The Role of Clinician in Variant Interpretation

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What Are the Indications for WES?

• Etiology not found with existing diagnostic modalities.
• Rare, clinically unrecognizable, or puzzling disorders.
• Numerous individual genetic tests would be required for evaluation.
• The condition is genetically heterogeneous (e.g. retinitis pigmentosa)
• Genetic testing is not clinically available to evaluate the suspected diagnosis.
5 answers that family seeks to know about any condition involving themselves or their family members:

- What is the diagnosis?
- How did it happen?
- Who else in the family might be at risk?
- What can be expected in the future?
- Is there any treatment or cure?
Why Is It Important to Find the Genetic Cause?

- It can end an expensive, potentially invasive, and stressful diagnostic odyssey.
- Determine prognosis.
- Discontinuation of additional planned studies
- Screening patients for additional manifestations
- It can lead to a specific treatment or management strategy.
- Identification of disease in at risk family members
- Reproductive planning
“Forward Genetics” or “Genomics”

- **Forward genetics** refers to a process where studies are initiated to determine the genetic basis of observable phenotypic variation.

Challenges: broad dd; lethal genetic variants and mild and atypical phenotypes are not assessed.
The utility of the traditional medical genetics diagnostic evaluation in the context of next-generation sequencing for undiagnosed genetic disorders (Duke University)

Genet Med. 2014 Feb;16(2):176-82.

- The **diagnostic yield** of traditional, comprehensive clinical evaluation and **targeted** genetic testing in 500 pts

<table>
<thead>
<tr>
<th>Category</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic cause</td>
<td>46% (72% 1st visit)</td>
</tr>
<tr>
<td>Non genetic cause</td>
<td>7.8%</td>
</tr>
<tr>
<td>No diagnosis</td>
<td>46.2%</td>
</tr>
<tr>
<td>Cost per subsequent</td>
<td></td>
</tr>
<tr>
<td>successful genetic diagnosis</td>
<td>$25,000.</td>
</tr>
</tbody>
</table>

- For those remaining **undiagnosed**, next-generation sequencing may be clinically and economically beneficial.
Reverse genetics refers to the study of phenotypes associated with a specific genetic change. It enables clinical characterization of previously unknown genetic/genomic disorders.

Challenges: VUSs, to what extent should we investigate the phenotype?
REFERRAL FORM FOR WHOLE EXOME SEQUENCING

Child’s Name: ____________________________  Date of Birth: ____________________________
Parents/Guardians’ Names: ____________________________
Contact Phone Number(s): ____________________________

This form is confidential and will become part of the patient’s medical record.

Referring MD: ____________________________  Primary Care Physician: ____________________________
Phone: ____________________________  Phone: ____________________________
Fax: ____________________________  Fax: ____________________________

To refer a patient for whole exome sequencing, please complete the following:

- Complete this 2-page form in its entirety.
- Provide printed copies of the patient’s pertinent medical records. Printed medical records are integral to the process of exome sequencing as these are provided to the lab to allow detailed phenotyping of the patient and direct the analysis. Providing detailed clinical information increases the likelihood of detecting a relevant gene mutation with exome sequencing.
  - Most recent clinic note from all specialists
  - Radiology reports (e.g. brain MRI, skeletal survey, ultrasounds)
  - Previous laboratory studies (e.g. metabolic and biochemical testing, CSF studies)

  - Note: please do not send copies of routine labs such as CBCs and CMPs unless you believe
Pretest Counseling

- To maintain **realistic expectations** for finding the causative mutation.
- To discuss the possibility of **VUSs**.
- To alert the patient or family that in most cases, a positive result is **unlikely to change treatment or management decisions** or to improve the **prognosis**.
- To discuss that **incidental (secondary) findings** unrelated to the reason for testing may be found and reported.
NGS: Exome Sequencing

- All coding regions of the genome (~1% of the genome)
  - ~85% of recognized disease-causing mutations.

- Current estimates of exome coverage through NGS are between 90 and 95%. The depth of coverage for an exome is not uniform.

- ES is used for detecting variants in known disease-associated genes as well as for the discovery of novel gene-disease associations.
Primary Findings

- This term is used to describe variants in a gene or genes that are relevant to the diagnostic indication for which the sequencing was ordered (e.g., a pathogenic variant in *MECP2* in a girl with loss of developmental milestones).
Primary Findings (variant-level classification)

- **Pathogenic**
  - Reported

- **Likely pathogenic**
  - Reported

- **VUS**
  - Reported

- **Likely benign**
  - Not Reported

- **Benign**
  - Not Reported
Case-Level Classification by the Lab

• A synthesis of all the molecular data in a single subject specifying whether the test results provide a molecular diagnosis.

  • *Definitive*
  • *Possible*
  • *Candidate*
  • *Incidental*
Incidental (or Secondary) Findings

(Genet Med 2013:15(7):565–574)

• Unexpected results that are not related to the indication for ordering the sequencing but that may nonetheless be of medical value or utility to the patients and families.

• Disorders for which preventive measures and/or treatments are available; can be asymptomatic.

• Known Pathogenic (KP) and Expected Pathogenic (KP or LP)

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Gene</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hereditary breast and ovarian cancer</td>
<td>BRCA1, BRCA2</td>
<td>AD</td>
</tr>
<tr>
<td>Li–Fraumeni syndrome</td>
<td>TP53</td>
<td>AD</td>
</tr>
<tr>
<td>Peutz–Jeghers syndrome</td>
<td>STK11</td>
<td>AD</td>
</tr>
<tr>
<td>Lynch syndrome</td>
<td>MLH1, MSH2, MSH6, PMS2</td>
<td>AD</td>
</tr>
<tr>
<td>Familial adenomatous polyposis</td>
<td>APC [BMPR1A, SMAD4 juvenile polyposis syndrome]</td>
<td>AD</td>
</tr>
<tr>
<td>MYH-associated polyposis;</td>
<td>MUTYH</td>
<td>AR</td>
</tr>
<tr>
<td>Von Hippel–Lindau syndrome</td>
<td>VHL</td>
<td>AD</td>
</tr>
<tr>
<td>Multiple endocrine neoplasia type 1</td>
<td>MEN1</td>
<td>AD</td>
</tr>
<tr>
<td>Multiple endocrine neoplasia type 2</td>
<td>RET</td>
<td>AD</td>
</tr>
<tr>
<td>Familial medullary thyroid cancer</td>
<td>RET</td>
<td>AD</td>
</tr>
<tr>
<td>PTEN hamartoma tumor syndrome</td>
<td>PTEN</td>
<td>AD</td>
</tr>
<tr>
<td>Hereditary paraganglioma–pheochromocytoma syndrome</td>
<td>SDHD, SDHAF2, SDHC, SDHB</td>
<td>AD</td>
</tr>
<tr>
<td>Tuberous sclerosis complex</td>
<td>TSC1, TSC2</td>
<td>AD</td>
</tr>
<tr>
<td>WT1-related Wilms tumor</td>
<td>WT1</td>
<td>AD</td>
</tr>
<tr>
<td>Neurofibromatosis type 2</td>
<td>NF2</td>
<td>AD</td>
</tr>
<tr>
<td>Phenotype</td>
<td>Gene</td>
<td>Inheritance</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>Marfan syndrome, Loeys–Dietz syndromes, and familial thoracic aortic</td>
<td><strong>FBN1, TGFBR1/2, SMAD3, MYLK, MYH11</strong></td>
<td>AD</td>
</tr>
<tr>
<td>thoracic aortic aneurysms and dissections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ehlers–Danlos syndrome, vascular type</td>
<td><strong>COL3A1</strong></td>
<td>AD</td>
</tr>
<tr>
<td>Hypertrophic cardiomyopathy, dilated cardiomyopathy</td>
<td><strong>MYBPC3, MYH7, TNNT2, TNNI3, TPM1, MYL3, ACTC1, PRKAG2, GLA, MYL2, LMNA</strong></td>
<td>AD, X-linked</td>
</tr>
<tr>
<td>Catecholaminergic polymorphic ventricular tachycardia</td>
<td><strong>RYR2</strong></td>
<td>AD</td>
</tr>
<tr>
<td>Arrhythmogenic right-ventricular cardiomyopathy</td>
<td><strong>PKP2, DSP, DSC2, TMEM43, DSG2</strong></td>
<td>AD</td>
</tr>
<tr>
<td>Romano–Ward long QT syndrome types 1, 2, &amp; 3, Brugada syndrome</td>
<td><strong>KCNQ1, KCNH2, SCN5A</strong></td>
<td>AD</td>
</tr>
<tr>
<td>Familial hypercholesterolemia</td>
<td><strong>LDLR, APOB, PCSK9</strong></td>
<td>SD, AD</td>
</tr>
<tr>
<td>Malignant hyperthermia susceptibility</td>
<td><strong>RYR1, CACNA1S</strong></td>
<td>AD</td>
</tr>
<tr>
<td>OTC deficiency, Wilson disease</td>
<td><strong>OTC, ATP7B</strong></td>
<td>X-Linked; AR</td>
</tr>
</tbody>
</table>
Actionable Genetic Variants

- These are variants in specific genes that result in recommendation(s) that are supported by evidence and would be expected to avoid significant morbidity and mortality.
How labs can filter huge amounts of variants to find one match that can fit with patient’s phenotype that is usually consistent with a rare disease.
Pathogenicity of Genetic Variants

- Low population minor allele frequency (MAF)
- A *de novo* mutation (if the phenotype is sporadic in the context of a dominant disorder)
- Novel or very rare truncation variants
- Co-segregation of variant and disorder within families
Pathogenicity of Genetic Variants

- Presumed inheritance pattern (e.g., biallelic if recessive) and consanguinity (e.g., homozygous variants)
- Algorithmic scores for in silico assessment of protein function or splicing impact and conservation.
- Gene expression pattern and biological pathway analysis.
<table>
<thead>
<tr>
<th>Study</th>
<th># of patients</th>
<th>Molecular diagnosis rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baylor</td>
<td>2000</td>
<td>25.2%</td>
</tr>
<tr>
<td>UCLA</td>
<td>814</td>
<td>26%</td>
</tr>
<tr>
<td>Ambry</td>
<td>500</td>
<td>30%</td>
</tr>
</tbody>
</table>

- Trio sequencing and focusing on specific disease subgroups can raise the diagnostic rate

*JAMA. 2014;312(18):1870-1879.*

Clinician’s Role in Optimal Interpretation of clinical ES results.

- Diagnostic course after WES has been completed by the laboratory
- The role of the medical geneticist in interpretation of results
- Auxiliary studies performed to determine pathogenicity of genetic variants
Clinician’s Role in Optimal Interpretation of clinical ES results.

- Exomes for 155 probands (128 trios; 86% outpatient) were ordered between March 2012 and January 2015.
- Retrospective chart review and interview with the ordering medical geneticists and genetic counselors.
- Laboratories reported genetic variants as pathogenic, likely pathogenic, or VUS, and incidental findings.
Four Possible Outcomes Based on Phenotypes

- Definitively related
- Possibly related (requires further clinical evaluation or enrolling the patient in a research study).
- Potential candidate genes
- Cause is not apparent, on the basis of current knowledge.
Clinical Assessment of WES Findings

- Exome findings were confirmed or reclassified as needed as **definitively, likely, possibly, or unlikely** causative of the patient’s symptoms
  - Based on:
    - The molecular data (ExAC, ClinVar, etc.)
    - Geneticist’s clinical assessment
- Clinical impression was then categorized as **concordant or discordant** with the laboratory’s classification
Clinical Assessment of ES Findings

• Clinical phenotype of the patient compared to published syndrome.
• Follow up biochemical, radiological or functional studies
• Segregation analysis of the disease
• The most likely mode of inheritance in the family
Clinical Assessment of ES Findings

- Additional molecular studies (such as single gene re-sequencing or deletion/duplication studies)
- Subsequent publication of new syndrome in similarly affected patients, or discovery of a new genetic syndrome in the index patient.
- Functional analysis of variants on research basis
Total
(155; 100%)  

Neurological
(103; 66%)

MCA
(15; 10%)

Mixed, Neurological plus
(16; 10%)

Others
(21; 14%)

Immunology (5)
Ophthalmology (5)
Cardiology (2)
Metabolic (2)
Confirmation of clinical diagnosis (1)
Connective tissue (1)
Endocrinology (1)
ENT (1)
GI (1)
Psychiatry (1)
Vascular (1)

Neurological plus MCA
(6; 4%)
Hematologic/Oncologic
Renal
Pulmonary
Allergy/immunologic/infectious
Endocrine
Metabolic/biochemical/mitochondrial
Craniofacial
Genitourinary/obstetric
Dermatologic/dental/hair
Audiologic/otolaryngologic
Gastrointestinal
Cardiovascular
Musculoskeletal/structural

Progressive phenotype
Ataxia
Psychiatric or other behavioral
Autism spectrum disorder
Movement Disorder
Seizures/epilepsy
Brain MRI positive
Intellectual disability and/or developmental...
## Diagnostic Yield by Demographic and Phenotypic Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Diagnostic Yield (%)</th>
<th>p-value</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>36.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Females vs. Males</strong></td>
<td></td>
<td>0.098</td>
<td>1.72 (0.9-3.3)</td>
</tr>
<tr>
<td>Craniofacial</td>
<td>64.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other phenotypes</td>
<td>40.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Craniofacial vs. Others</strong></td>
<td></td>
<td>0.085</td>
<td>2.65 (0.84-8.32)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>46.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed ethnicity</td>
<td>14.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Caucasian vs. Mixed</strong></td>
<td></td>
<td>0.021</td>
<td>5.2 (1.1-24.2)</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>52.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal OFC</td>
<td>35.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Microcephaly vs. Normal OFC</strong></td>
<td></td>
<td>0.14</td>
<td>1.72 (0.8-3.4)</td>
</tr>
</tbody>
</table>
The diagnostic laboratory reported 237 genetic variants (~1.5 variants/patient; 0 to 6)

55 subjects (36%) obtained a definitive diagnosis according to the molecular laboratory

10% incidental findings: BRCA2 (2), FBN1, LDLR (2), MLH1, MYBPC3 (4), MYH7, RET, SCN5A, TTN (2).
Variant Classification and Interpretation - Clinician

A

- Negative: 19%
- Candidate: 6%
- Possible: 39%
- Definitive: 36%

B

155 Total

- Promoted
- Demoted
- No Change

Laboratory:
- 56 (36%)
- 5 (10%)

Clinical Geneticist:
- 67 (43%)
- 16 (25%)
<table>
<thead>
<tr>
<th>Reason</th>
<th>Demoted</th>
<th>Promoted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical phenotype of the patient compared to published syndrome</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Segregation analysis of the disease</td>
<td>3*</td>
<td>0</td>
</tr>
<tr>
<td>Follow up biochemical / functional studies</td>
<td>2*</td>
<td>2</td>
</tr>
<tr>
<td>Molecular studies (single gene resequencing)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Subsequent publication of new syndrome in other patients</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Discovery of a new genetic syndrome in these patients</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total Number of Cases</strong></td>
<td><strong>5</strong></td>
<td><strong>16</strong></td>
</tr>
</tbody>
</table>

* 2 cases are in both categories
## Publication of New Syndromes

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>DeSanto-Shinawi syndrome</td>
<td>WAC</td>
</tr>
<tr>
<td>COQ4-related disorder</td>
<td>COQ4</td>
</tr>
<tr>
<td>CTBP1-related disorder</td>
<td>CTBP1</td>
</tr>
<tr>
<td>GABRB2-related disorder</td>
<td>GABRB2</td>
</tr>
<tr>
<td>You-Hoover-Fong Syndrome</td>
<td>TELO2</td>
</tr>
</tbody>
</table>
Re-Analysis of Exome

- Reanalysis of the exome in 14 cases: no change (7), new definitive diagnosis (4), demoted (1), efforts to define a new syndrome (2)
Interesting Data

• 48% definitive cases had mutations in genes described in 2011 or later.
• 11% are being described as new genetic syndromes
• 7.5% had definitive variants in two genes resulting in “blended phenotypes”
• 34% of variants were *de novo*
The Effect of Exome Results on Auxiliary Tests

- 84 Subjects (54%)
  - Molecular studies: 37 (24%)
  - Imaging studies: 29 (19%)
  - Biochemical and/or chemistry tests: 22 (14%)

- 12/84: follow up studies for incidental finding
- 19 probands or family members needed echo
- Cancer surveillance protocols were initiated in 8 probands or related family members
- 3 families used results for prenatal or PGD
The Effect of Exome Results on Management

- Clinical care was directly altered due to primary WES findings in 12%:
  - Discontinuation of levothyroxine
  - Cardiac ablation (WPW)
  - Prophylactic thyroidectomy and Hirschprung’s diagnosis
  - Neuropsychology evaluation & atomoxetine
  - Orthopedics referral and repair of scoliosis
  - Amantadine trial for ataxia telangiectasia
  - Methylene blue and vitamin C
  - Serine prescription for serine-responsive seizures
Case 1: 2 Siblings with Unknown Syndrome

- 6-year-old Caucasian female initially referred to genetic at ~2 yo
- Speech and motor delay; walked at 21 months. Two-word phrases at 32 months
- Hypotonia and chronic constipation
- Strabismus surgery; wears glasses for myopia and astigmatism.
Dysmorphic Features

• Broad forehead with mild frontal bossing, deep-set eyes, hypertelorism, depressed nasal bridge, flaring nares and bulbous nasal tip, thin upper lip, and a smooth philtrum.

• Eyebrows were bushy with mild synophrys

• Ears had thickened upper helices but otherwise well-formed and normally positioned
Diagnostic Work-up

- CMA, thyroid function tests, serum and urine amino acids, urine organic acids, acylcarnitine profile, total and free carnitine, ammonia, lysosomal enzymes, carbohydrate deficient transferrin, very long chain fatty acids (VLCFA), CK, aldolase, lactate and pyruvate.

- Brain MRI, hearing test, abdominal ultrasound, and electromyography; all revealed no abnormalities.
Siblings

- Her sister exhibited mild motor and speech delays, hypotonia and chronic constipation.
- Dysmorphic features including a broad forehead with mild frontal bossing, hypertelorism, depressed nasal bridge, and posteriorly rotated ears.
- Inverted nipples, right temporal scalp nevus, and keratosis pilaris were also noted.
WES Results

- Whole exome sequencing of the siblings revealed a heterozygous *de novo* W574X (c.1721G>A; p.Trp574Stop) pathogenic variant in exon 12 in *WAC*.

- The W574X pathogenic variant was not detected in the parents, strongly suggesting *germline mosaicism* in one of the parents.

- This pathogenic variant is predicted to cause loss of normal protein function either through protein truncation or nonsense-mediated mRNA decay.
<table>
<thead>
<tr>
<th></th>
<th>Pt. 1</th>
<th>Pt. 2</th>
<th>Pt. 3</th>
<th>Pt. 4</th>
<th>Pt. 5</th>
<th>Pt. 6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WAC mutation</strong></td>
<td>c.1721G&gt;A (p.Trp574Stop)</td>
<td>c.1721G&gt;A (p.Trp574Stop)</td>
<td>c.267_268dup (p.D90fs)</td>
<td>c.374C&gt;A (p.Ser125*)</td>
<td>c.1852C&gt;T (p.Gln618*)</td>
<td>c.112delA (p.Ser38Alafs*154)</td>
</tr>
<tr>
<td><strong>Sex/age</strong></td>
<td>F/6y</td>
<td>F/4y</td>
<td>F/3y</td>
<td>M/17mo</td>
<td>F/6y</td>
<td>F/10y</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td>Caucasian</td>
<td>Caucasian</td>
<td>Italian, Puerto Rican</td>
<td>Mexican</td>
<td>Caucasian</td>
<td>Caucasian</td>
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<tr>
<td><strong>DD (motor):</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Fine motor delay</td>
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<tr>
<td><strong>Walking</strong></td>
<td>21</td>
<td>21</td>
<td>30</td>
<td>No</td>
<td>30</td>
<td>12</td>
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<tr>
<td><strong>Walking (months)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>DD (language)</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Age of 1st words</strong></td>
<td>14</td>
<td>24</td>
<td>Nonverbal</td>
<td>Nonverbal</td>
<td>48</td>
<td>22</td>
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<tr>
<td><strong>Behavioral abnormalities</strong></td>
<td>ADHD, Anxiety</td>
<td>ADHD, Anxiety</td>
<td>None</td>
<td>Inconsolable agitation</td>
<td>Aggression, self-injurious behavior, autistic features</td>
<td>ADHD, anxiety, poor sleep</td>
</tr>
<tr>
<td><strong>Intelligence Quotient (IQ)</strong></td>
<td>98</td>
<td>80</td>
<td>too young to be tested</td>
<td>too young to be tested</td>
<td>N/A</td>
<td>79</td>
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<tr>
<td><strong>Therapeutic services</strong></td>
<td>PT, OT, ST, DT</td>
<td>PT, OT, ST</td>
<td>PT, OT, ST</td>
<td>PT, OT, ST</td>
<td>PT, ST</td>
<td>IEP, OT, ST</td>
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<td><strong>Hypotonia</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Pt. 1</td>
<td>Pt. 2</td>
<td>Pt. 3</td>
<td>Pt. 4</td>
<td>Pt. 5</td>
<td>Pt. 6</td>
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</tr>
<tr>
<td>GI</td>
<td>Constipation</td>
<td>Constipation</td>
<td>Difficulty Swallowing</td>
<td>Feeding difficulties, G-tube, constipation</td>
<td>Constipation, Feeding difficulties</td>
<td>Feeding difficulties, Intermittent loose stools</td>
</tr>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Congenital anomalies</td>
<td>Ankyloglossia</td>
<td>Bilateral branchial cleft cysts</td>
<td>None</td>
<td>Brain ventriculomegaly, orbital epidermoid cyst, kyphosis</td>
<td>Right pelvic kidney, webbing of index and first fingers</td>
<td>Dacryostenosis</td>
</tr>
<tr>
<td>Broad Forehead</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ears</td>
<td>None</td>
<td>Posteriorly rotated</td>
<td>Ear notched, preauricular pit, posteriorly rotated</td>
<td>Low set, posteriorly rotated</td>
<td>Simple folding, narrow auditory canals</td>
<td>None</td>
</tr>
<tr>
<td>Nose</td>
<td>Flat nasal bridge, bulbous nasal tip</td>
<td>Flat nasal bridge, bulbous nasal tip</td>
<td>Flat nasal bridge, bulbous nasal tip</td>
<td>Flat nasal bridge, bulbous nasal tip</td>
<td>Flat nasal bridge, bulbous nasal tip</td>
<td>Flat nasal bridge, malar hypoplasia</td>
</tr>
<tr>
<td>Eyes</td>
<td>Synophris, deep-set eyes, hypertelorism, strabismus</td>
<td>Hypotelorism, strabismus</td>
<td>Synophrys, ptosis, downslanted palpebral fissures</td>
<td>Deep-set eyes, bushy eyebrows</td>
<td>Synophrys, myopia, strabismus</td>
<td>Astigmatism, myopia</td>
</tr>
<tr>
<td>Mouth</td>
<td>Thin upper lip, smooth philtrum</td>
<td>Thin upper lip</td>
<td>Down-turned mouth, thin upper lip</td>
<td>Normal</td>
<td>Full lips</td>
<td>Down-turned mouth</td>
</tr>
<tr>
<td>Other</td>
<td>Thin, sparse hair, bitemporal narrowing</td>
<td>bitemporal narrowing</td>
<td>Brachycephaly, plagiocephaly,</td>
<td>Hirsutism, growth hormone</td>
<td>Pes planus, stiff ankles, broad-based</td>
<td>Brachycephaly</td>
</tr>
</tbody>
</table>
10p Deletions

- 9 individuals with 10p11.23 deletions encompassing WAC have been reported
- 1-10.6 Mb deletions
- Dysmorphic features, hyperactivity, congenital heart defects, and developmental delay. Their facial features included synophrys, thick eyebrows, short neck, deep set eyes, bulbous nose and full cheeks.
A 306 kb a smallest region of overlap (SRO) was identified, which contained only two genes, BAMBI and WAC.
Pathogenicity of WAC Mutations

- Low population minor allele frequency (MAF)
- A *de novo* mutation (if the phenotype is sporadic in dominant disorder).
- Truncation variants (3 nonsense, 2 frame shift)
- *In silico* assessment of protein function or splicing impact and conservation (truncation; nonsense-mediated mRNA decay)
- Gene expression pattern and biological pathway analysis.
Is It a Syndrome?

- 6 patients with 5 different pathogenic variants in \textit{WAC}
- Motor and language delay, behavioral abnormalities, dysmorphic features, hypotonia, and constipation/feeding problems.
- Typical facial features encountered in our patients include a \textit{broad forehead, synophrys, depressed nasal bridge with a bulbous nasal tip, and a thin upper lip.}
- Eye findings were also frequently noted.
Who Names Newly Discovered Disorders

- In the world of genetics, OMIM (Online Inheritance in Man) often does.
- OMIM (www.omim.org) is a comprehensive, authoritative collection of human genes and genetic phenotypes that is freely available and updated daily.
- Why not after gene name? 1) one gene can cause a number of diseases 2) gene names change from time to time.
A number sign (#) is used with this entry because of evidence that DeSanto-Shinawi syndrome (DESSH) is caused by heterozygous mutation in the WAC gene (615049) on chromosome 10p11. Some patients with an overlapping phenotype have a deletion at chromosome 10p12-p11 encompassing several genes and consistent with a contiguous gene deletion syndrome.

**Description**

DeSanto-Shinawi syndrome is a rare neurodevelopmental disorder characterized by global developmental delay apparent in infancy or early childhood and associated with characteristic dysmorphic facial features, such as broad forehead, depressed nasal bridge with bulbous nasal tip, and deep-set eyes. Most patients also have gastrointestinal and mild ocular abnormalities, as well as behavioral problems (summary by DeSanto et al., 2015).

**Clinical Features**

DeSanto et al. (2015) reported 6 children, including 2 sibs, of various ethnic origins, with global developmental delay noted in the first year of life and dysmorphic features. The patients ranged from 15 months to 11 years in age at the time of the report. Two patients were non-verbal, and the others showed delayed language acquisition. Behavioral abnormalities were variable but common, and included aggression, anxiety, and attention deficit-hyperactivity disorder, and autistic features. All had hypotonia and gastrointestinal difficulties, mainly feeding difficulties and constipation. Dysmorphic features included broad, prominent forehead, flat nasal bridge with bulbous tip and flaring nostrils, hypertelorism, synophrys, and strabismus. More variable
**WAC** (WW domain-containing adapter with a coiled-coil region)

- WAC encodes a 647 aa protein (mostly in the nucleus but also Golgi)
  - **WW domain** (AAs:129–162); interacts with RNA polymerase II
  - **Coiled-coil domain** (AAs:618-644); recruits E3 ligase for ubiquitination

- Expressed in all adult and fetal tissues but highest in whole adult brain and fetal liver.
WAC Functions

• Involved in multiple cell processes including
  – Transcription elongation regulation through promoting monoubiquiquination of histone H2B at Lys123
  – Autophagosome formation (autophagy)
  – Golgi biogenesis
  – Cell-cycle checkpoint activation in response to DNA damage
WAC mediates transcription-coupled H2B ubiquitination

WAC mediates the association of RNF20/40, an E3 ligase, and two E2 ubiquitin conjugases, RAD6A and RAD6B, with RNA polymerase II (Pol II) complex

Autophagosome formation
X-linked intellectual disability type Nascimento is a clinically distinct, probably underdiagnosed entity

- X-linked UBE2A (RAD6A) cause a syndrome with some overlapping findings as seen in patients with WAC mutations including moderate to severe ID, craniofacial dysmorphism (synophrys, prominent supraorbital ridges, deep-set eyes, depressed nasal bridge), hirsutism, motor delay, impaired/absent speech, and seizures.
Case 2-History

• A newborn female of mixed Caucasian-Hispanic ancestry born at term, AGA after uneventful pregnancy & delivery.

• The mother has been followed closely because of the death of the proband’s sister at 36 hours of life of uncertain cause, but with symptoms concerning for a metabolic disorder.

• After birth, the patient required CPAP but quickly improved.

• PE: generalized hypotonia; otherwise unremarkable
Case 2-History

• 8 hours of life: respiratory distress; lactic acidosis (lactate-19.5 mM). Lactate in CSF was 3.9 mM.

• Intubated and transferred from SCN to the NICU

• IV D10 to reverse her catabolic state

• Supplementations: carnitine, thiamine, CoQ10, riboflavin, hydroxycobalalmine, and biotin.
Case 2-History

- Developed **heart failure** requiring dopamine and milrinone infusions.
- Echo: $\downarrow$ systolic function and moderate dilatation of LV. Later developed **biventricular hypertrophy**.
- Showed some stabilization and improvement after 2 wks and was gradually weaned off her pressor support.
- Exhibited recurrent episodes of metabolic and hemodynamic decompensation with intermittent elevation of lactate levels.
Case 2-History

• **Seizures** on first day of life.

• Video EEG study at 26 hours of age was remarkable for frequent **subclinical seizures and background suppression** with poor variability, consistent with a diffuse encephalopathy.

• Brain MRI showed **small cerebellar size and diffuse T2 white matter hyperintensity** and MRS ↓ N-acetylaspartic acid (NAA) and ↑ lactate peak.

• At 2 mo she decompensated and developed severe lactic and respiratory acidosis and died after her parents elected to redirect her care.
Proband’s Deceased Sister

- Born at term AGA; Apgar scores of 2 and 8, at 1 and 5 min.
- CPAP for breathing difficulties, which quickly improved.
- At 5 hours of age, increasing respiratory distress; metabolic acidosis with pH in venous blood gas at 7.31, bicarbonate of 16.6 and base excess (BE) of -8.3.
- She was transferred to the NICU in OSH.
- Stable but developed a sudden apneic/gasping episode at 30 hours age requiring intubation.
Proband’s Deceased Sister

- BG immediately after this event: pH of 6.54, bicarb of 3.5 and BE of -36.6. Lactate was 22 mM and ammonia was 285 uM.
- Echocardiography showed “poor contractility” and CXR cardiomegaly.
- Head CT was unremarkable.
- She was placed on medical cardiac support and antiepileptics.
- Died at 36 hours of life.
- Metabolic studies were not performed for this patient.
- NBS was normal.
Molecular and Metabolic Workup

- Karyotype, CMA, mtDNA seq and Mitome panel, PC, D2HGDH, IDH2, mtDNA content in muscle all were normal.

- Respiratory chain analysis (muscle): functional deficiencies in complex II (succinate dehydrogenase; 10%) and complex II/III (succinate cytochrome C reductase; 14%).

- CoQ10 level was 5.2 ug/g (16% of normal mean values).
WES Results

• 2 compound heterozygous missense mutations in the COQ4 gene.

• The first mutation, c.245T>A (p.Leu82Gln), was paternally inherited and the second, c.473G>A (p.Arg158Gln) was maternally inherited.

• Targeted mutation testing on DNA extracted from placental blocks of her sister still pending.
## Mutations in Patients with COQ4 deficiency

<table>
<thead>
<tr>
<th>Case Number</th>
<th>Mutation</th>
<th>Mutation type</th>
<th>Inheritance</th>
<th>Location</th>
<th>Depth of exome reading for COQ4 gene in proband</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>p.L82Q (het)</td>
<td>Missense</td>
<td>Paternal</td>
<td>Exon 3</td>
<td>780 of 805 bases (96.89%) covered at 10x</td>
</tr>
<tr>
<td></td>
<td>p.R158Q (het)</td>
<td>Missense</td>
<td>Maternal</td>
<td>Exon 5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>p.R240C (hom)</td>
<td>Missense</td>
<td>Maternal &amp; Paternal</td>
<td>Exon 7</td>
<td>100% covered at 10x</td>
</tr>
<tr>
<td>3</td>
<td>p.D68H (het)</td>
<td>Missense</td>
<td>Maternal</td>
<td>Exon 2</td>
<td>803 of 805 bases (99.75%) covered at 10x</td>
</tr>
<tr>
<td>4</td>
<td>p.R240C (hom)</td>
<td>Missense</td>
<td>Maternal &amp; Paternal</td>
<td>Exon 7</td>
<td>803 of 805 bases (99.75%) covered at 10x</td>
</tr>
</tbody>
</table>

Because family 2 and 4 were Ashkenazi Jewish, we screened 1047 healthy Ashkenazi Jewish adults for the Arg420Cys and determined that 7 were carriers for a carrier frequency of 0.7%. 
<table>
<thead>
<tr>
<th></th>
<th>Family 1</th>
<th>Family 2</th>
<th>Family 3</th>
<th>Family 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td>Female</td>
<td>Female</td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td>Caucasian-Hispanic</td>
<td>Ashkenazi Jewish</td>
<td>Caucasian</td>
<td>Ashkenazi Jewish</td>
</tr>
<tr>
<td><strong>Hypotonia</strong></td>
<td>+</td>
<td>n/a</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Feeding</strong></td>
<td>+</td>
<td>tye</td>
<td>tye</td>
<td>+</td>
</tr>
<tr>
<td><strong>difficulties</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lactic acidosis</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Lactate (blood)</strong></td>
<td>19.5</td>
<td>22</td>
<td>n/a</td>
<td>2.2</td>
</tr>
<tr>
<td><strong>Lactate (CSF)</strong></td>
<td>3.9</td>
<td>n/a</td>
<td>n/a</td>
<td>2.7</td>
</tr>
<tr>
<td><strong>Brain (MRI or Cerebellar</strong> hypoplasia; T2 white matter hyperintensity</td>
<td>n/a</td>
<td>n/a</td>
<td>Cerebellar and brainstem hypoplasia, and microdysgenesis</td>
<td>Cerebellar hypoplasia</td>
</tr>
<tr>
<td><strong>Cardiomyopathy</strong></td>
<td>+ Dilated/hypertrophic</td>
<td>+ Poor contractility</td>
<td>+ Hypertrophic Prenatally diagnosed</td>
<td>+ Hypertrophic</td>
</tr>
<tr>
<td><strong>Seizure</strong></td>
<td>+</td>
<td>Suspected</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>EEG</strong></td>
<td>Seizures, background suppression, poor variability</td>
<td>n/a</td>
<td>Burst suppression pattern</td>
<td>n/a</td>
</tr>
</tbody>
</table>

**Age of Death**
- Family 1: 2 mo
- Family 2: 36 hrs
- Family 3: 3 d
- Family 4: 19 mo, 10 wks, 7 wks
Coenzyme Q (CoQ) (Ubiquinone)

- Essential for the function of mitochondrial respiratory chain complexes: shuttling electrons from complex I (NADH:CoQ reductase) and complex II (succinate:coenzyme Q reductase) to complex III (CoQ:cytochrome c reductase).
Coenzyme Q (CoQ) (Ubiquinone)

- It has a quinone ring and an isoprenoid tail.
- The length of the tail of CoQ is species dependent, and in humans the tail length is 10 and therefore the CoQ is designated as CoQ10.
The Biosynthesis of CoQ

- The biosynthesis of CoQ is complex and incompletely understood, but yeast data indicate that it involves at least 12 proteins, encoded by corresponding COQ genes, all of which have mammalian homologues.
- In order to be functional, these proteins are assembled in yeast into a multi-subunit complex.
- The products of COQ genes have a stabilizing function of each component for the multi-subunit CoQ complex.
COQ4

• In yeast, *COQ4* encodes a 335 amino-acid polypeptide peripherally associate with the matrix face of the mitochondrial inner membrane.

• Mitochondrial-targeting sequence at N-terminus.

• CoQ4 protein in yeast is hypothesized to be a core component of multisubunit complex required for CoQ biosynthesis and plays an essential role in organizing and stability of CoQ biosynthetic complex.
CoQ10 Deficiency

• Mutations in genes involved in CoQ biosynthesis cause primary CoQ deficiency, severe multisystem disorders presenting as progressive encephalomyopathy and nephropathy

• Treatable condition with high dose CoQ10
COQ4 Mutations Cause a Broad Spectrum of Mitochondrial Disorders Associated with CoQ₁₀ Deficiency


A

F1: family of S1
I: 1
II: 1
II: 2
II: 3 (S1)
II: 4

F2: family of S2
I: 1
II: 1 (S1)
II: 2
II: 3 (S1)
II: 4

F3: family of S3 and S4
I: 1
II: 1 (S3)
II: 2
II: 3 (S4)
n.d.

F4: family of S5
I: 1
II: 1 (S5)
II: 2
II: 3 (S5)
II: 4

B

NM_016035 (1,597 bp)

exon

CDS

UTR

Intron

100 bp

1,000 bp

amino acid

NP_057119.2 (265 aa)

mitochondrial targeting sequence

Homo sapiens (NP_057119.2)
Mus musculus (NP_648008.1)
Caenorhabditis elegans (NP_491246.4)
Drosophila melanogaster (NP_730270.1)
Schizosaccharomyces pombe (NP_593130.1)
Neurospora crassa (XP_964516.)
All slides for Case 3 (mosaicism) were provided by Dr. Catherine Cottrell
Case 3 - Patient Indication

- 5 mo boy with congenital hemihypertrophy, capillary hemangioma, and syndactyly
- **Right hemihypertrophy** – associated with a vascular malformation most prominent on his right scalp and face but extending onto the body as well
- **Syndactyly** - bilateral 2-3 toe and right 3-4 syndactyly of the fingers
- No macrocephaly - but Brain MRI showed asymmetrically enlarged right cerebral hemisphere and the right lateral ventricle consistent with **right Megalencephaly**.
- Features are consistent with MCAP syndrome (megalencephaly capillary malformation syndrome)
- GPS - **PIK3CA** gene test (PB and buccal swab)
**PIK3CA – NGS analysis**

- No *PIK3CA* hotspots mutation
- *PIK3CA* p.P104L – 37% variant allele frequency in buccal swab and ~1% in PB
- p.P104L – reported as a somatic alteration in cancer (COSMIC database)
- Not described in MCAP / overgrowth syndromes
- Not reported in dbSNP and EVS databases
- *In silico* analysis (Condel / Polyphen)- deleterious and probably damaging
- “A mosaic variant was detected within the PIK3CA gene” (post-zygotic mutation)
Buccal swab – P104 coverage
PB – P104 coverage
SOMA Gene Set

• Clinical testing for **11 genes** at Genomics and Pathology Services at Washington University in St. Louis (GPS@ WUSTL)

• **AKT1, AKT2, AKT3, GNAQ, MTOR, PIK3CA, PIK3R2, PTEN, RASA1, TSC1** and **TSC2**

• Acceptable specimen types:
  – fresh tissue
  – formalin-fixed paraffin embedded tissue
  – buccal swab samples
SOMA NGS Methodology

- Target-hybrid Capture approach
- cDNA baits designed for exonic regions
- Minimum DNA input 50ng
- Paired-end 101bp sequencing- HiSeq
SOMA Assay Sensitivity

- Increasing sensitivity at increasing depth
- VarScan 2.3.6
- Avg unique coverage across capture space ~1300x
MCAP

• Megalencephaly-capillary malformation syndrome

• Is characterized by
  – megalencephaly (MEG) or hemimegalencephaly (HMEG) associated with neurologic findings of hypotonia, seizures, and mild to severe intellectual disability
  – cutaneous capillary malformations with focal or generalized somatic overgrowth
  – Additionally, digital anomalies (syndactyly, polydactyly), cortical malformations – most distinctively polymicrogyria (PMG), and variable connective tissue dysplasia
Characteristic brain MRI of MCAP syndrome in three individuals (A-D, E-H, and I-L). Note: Megalencephaly with a prominent forehead (A, E, I); cerebellar tonsillar ectopia with a large cerebellum and crowded posterior fossa (A, E, I); ventriculomegaly (G) and hydrocephalus (J, K, L); and bilateral perisylvian polymicrogyria (B, D, F, G, H, K, and L).

MCAP

- MCAP syndrome is one of the common overgrowth syndrome - >150 individuals have been reported
- Somatic mutations in PIK3CA have been identified - majority arise postzygotic (thus mosaic) – need to test the affected tissue or more than one tissue

Identification of PIK3CA mutations in affected cells and tissues
PI3K-AKT-mTOR pathway

**PI3K-AKT Signaling Pathway**

**PIK3CA-Related Overgrowth Spectrum (PROS)**
- Macrodactyly
- Hemihiperplasia
- Multiple Lipomatosis (HHML)
- Fibroadipose overgrowth (FAO)
- Muscle Hemihipertrophy
- Facial Infiltrating Lipomatosis
- CLOVES
- Megalencephaly - Capillary Malformation (MCAP)
- Skin disorders:
  - Epidermal nevi
  - Seborrheic keratoses
  - Benign lichenoid keratoses

**Cell cycle/apoptosis regulation, metabolism, angiogenesis**

- Proteus Syndrome (AKT1)
- Lipodystrophy syndrome - Hypoglycemia (AKT2)
- Hemimegalencephaly and Megalencephaly-polymicrosyria-polydactyly-hydrocephalus (MPPH) (AKT3)
- Bannayan - Riley - Ruvalcaba and Cowden and Type II Segmental Cowden syndrome
- Lhermitte–Duclos disease

**RTK**

**PI3K**

**PIP3**

**PI3K**

**PTEN**

**AKT**

**Thr308**

**Ser473**

**PIK3CA**

**PIP3**

**PDK1**

**mTOR2**

**mTOR1**

**TSC1**

**TSC2**

**Cell cycle/apoptosis regulation, metabolism, angiogenesis**
SCN1A and Dravet syndrome
Post-Zygotic Mutation

- Necessity of sequencing affected tissue
Figure 1. The timing of postzygotic mutation influences the distribution of mutant cells in the individual. (A) Mutations that occur during the first mitosis result in approximately half of the individual being affected. Individuals with CHILD syndrome (congenital hemidysplasia with ichthyosiform erythroderma and limb defects) have been observed with this striking pattern (see Figure 2A). (B) Mutations that occur before left-right determination can affect both sides of the individual, including one or both gonads. (C) Mutations that arise after the determination of the two sides of the embryo can be confined to only one side of the individual. Only one gonad is likely to be affected. (D) Mutations that occur after differentiation of primordial germ cells (PGCs) will be absent from somatic tissues. Thus, molecular investigations to detect such gonadal mosaicism must involve direct observation of germ cells. For males, this process is relatively straightforward, but for females it involves invasive biopsy of potentially both ovaries.

Necessity of assaying affected tissue
Phenotypic Variability

- GNAQ variant - p.R183Q
- Non-syndromic port-wine stain

- Sturge-Weber Syndrome
  - Seizures
  - Intellectual disability
  - Glaucoma

- KRAS, HRAS, NRAS variants
- Isolated nevus sebaceous

- Schimmelpenning-Feuerstein Mims (Linear nevus sebaceous syndrome)
  - Seizures
  - Intellectual disability
  - Hemimegalencephaly
  - Ocular/Skeletal abnormalities

http://epostersonline.com/acmg2016/node/1279
Common Ground: Utility of NGS

- **Cancer Somatic Profiling**
  - Admixture of tumor and normal cells
  - Sub-clonal tumor populations

- **Overgrowth Syndrome Testing**
  - Post-zygotic mutations
  - Mutations limited to affected tissue

Shared Mutational Pathways – PI3K/AKT/mTOR

Limitations in Specimen Material Available

Low Variant Allele Frequencies Observed

High Depth of Coverage Necessary

NGS Technology
Conclusions

• Our results emphasize the clinical utility of WES
• There is a significant role of the medical geneticist in the diagnostic process of patients undergoing WES
• The partnership of the clinician with the molecular laboratory can increase the diagnostic yield by 8%.
• Receiving exome results can be the beginning of a continuing exploration process rather than the end of the “diagnostic odyssey.”
Thank You