Ordering the Right Tests
Genetics in Primary Care

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Thursday, May 31
12:00 - 12:30pm Central

Time Out for Genetics Webinar Series
Presented by the Genetics in Primary Care Institute

Learning Objectives

1. Describe different types of genetic testing and review advances in testing technology
2. Demonstrate the role of genetic testing in primary care
3. Discuss ethical principles involved in genetic testing in pediatrics
### Genetic Testing

<table>
<thead>
<tr>
<th>Screening</th>
<th>vs</th>
<th>Diagnostic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testing done on a particular population</td>
<td>Testing done on individuals</td>
<td></td>
</tr>
<tr>
<td>Individuals are asymptomatic</td>
<td>Individuals are often symptomatic</td>
<td></td>
</tr>
<tr>
<td>Not designed to diagnose, simply to identify individuals at a higher risk</td>
<td>Individuals may have had a positive screening test</td>
<td></td>
</tr>
<tr>
<td>May lead to diagnostic tests</td>
<td>May lead to treatment options</td>
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### Genetic Screening Test Examples

- Family history
- Prenatal screening tests
- Newborn screening tests
- M-CHAT/M-CHAT screening test for Autism
Newborn Screening

Screening

Diagnostic

Genetic Testing in Newborn Screening

- Initial testing in newborn is verified by reflex genetic testing in some conditions
- Formal informed consent is not usually obtained
- Genetic testing may uncover such things as non-paternity
Case Study #1

- A couple has carrier screening for cystic fibrosis (CF) during the pregnancy
- The mother is found to have a common CF mutation and the father’s screening test is negative
- The baby was diagnosed with meconium ileus at birth and follow-up sweat test was positive for CF
- Further testing revealed that the father had a rare CF mutation that was not included in the screening test

Case Study #2

- A newborn infant has a positive newborn screen for CF
- Reflex genetic testing reveals two different CF mutations
- Both parents had prenatal carrier screening for CF; only the mother was found to be a carrier
- Further testing revealed that the father was not the birth father
Take Home Points: Newborn Screening

- Newborn screening test includes many genetic disorders
- Pediatricians need to understand the nature of the testing and when to refer for genetic counseling
- Ideally the parents will have discussed the implications of newborn screening testing prior to delivery

Diagnostic Genetic Testing

- Karyotype
- Fluorescence In Situ Hybridization
- FISH Image
- Microarray
Whole Genome Microarray
Array Comparative Genomic Hybridization (aCGH)

Reference DNA

Mix
Block repeated sequences

Test DNA

Hybridize

Microarray with oligonucleotides

“Bread and Butter” Microarrays

• Whole genome oligonucleotide array – 105,000 probes
• Detects deletions or duplications that are 200 kb overall and 50 kb in targeted regions (high resolution karyotype detects 2-5 Mb)
• Not able to detect translocations
Whole Genome Oligonucleotide Microarray

- Targeted areas of interest
- Whole genome coverage
- Targeted areas of interest

Comparison

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Individual FISH test</th>
<th>Whole Genome Microarray</th>
</tr>
</thead>
<tbody>
<tr>
<td>Can detect whole chromosome differences, translocations, and large deletions or duplications</td>
<td>Only looks at specific areas for deletions or duplications</td>
<td>Can detect very small duplications or deletions and high level mosaicism</td>
</tr>
</tbody>
</table>
Take Home Points: Karyotype vs Microarray

- Both karyotypes and microarrays pick up missing or added chromosomes (i.e., Turner syndrome or Down syndrome)
- Microarrays pick up smaller deletions and duplications than karyotypes
- Karyotypes pick up translocations, microarrays do not
- Neither pick up sequence changes

Case Study #3

- Newborn term infant – born to a G3P3 mom
- Initially had grunting and went to NICU
- Noted to have epicanthal folds, 5th finger clinodactyly, 3-4 syndactyly of the toes and mildly increased nuchal skin
- No clear syndrome diagnosis
- Karyotype sent and was normal
Case Study #3 - Karyotype

Case Study #3 – Follow-up

- Hypsarrhythmia dx at 6 months, treated with Keppra without recurrence
- Referred for OT, PT, and speech
- At 13 months is walking and saying “Mama” and “Dada”
- Parents note that people keep asking them if he has Down syndrome
- Referred to the genetics clinic
Case Study #3
Microarray (aCGH) Results

- Found to have a duplication of the terminal end of the long arm of chromosome 21 that was inserted into the short arm of chromosome 21

Karyotype was reviewed and the duplicated material was found to have a similar structure to an expanded satellite region.
Variants of Unknown Significance: The Dreaded VUS

With aCGH (microarrays) you may get a result which is reported out as of “unknown significance”

Comparing to parental samples may help

Looking at the specific change and its possible effect on the resultant protein may help

Bottom line: parents want to know what it means

Very important to counsel parents about VUS BEFORE testing
Newer than Microarrays

• Next Generation Sequencing
  – A massively parallel sequencing technology in which millions of overlapping “reads” of DNA sequences are done simultaneously
  – Creates an enormous amount of data to be analyzed
  – Can detect single gene mutations including nonsense, missense, splice-site, and frameshift mutations
  – Used in cardiovascular diagnosis and cancer as well as childhood syndromes

Newer than Microarrays

• Exome Sequencing
  – Now being offered clinically
  – Parallel sequencing of at least 98% of the coding sequences
  – Recommended when all other diagnostic testing has been negative
  – Cost is still prohibitive for most situations
  – Recommend sequencing the child and both parents together to address changes that are not clear
  – Findings must be confirmed using a second method
Cost

- Microarray: $1500
- Autism Panel: at least $5000
- XLID panel: $5000 - 6000
- Cardiology panels: $3200 each
- Exome sequencing: $9000

So how do you choose?

Practice Guidelines can be helpful.
Genetic Testing in Developmental Delay and Autism

- After a normal karyotype, microarrays identify an abnormality in:
  - 15% of individuals with intellectual disability who have a normal karyotype
  - 7% of individuals with autism
  - 27% of individuals with autism with additional abnormalities

- Karyotype is no longer being recommended as part of the first line testing
Clinical Genetic Testing: Patients with unexplained DD, MR, MCA, ASD*

NORMAL
- No clinically significant copy number change
- Known benign CNVs

Chromosomal Microarray
- Targeted, clinically relevant region or gene
- Backdrop region (size, gene content)

ABNORMAL

Further clinical evaluation and testing, as indicated:
- Single-gene testing
- Other molecular test panels

Variant of Uncertain Clinical Significance (VUS)

Parental samples required for clinical interpretation (FISH, Amp, G-bands, MLPA)

Follow-up Analysis:
- Parents: Confirmation of deleted or duplicated (FISH, G-band, MLPA)
- Parents: Inherited or de novo
- Recurrence risk (FISH, G-band)

Parental Result:
- Unbalanced parent, unaffected
- Balanced parent, affected
- Balanced carrier, affected
- Balanced carrier, unaffected

Final Result:
- ABNORMAL (PHES
- FAMILIAL (PHES
- ABNORMAL (PHES
- ABNORMAL (PHES

* Excludes patients with recognizable syndromes (e.g., Down syndrome), family history of a chromosomal rearrangement or multiple mendelian

Genetic Testing in Autism

Steps in genetic clinical and molecular diagnosis of ASD

Step 1. Clinical Genetic Evaluation
- Thorough family and personal history
- Physical and morphological exam (by a clinical geneticist)

Step 2. Laboratory Genetic Testing
- Complex ASD, but no recognizable genetic syndrome
- Essential ASD
- ASD and recognizable genetic syndrome
  - A-CGH/karyotype
  - Specific genetic test

Step 3. Family counselling based on genetic results

Figure 1. Proposed genetic diagnostic itinerary for autism spectrum disorders.

Genetic Testing in Developmental Delay and Autism

- Pediatricians need to decide which course of action to take:
  - refer all children with developmental delay and/or autism to genetics for pre-test counseling
  OR
  - obtain consent and refer only abnormal results

Additional Points...

- What can a pediatrician do prior to a genetics referral?
  - Most importantly, take a family history and provide accurate prenatal and birth information when available
  - Refer for developmental testing as particular developmental findings can help guide the genetic testing
  - Note any repeated illnesses
Additional Points...

• What about in a terminal or emergency situation?
  – If a child with an undiagnosed condition is dying, it may be helpful to obtain the following samples for testing
    • Skin for fibroblast culture
    • Blood in a purple top (EDTA) tube for DNA testing
    • Urine and blood for metabolic testing
    • In the absence of blood, a newborn screening blood spot filter paper can be used for metabolic testing
  – If a child is lethargic and you suspect a metabolic condition, get blood samples before treatment

Ethics of Genetic Testing in Children
2001 – AAP Committee on Bioethics

- Genetic tests require informed consent
- No carrier testing or screening in children or adolescents
- Genetic testing for adult-onset conditions generally should be deferred until adulthood
- PCPs need to provide the necessary information and counseling

Direct-to-Consumer Genetic Testing

American College of Medical Genetics (2008) — minimal requirements for direct-to-consumer genetic testing

- Knowledgeable professional ordering and interpreting
- Fully informed about what test says or doesn’t say about health
- Scientific evidence should be straightforward for the consumer
- Appropriate lab accreditation
- Privacy concerns addressed
It’s never that simple…

• False positives do occur

• It is difficult for parents to understand why a restriction is placed on their decision making rights for their children

• Pediatricians often find it difficult to consult parents on the ethical and legal implications of genetic testing

What about adult-onset conditions?

• You take a family history and find out that one of the parents has a genetic condition that does not become symptomatic until adulthood
  – What is your responsibility to the parent?
  – What is your responsibility to the child?
  – What if they are in conflict?
2011 – AAP Committee on Bioethics

• Special Article on Testing Children for Adult-Onset Genetic Diseases (E. Charlisse F. Caga-anan, JD, et al.)

• Presented cases that illustrated the complexity of the decision-making around genetic testing in adolescents for adult onset conditions

• Did not come to any clear decision or change in the recommendations from 2001

Where do you find information about genetic testing?

• [www.genetests.org](http://www.genetests.org)
  - Contains information about the condition (GeneReviews) as well as testing (GeneTests)

  - GTR is The Genetic Testing Registry and includes clinical utility and analytical and clinical validity information. The GTR also contains pharmacogenetic tests
  - The GTR was developed at the National Center for Biotechnology Information (NCBI). NCBI also hosts and supports GeneTests and GeneReviews. The GTR and the GeneTests laboratory directory will overlap for approximately 1 year, at which time the GeneTests laboratory directory will be phased out.
Take Home Points: Ethics of Genetic Testing

- Genetic testing may have implications for other family members
- Consensus opinion of the AAP is still to protect the autonomy of the child
- Therefore, for carrier testing or testing for conditions in which the onset of disease does not occur until adulthood, the decision to test a child may not rest on the parents

In Summary...

- Genetic testing changes rapidly and primary care physicians need to be aware of current testing practices
- Primary care pediatricians may encounter genetic testing results from a variety of places
- Genetic testing in children for carrier status or for adult-onset conditions should be avoided
Questions?

Thank you for your participation!

For more information, please contact
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847/434-4738

www.medicalhomeinfo.org/GPCI.aspx
Time Out for Genetics

Registration is now open for

“Genetics Evaluation, Referrals, and More — What To Do Next”

Thursday, June 28
12:00 - 12:30pm Central

https://www2.gotomeeting.com/register/930230930