Genome Sequencing:
What it means to you and your patients today

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Objectives

- Understands the scope of normal and disease-associated genomic variation
- Describe essential elements of genomic testing: sequencing, variant identification, and analysis
- Understand how genomic testing may impact patient care
Disclosures

- Research and programmatic funding
  - NICHD, NHGRI
  - Giannini Foundation
  - Black and Veatch
  - Pat and Gil Clements Foundation
  - KCALSI Patton Trust
There are ~8000 genetic (inherited) diseases.
- affect 1 US child in 30
- cause 1 in 6 children’s hospital admissions
- the causative genes are known for >5000 diseases
- diagnosis often takes years
- diagnosis often impacts treatment, always impacts families

The Hard Facts
Human Genome =
6.4 billion nucleotides in pairs
Equal to typing 60 words/min x 8hrs/day x 50 years

Human Exome =
1-2% of genome
19,000 genes
Gene → Protein

DNA

Transcription start site  Introns  Exons  Transcription stop site

Promoter

Potential regulatory elements

Intron sequences removed during splicing

Transcription

Splicing

Initial transcription product

Finished transcription product containing only exons

Translation

5' UTR

Initial translation product (amino acid chain)

3' UTR

Posttranslational modification

Finished protein
Alignment
- coverage
- depth of sequencing
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<th>CGCTTTTTCCATATAGATAGAGATGAGACGGTCTTTTCTTGTGAGTCTG</th>
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</table>

Variant detection

call 129990 C>A
Human Variation < 0.5%
(0.4% from CNVs, <0.1% from SNVs)

= 16 million base pairs
NGS for clinical diagnosis

1. Sequencing
2. Alignment and Variant Detection
3. Variant Calling and Characterization
4. Analysis
Genomics Can:

- End the diagnostic odyssey
- Identify genetic diagnoses in genes for which no other test exists
- Discover new genetic disorders
- Enable powerful treatment options
- Illuminate common complex disorders
100 families with NDD

![Pie charts showing distribution of diagnoses and genotypes among families with NDD.]

**Exome Sequencing (months)**

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<th>Mean</th>
<th>Range</th>
<th>Rapid Genome Sequencing (days)*</th>
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<td>Mean</td>
<td>Median</td>
<td>Range</td>
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<tr>
<td>Symptom Onset</td>
<td>6.6</td>
<td>0-90</td>
<td>8.2</td>
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<tr>
<td>Enrollment</td>
<td>83.8</td>
<td>0-90</td>
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<tr>
<td>Molecular Diagnosis</td>
<td>95.3</td>
<td>0-90</td>
<td>107.5</td>
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Tag Archives: MAGEL2

Genome Sequencing: Exploring the Diagnostic Promise
Posted on December 16, 2014 by Dr. Francis Collins

At the time that we completed a draft of the 3 billion letters of the human genome about a decade ago, it would have cost about $100 million to sequence a second human genome. Today, thanks to advances in DNA sequencing technology, it will soon be possible to sequence your genome or mine for $1,000 or less. All of this progress has made genome sequencing a far more realistic clinical option to consider for people, especially children, who suffer from baffling disorders that can't be precisely diagnosed by other medical tests.

While researchers are still in the process of evaluating genome sequencing for routine clinical use, and data analysis continues to be a major challenge, one area of considerable promise centers on...
Genomics Can:

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6 yo with global delays, autism, craniosynostosis
Clinical course

- Diagnosed with Familial Hyperinsulinemia
- By 18 months - hospitalized 10 x for vomiting, FTT, and hypoglycemia
- Postnatal microcephaly;
- MRI - prominent sulci and enlarged vents
- Global delay (army crawl, nonverbal)
- Metopic synostosis
- Irritability – hours of screaming
- SIB
“I was … blaming myself for the diagnosis. After getting the gene result, we were able to move past that.”

BAINBRIDGE-ROPERS SYNDROME: A newly discovered, and extremely rare, genetic cause of developmental disability
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10 year old with regression, seizures and autism

* at 2, 6, and 10 years of age

Children's Mercy
KANSAS CITY
History

- Well infant - alert, vocal, good eye contact
- Seizure disorder by 6 mo
- Regression between 18 mo and 2-1/2 yrs in all developmental domains.
  - Socially withdrew
  - Gait became unsteady and wide based
- By 10 was nonverbal, spastic, wheelchair dependent, no self-care skills
Etiologic evaluation

- Sedated ABR, Brain MRI normal
- EEG demonstrated left hemisphere epileptogenic focus, atypical background activity with slowing.
- Normal karyotype, microarray, Angelman syndrome, MECP2 sequencing and deletion testing, extensive metabolic workup
X:15349984-15349985 -> C

Gene – PIGA

Classification
Category 2 frameshift

CMH MAF 2 / 2297 samples
The Phenotype of a Germline Mutation in PIGA: The Gene Somatically Mutated in Paroxysmal Nocturnal Hemoglobinuria

Jennifer J. Johnston,1 Andrea L. Gropman,2 Julie C. Sapp,1 Jamie K. Teer,1 Jodie M. Martin,2 Cyndi F. Liu,3 Xuan Yuan,3 Zhaohui Ye,3 Linzhao Cheng,3 Robert A. Brodsky,3 and Leslie G. Biesecker1,4,*

aa37 ATG
Genomics Can:

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- Identify genetic diagnoses in genes for which no other test exists
- Discover new genetic disorders
- **Enable powerful treatment options**
- Illuminate common complex disorders
6 month old – inpatient code

- Admitted after eval by developmental ped
- h/o hypotonia and ALTEs
- Inpatient code night of admission
- Work-up nondiagnostic
6 month old – inpatient code

- StatSeq: Compound het variants in *SLC25A1*
- Mitochondrial malate/citrate transporter
- 8/13 patients deceased by 8 mo of age
- Na-K-citrate treatment changed biomarkers and clinical course
**Clinical Tests**
- Whole Genome
- Custom panels
- TaGSCAN

**Research Tests**
- Whole Exome
- Whole Genome
- RNA
- Methylome
- Single cell sequencing
- Pharmacogenomic method development
Clinical Limitations and Challenges

- Expert interpretation is a bottleneck
- Integration of results into clinical care
  - Patient and clinical understanding
  - Data storage, dynamic nature of results
- Incidental findings
- WES/WGS are not stand alone tests
  - currently unable to detect med-large insertions, deletions, and rearrangements
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Monogenic disorders vs common complex traits
Tourette Syndrome
Family with multiple affected

Found two related variants of interest
  - private mutation in a nerve growth factor receptor
  - SNP in nerve growth factor
ROR1

NGF
Male NGF/ROR1 double deletion mice display a robust aberrant movement disorder

Genotype Comparison at 40 Weeks of Age

- **Male WT**
- **Male ngf+ Het**
- **Male ror+ Het**
- **Male ngf/ror Double Het**

Frequency (Hz)
0 5 10 15 20 25
Power
0.00 0.02 0.04 0.06 0.08 0.10 0.12 0.14

Inset:
- **387**
- **388**

Frequency (Hz)
0 5 10 15 20 25
Power
0.00 0.02 0.04 0.06 0.08 0.10 0.12 0.14
Male NGF/ROR1 double deletion mice show unusual home cage behaviors

Wild-type male home cage
• Visible Nest
• No food on floor

ngf/ror1 double Het male home cage
• No visible nest
• Unusual large amount of crushed food pellets on floor of cage
References

- Kononoff et al. Histamine H3 receptor regulates sensorimotor gating and dopaminergic signaling in the striatum. *J Pharmacol Exp Ther.* 2016 Mar 4


