General testing is an important diagnostic tool available to primary care providers. In order to choose genetic tests, primary care providers (PCPs) must understand two basic categories of genetic variation and the types of genetic tests used to detect them. This information was presented during the webinar, “Genetic Testing in Primary Care,” held December 2013. The webinar, which featured Lee Zellmer, MS, CGC, was part of the Integrating Genetics into Your Practice webinar series hosted by the Genetics in Primary Care Institute (GPCI).

**Understanding Genetics: Basic Review**

While a PCP does not perform genetic testing, he or she must have a strong basic understanding of genetics in order to know:

- What can happen genetically to create variations
- What genetic changes or variations to look for during diagnosis
- Which tests to order
- How to interpret test results

To get started, the following is a review of key concepts:

- **DNA** – Chemical structure made up of four bases (A, C, G, and T).
  - DNA is converted into RNA and then translated into protein.
  - DNA bases are “read” in groups of three.
  - Each codon (three bases) is specific for a single amino acid.
- **Gene** – A stretch of DNA sequence needed to make a functional product. Each gene has untranslated parts that help with processing.
- **Chromosome** – A nuclear DNA strand wound tightly with proteins to form an independent structure.
- **Genome** - An individual’s complete DNA sequence, stored on 46 chromosomes.
- **Exome** – The portion of the genome that encodes proteins and gene products.

**Categories of Genetic Change: Dosage and Sequencing**

There are two primary categories of genetic change: dosage and sequencing.

**Dosage:** Correct gene dosage is critical for typical human development. When there is an “overdose” (extra genetic material), or an “underdose” (a deletion), disease may occur. Dosage disorders can affect many genes at once and can vary significantly in size.

Some dosage disorders are caused by “gene inactivation.” With inactivation, the genetic material is present, however, it has been inactivated through a chemical process called methylation and therefore, that genetic material cannot be used. While there are many normally methylated genes in everyone, methylation of genes that should normally be active can cause a dosage disorder as if it was a deletion.
Sequencing: Because the sequence within DNA provides the coding for genetic development and function, a sequence variation can cause mutation or disease. Sequence changes usually only affect one gene and most disease-causing sequence changes, or mutations, occur in the coding region, resulting in change to the protein structure.

Testing for Dosage Disorders

There are a number of tests that can be used to identify dosage disorders. Because some are better at detecting large dosage changes and others identify smaller, more pinpointed changes, a combination of tests is often necessary to make a final diagnosis.

The following is a summary of tests used to detect large dosage changes:

- **Karyotype (chromosome analysis)** - performed using microscopes to look at cells during metaphase when they are easiest to see.
- **FISH Analysis** - a targeted technique to look for the presence, absence, and/or relative location of a specific chromosomal area. (FISH is not used to "fish" for a diagnosis!)
- **Microarray CGH** - used to detect sub-microscopic dosage changes but does not look inside individual genes.

Tests used to detect small dosage changes include

- **Exon-level targeted Microarray** - can see dosage changes within exons of a specific gene. Microarray compares the amount of probe from your patient with that of a control on a particular chromosomal region.
- **Methylation Analysis** - looks at genes that have the translation mechanism physically blocked to make the gene inactive. (Also known as epigenetics.)

The following shows examples of some dosage disorders and how they can be detected.

<table>
<thead>
<tr>
<th>Name</th>
<th>Region</th>
<th>Type</th>
<th>Test to detect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Down syndrome</td>
<td>21</td>
<td>Duplication</td>
<td>Karyotype</td>
</tr>
<tr>
<td>Turner</td>
<td>X</td>
<td>Deletion</td>
<td>Karyotype</td>
</tr>
<tr>
<td>DiGeorge/VCF</td>
<td>22q11.2</td>
<td>Deletion</td>
<td>FISH/CGH</td>
</tr>
<tr>
<td>Wolf-Hirschhorn</td>
<td>4p16</td>
<td>Deletion</td>
<td>FISH/CGH</td>
</tr>
<tr>
<td>Potocki-Lupski</td>
<td>17p11.2</td>
<td>Duplication</td>
<td>FISH/CGH</td>
</tr>
<tr>
<td>Pallister-Killian</td>
<td>12p</td>
<td>Triplication</td>
<td>Karyotype</td>
</tr>
<tr>
<td>Cat-eye</td>
<td>22q11.1</td>
<td>Triplication</td>
<td>Karyotype</td>
</tr>
<tr>
<td>Steroid sulfatase deficiency (XL ichthyosis)</td>
<td>STS gene on Xp22</td>
<td>Deletion</td>
<td>FISH/CGH</td>
</tr>
<tr>
<td>Duchenne/Becker muscular dystrophy</td>
<td>Parts of the DMD gene</td>
<td>Deletion/duplication</td>
<td>Targeted array</td>
</tr>
</tbody>
</table>
Finally, the following compares the detectable range of each type of test.

**Testing for Sequence Disorders: DNA Sequencing**
DNA sequencing is considered the “gold standard” for DNA testing because it involves spelling out the specific DNA code for a pre-determined coding region.

However, DNA sequencing is not 100% accurate. Limitations include the following:
- You only get data on what you sequence (i.e., the coding region), so you can miss something if it is not in the region you select
- You can only sequence what is there (no large deletions)
- The clinical significance of many sequence variants is unknown

**Ordering and Interpreting Tests**
When ordering tests, it is important to first consider what diagnosis is likely to be found and whether change is typically found as a dosage or sequencing change. The selection of the test can then be made based on whether the location of the expected genetic change is known, and the size of that expected change. In addition, the following points should be considered:
- Most genetic diseases can be caused by either sequencing or dosage errors.
- Result reports include cytogenetic and molecular genetic nomenclature that gives detail about things such as whether there is a translocation, the testing method used, what is normal, and where the change is found.
- Not all genetic changes cause disease. In fact, there are many polymorphisms (normal variations) in the genome, in both dosage and sequence.
- In recessive conditions, you need both copies of the gene to be altered in order to show symptoms (many times testing only reveals one mutation).
Conclusions

- Karyotype, FISH, microarray, methylation testing, exon-level array, and sequencing all detect different sizes and types of mutations.
- The correct test depends on disease/gene suspected; often more than one test is required.
- Interpretation is guided by type of mutation, clinical scenario, family studies – and may be unclear despite best efforts.
- Genetic testing and interpretations may be complicated. Contact your friendly lab genetic counselor for help!

About the Presenter

Ms Zellmer is an ABGC-certified genetic counselor, having received her Master’s degree in Genetic Counseling in 2002 from the University of California, Irvine.

She spent the first years of her career coordinating a large research study, looking into the genetic causes of autism spectrum disorders. She then moved into the field of genetic testing, working as a Genetic Coordinator at what was then Genzyme Genetics, then as a clinical genetic counselor at Children’s Mercy Hospital. She returned to the laboratory in 2011 as Children’s Mercy Hospital’s first Laboratory Genetic Counselor.

In addition to overseeing and facilitating all the molecular genetic testing at her institution, she is currently focused on improving genetic test utilization at Children’s Mercy Hospital and as part of a larger consortium of pediatric institutions nationwide.

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.

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