus callosum, cortical atrophy with ventriculomegaly, cerebral hemorrhage, intracranial calcifications, delayed myelination
  - *NOTCH1, DOCK6, DLL4, EOGT, RBPJ* and *ARHGAP31*
- Aortic valve disease (MIM: 109730), autosomal dominant
  - Bicuspid aortic valve

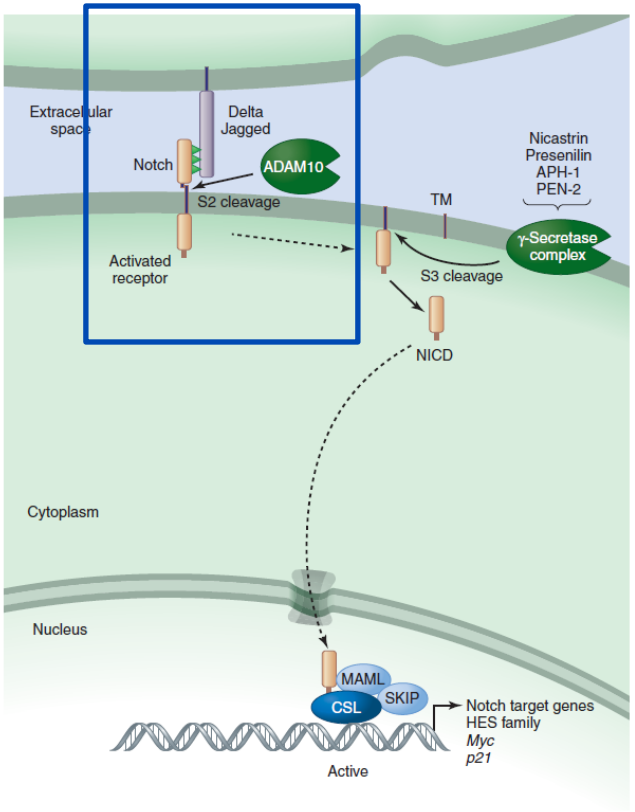


Figure 1. Notch signaling (simplified view).

Notch protein is transmembrane receptors which regulate cell decision during development

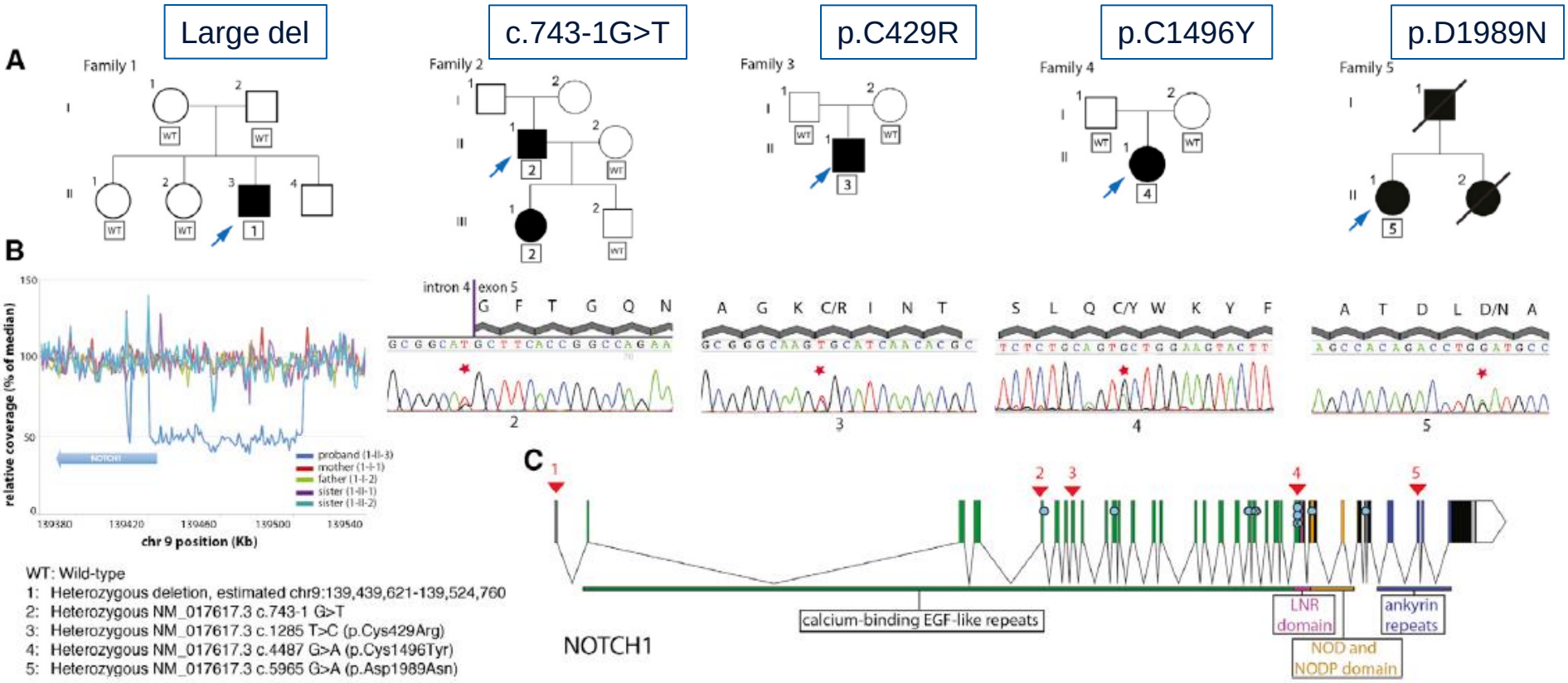
Cold Spring Harb Perspect Biol 2012;4:a011213

Loss of Function Mutations in *NOTCH1* cause Adams-Oliver Syndrome

REPORT

Mutations in *NOTCH1* Cause Adams-Oliver Syndrome

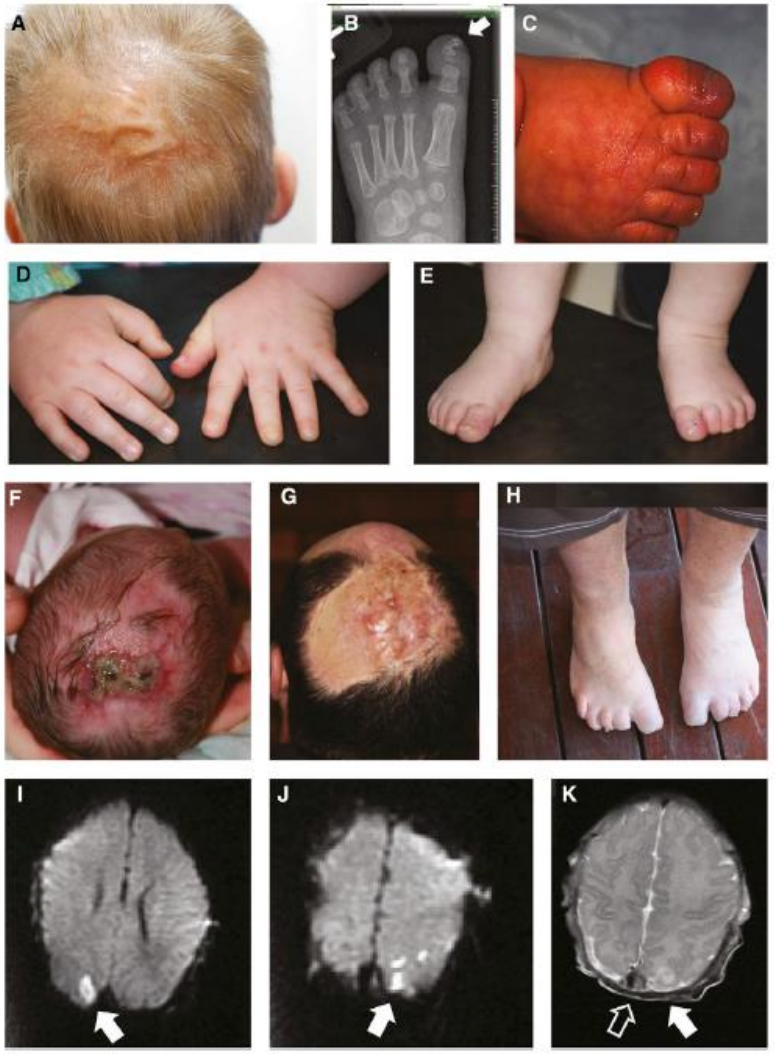
Anna-Barbara Stittrich,[1,9] Anna Lehman,[2,9] Dale L. Bodian,[3,9] Justin Ashworth,[1] Zheyuan Zong,[2]



WT: Wild-type
1: Heterozygous deletion, estimated chr9:139,439,621-139,524,760
2: Heterozygous NM_017617.3 c.743-1 G>T
3: Heterozygous NM_017617.3 c.1285 T>C (p.Cys429Arg)
4: Heterozygous NM_017617.3 c.4487 G>A (p.Cys1496Tyr)
5: Heterozygous NM_017617.3 c.5965 G>A (p.Asp1989Asn)

Am. J. Hum. Genet. 95: 275-284, 2014.

Characteristic Features of Adams-Oliver Syndrome



- Scarred aplasia curtis lesion of the scalp (Fig A, F, G)
- Calcific deposits in the subcutaneous tissue of first toe (Fig B) and terminal transverse defect of the toes and marmorata in infancy (Fig C, H)
- Distal hypoplasia of the digits of toes and hands (Fig D, E)
- Brain MRI showed infarcts and partial thrombus (Fig I, J, K)

Am. J. Hum. Genet. 95: 275-284, 2014.

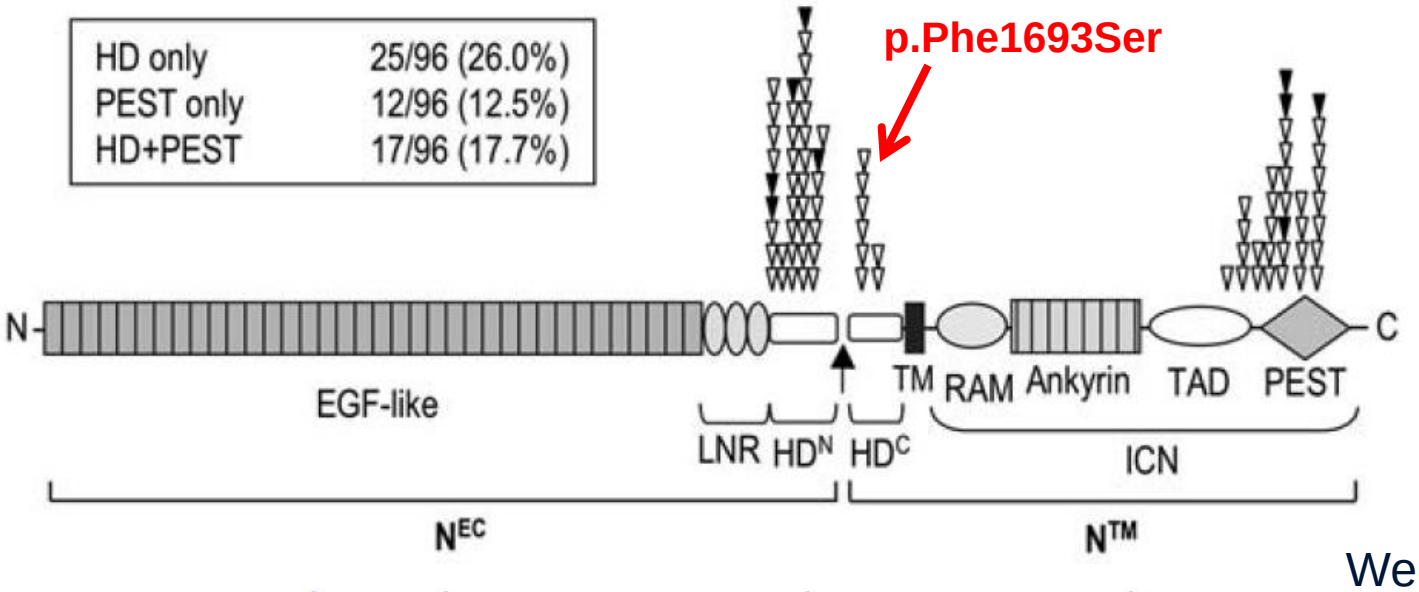
Questions?

- Does the patient have Adams-Oliver? (No)
- Does the de novo mutation in *NOTCH1* cause patient's phenotype? (Don't know)
- Possible different disease-causing mechanism to cause a new syndrome? (Possible)

NOTCH1 Gain of Function Mutation Hypothesis

Activating Mutations of *NOTCH1* in Human T Cell Acute Lymphoblastic Leukemia

Andrew P. Weng,[1*†] Adolfo A. Ferrando,[2*] Woojoong Lee,[1] John P. Morris IV,[2] Lewis B. Silverman,[2] Cheryll Sanchez-Irizarry,[1] Stephen C. Blacklow,[1] A. Thomas Look,[2] Jon C. Aster[1‡]



Weng, et al. 2004

p.Phe1693Ser Has been Observed in ALL

Human Cancer Biology

ETV6-NCOA2: A Novel Fusion Gene in Acute Leukemia Associated with Coexpression of T-Lymphoid and Myeloid Markers and Frequent *NOTCH1* Mutations

Sabine Strehl,[1] Karin Nebral,[1] Margit König,[1] Jochen Harbott,[4] Herbert Strobl,[2] Richard Ratei,[5] Stephanie Struski,[6] Bella Bielorai,[7,8] Michel Lessard,[6] Martin Zimmermann,[9] Oskar A. Haas,[3] and Shai Izraeli[7,8]

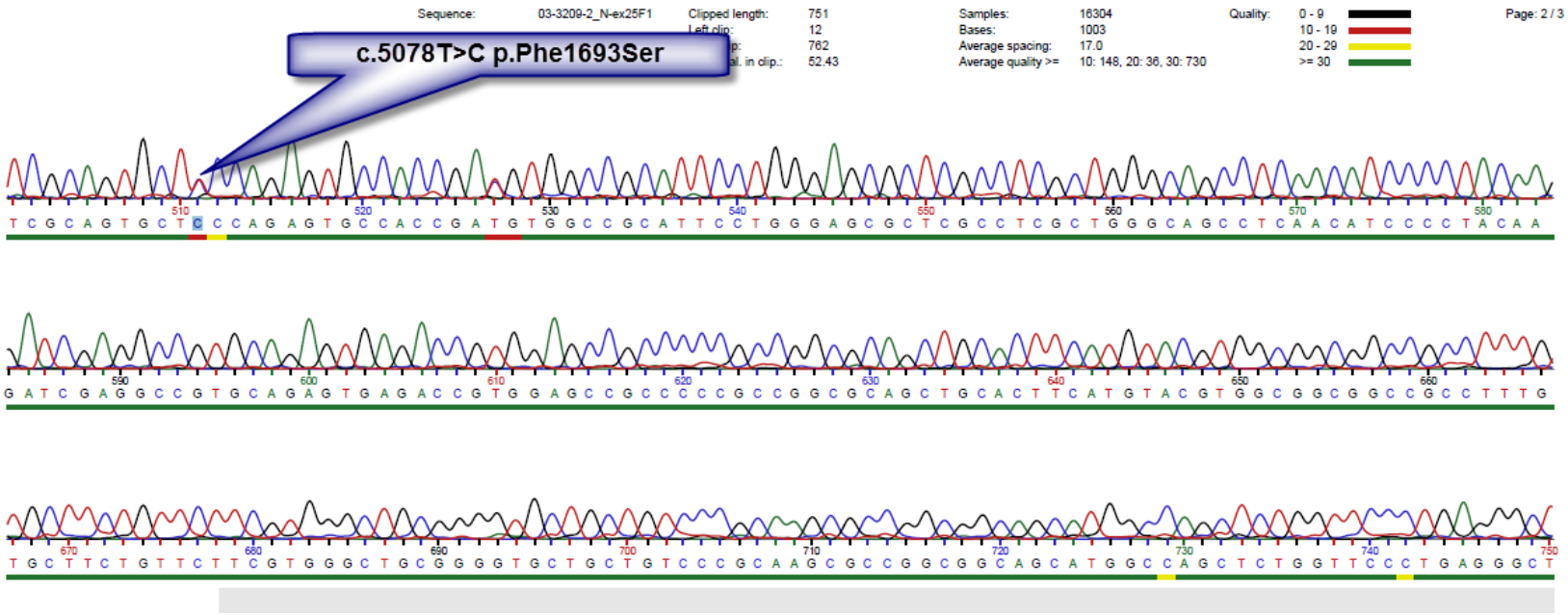
Table 4. *NOTCH1* mutations in *ETV6-NCOA2* leukemia

Case	Domain	SNP	Nucleotide change	Amino acid change
1	HD	c.5097 C/T	—	—
	PEST	—	c.7403C>A	p.S2468X
2	HD	c.5097 C/T	c.5081C>T	p.F1694S
	PEST	—	c.7007_7008insT	p.2336 EHTGPLPAAWHGRPAAQ
3	HD	—	c.5036T>C	p.L1679P
	PEST	—	—	—
4	HD	—	—	—
	PEST	—	—	—
6	HD	—	—	—
	PEST	—	c.7544_7545delCT	p.2515 RVP

Abbreviations: SNP, single nucleotide polymorphism; HD, heterodimerization.

Clin Cancer Res. 2008 Feb 15;14(4):977-83.

Confirmed p.Phe1693Ser



Strehl Sabine, et al 2008

Hedgehot/NOTCH involving develop Hirshsprung Disease

Hedgehog/Notch-induced premature gliogenesis represents a new disease mechanism for Hirschsprung disease in mice and humans

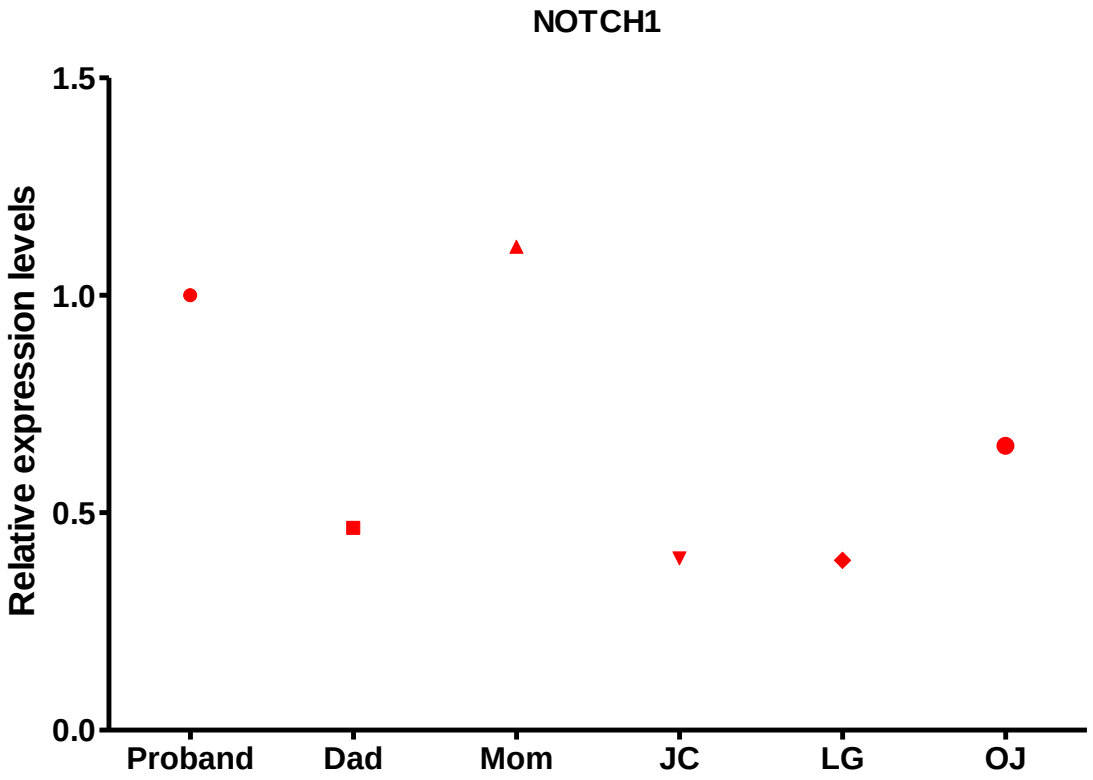
Elly Sau-Wai Ngan,[1,2] Maria-Mercè Garcia-Barceló,[1,2] Benjamin Hon-Kei Yip,[1,2,3] Hiu-Ching Poon,[1] Sin-Ting Lau,[1] Carmen Ka-Man Kwok,[1] Eric Sat,[1] Mai-Har Sham,[2,4] Kenneth Kak-Yuen Wong,[1,2] Brandon J. Wainwright,[5] Stacey S. Cherny,[3] Chi-Chung Hui,[6,7] Pak Chung Sham,[2,3] Vincent Chi-Hang Lui,[1,2] and Paul Kwong-Hang Tam[1,2]

[1]Department of Surgery, [2]Centre for Reproduction, Development and Growth, [3]Department of Psychiatry, and [4]Department of Biochemistry, Li Ka Shing Faculty of Medicine, University of Hong Kong, Pokfulam, Hong Kong, China. [5]Institute for Molecular Bioscience, The University of Queensland, St. Lucia, Australia. [6]Program in Development and Stem Cell Biology, The Hospital for Sick Children, University of Toronto, Toronto Medical Discovery Towers, Toronto, Ontario, Canada. [7]Department of Molecular and Medical Genetics, University of Toronto, Toronto Medical Discovery Towers, Toronto, Ontario, Canada.

J Clin Invest. 2011 Sep;121(9):3467-78.

Next Step: Functional Study

- Mutagenesis and NOTCH1 gene expression.



Case 3: Re-analysis

Slides courtesy of Wei Shen, PhD

Clinical Information

- 8 yo Hispanic boy
- **Neurologic:** severe global DD, chorea, history of an intractable seizure disorder
- **Brain MRI:** mild bilateral perisylvian cortical dysplasia, nodular heterotopia
- **Dysmorphic features:** microcephaly, wide-spaced eyes, downturned corners of the mouth, U-shaped contour to the mouth with micrognathia
- **Skeletal:** hip dysplasia
- **EEG**: hypsarrhythmia
- **GI:** dysphagia, constipation
- **Growth parameters**: Wt 36%, Ht 22%, OFC 0%.
- **Surgeries:** device closure of PDA, repair of coronal hypospadias, bilateral tubes and revision
- **Previous normal testing**: karyotype and microarray, *TTP*, *CDKL5 MECP2*, *ARX* sequencing and del/dup, hearing test, purine panel, CDC transferrin, CSF studies (lactic acid, glucose, protein, amino acids), very long chain fatty acids,, mucopolysaccharides screen, lactic acid, plasma amino acids, acylcarnitine profile, urine organic acids, total carnitine, and lipid profile.
- **Family History:** maternal first cousin with seizures controlled by medication. Two maternal great aunts have severe intellectual disabilities and one is paralyzed from the waist down. No symptoms in mother.

- Proband ONLY

Negative Exome

- No strong candidate gene/variant identified
- Some variants to discuss

Gene	Transcript	Type	Zygosity	DNA alteration	Protein alteration	Inheritance mode	Human disease	Classification
CSTB	NM_000100	nonsense	het	c.C136T	p.Q46X	Autosomal recessive	Progressive myoclonic epilepsy 1A	Pathogenic
POLR3B	NM_018082	missense	het	c.G2158A	p.V720I	Autosomal recessive	Hypomyelinating leukodystrophy-8	VUS
GRID2	NM_001510	missense	het	c.A101G	p.D34G	Autosomal recessive	Spinocerebellar ataxia- 18	VUS
STARD9	NM_020759	missense	het	c.G986A	p.R329Q	Autosomal recessive	Okamoto et al (PMID 28777490)	VUS
	NM_020759	missense	het	c.C6955T	p.R2319W			VUS
TIMM17B	NM_001167947	missense	hemi	c.G304A	p.A102T	X-linked	Unknown	VUS

Compound Heterozygous Variants in STARD9

Gene	Transcript	Type	Zygosity	DNA alteration	Protein alteration	Inheritance mode	Human disease	Classification
STARD9	NM_020759	missense	het	c.G986A	p.R329Q	Autosomal recessive	Okamoto et al 2017 (PMID 28777490)	VUS
	NM_020759	missense	het	c.C6955T	p.R2319W			VUS

- *STARD9* gene encodes a protein that belongs to the kinesin-3 family. It associates with mitotic microtubules and regulates spindle pole assembly (Torres et al., 2011).

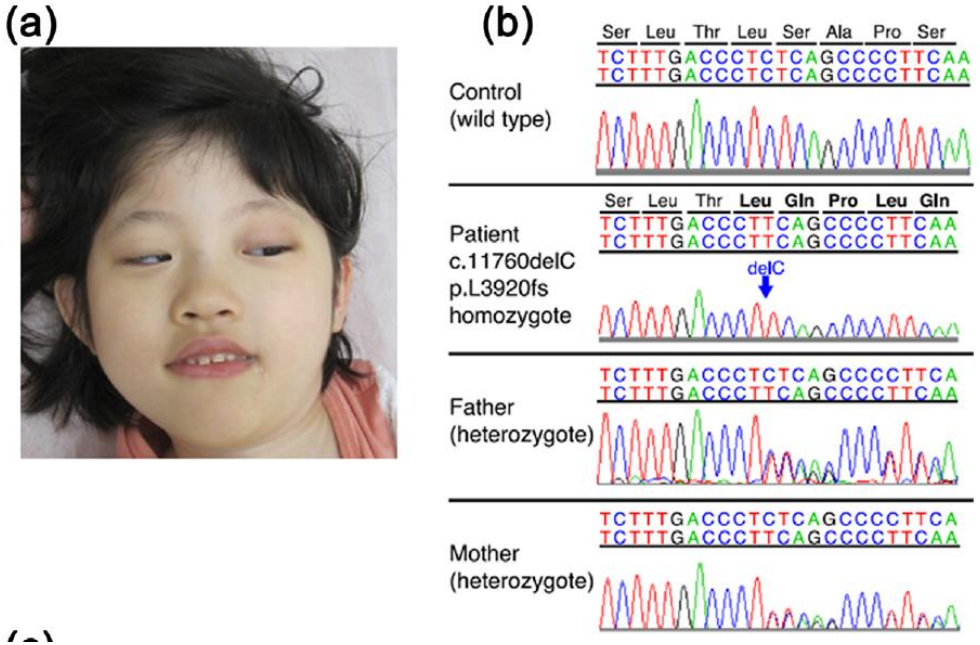
- Okamoto, et al., 2017 (PMID 28777490, Epub ahead of print on Aug 4, 2017) identified a homozygous pathogenic frame-shift variant in the *STARD9* gene via WES in one patient with severe intellectual disability, dysmorphic features, generalized tonic seizure, acquired microcephaly, cortical blindness, and sleep apnea.

ORIGINAL ARTICLE

WILEY American Journal of Medical Genetics Part A

A novel genetic syndrome with *STARD9* mutation and abnormal spindle morphology

Nobuhiko Okamoto[1,2] | Yuki Tsuchiya[3,4] | Fuyuki Miya[5,6] | Tatsuhiko Tsunoda[5,6] | Kumiko Yamashita[7] | Keith A. Boroevich[6] | Mitsuhiro Kato[8] | Shinji Saitoh[9] | Mami Yamasaki[10] | Yonehiro Kanemura[11,12] | Kenjiro Kosaki[13] | Daiju Kitagawa[3,4]



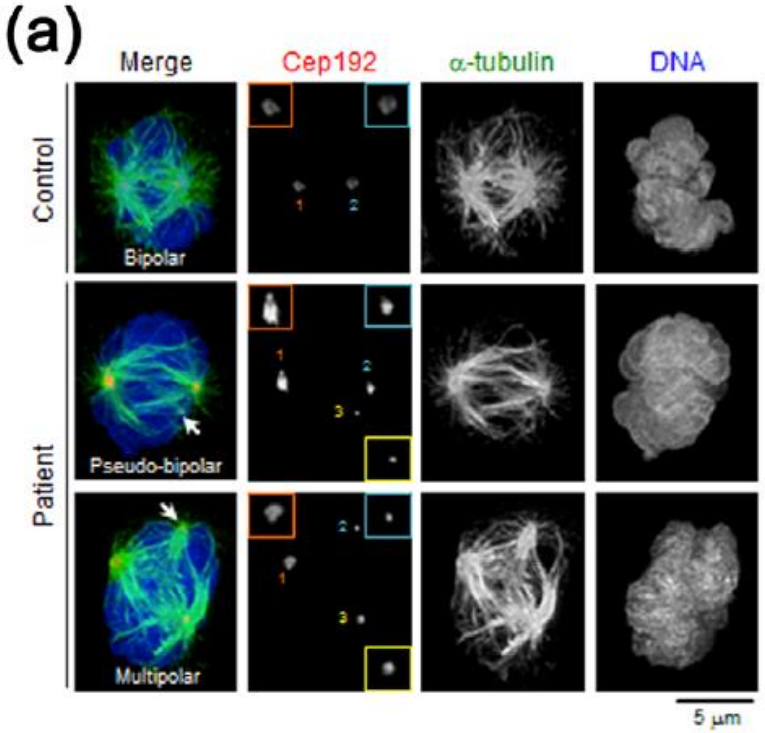
Clinical Report
6 yrs female

- **Neurologic**: Server DD, Seizure, less/no speech, cortical blindness, and sleep apnea
- **MRI**:
- **Dysmorphic features**: microcephaly, sparse eyebrow, epicanthal fold,
- **Muscle**: hypotonia, deep tendon reflexes were absent
- **Growth parameters**: height 99cm (-4.0SD), weight 11.7kg (-2.8SD), OFC47.0cm (-2.2SD)
- **GI**: poor feeding

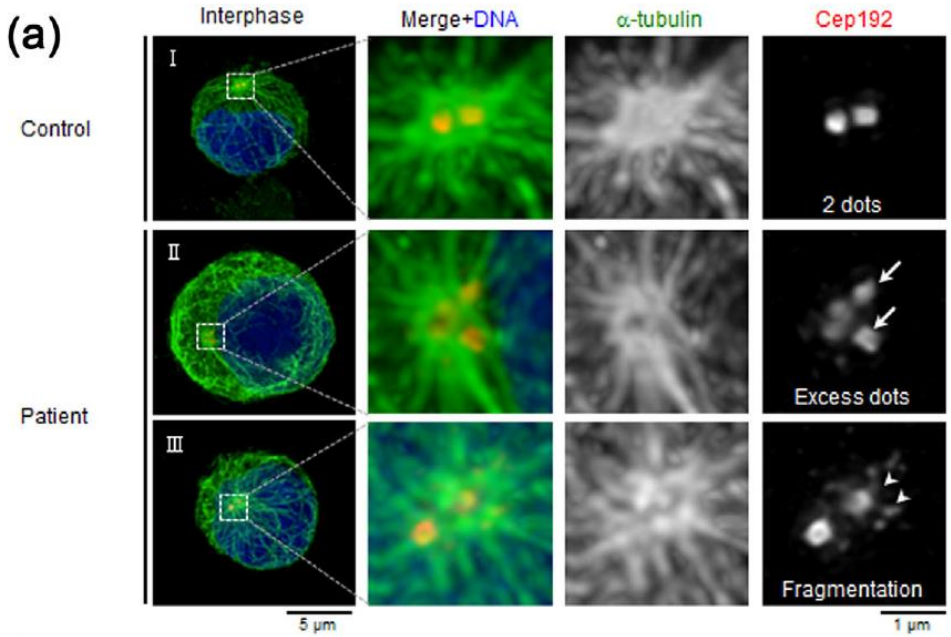
Mutation: homozygous of c.1176odelC, p.L3920fs in *STARD9*

Abnormal Spindle Morphology and Increase # of Centrosomes.

Abnormal spindle morphology



Increased number of centrosomes and fragmentation



Okamoto et al. 2017

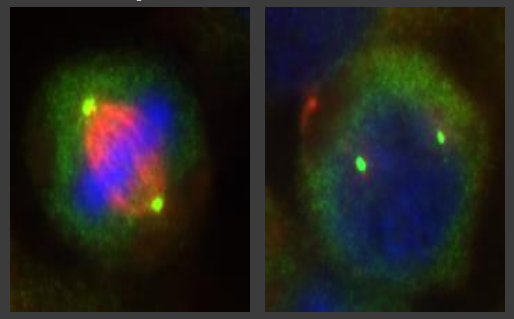
Research Collaboration with HCI

Dr. Katherine Ullman, Dollie LaJoie and Dr. Reha Toydemir

1) Initial antibody test on adherent HeLa cells (no smear gel) – CEP192 antibody works nicely

Metaphase Prometaphase

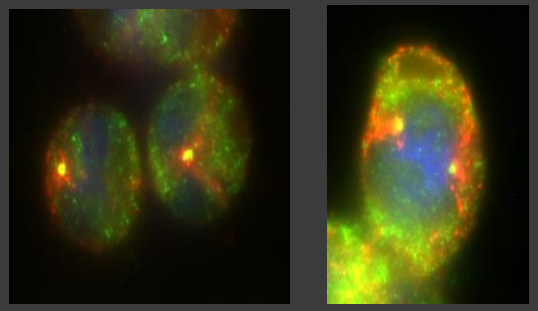
CEP192
Tubulin DNA



2) Optimized conditions using trypsinized HeLa cells (to mimic suspension cells) in smear gel:

Recently divided daughter cells Prometaphase

CEP192
Tubulin DNA

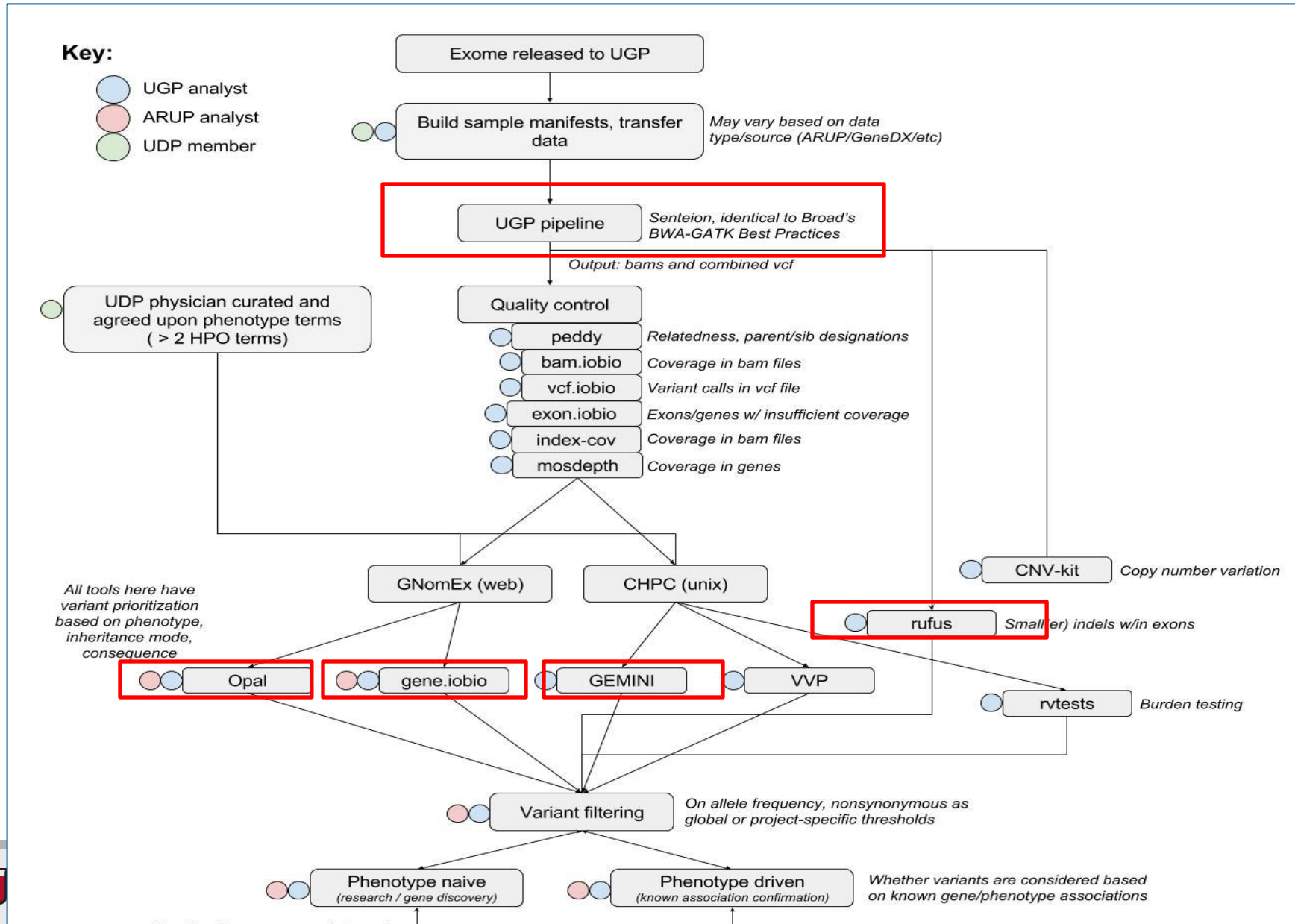


Re-analysis of Negative Exomes

- Re-analysis increased 10% of positive yield
- When to re-analysis exome? 6 months, one year or two years?
- Which bioinformatics pipeline to use?

Re-Analysis of Negative Exome Workflow

Collaboration with UGP, Drs. Gabor Marth and Matt Velinder



Summary

- Multidisciplinary team of UDP program developed a path to patient care: right patient, right diagnosis leading to right treatment
- It accomplished a goal to identify specific needs of rare disease clinic research: more collaboration, new gene discovery, exposure to patients and families and potential drug targets.

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