Genetic Testing
From Basic To COMPLEX

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• I HAVE NO RELEVANT FINANCIAL RELATIONSHIPS WITH THE MANUFACTURERS(S) OF ANY COMMERCIAL PRODUCTS(S) AND/OR PROVIDER OF COMMERCIAL SERVICES DISCUSSED IN THIS CME ACTIVITY

• I DO NOT INTEND TO DISCUSS AN UNAPPROVED/INVESTIGATIVE USE OF A COMMERCIAL PRODUCT/DEVICE IN MY PRESENTATION.
Objectives

- **Knowledge**
  - List four methods of genetic testing

- **Application**
  - Practice how to choose right genetic test

- **Analysis**
  - Distinguish benefits and limitations between two genetic testing methods

- **Synthesis**
  - Propose models of integrating genetic testing into primary care practice

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Responding with Poll Everywhere

- **Web voting**
- **Text voting**

[Images of Poll Everywhere interfaces]
Knowledge

Genetic Testing Methods

- Sequencing
- Microarray
- Targeted mutation analysis
- Methylation analysis
Genetic Testing Analogies

46 chromosomes

6 billion letters
Chromosome testing is like looking for chapters in a book

Microarray testing looks closer to see if pages with the recipe(s) are missing/extra
Sequencing is like reading

- Single gene sequencing – reading one gene (one recipe)
- Multi gene panel sequencing – reading a group of genes (group of recipes) at the same time
- Exome sequencing – reading the protein coding regions of all 20,000 exons (20,000 recipes) at the same time
- Genome sequencing – reading all the protein coding and non-coding regions of genome such as exons and introns (recipes and the “blank” pages in between) at the same time
Mutation vs Variation

The cat in the hat
The cat in the zat
The cat in the mat
### ACMG Variant Classification

<table>
<thead>
<tr>
<th>Classification</th>
<th>Evidence</th>
<th>Report status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogenic</td>
<td>Mutation known to be associated with disease</td>
<td>Yes</td>
</tr>
<tr>
<td>Likely pathogenic</td>
<td>Mutation known to be associated with disease, but not sufficient evidence</td>
<td>Yes</td>
</tr>
<tr>
<td>Uncertain significance</td>
<td>Does not meet criteria to be benign or pathogenic</td>
<td>Usually</td>
</tr>
<tr>
<td>Likely benign</td>
<td>Does not meet criteria to be benign or pathogenic and functional studies argue against damaging effect</td>
<td>No</td>
</tr>
<tr>
<td>Benign</td>
<td>Variation present at greater than 5% frequency in the general population</td>
<td>No</td>
</tr>
</tbody>
</table>

### Whole Exome vs Whole Genome

<table>
<thead>
<tr>
<th></th>
<th>WES</th>
<th>WGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein coding regions of genome - 20,000 genes or 1% genome</td>
<td>Coding and non coding regions</td>
<td></td>
</tr>
<tr>
<td>96%-99% coverage of exons</td>
<td>Uniform coverage</td>
<td></td>
</tr>
<tr>
<td>85% of mutations are in the exome</td>
<td>15% mutations in intron/exon boundary or introns</td>
<td></td>
</tr>
<tr>
<td>~200,000 variations</td>
<td>2-4 million variations</td>
<td></td>
</tr>
<tr>
<td>Results in 2-4 months*</td>
<td>Results in 4-6 months*</td>
<td></td>
</tr>
<tr>
<td>Incidental findings 3%</td>
<td>Incidental findings 3%</td>
<td></td>
</tr>
</tbody>
</table>
Methylation analysis: is the gene “turned on” or “off”?

Targeted mutation analysis

Trinucleotide Repeat Disorders

A trinucleotide is a group of 3 chemical bases within a gene. Trinucleotides code for amino acids. Amino acids are the building blocks of proteins.

**TRINUCLEOTIDE**  CGG

- Normal number of repeated trinucleotide segments within a gene — Normal Protein Function.
  
  - CGG CGG CGG CGG

- Moderately expanded number of repeated trinucleotide segments — Normal Protein Function.
  
  - CGG CGG CGG CGG CGG CGG CGG

- Fully expanded number of repeated trinucleotide segments — Abnormal or Absent Protein.
  
  - CGG CGG CGG CGG CGG CGG CGG CGG CGG CGG
Non Invasive Prenatal Screening

Analyze cell free fetal DNA
- Massively parallel shotgun sequencing (MPSS)

10%-15% of cell free DNA circulating in maternal blood is from fetus (placenta)

Quantitative differences in chromosome fragments in maternal blood can be used to distinguish fetuses affected with Trisomy 13, 18, 21

Non Invasive Prenatal Screening

Testing can be done after 10 weeks

Results 7 - 10 days

Indications
- AMA
- Abnormal serum screen
- Personal or family history of aneuploidy
- Abnormal ultrasound
Non Invasive Prenatal Testing

Detection rate for trisomy 21 and 18
- 99% detection rate
- <1% false negative rate
- 0.2% false positive rate

Detection rate for trisomy 13
- 79%-92% detection rate
- <1% false positive rate

Comparing Down syndrome Screening Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Detection Rate</th>
<th>FPR</th>
<th>PPV high risk population (1/100)</th>
<th>PPV low risk population (1/500)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quad screening</td>
<td>80%</td>
<td>5%</td>
<td>17%</td>
<td>4%</td>
</tr>
<tr>
<td>Non invasive prenatal screening</td>
<td>98.6%</td>
<td>0.2%</td>
<td>91%</td>
<td>67%</td>
</tr>
</tbody>
</table>

Positive Predictive Value – dependent on the prevalence of the condition

PPV for microdeletion conditions 5%-10%
Interpretation of NIPS results

Positive results are “near diagnostic”
- should be confirmed with CVS, amnio or postnatal testing

Negative results – highly sensitive and specific but not 100%

Terminology

1 base pair
1000 bp = 1 kilobase pair
1000 kb = 1 megabase pair
1 Mb = 1 million bp

Size of Human genome
3 billion base pairs
Genetic Test Resolution

- **Karyotype**
  - ~ 4 Mb

- **FISH**
  - 40 bp – 250 kb

- **Array Comparative Genomic Hybridization**
  - 20 bp - 100 kb

- **Sequencing**
  - ATGC
  - 1 base pair
Karyotype

**BENEFITS**
- Detect
  - Deletion
  - Duplications
  - Trisomies
  - Unbalanced translocations
  - Balanced translocations
  - Inversions

**LIMITATIONS**
- Detect copy number variations ~ bigger than 4 Mb

“FISH” using Probes

**BENEFITS**
- Cell growth not required
- Rapid screening aneuploidies (24h)
- Mosaicism
- Detect known deletions

**LIMITATIONS**
- Sensitivity limited by size of probe
- Requires clinical suspicion of a condition
FISH or CHIPS?

“FISH”ING
ONE FISH AT A TIME

MICROARRAY “CHIP”
CASTING A WIDE NET

Array CGH Diagnostic Applications:
Characterization of Microdeletions

<table>
<thead>
<tr>
<th></th>
<th>Normal Chromosome</th>
<th>Large Deletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log2 Ratio</td>
<td>1.2</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>0.9</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>-0.3</td>
<td>-0.6</td>
</tr>
<tr>
<td></td>
<td>-0.6</td>
<td>-0.9</td>
</tr>
<tr>
<td></td>
<td>-0.9</td>
<td>-1.2</td>
</tr>
</tbody>
</table>
Array CGH will **not** detect balanced alterations
COPY NEUTRAL HOMOZYGOSITY;

Identical alleles at polymorphic sites

<table>
<thead>
<tr>
<th>Allele</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>2</td>
</tr>
<tr>
<td>AB</td>
<td></td>
</tr>
<tr>
<td>BB</td>
<td></td>
</tr>
</tbody>
</table>

AATCGCGTACGGTTTAGCGCAGGATGT
AATCGCGTACGGTTTAGCGCAGGATGT

Chromosome 10: 97Mb Interval Total

IDENTITY BY DESCENT
PEDIGREE
WITH HIGHEST
LEVEL OF IBD =
953 MB LCSH

MLD
Asperger syndrome
Autism, DD,
Speech Problems

Asperger syndrome, DD

Proband
IQ=60
Autism, DD,
Speech Problems

Non-dysmorphic;
IQ=70-90, but no
significant genetic issues

All: Non-dysmorphic;
IQ=70-90, but no
significant genetic issues

Application

- Practice how to choose right
genetic test

- Genereviews.org

- AAP Guidelines on Healthcare
  Supervision
  http://pediatrics.aappublications.org/
  cgi/collection/committee_on_genetics?page=1
How to interpret results?

Analysis
Components of Informed Consent

- Variable expression
- Incomplete penetrance
- Does not detect all variations
- Interpretation of negative result
- Variants of uncertain significance
- Familial implications
- Reproductive implications
- Option to participate in research
- Incidental findings
- Insurance discrimination

Points to consider

- Medical status of infant/child
  - Turn around time for results
  - Tiered genetic testing strategy vs “comprehensive”

- Specific features vs broad differential
  - Targeted mutation analysis vs whole exome

- Cost and insurance coverage
Synthesis

KU Wichita Pediatrics
Primary Genetic Evaluation Clinic

Geneticist
- Dysmorphology exam
- Review disorder-specific physical exam
- Provide disorder-specific recommendations

Pediatrician
- Disorder-specific physical exam
- Overall health assessment
- Provide overall health recommendations

Genetic counselor
- Analyze family and medical history
- Coordinate genetic testing
- Provide genetic counseling

Distant site (UAMS): geneticist location
Local site (KUSM-W): pediatrician and genetic counselor on site with patient
Challenges

National average 3.5 geneticists/million population
Kansas <1 geneticists/million population
Genetics clinics located
  - Wichita
  - Kansas City
KU School of Medicine-Wichita
Wesley Pediatric Subspecialists

Diagram:
- Referrals triaged (n = 365)
  - Seen first by pediatrician/geneic counselor (n = 115)
  - Seen first by geneticist (n = 149)
  - Further evaluation with geneticist (n = 80)
    - Positive genetic testing, referred for discussion regarding management
    - Negative genetic testing, or did not meet criteria for genetic testing, but features require further evaluation
  - Did not require evaluation with geneticist (n = 34)
  - Total scheduled with geneticist (n = 221)
    - Follow-up with genetic counselor (n = 19)
    - Follow-up with developmental pediatrics, cardiology, neurology, or endocrinology (n = 13)
    - Inconsistent information; referred back to primary care (n = 2)
Outpatient Telegenetics Clinic

Results – Patient Satisfaction with Telegenetics

Before today’s visit, what made it hard to get the specialty services your child needs?

The information I received before the visit helped me understand what was going to happen.

The equipment worked well.

The use of technology did not get in the way with being able to have a good conversation with the specialist.

Despite the obstacles to receiving care in person, I would STILL prefer to travel to see the specialist.

If you had not been able to use telemedicine to get specialty care today, what would you have done?

71 patients

All patients

- Satisfied - highly satisfied

More convenient/less gas

I really like the telemedicine, it is really nice not having to travel

I hope the other specialist begin to appreciate this technology. We live in great times!

Awesome love the people here

Thank you for providing us with wonderful care that is so easily accessible
Results – Patient Satisfaction with Pediatrician/GC

30 responses

All patients –
- Satisfied or highly satisfied

The information I received before the visit helped me understand what was going to happen

Yes, even though we didn’t have genetic doctor here I got the information we needed

The pediatrician and genetic counselor responded to all of my questions

I feel very confident in the pediatrician and counselor to handle my child’s case

I feel confident in the quality of care provided today

I feel confident in the recommendations from today’s visit

I would recommend the genetics clinic to other families

Overall I was satisfied with the visit today

Heartland Genetics Network

- KU Wichita Pediatrics Subaward to work with PCP’s and communities to
- Increase access to genetic services in remote sites
- Genetics education for primary care providers
- Support families of children with special health care needs
Review Objectives

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Resources

GeneReviews
AAP Guidelines on Healthcare Supervision
http://pediatrics.aappublications.org/collection/committee-genetics

Genetics in Primary Care
http://www.geneticsinprimarycare.org/Pages/default.aspx
THANK YOU!

Direct clinic number for appointments– 316-962-2153
Fax – 316-962-2147
Pager – 316-962-3030
Shobana.kubendran@wesleymc.com

Resources

GeneReviews

AAP Guidelines on Healthcare Supervision
- [http://pediatrics.aappublications.org/cgi/collection/committee_on_genetics?page=1](http://pediatrics.aappublications.org/cgi/collection/committee_on_genetics?page=1)
References


ACMG Board of Directors. (2012). Points to consider in the clinical application of genomic sequencing. *Genetics in Medicine, 14*(8), 759-761.