Diagnostic Opportunities for Rare Disease with NGS

Sarah Sawyer, PhD, MD, FRCPC, FCCMG
Shenzen, China
NGS is changing the pace of diagnosis, reducing diagnostic odyssey for patients

Clinical opportunity to increase diagnostic rate for rare disease patients

Diagnosis for patient with rare disease is often laborious, time consuming, and frustrating for families
FORGE; Finding of rare disease genes

67 Novel genes

95 Known genes

Dr. Kym Boycott
Dr. Jacek Majewski
FORGE steering committee
Jeremy Schwartzentruber
Chandree Beaulieu
FORGE: 264 Projects >500 families

67 Novel genes

96 Known genes

100 diagnoses

PIK3R1

POMC, NNT, BRCA1, EFNB1, RAB3GAP1, SACS, ALG3, RTTN, MLL2, RPE65, SLC45A2, G6PC3, LRPS5, IGHMBP2, CYP26C1, PLCB4, TERT, IDS, PRPS1, TRPV4, CORO1A, HSD17B4, OFD1, CEP290, CC2D2A, GRIN2A, EFTUD2, GNE, NDUFS2, SPTAN1, CHM, MUSK, C12orf65, MTO1, MUSK, SYNGAP1, TMPRSS6, ABCD1, PLA2G6, PYCR1, ZMYND10, SPAG1, LRRC6, MYOC, WDR36, NTF4, ASAH1, ATM, KAT6B, OTX2, COX10, SLC25A1, ALDH6A1, COL11A1, GRIN2A, AICDA, EP300, FRAS1, WNT5A, RARS2, PMM2, COQ9

SRCAP
Opportunities for patients with atypical presentations and heterogeneous disorders

- Two Diagnoses
- Limited access to testing
- Too rare
- Identified while in pipeline
- Missed by another method
- Atypical presentation
- Heterogeneity
Two disorders in one patient: Fitzsimmons Syndrome

Hereditary spastic paraparesis and brachydactyly

Dr. Mohnish Suri, Dr. Christine Armour, Taila Hartley
Trichorhinophalangeal syndrome, type III & Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS)


TRPS1:c.276C>T:p.R921X
Ultra Rare: Neo-natal onset primary Coenzyme Q$_{10}$ deficiency due to mutations in *COQ9*

Lactic acidosis, hypotonia, cardiomyopathy, hypoplasia of corpus callosum, subventricular cysts around lateral ventricles and clenched hands.

Early death

Mitochondrial disorder suspected

**COQ9**: c.521+2T>C and c.711+3G>C

Neonatal presentation of coenzyme Q$_{10}$ deficiency

*Shamima Rabman, MRCP, Iain Hargreaves, PhD, Peter Clayton, MD, FRCP, and Simon Heales, PhD, MRCPath*
Ultra Rare (n=5)

Mutations in *ALDH6A1* encoding methylmalonate semialdehyde dehydrogenase are associated with dysmyelination and transient methylmalonic aciduria.

- 3 reported cases with molecular confirmation
- Variably elevated lactates
- Delayed myelination
- Developmental delay

A. 13 months  
B. 21 months  

Dr. Michael Geraghty,  
*Orphanet J Rare Disease* 2013
Atypical Presentation of a known disorder

Presented with absence and atonic seizures with sudden falls at age 10

Frequent myoclonic jerks of upper and lower extremities

Seizures reached >100 per day, classified as myoclonic-absence epilepsy

Bilateral sensorineural hearing loss
Spinal muscular atrophy with progressive myoclonic epilepsy (SMA-PME): **ASAHI1**

![Genetic diagram and protein expression analysis](image)
Atypical Presentation of a known disorder

- Microcephaly
- TEF
- Cleft palate
- Choanal atresia
- Deafness
- Heart defect
- Normal $CDH7$
- CHARGE-like
Haploinsufficiency of a Spliceosomal GTPase Encoded by \textit{EFTUD2} Causes Mandibulofacial Dysostosis with Microcephaly

Matthew A. Lines,\textsuperscript{1} Lijia Huang,\textsuperscript{1} Jeremy Schwartzentruber,\textsuperscript{2} Stuart L. Douglas,\textsuperscript{1} Danielle C. Lynch,\textsuperscript{1} Chandree Beaulieu,\textsuperscript{1} Maria Leine Guion-Almeida,\textsuperscript{3} Roseli Maria Zechi-Ceide,\textsuperscript{3} Blanca Gener,\textsuperscript{4} Gabriele Gillessen-Kaesbach,\textsuperscript{5} Caroline Nava,\textsuperscript{6} Geneviève Baujat,\textsuperscript{6} Denise Horn,\textsuperscript{7} Usha Kini,\textsuperscript{8} Almuth Caliebe,\textsuperscript{9} Yasemin Alanay,\textsuperscript{10,11} Gulen Eda Utine,\textsuperscript{10} Dorit Lev,\textsuperscript{12} Yiwen Kehl,\textsuperscript{13} Arthur W. Grix,\textsuperscript{14} Dietmar R. Lohmann,\textsuperscript{15} Ute Hehr,\textsuperscript{16} Detlef Böhm,\textsuperscript{13} Jacek Majewski,\textsuperscript{18} Dennis E. Bulman,\textsuperscript{19} Dagmar Wieczorek,\textsuperscript{15,20} and Kym M. Boycott\textsuperscript{1,20,*}

\textbf{EFTUD2} deletion

FORGE Canada Consortium
Mandibulofacial dysostosis and microcephaly-\textit{EFUTD2}

Phenotypic spectrum not well understood

Three patients separately identified with mutations in this gene!

Need for non-biased methods for diagnosis
Missed by another method: Emery-Dreifuss-like (*COL6A1*)

**FINAL DIAGNOSIS:**
Biopsy, Skeletal muscle (immuno only) - **Immunopositive** for alpha-dystroglycan and **collagen VI**

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**Staff Pathologist**

***Electronically Signed Out by***

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**CLINICAL HISTORY**
No clinical history provided with specimen.

**TISSUE SUBMITTED:**
7 Unstained frozen slides

**GROSS DESCRIPTION:**
Received at the request of the Children's Hospital of Eastern Ontario are 7 frozen slides for Immunostaining.

**MICROSCOPIC DESCRIPTION:**
**IMMUNOFLUORESCENCE**
The muscle cells show sarcolemmal immunopositivity for alpha-dystroglycan and Collagen VI, the latter is normally co-localised with laminin.

End of Report
Missed by another method:
Emery-Dreifuss *COL6A1*

<table>
<thead>
<tr>
<th>Project# And Disorder</th>
<th>Gene name</th>
<th>Patient ID</th>
<th>Mutation Type</th>
<th>Mutation</th>
<th>If match with Exome Sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4R_468 Emery-Dreifuss like</td>
<td>COL6A1</td>
<td>Affected (CH0048)</td>
<td>Het Splicing</td>
<td>COL6A1(NM_001848:exon14:c.1003-3C&gt;G)</td>
<td>Yes</td>
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<tr>
<td></td>
<td></td>
<td>Mother (CH0047)</td>
<td>Normal</td>
<td>Normal</td>
<td>NA</td>
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<tr>
<td></td>
<td></td>
<td>Father (CH0049)</td>
<td>Normal</td>
<td>Normal</td>
<td>NA</td>
</tr>
</tbody>
</table>

*COL6A1(NM_001848:exon14:c.1003-3C>G)*

**De Novo**

**Affected**

**Mother**

**Father**
Genetic Heterogeneity: Ataxia

Feature of >100 neurological disorders with childhood onset

Standard-of-care genetic testing for patients with ataxia: SCA panel (repeats), Friedreich’s ataxia (FXN), ARSACS (SACS)

Retrospectively selected all FORGE Canada projects that included cerebellar ataxia as a feature; 28 families
Diagnosed 11 out of 28 = 39%

<table>
<thead>
<tr>
<th>DISORDER</th>
<th># AFF</th>
<th>GENE</th>
<th>DISORDER</th>
</tr>
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<tbody>
<tr>
<td>Congenital cerebellar atrophy</td>
<td>2</td>
<td>PMM2</td>
<td>Congenital disorders of glycosylation</td>
</tr>
<tr>
<td>Holmes syndrome</td>
<td>2</td>
<td>RNF216</td>
<td>No change</td>
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<tr>
<td><strong>AR spinocerebellar ataxia childhood onset</strong></td>
<td>1</td>
<td>SACS</td>
<td>ARSACS</td>
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<tr>
<td>Perrault syndrome</td>
<td>2</td>
<td>HSD17B4</td>
<td>D-bifunctional protein deficiency</td>
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<tr>
<td>Ataxia with cognitive impairment</td>
<td>2</td>
<td>SETX</td>
<td>AR spinocerebellar ataxia</td>
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<tr>
<td>Cerebellar atrophy</td>
<td>3</td>
<td>HSD17B4</td>
<td>D-bifunctional protein deficiency</td>
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<tr>
<td>Marinesco Sjogren</td>
<td>1</td>
<td>RAB3GAP1</td>
<td>Warburg Micro syndrome</td>
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<tr>
<td>Ataxia, DD, seizures</td>
<td>1</td>
<td>SYNGAP1</td>
<td>Intellectual disability</td>
</tr>
<tr>
<td><strong>Ataxia</strong></td>
<td>1</td>
<td>SACS</td>
<td>ARSACS</td>
</tr>
<tr>
<td>Epilepsy with ataxia</td>
<td>3</td>
<td>KCTD7</td>
<td>Progressive myoclonic epilepsy</td>
</tr>
<tr>
<td>DD and hypotonia</td>
<td>1</td>
<td>PLA2G6</td>
<td>Neurodegeneration with brain iron accumulation</td>
</tr>
</tbody>
</table>
Exome Sequencing as a Diagnostic Tool for Pediatric-Onset Ataxia


Ataxia exome panel: 332 genes
Lessons learned-what works!

1. WES diagnosed patients with disorders with significant **genetic heterogeneity**

2. Relatively non-biased approaches to testing identified patients with **atypical presentations** and **ultra- rare disorders**
Lessons learned-what works!

1. WES diagnosed patients with disorders with significant genetic heterogeneity.

Therapies were adjusted or initiated for 6 patients given a clinical diagnosis.
A molecular diagnosis provides an opportunity to treat patients with rare disease.

Therapies were adjusted or initiated for 6 patients given a clinical diagnosis.

~6%
Intractable Epilepsy

de novo GRIN2A mutation

Subunit of N-methyl D-aspartate
Mediates excitatory transmission in the CNS

Adjusted therapy: Topiramate enhances GABA evoked current

10 months of significant seizure reduction

Dr. David Dyment, Epilepsia in press
Cerebral folate transporter deficiency

Seizures and intellectual disability, onset at 2y
Extensive white matter damage
Homozygous mutations in FOLR1
Rx: Improved seizure control on folic acid
WES staged approach

Run proband

Filter with HPO, OMIM, panels

Hit in known disease gene

Validation and Report

No hit

WES family members
Clinical implementation for Canada

- Defined Diagnostic Utility: CCMG Position Statement
- Approach to Incidental Findings: CCMG Best Practice Guidelines
- Economic Impact
- Education
Impact of diagnosis on the health care system

1. Shorten the diagnostic odyssey
2. Some disorders are treatable!