Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
www.traineecouncil.org

TITLE: Prader-Willi and Angelman Syndromes

PRESENTER: Christina Lockwood

Slide 1:
Hello, my name is Christina Lockwood. I am an Assistant Professor of Pathology and the Medical Director of Molecular Diagnostics at Washington University School of Medicine in St Louis. Welcome to this Pearl of Laboratory Medicine on "Prader-Willi and Angelman Syndromes."

Slide 2:
As the molecular mechanism responsible for most cases of Prader-Willi and Angelman Syndromes involves abnormal genomic imprinting, a brief introduction to imprinting is important. I will then delineate the clinical features and molecular mechanisms responsible for Prader-Willi and Angelman syndromes. Conveniently, a single laboratory test can identify both Prader-Willi and Angelman syndromes. Thus, discussion of laboratory testing will be postponed until the end of this Pearl.

Slide 3:
Humans inherit two complete sets of non-sex chromosomes, one from the father and one from the mother. Genes on these chromosomes are typically expressed from both the maternal and paternal copies. However, a small minority of genes are specially marked or imprinted such that only the copy inherited from one parent is active. Whether it is the maternal or paternal copy that is active depends on the specific gene involved.

Imprinting is a reversible process established before fertilization. Significantly, imprinting does not change the primary DNA sequence, but rather utilizes heritable epigenetic marks or modifications.

Epigenetic marks include DNA methylation and histone modifications. Appropriate gene expression depends on the interaction between epigenetic marks and epigenetic regulators, such as non-coding RNAs. DNA methylation is the best-studied epigenetic mechanism for imprinting. Errors in imprinting are responsible for PWS and Angelman syndromes and molecular laboratory testing for these syndromes has been able to exploit the differential methylation found in these cases.

Slide 4:
Prader-Willi syndrome is a rare genetic disorder that was first described by Andrea Prader, Heinrich Willi, and Alexis Labhart in 1956. Both males and females are equally affected by this multi-system genetic disorder. The prevalence of PWS is approximately 1:25,000 across multiple populations. The chromosomal region responsible for PWS is called the critical region, which occurs on the long arm of chromosome 15, designated 15q11-q13.

Slide 5:
PWS is a complex, multi-system disorder and many features are nonspecific. Thus, consensus clinical criteria for diagnosis were developed to help identify patients who should undergo specialized genetic testing. Laboratory testing can readily identify greater than 98% of PWS cases.

PWS can be broadly divided into 2 distinct clinical phases. The first is severe hypotonia in infancy such that babies are described as "floppy" and exhibit poor suck. Global developmental delay begins to emerge in toddlers and children 2-6 years of age. There is later onset in early childhood of excessive eating and the gradual development of morbid obesity. Adolescents and adults exhibit reduced function of the sex organs and behavior problems, including temper tantrums and obsessive-compulsive features.

Note that the physical characteristics associated with PWS, including narrow nasal bridge, downturned mouth with thin upper lip, and overall short stature, are not included in these guidelines because they may not be observed in all patients and are frequently found in other disorders.

Slide 6:
The genetic basis of PWS is heterogeneous. In unaffected individuals, specific genes in the PWS critical region are expressed only from the paternally inherited chromosome. The maternal contribution is silenced by methylation, as indicated by the black circle on this schematic.

Approximately 70% of PWS cases are caused by a deletion encompassing the critical region on the paternally contributed chromosome. The maternal chromosome is unable to compensate because methylation inactivates these genes.

In 25-30% of PWS cases, there is uniparental disomy or UPD of the maternal contribution. UPD refers to the inheritance of two copies of a chromosome or chromosomal region from one parent and none from the other. In PWS, the presence of two maternal copies of chromosome 15q11-q13 has the same effect as deletion of the paternal critical region. There are two maternal copies of the critical region, but their methylation means that proper gene expression is overall missing.

UPD can be caused by several different mechanisms, including trisomy rescue and gamete complementation, which increase in frequency with advanced maternal age. This is likely due to long-term meiotic arrest of maternal eggs, which increases the risk of non-disjunction. Interestingly, countries where childbearing age is rising have seen a higher proportion of PWS that are caused by UPD of maternal chromosome 15.

A substantial consideration for any genetic disorder is recurrence risk. If a child with PWS is caused by paternal deletion or UPD, there is typically a less than 2% recurrence risk for siblings. Additional studies may be necessary for confirmation of recurrence risk, as described later.

An imprinting center defect is a much less commonly encountered mechanism causing PWS. These may be paternal epigenetic disruptions or specific microdeletions in the *SNRPN* gene. Depending on the imprinting center defect, it may or may not be heritable.

Slide 7:

PWS management requires a coordinated team approach by multiple specialists. Treatment is aimed at behavior modification and diet restriction to reduce central obesity. Due to their reduced basal state, individuals with PWS require only half of the calories of their unaffected peers and dietician guidance is essential.

Short stature can also be significant. If left untreated, males with PWS grow to a mean height of 5 feet, 4 inches and females grow to 4 feet, 11 inches. In 2000, the FDA approved the treatment of PWS with recombinant growth hormone. As expected, it augments adult height, but has other benefits. Recombinant growth hormone treatment also improves muscle tone and therefore motor development, enhances bone mineral density, and increases lean body mass relative to fat mass.

The reduced function of gonads is challenging for PWS adolescents undergoing puberty. These effects can be reduced by exogenous introduction of sex steroids with testosterone for males and estrogen for females.

Slide 8:

Angelman syndrome is a rare genetic disorder that was first described by Harry Angelman in 1965. Approximately 1:12,000 individuals have Angelman syndrome and males and females are equally affected. Although the same general chromosomal region, 15q11-q13, is involved, the phenotype of Angelman patients is completely different than PWS.

Slide 9:

The clinical signs and symptoms associated with Angelman syndrome can vary significantly from one patient to another. Those listed here are almost always present in Angelman syndrome patients, who frequently have a normal prenatal and birth history. During infancy, developmental delay begins to be apparent by 6-12 months. However, developmental delay is non-specific and the unique clinical features of Angelman syndrome may not become apparent for years.

Speech impairment is substantial with many patients using only a few words or lacking speech altogether. Receptive language and nonverbal communication skills are better would be expected with such severely limited speech.

The combination of two features is characteristic of Angelman syndrome. These are gait ataxia and/or shaking of the limbs plus an inappropriately happy demeanor with frequent smiling and laughter. Historically, Angelman syndrome was referred to as "happy puppet" syndrome as a result of these symptoms.

Slide 10:
Other clinical indications of Angelman syndrome found in greater than 80% of patients include delayed or disproportionately slow growth in head circumference, which is referred to as microcephaly, seizures that frequently begin before 3 years of age, and characteristic EEG patterns.

Abnormal physical features may be present in Angelman syndrome patients, such as a wide mouth with wide-spaced teeth and protruding tongue. However, they are non-specific, present in fewer than 80% of patients, and don't typically point clinicians to Angelman syndrome.

Slide 11:
The genetic basis of Angelman syndrome is even more heterogeneous than PWS. In unaffected individuals, specific genes on chromosome 15q11-q13 are expressed only from the maternally inherited chromosome. The paternal contribution is silenced by methylation, as indicated by the black circle. Note that the methylated genes are distinct from those affected by PWS.

Approximately 70% of Angelman syndrome cases are caused by a deletion in this imprinted region on the maternally contributed chromosome 15. The paternal chromosome is not sufficient because it is silenced by methylation.

In 3-7% of Angelman syndrome cases, there is uniparental disomy or UPD of the paternal contribution, analogous to the maternal UPD that can cause PWS. In Angelman syndrome, the presence of two paternal copies of chromosome 15q11-q13 has the same effect as the previously described maternal deletion. Because both paternal regions are methylated, proper gene expression is missing.

An additional 10% of Angelman syndrome cases are caused by a maternal mutation in *UBE3A* gene. These mutations typically result in premature protein truncation and can occur throughout the gene.

A much less commonly encountered mechanism causing Angelman syndrome is an imprinting center defect, which is responsible for 2-3% of cases. This defect may be a maternal epigenetic disruption or a specific microdeletion in the imprinting center. Depending on the imprinting center defect, it may or may not be heritable.

The final 10% of Angelman syndrome cases have no identifiable molecular abnormality. These individuals may have mutations in other genes, cryptic chromosomal rearrangements, or changes in regulatory elements. Elucidating the cause of these cases is an active area of investigation.

Slide 12:
Multiple methods exist for laboratory testing for PWS and Angelman syndrome. Professional guidelines advocate the use of DNA methylation analysis as an initial test. This is particularly advantageous because methylation analysis can detect *any* cause of PWS or Angelman syndrome that disrupts normal methylation and most specimens that are tested in a clinical lab are negative.

The most recent 2013 CAP proficiency testing for molecular methylation analysis of PWS and Angelman shows the breadth of techniques that are available for testing. While most laboratories utilize methylation specific-PCR, others employ multiplex ligation probe-dependent amplification or even

Southern blot hybridization. Each method has advantages and disadvantages. The ability to detect most causes for both syndromes with diverse methodology is particularly valuable.

By cytogenetic methods, large deletions can be detected by fluorescent in situ hybridization (FISH) and some types of UPD can be detected by a SNP array, which is one specific type of chromosomal microarray. FISH is an appealing assay given the rapid turnaround time; however, it will not identify UPD cases. SNP arrays may be able to detect UPD, but they have a longer turnaround time and are both cost-prohibitive and labor-intensive.

Slide 13:
Southern blot hybridization, methylation specific-PCR, and MLPA are all recommended as first-tier tests because they detect abnormal methylation.

In PWS, causes of aberrant methylation include deletion of the paternal contribution, maternal UPD, and some types of imprinting defects, which combine for >98% of PWS cases.

In Angelman syndrome, methylation is disrupted by deletion of the maternal contribution, paternal UPD, and some types of imprinting defects, which combine for >80% of AS cases.

Further resolution of the underlying molecular mechanism is important for determining recurrence risk and different methods are used for differentiation. For example, large deletions can be detected by fluorescent in situ hybridization. FISH is an appealing assay given the rapid turnaround time; however, it will not identify UPD cases. Microsatellite analysis with polymorphic DNA markers is a molecular technique that can be used to detect uniparental disomy, which is more prevalent in PWS than Angelman syndrome. Sequencing of *UBE3A* may also be required in cases of suspected Angelman syndrome where *UBE3A* mutations account for approximately 10% of cases.

Slide 14:
Practice guidelines for diagnostic testing in PWS and Angelman syndrome have been issued by European professional societies. There are different causes for these syndromes and some methods will detect only deletions or UPD. Methylation analysis is advocated as the first test because it can detect both deletions and UPD.

- If methylation is normal, PWS is highly unlikely, but Angelman syndrome is not excluded.
- If Angelman syndrome is suspected, further testing by *UBE3A* sequencing should be undertaken.
- FISH will identify large deletions, which account for a majority of both syndromes.
- If normal, DNA microsatellite analysis using specific polymorphic markers will differentiate between UPD and biparental inheritance.
- Imprinting center defects caused by a deletion may be detected by clinically available assays, but epigenetic modifications are typically available only on a research basis.

Slide 15:
Since assessment of the recurrence risk depends on the molecular mechanism causing PWS or Angelman syndrome, abnormal methylation analysis should be further investigated. This information is important for genetic counseling of both the affected nuclear family and extended family members who may be carriers.

Most genetic mechanisms for PWS or Angelman syndrome occur *de novo*. These include large deletions, UPD, and most types of imprinting defects. Since these changes are typically not heritable, they are associated with a very low recurrence risk.

In Angelman syndrome, *UBE3A* mutations can be either inherited or *de novo*. If the mutation is present in the mother, the risk to siblings of the proband is 50%.

Imprinting defects are most commonly not inherited; however, approximately 10% have an inherited imprinting center deletion. In such cases, the risk to siblings is 50%.

Slide 16: Summary

To summarize, PWS and AS are imprinting disorders of chromosome 15q11-q13.

- PWS is most commonly caused by deletion of the paternal contribution
- Angelman syndrome is most commonly caused by deletion of the maternal contribution

Molecular methylation analysis is recommended as a first-line test for both PWS and Angelman syndrome.

Establishing the underlying cause of PWS or Angelman syndrome is important for genetic counseling and assessment of recurrence risk.

- Additional testing is often required to determine the genetic mechanism
- An algorithmic approach is recommended for optimal utilization

Slide 17: References

Slide 18: Disclosures

Slide 19: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "Prader-Willi and Angelman Syndromes." My name is Christina Lockwood.