**Whole Exome Sequencing in the Clinical Sphere**

Black, white and a lot of gray.

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8 year old male with history of developmental delay, coarse features, and short stature.

Clinical Genetics: An Introduction

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Chief complaint is developmental delay and short stature:
1. How severe are these features? What is the tempo?
2. When did the features become noticeable?
3. Any loss of skills?
4. Does the child have other health concerns?
5. Has the patient been evaluated by other specialty services?
6. Are there previous imaging studies available?
7. Any environmental exposures to consider?
8. Prematurity, lead exposure, prenatal drug exposure

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History
Recurrent pregnancy loss?  
Birth defects?  
Symptoms similar to the patient’s?  
Consanguinity?  

Family history  

Pedigree Analysis  

Undetermined
Physical Exam

- Excessive Body Hair-Hirsutism
- Small toenails/fingernails
- Coarse facial features
- Short stature -3SD for age
- Heart murmur

In summary, you have a child with developmental delay, short stature and features not typical for family and notable hirsutism. No notable family history. What do you order?

- Single gene sequencing test with del/dup studies
- Gene panel (ID panel? Syndromic panel?) plus del/dup for all genes in the panel?
- CMA
- Exome Sequencing
- Whole genome sequencing

Diagnostic Work-up

High Throughput Sequencing
~1% of the genome is in protein encoding exons, containing 85% of known mutations

The exome

Introduction of the “the exome” 2010

Exome sequencing identifies the cause of a mendelian disorder

The exome in research — identification of genes for known disease in groups of patients with clinically defined phenotype
Transition to the clinic (2011):
The “n of 1” problem.

Report is in 3 sections:
- Known disease causing variants
- Possible disease causing variants
- Incidental Findings

What does the clinical exome report?

The Black: 2 week old female with microcephaly, agenesis of the corpus callosum, multiple dysmorphic features, congenital heart disease, an aplastic cystic kidney and multiple skeletal anomalies.
The Black: 8 yo male with developmental delay, frequent infections, hyper-IgE and eczema. Male sibs with developmental delay and similar facial appearance.

Yang et al, JAMA Oct 2014
- 2000 Clinical exomes sent to Baylor
- 25.2% all phenotypes (50% novel mutation in known gene)
- 36% for neurological/20.1% for non-neurological
- 4% with 2 single gene disorders
- 30% with syndromes that were molecularly defined since 2011.

Neidich et al, ACMG abstract 2013
- 788 Clinical exomes sent to GeneDx, proband + at least 1 in 77%
- 30.1% all phenotypes (better if trio than proband alone)
- 59% had variants suggestive of diagnosis but not definitive
- 10% had no reportable variant (potential or definitive)

Diagnostic yield of clinical exomes (laboratory experience)

Iglesias et al, Genetic Med June 2014
- 115 exomes sent by a single center
- 32.1% success rate
- 34% for developmental delay
- 33.5% for multiple congenital anomalies

Atwal et al, Genetic Medicine, May 2014
- 35 exomes sent by a single center
- 22.8% success
- One benign variant called pathogenic

Diagnostic yield of clinical exomes (Clinical practice experience)
Transition to the clinic:
What the clinical report won’t show you.

The White: 8 yo male with intellectual disability, dysmorphic features, short stature, and hypoplastic 5th toenails

The White: 4 yo with recurrent infections. Concern for immunodeficiency with T and B cell effects and hypogammaglobulinemia. +hemolytic anemia, now s/p splenectomy.
Because new genes are being continually identified by exome methods, the “known” genes for various syndromes are constantly changing. You may not get a diagnosis this year but re-analysis of the same data next year may reveal an answer. Most clinical laboratories offer but how often and for what duration varies between labs.

Into the future: re-analysis

Stop diagnostic odyssey — testing can take up to 6 months for results but may be faster and cheaper than gene by gene analysis depending on how long it takes to get to the answer.

Family planning — recurrence risk should be discussed at counseling and should include description of the whole spectrum of options in reproductive planning.

Treatment — in some cases, specific screening protocols or treatments can be initiated based on the knowledge of the genetic diagnosis

Positive outcomes of exome sequencing

Teaching us what we don’t know: Exome sequencing extends the phenotype of known syndromes
The Gray: 7 yo girl with seizures and significant developmental delay, as well as facial features not typical for family.

- 4 variants in 4 genes described
  - All inherited from an unaffected parent
  - EHMT1 gene associated with Kleefstra syndrome (seizures plus intellectual disability and dysmorphisms)
  - Disease previously reported as being inherited from a mosaic mother (AD). This variant was ~50/50.
  - Email with Dr. Kleefstra identified this variant in 4/4403 African Americans (not reported in clinical reports).
  - GABRG2 causes seizure disorders, usually associated with febrile seizures
  - Also inherited from asymptomatic mother
  - Less severe phenotype than patient's
  - Primary contributing variant

The gray: Variants of unknown significance

The gray: 5 month old with h/o neonatal hypotonia and hypoglycemia now with increasing hypertonia
Looking a bit closer:
hemizygous variant in AIFM1, at
position c.133_134 del
Apoptosis Inducing Factor

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hemizygous variant in AIFM1, at
position c.133_134 del
Apoptosis Inducing Factor (X-linked)

It is commonly accepted that up to 10% of children have a social father who is not their biological father.
Non-maternity can also occur in cases of in vitro fertilization.
This complicates interpretation of exome results and can be a source of embarrassment and added stress at the time of result delivery.

The gray: Non-paternity
The gray: partial diagnosis (1)
6 year old boy with partial albinism, growth failure, ADHD, frequent infections and rectal prolapse. Younger brother with albinism and autism.

OCA2 causes ocular cutaneous albinism in an AR pattern but in ~30% of Caucasians, a second hit is not identifiable.

The gray: partial diagnosis (2)
10 mo old infant with severe global developmental delay and multiple seizure types.

- SCN1A (Dravet Phenotype): normal early development with later plateau and loss of skills. Patient had no “normal” period.
- SCN2A variant is maternally inherited but other changes in this gene are associated with seizure. Mother is asymptomatic.
- ? Modifier vs benign variant? Unnamed 3rd gene?

The gray: Infant with lethal neurologic phenotype. Unexpected incidental finding also carried by a parent.
ACMG list of incidental findings to be reported for exome sequencing
### Pre-symptomatic Testing of Minors for Adult Onset Conditions

<table>
<thead>
<tr>
<th>Condition Description</th>
<th>Prior to 2013</th>
<th>2013 and after</th>
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<tbody>
<tr>
<td>Symptomatic</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Pre-symptomatic, childhood onset, treatable</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Pre-symptomatic, childhood onset, not treatable</td>
<td>Gray area</td>
<td>Yes</td>
</tr>
<tr>
<td>Pre-symptomatic, adult onset, treatable</td>
<td>No</td>
<td>Yes</td>
</tr>
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<td>Pre-symptomatic, adult onset, not treatable</td>
<td>No</td>
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</tr>
</tbody>
</table>

#### The Gray: One hit in a recessive condition.
Toddler with developmental delay and FTT.

#### Gray: One hit in a recessive condition.
One of the called variants in a myopathy gene thought by his neuro-muscle specialist to be consistent with his presentation. Call to the sequencing lab revealed coverage for exon 1 and 3 was 0% and 8 and 9 was 50% (7x). Coverage not reported on standard report.
Additional challenges for variants of unknown significance since full phenotype is not known.

Designer baby?

Other testing environments: prenatal

Brigham and Women’s Hospital:
Delivery of newborn genomic results to parents and caregivers, ethical impact and optimization of delivery.

Children’s Mercy Hospital (KC):
Exome sequencing in NICU babies with goal of results returned in 50 hours.

UCSF:
Use of exome sequencing as a method of NBS for disorders currently screened for and others that are not currently screened for, but where newborns may benefit from screening.

UNC Chapel Hill:
The researchers will sequence the exomes of healthy infants and infants with known conditions (PKU, CF, etc). Identify the best ways to return results to doctors and patients, exploring the ethical, legal and social issues.

Other testing environments: Newborn screen

Other testing environments: Adult onset/pre-symptomatic testing
List price $5000-9000
Charge to Insurance of $20,000
Varying coverage and out of pocket costs

Costs and insurance

What are the results?
What do we need to do for the patient?
What are recurrence risks?
What are the risks to the patient themselves, siblings, extended family?

After the test: return of information

Clinical genetics, next-gen

- Good history and physical is still key.
- If more than 1-2 items on your differential, consider exome (after CMA).
- Correct diagnosis requires the interaction between the lab, multiple physicians, and often researchers.
- A test done today may not reveal the answer for your patient if the gene for their condition is discovered tomorrow.
- Exome data must be revisited over time to learn the most from it.
Once per month at SLCH
6 patients per month referred by any physician in the region
Clinical exome sequencing with the option of research investigation of raw data from “negative” exomes to fuel discovery.