Genetic testing is an important tool in the screening and diagnosis of many conditions. New technology is allowing pediatricians to not only predict or identify genetic conditions, but in many cases, identify the genetic root. It is important for pediatricians to be aware of the types of tests available and the benefits and limitations of each, in order to refer patients, answer parent questions and provide follow-up as the patient’s medical home.

There are many useful resources to support pediatricians in this process, and all of this information was presented during the webinar, “Overview of Genetic Testing and Screening,” held in October 2013. The webinar, which featured Leah Burke, MD, FAAP, FACMG, was part of the Integrating Genetics into Your Practice webinar series hosted by the Genetics in Primary Care Institute (GPCI).

The difference between diagnostic and screening genetic tests

The basic differences between genetic testing intended to screen versus diagnose include:

<table>
<thead>
<tr>
<th>Screening</th>
<th>Diagnostic</th>
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<tbody>
<tr>
<td>Testing done on a particular population</td>
<td>Testing done on individuals</td>
</tr>
<tr>
<td>Individuals are asymptomatic</td>
<td>Individuals are often symptomatic</td>
</tr>
<tr>
<td>Not designed to diagnose, simply to identify individuals at higher risk</td>
<td>Individuals may have had a positive screening test</td>
</tr>
<tr>
<td>May lead to diagnostic tests</td>
<td>May lead to treatment options</td>
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Genetic screening is done in order to predict the development of certain genetic conditions. The most basic, yet often the most informative screening method is family history. Other screenings include prenatal screening tests, newborn screening tests, and the M-CHAT screening for autism.

Types of diagnostic genetic testing

There are three primary genetic testing methods used today:

**Karyotype**

Karyotype is a process of chromosome analysis that allows cytogenetic technologists to analyze chromosomes from a patient sample to identify whole chromosome differences, translocations (rearrangements of chromosomes), and large deletions or duplications. In this process, the technician examines a patient sample (typically blood) under a microscope to photograph, arrange and analyze the material. With the aid of software, the trained technician counts and rearranges the chromosomes in size order, then analyzes them by comparing them to published idiograms.
FISH: Fluorescence in Situ Hybridization

FISH is used to test for the presence or absence of specific stretches of DNA. During this process, technicians tag the areas in question with a fluorescent dye. They then mix reference DNA with patient samples to see where they don’t match, indicating a deletion or duplication of a specific region. Key considerations include:

- FISH tests only specific areas of DNA, so the area of interest must be known
- Testing can be done on either metaphase or interphase chromosomes
- Works best for identifying deletions

Microarray

Whole Genome Microarray Comparative Genomic Hybridization (CGH) makes it possible to look for much smaller duplications or deletions within all of the chromosomes. During this process, reference DNA is mixed with the patient DNA, hybridized, and then analyzed to identify differences using thousands of oligonucleotide probes. The probes detect copy number variants (CNVs) anywhere in the genome, including CNVs as small as 500bp-15Kb in targeted locations, or 15kb to the length of a full chromosome in the rest of the genome.

In addition to CGH Microarray, Single Nucleotide Polymorphism (SNP) arrays can now be studied on the same sample. SNPs represent a single letter change in linked DNA, revealing the basis of individual variation. A SNP array can identify Loss of Heterozygosity (LOH), which is indicative of certain conditions such as cancer tumor tissues or uniparental disomy, which can cause syndromes such as Beckwith-Wiedemann, Prader-Willi, Angelman and Russell-Silver. In addition, LOH can indicate a higher risk for recessive conditions in that area of the DNA.

These three tests are the primary means for diagnostic genetic testing.
Genetic testing in pediatrics: Four case studies

Case #1 – Down syndrome with a normal karyotype
A newborn term infant presented with respiratory distress, epicanthal folds, 5th finger curving, 3-4 webbing of the toes, and mildly increased nuchal skin. There was no clear syndrome diagnosis, so the karyotype was sent and returned normal. At 13 months, the parents noted that people kept asking if he had Down syndrome. Microarray testing showed a duplication of the terminal end of the long arm of chromosome 21 within the short arm of the chromosome. This material was not apparent from the karyotype because the duplicated material had a similar structure to an expanded satellite region of the short arm of chromosome 21, which varies in the general gene pool and therefore cannot be compared to an ideogram to detect differences. However, the microarray detected the specific duplication and confirmed Down syndrome.

Case #2 – Parental consanguinity
A four-day old infant presented with severe intrauterine growth restriction, cleft palate, and unusual facial features. A whole genome microarray (CGH+SNP) was sent. Results showed no deletion or duplication, but long stretches of LOH, indicating that the phenotype was most likely caused by homozygous mutations of one or more recessive genes that had reacted together to cause the genotype. While the family history had provided no indication of paternity, the genetic testing indicated the parents were first or second degree relatives.

Case #3 – Testing for specific or common mutations
A baby was diagnosed with meconium ileus, a sign of Cystic Fibrosis (CF), at birth. Sweat chloride testing was positive for CF. The couple had had carrier screening for CF during pregnancy and the mother had been found to have a common CF mutation, however the father’s screening test was negative. The newborn screening test was also positive for CF. However, reflex testing only revealed the one mutation found in the mother. Because standard newborn screening programs use a panel of the most common mutations as a reflex test, the baby had sequencing done of the whole CF gene and was found to have a second rare mutation. The father was subsequently found to carry the rare mutation.

Case #4 – Paternity
A newborn had a positive screen for CF and subsequent reflex genetic testing revealed two different CF mutations. Both parents had had prenatal carrier screening for CF and only the mother was found to be a carrier. The parents were retested and the screen results were confirmed. The implications for paternity were discussed with the couple.
Newborn screening: The most widely used genetic screening
The standard newborn screening panel screens for 29 conditions, 28 of which are caused by genetic differences. It is truly the most widely used genetic screening. Initial testing on bloodspots looks for biochemical differences caused by the condition. In some cases, a positive chemical marker initiates automatic reflex testing. For example, in the case of a positive biochemical measurement in the CF screen, the sample would be automatically reflex tested for the most common CF mutations. In the case of automatic reflex testing, pediatricians are often notified of a positive screen result, but at that point, reflex testing is already being done. Because it is a part of a public health initiative, formal informed consent is not usually obtained. Therefore, ideally, the parents will have discussed the implications of newborn screening prior to delivery with a health care provider. In some states, however, referral for genetic follow-up testing is made through the medical home. As a result, it is important for pediatricians to understand the nature of testing and when to refer for genetic counseling.

A final method for mutation testing: Gene sequencing
- **Gene sequencing** is done by amplifying the patient DNA using non-labeled deoxy nucleotides and fluorescently labeled dideoxy nucleotides. Each time a labeled dideoxy nucleotide is incorporated, the copying stops leaving segments of various lengths ending in the fluorescently labeled nucleotides. The segments are then sorted by size using the colors and then reported out as wavelengths. This method of sequencing individual DNA strands can be very time consuming.
- **Next Generation Sequencing** allows technicians to sequence multiple – even millions of – genes simultaneously. The process can detect single gene mutations and is often used in cardiovascular and cancer diagnosis, as well as in childhood syndromes. Because of the volume of data, analysis can take some time.
- **Whole Exome Sequencing** is now being offered clinically and is recommended when all other testing has been negative. The process offers parallel sequencing of at least 98% of the coding sequences and it is recommended that sequencing is done for both the child and both parents together to address changes that are not clear. However, this process is still cost prohibitive in most situations and findings must be confirmed using a second method.

An occupational hazard: Variants of Unknown Significance (VUS)
In some cases, genetic testing technology has surpassed our clinical knowledge, allowing the discovery of occasional variations of which the significance is unknown. Comparing to parental samples and looking at the specific change and its possible effect on the resultant protein may help. However, whatever the finding, parents want to know what it means. In addition, with some methods such as whole exome sequencing, for example, you may get answers to questions you weren’t asking – for example, finding a cancer mutation in a child you were testing due to physical or developmental differences. Professional recommendations about whether and how to report VUS and unrelated findings have been developed, however, they are still being debated. Ultimately, it is very important to counsel parents about VUS and other issues prior to genetic testing.

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**Genetic testing resources**

The following online resources are available to help practitioners identify and learn about conditions, identify appropriate testing, provide information to patient families, and more.

**GeneTests.org** provides gene reviews and a summary of conditions, as well as lab and clinical directories and links to other online resources such as OMIM and gene reviews. Features include

- **Specific information on syndromes** – Type the name of a syndrome and get a list of syndromes with related tests
- **Links to Gene reviews** – Read in-depth reviews of specific genetic conditions
- **Panel directories** – Find lists of multiple gene panels available to test entire pathways as well as listings of specific labs that do those panels
- **Illustrated glossary** – Search for terms that might be helpful to understand when reviewing specific gene reviews

**Ncbi.nlm.nih.gov/omim**

- **OMIM** – Review lists of more conditions than what is covered in the gene reviews according to what is known regarding their genetic cause. OMIM also lets you search by symptom.

**Ncbi.nlm.nih.gov/gtr**

- **Genetic Testing Registry** – Find conditions and phenotypes by searching for disease names, traits, drugs, proteins, and analytes.

**Ghr.nlm.nih.gov**

- **Genetics Home Reference** – Pull 1-2-page, consumer-friendly summaries on specific conditions. This is a good resource for primary care providers to share with parents.

Images used in this fact sheet came from the Digital Media Database at the National Human Genome Research Institute.

**About the Presenters**

Dr Burke is a pediatrician, clinical geneticist, and educator. She is an active faculty member in the College of Medicine at the University of Vermont as a course director and lecturer. Through the New England Genetics Collaborative, she has led the development of an online resource for educators of children with genetic and metabolic conditions. She also serves as the Chair of the AAP Section on Genetics and Birth Defects and is a chapter author of the recent American Academy of Pediatrics publication *Medical Genetics in Pediatric Practice.*

**About GPCI**

The GPCI was established to increase primary care providers’ knowledge and skills in the provision of genetic-based services. The GPCI is a cooperative agreement between the US Department of Health and Human Services, the Health Resources & Services Administration, the Maternal & Child Health Bureau and the American Academy of Pediatrics.

For additional information on the GPCI, contact Natalie Mikat-Stevens, MPH, Manager, Genetics in Primary Care Institute, Division of Children with Special Needs, AAP, at 847/434-4738.

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