Measuring the Value of Whole Exome Sequencing – Beyond the Numbers

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Disclosure

• None
Overview of Whole Exome Sequencing

- Traditional Genetic Testing
  - Single Gene Analysis
  - Panel testing (group of genes based on condition)

- Whole Exome Sequencing
  - Analysis of Exome
    - 20,000 genes
Terminology

• Genes – The chapter
• Exons – The readable portion of the genes (The words)
• Introns – The non-read part of our genome (The white space on the page)
• Variants – The “mutations” or misspellings of the genes. Not all variants are harmful!
Which Patients Are Offered WES?

- Multiple medical problems suggestive of an underlying genetic condition
- Multiple conditions in the differential
- Those who have already had a number of unrevealing genetic tests
Key topics for discussion with the family

- Broad range of diagnostic possibilities
  - More uncertainty about possible findings
  - More than just “positive” and “negative”
- Unexpected/unintended results
  - Secondary findings
  - Non paternity
- Impact on family members
  - Carrier information
  - Secondary findings
- Privacy/Insurability
  - GINA – Genetic Information Nondiscrimination Act
Test Logistics

Blood is drawn → DNA extracted → DNA sequenced → Bioinformatics → 70,000 variants

- WES team custom filters
- 100-300 variants for manual review
- 0-10 variants selected for reporting
**CLINICAL INFORMATION SUMMARY**

Based on information from the ordering provider, the patient’s clinical features and history include ataxia, spasticity, cataplexy, vertical gaze abnormality, dysarthria, intellectual disabilities, immunodeficiency, mild splenomegaly, dysmorphic features, and cryptorchidism.

Samples were also received from the patient’s reportedly unaffected biological mother and father.

**RESULT SUMMARY**

**LIKELY CAUSATIVE VARIANTS:** None identified.

**POSSIBLY RELEVANT VARIANTS:** Seven variants of uncertain significance (VUS) were identified.

**VARIANTS IN GENES OF UNCERTAIN SIGNIFICANCE (GUS):** No reportable variants identified.

**SECONDARY FINDINGS:** No reportable variants identified.

A consultation with a genetics professional is recommended. Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Evaluation for additional clinical features and appropriate screening and/or management measures should be considered.

**RESULTS: LIKELY CAUSATIVE VARIANTS**

No variants highly suspected of causing the patient’s reported clinical features were identified. However, this does not rule out a genetic cause for the patient’s clinical history. A genetic consultation is recommended.

**RESULTS: POSSIBLY RELEVANT VARIANTS**

<table>
<thead>
<tr>
<th>Gene (Transcript)</th>
<th>Variant</th>
<th>Genomic Location</th>
<th>Condition</th>
<th>Inheritance Pattern</th>
<th>Zygosity</th>
<th>Parental Origin</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNMT1</td>
<td>c.4930G&gt;C</td>
<td>chr16:10245317</td>
<td>autosomal dominant perihellar ataxia, deafness, and narcolepsy (ADASAN) (MIM604121); hereditary sensory neuropathy type IC (HSN1) (MIM614118)</td>
<td>Autosomal dominant</td>
<td>Heterozygous</td>
<td>De Novo</td>
<td>Uncertain Significance</td>
</tr>
<tr>
<td>RHBDL3</td>
<td>c.2687A&gt;T</td>
<td>chr8:84210508</td>
<td>Immunodeficiency-15 (MIM615502)</td>
<td>Autosomal recessive</td>
<td>Heterozygous</td>
<td>Paternal</td>
<td>Uncertain Significance</td>
</tr>
</tbody>
</table>
How to define “value”? Value to whom?

• Value to the medical system
  • Cost benefit
  • Better ability to give patient-directed care, to anticipate future medical needs and avoid unnecessary interventions

• Value of a diagnosis to the family
  • Putting an end to other testing
  • Change in outcome
    • Medical care (Treatment and/or screening for associated health problems)
    • Guidance for educators
  • Removing a burden to families
    • Uncertainty
    • Hassle living without a diagnosis (filling out paperwork/requesting services)
    • Emotional
      • Guilt, shame, worry, grief, isolation/loneliness

• Research/Education Value
  • New gene discovery
  • Value of reanalyzing old data with new information
Cost benefit

• Cost of WES is $5,000-$12,000
• 25-30% diagnosis rate
• Can replace other sequencing tests
• Cannot replace some forms of testing*
  • Deletion/duplication studies
  • Biochemical testing
  • Imaging/non-genetic studies
  • *However, if a diagnosis is known, it may mean that other testing (both genetic and non-genetic) may not need to be performed.
Cost-based analysis of WES

Cost per Raw Megabase of DNA Sequence

Moores Law

NIH National Human Genome Research Institute
genome.gov/sequencingcosts


$10K $1K $100 $10 $1 $0.1
Cost-based analysis of WES

- 2017 prospective study

- 40 infants, no previous genetic testing

- 56% with positive diagnoses

*Figure 1  Diagnostic trajectory and resulting diagnostic yield and costs per patient for standard care and for integrating singleton whole-exome sequencing (WES) using three models.* In model 1, standard investigations are exhausted first, including all planned tests, resulting in an additional six diagnoses (a total of 13), and WES is performed as a last resort for patients who remain undiagnosed. In model 2, WES replaces some investigations, particularly gene sequencing tests, complex biochemical tests, and invasive tests. In model 3, WES replaces most investigations. Dx, diagnosis.
Cost-based analysis of WES

- Cost of WES continues to drop
- When used earlier in the workup, diagnosis rate appears to be higher
- Can replace the addition of many single-gene sequencing studies
- Ability to reanalyze

**Number of genes annotated in HGMD**

![Bar chart showing the number of genes annotated in HGMD from 2000 to 2015.](chart.png)
Patient Examples
Patient #1 – 20-Year-Old Male

- Primary features:
  - Seizures
  - Profound developmental delay, autism, averbal.
  - Optic nerve hypoplasia/diminished vision with possibility of septo-optic dysplasia
  - Scoliosis, Pectus carinatum, dysmorphic face
  - Hypotonia

- Extensive metabolic/genetic work up was unrevealing
- Family history noncontributory
## Likely Causative

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<thead>
<tr>
<th>Gene</th>
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<th>Zygosity</th>
<th>Origin</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR2F1 005654.5</td>
<td>257G&gt;T Cys86Phe</td>
<td>5:9290986</td>
<td>Bosch-Boonstra-Schaaf optic atrophy</td>
<td>AD</td>
<td>Het</td>
<td>De novo</td>
<td>Likely path</td>
</tr>
</tbody>
</table>
Bosch-Boonstra-Schaaf optic atrophy syndrome

- Autosomal Dominant condition
- Characterized by
  - Optic atrophy
  - Intellectual disability
  - Nonspecific dysmorphic features
  - Hypotonia
  - Seizures
  - Autism spectrum disorders

![Diagram of gene regions and mutations]

C86F
Value to the family:

• End to 20 year odyssey
  • New diagnosis of Bosch-Boonstra-Schaaf optic atrophy
• De Novo, no risk to other family members
• Patient support group
• Contact with Dr. Schaaf
Value to payers: Potential cost savings

Red = tests that would have been duplicated by WES and would not have been necessary if WES was performed.
Green = tests that were done looking for a diagnosis that one could argue would not have been done if diagnosis was known.

- Chromosome microarray analysis
- PTEN gene analysis
- Spinal fluid analysis
- EEG
- EKG
- Brain MRI
- Skeletal survey abnormal – delayed bone age
- Organic acids
- Creatine disorders panel
- Plasma lactate/pyruvate
- Screen for Smith-Lemli-Opitz syndrome
- Screen for congenital disorder of glycosylation
- Plasma amino acids
- Peroxisomal panel
- Acylcarnitine profile
- DNA testing for fragile X
- DNA testing for TGFBR1 and TGFBR2 genes
- DNA testing for UBE3 gene
- Prader-Willi/Angelman syndrome methylation assay
- Infantile spasms panel (ARX, CDKL5, FOXG1, MECP2)
- Mitochondrial DNA testing
- Chromosome analysis on skin biopsy
- Skin biopsy - Electromicroscopy was negative for presence of signs suggestive of storage disorder or ceroid lipofuscinosis
- Sweat test (for CF w/u d/t poor growth)
- Echocardiogram
Patient #2 – 17-year old female

• Main features:
  • Encephalopathy with dystonia/spasticity, intellectual disability and seizures
    • Normal growth and development until seizures began around the age of 2. Since then, worsening intellectual disability.
  • Abnormal brain MRI
    • Symmetric signal abnormality in the bilateral putamen and caudate heads, with evidence of associated necrosis
    • Per radiology note findings are indeterminate and most likely represent a long-standing toxic/metabolic abnormality, but could also be due to an old ischemic, infectious, or inflammatory insult
• Family history
  • Consanguineous union (parents are first cousins)
  • 7 siblings, including an 11 year old brother with “similar but more mild” symptoms
## Likely Causative

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<tr>
<td>SLC19A3</td>
<td>1246A&gt;G Thr422Ala</td>
<td>2:228552932</td>
<td>Thiamine metabolism dysfunction syndrome [607483]</td>
<td>AR</td>
<td>Homo</td>
<td>Mat/Pat</td>
<td>Path</td>
</tr>
</tbody>
</table>
Thiamine Metabolism Dysfunction syndrome

- Autosomal recessive condition
- c.1246A>G / p.Thr422Ala is a founder variant in Saudi Arabia
- Features include onset in childhood, often triggered by febrile illness
  - Present with confusion, seizures, external ophthalmoplegia, dysphagia, and sometimes coma and death
  - MRI findings may show bilateral lesions of the basal ganglia
- Treatment through oral thiamine and biotin
  - This slows progression, allows patients to improve, and may, if started early enough, allow for much more normal life
  - Even brain imaging was seen to be improved after treatment
  - If a patient is known to be at risk, treatment recommended prior to symptoms presenting
Value to the patient/family:

- Treatment to be started ASAP
- Risk to siblings 1 in 4
  - Testing to be recommended for all siblings
  - Treatment to be started for the 11 year old brother
Added value outside of the immediate family:

• Reduced cost of medical care/educational needs
• Better ability of affected individuals to contribute to society
Patient 3: Newborn female

- G4,P3→4, Normal pregnancy
- Baby born with contractures, aplasia of the scalp
  - Seizures began on day 1
  - Cardiomyopathy identified
- Suspected chromosomal abnormality, Adams-Oliver syndrome
- At 3 months of age, WES ordered
- Diagnosis of ALG1-related Congenital Disorder of Glycosylation
ALG1-related Congenital Disorder of Glycosylation

- Autosomal recessive
- Poor prognosis
- Features:
  - Severe neurological dysfunction including psychomotor retardation, seizures, abnormal MRI findings, joint contractures, blindness, microcephaly
  - Associated with rapid progression and early death
  - No treatment
Value to the family:

• Removed the scenario where she “recovers” from this
• Ability to make decisions about end-of-life care
  • Allowed for the main priority to be to remove her pain
  • Baby passed away within two days of the diagnosis
• The family spoke of the value of this diagnosis, even at her funeral.
  • Gave her life “meaning” in that this could prevent other family members from going through this experience
Value outside of the immediate family:

- NICU care ~$3,500/day
- Allows the medical care providers to better care for the patient
- May protect the hospital from blame by the grieving families
Patient 4: 9 year old female

- Main features:
  - Mild global delay, mild dysmorphic facial features, behavioral abnormalities
- Family history non-contributory
Results

• Identified a protein-truncating de novo variant in a gene called ZNF148
  • No information about this gene
  • No animal models

• Classified as a VUS (Variant of Uncertain Significance) in a GUS (Gene of Uncertain Significance)
Results

• 1 week later…

  • Four patients, all found on WES to have protein truncating variants in exon 9 of ZNF148
  • Not a consistent phenotype – all had intellectual disability/developmental delay, growth problems (one is normal height after growth hormone supplementation) and agenesis/thinning of the corpus callosum. Otherwise variable – two with multicystic kidneys and heart abnormalities (one of whom died at the age of 6 days). The three surviving are all female and ages 6, 11, and 7.
OMIM - Updated Entries:

New gene/phenotype relationship(s) cataloged in OMIM:

617260 GLOBAL DEVELOPMENTAL DELAY, ABSENT OR HYPOPLASTIC CORPUS CALLOSUM, AND DYSMORPHIC FACIES; GDACCF
A number sign (#) is used with this entry because of evidence that global developmental delay, absent or hypoplastic corpus callosum, and dysmorphic facies (GDACCF) is caused by heterozygous mutation in the ZNF148 gene (601897) on chromosome 3q21.
# New Results

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</tr>
</thead>
<tbody>
<tr>
<td>ZNF148</td>
<td>2334_2335del Arg778Serfs*8</td>
<td>3:124951235_124951236</td>
<td>Global developmental delay, absent or hypoplastic corpus callosum, and dysmorphic facies (MIM#617260)</td>
<td>AD</td>
<td>Het</td>
<td>De Novo</td>
<td>Likely Path</td>
</tr>
</tbody>
</table>
Value to the family:

• A diagnosis and explanation for the child’s delays and behavior problems
• May help a child with “borderline” needs obtain additional services
• Evaluation of the heart and kidneys could be recommended
Value beyond the individual patient:

- New gene discovery!
  - Given WES’ power to test many genes at once, new gene discoveries like this can happen
  - Tools exist to help laboratories/clinicians identify gene/disease relationships
    - GeneMatcher

- Ability to diagnose conditions that have few outward features or are mild/nonspecific.
- Ability to reanalyze old data
  - “Sequence once, reanalyze often”

Take home messages

• Model of genetic care and rare disease diagnosis is changing

• Whole Exome Sequencing offers testing on a scale that is far larger than traditional genetic testing

• While the cost of WES is higher than a single traditional test, that cost is coming down, and one needs to consider that it may replace/make unnecessary multiple other studies.

• The value of a diagnosis goes beyond dollars and cents.

• As technology improves, new genes are discovered, and patients are tested earlier in the process, we expect the value of WES to also improve.
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• Teresa Kruisslebrink, MS CGC
References


Questions or requests…
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