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

Diagnostic Odyssey: 7 years
Clinical:
- Progressive, neurodegenerative
- Many tests, several invasive
- Seen at multiple institutions
- Started at age 5 years, now 12

Diagnostic Odyssey: 7 years

Value of diagnosis: answering the family’s key questions

- What is this?
- What will happen now?
- How do we treat it?
- Why did it happen?
- What will happen to my family?

End the odyssey
Outcomes
Care (cure)
Cause
Risk
Penelope - Undiagnosed Disease Program

ORIGINAL RESEARCH ARTICLE

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

William A. Edgar, MD1, MS, CRNP2,3,4,5, Lynne Wolfe, PhD, CRNP27,8,9, Steven K. Hwu, MD, MSc3,4,5, David L. Landis, MD1, Karin Fuentes Fajardo, MD2, Andrea Gropper, RN, BS5,6, Michele Nehrhofer, RN1, Sandra Yang, MS12, Andrea Gropper, RN, BS5,6, Michelle Williams, RN, CRNP2,3,4,5, David Adams, MD, PhD1,3

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.


Key Words: neurological disorders; rare disease; SNP arrays; undiagnosed disease; whole exome sequencing
Our journey to Penelope

Understanding what is important: begin from the end

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**

E Clark, Chair, Department of Pediatrics

**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)

**Consultants**

Neuromuscular
Hematology-oncology
Behavioral health

Endocrinology
Social work

**Executive Sponsor**

E Clark, Chair, Department of Pediatrics

**Core Team**

Team lead (L Botto)
Cardiogenetics (S Bleyl)
Dysmorphology (J Carey, D Viskochil)
Biochemical Genet (N Longo)
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Gastroenterology (S Guthery)
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Molecular Genetics (R Mao, P Bayrak-Toydemir)

**Front Line**

Parents
Clinical coordinators
Clinicians

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

Analyze

- Exome
- Bioinformatics
- Expand testing, RNA
Process (simplified)

Diagnose & Care
Team evaluation
• Diagnosis made?
• Care Plan
• Family binder
• Family Result Visit
• Referrals
• Follow up
Visual Summary (1)
Genetic Findings and Relation to Clinical Findings

Visual Summary (2)
Clinical Evaluation and Plan
**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

**Analyze**

- Exome
- Bioinformatics
- Expand testing, RNA

**Diagnose & Care**

Team evaluation

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

- Research
- Matching
- Follow up
Clinical:
- Progressive neurodegenerative condition
- Many genetic tests, imaging, invasive tests
- Seen at NIH and multiple institutions

**Diagnostic Odyssey: 7 years**

<table>
<thead>
<tr>
<th>Predicted Class</th>
<th>Gene</th>
<th>Chrom</th>
<th>Nucleotide Change</th>
<th>Protein Change</th>
<th>Zygosity</th>
<th>Effect</th>
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<tbody>
<tr>
<td>Disease Associated</td>
<td>PRPS1</td>
<td>chrX</td>
<td>c.344G&gt;C</td>
<td>p.Val112Leu</td>
<td>hemizygous</td>
<td>Missense</td>
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<tr>
<td>Uncertain Significance</td>
<td>CFH</td>
<td>chr1</td>
<td>c.2965T&gt;G</td>
<td>p.Cys989Gly</td>
<td>heterozygous</td>
<td>Missense</td>
</tr>
</tbody>
</table>

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
- Slowly progressive neuropathy
- Vision loss and deafness variable

Deafness XL
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.
Clinical:
- Progressive neurodegenerative condition
- Many genetic tests, imaging, invasive tests
- Seen at multiple institutions

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
- Charcot Marie Tooth type 5 (CMTX5) – Arts syndrome overlap: PRPS1
  - *Diagnosis-driven treatment (SAM +/- riboside), connected with other center*
- UDP Galactose transporter deficiency (CDG IIIm): 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
- 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
Lesson 4: journey to better value continues

- **Standard clinical + no/piecemeal testing**
  - Less effective
  - Lower cost

- **Standard Clinical + WES**
  - More effective
  - Higher cost

- **Standard Clinical + piecemeal testing**
  - Less effective
  - Lower cost

- **Diagnosis and Care Team + (early) WES**
  - More effective
  - Higher cost

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

Expert Panel creates a follow-up plan, program coordinator organizes clinical consultations

Patients

Expert Panel Review and decide a possible diagnosis

Present results to Expert Panel, discuss patient management, available treatment or trials, and family consultation

VUS need in vivo/in vitro functional study, mRNA study, animal model

Confirmed pathogenicity

Remaining undiagnosed

Re-analysis the undiagnosed cases

10% Positive

Yes

Positive

No

Perform Whole Exome Sequencing

10% Positive

Yes

Positive

No

Re-analysis the undiagnosed cases

Confirmed pathogenicity

Remaining undiagnosed

Expert Panel creates an assessment plan, program coordinator organizes administration, clinical consultations, and investigations

*
61% Positive Yield

ARUP first 300 exomes

- Negative: 60%
- Uncertain: 0%
- Positive: 40%

Penelope

- Positive: 70%
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

Paired-End Reading (2X100 bp)

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

QA matrix:
>100 mean
10X coverage, >95%
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)
Clinical Information Cont

- Family: Not remarkable, no consanguinity, a healthy 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

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

De novo: 50 hits, 3 confirmed by Sanger

GNAS, BNIP1, STY16

HGMD Matched Variants: 32

All benign

AR/AD analysis: 1% freq, 3% ARUP freq 410

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

SNVs: 746

Variants (SNV)s in targeted genes: 268,067
GNAS De Novo

- Chr20(GRCh37): g.57484255_57484258del; NM_000516.4 c.565_568del; p.Asp189fs
Fig. 1.
Distribution of GNAS mutations in POH and other conditions of progressive HO. Exons for the GNAS gene (Gsa 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.
GNAS c.565_568del Reported

- This variant has been reported in patients:
  - pseudohypoparathyroidism type 1A (PHP Ia) / pseudopseudohypoparathyroidism (PPHP) (Ahmed 1998)
  - Pseudopseudohypoparathyroidism (PPHP) (Walden 1999)
  - 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

**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

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
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

SNVs: 749

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
de novo

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

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

Notch protein is transmembrane receptors which regulate cell decision during development
Loss of Function Mutations in NOTCH1 cause Adams-Oliver Syndrome

Mutations in NOTCH1 Cause Adams-Oliver Syndrome

Anna-Barbara Stittrich, Anna Lehman, Dale L. Bodian, Justin Ashworth, Zheyuan Zong.

A. Family 1: Large del
   Family 2: c.743-1G>T
   Family 3: p.C429R
   Family 4: p.C1496Y
   Family 5: p.D1989N

B. Relative coverage (% of median) vs. chr9 position (kb)

C. NOTCH1: calcium-binding EGF-like repeats

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)

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. Aster1‡

<table>
<thead>
<tr>
<th>Mutation Type</th>
<th>Number of Cases</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>HD only</td>
<td>25/96 (26.0%)</td>
<td></td>
</tr>
<tr>
<td>PEST only</td>
<td>12/96 (12.5%)</td>
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<tr>
<td>HD+PEST</td>
<td>17/96 (17.7%)</td>
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\[ p.Phe1693Ser \]

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 Izraeli7,8

Table 4. NOTCH1 mutations in ETV6-NCOA2 leukemia

<table>
<thead>
<tr>
<th>Case</th>
<th>Domain</th>
<th>SNP</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
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<tbody>
<tr>
<td>1</td>
<td>HD</td>
<td>c.5097 C/T</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>HD</td>
<td>c.5097 C/T</td>
<td>—</td>
<td>p.S2468X</td>
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<tr>
<td></td>
<td>PEST</td>
<td>—</td>
<td>c.7403C&gt;A</td>
<td>p.F1694S</td>
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<tr>
<td>3</td>
<td>HD</td>
<td>—</td>
<td>c.5081C&gt;T</td>
<td>p.2336 EHTGPLPAW8HGPRPAAQ</td>
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<tr>
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<td>HD</td>
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<td>c.7007_7008insT</td>
<td>p.L1679P</td>
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<td>HD</td>
<td>—</td>
<td>c.5036T&gt;C</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>HD</td>
<td>—</td>
<td>—</td>
<td>p.2515 RVP</td>
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<tr>
<td></td>
<td>PEST</td>
<td>—</td>
<td>c.7544_7545delCT</td>
<td>—</td>
</tr>
</tbody>
</table>

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

Confirmed p.Phe1693Ser

c.5078T>C p.Phe1693Ser

Strehl Sabine, et al 2008
Hedgehog/NOTCH involving development of Hirschsprung 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 Tam1,2

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

<table>
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<tr>
<th>Gene</th>
<th>Transcript</th>
<th>Type</th>
<th>Zygosity</th>
<th>DNA alteration</th>
<th>Protein alteration</th>
<th>Inheritance mode</th>
<th>Human disease</th>
<th>Classification</th>
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</thead>
<tbody>
<tr>
<td>CSTB</td>
<td>NM_000100</td>
<td>nonsense</td>
<td>het</td>
<td>c.C136T</td>
<td>p.Q46X</td>
<td>Autosomal recessive</td>
<td>Progressive myoclonic epilepsy 1A</td>
<td>Pathogenic</td>
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<tr>
<td>GRID2</td>
<td>NM_001510</td>
<td>missense</td>
<td>het</td>
<td>c.A101G</td>
<td>p.D34G</td>
<td>Autosomal recessive</td>
<td>Spinocerebellar ataxia- 18</td>
<td>VUS</td>
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</tbody>
</table>
## Compound Heterozygous Variants in STARD9

<table>
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<tr>
<th>Gene</th>
<th>Transcript</th>
<th>Type</th>
<th>Zygosity</th>
<th>DNA alteration</th>
<th>Protein alteration</th>
<th>Inheritance mode</th>
<th>Human disease</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NM_020759</td>
<td>missense</td>
<td>het</td>
<td>c.C6955T</td>
<td>p.R2319W</td>
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</tbody>
</table>

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

Nobuhiko Okamoto1,2, Yuki Tsuchiya3,4, Fuyuki Miya5,6, Tatsuhiko Tsunoda5,6, Kumiko Yamashita7, Keith A. Boroevich6, Mitsuhiro Kato8, Shinji Saitoh9, Mami Yamasaki10, Yonehiro Kanemura11,12, Kenjiro Kosaki13, Daiju Kitagawa3,4

(a)

(b)

Control (wild type)

Patient c.1176delC, p.L3920fs homozygote

Father (heterozygote)

Mother (heterozygote)

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

Clinical Report

6 yrs female

- **Neurologic**: Sensorimotor delay, 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), OFC 47.0cm (-2.2SD)
- **GI**: Poor feeding
Abnormal Spindle Morphology and Increase # of Centrosomes.

Abnormal spindle morphology

Increased number of centrosomes and fragmentation

Okamato 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

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

Metaphase  Prometaphase

Recently divided daughter cells  Prometaphase
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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EC Clark

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Gabor Marth, Matt Velinder

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ARUP Genomics Lab and Biocomputing Group

ARUP Laboratories