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TITLE: Biotinidase Deficiency

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Slide 1:

Hello, my name is Anna Scott. I am a biochemical genetics laboratory director at Seattle Children's Hospital. Welcome to this Pearl of Laboratory Medicine on "**Biotinidase Deficiency**."

Slide 2: Lecture Overview

For today's Pearl, I will start with background information about biotinidase including its role in metabolism and clinical features. Then we will discuss different clinical assays that can detect and diagnose the enzyme deficiency. Finally, I will touch on biotinidase as it relates to newborn screening.

Slide 3: Background

Biotinidase deficiency is an inborn error of metabolism, specifically affecting biotin metabolism. Biotin is also known as vitamin B7. Most free biotin is absorbed through the gut from food. This vitamin is an essential cofactor for four carboxylase enzymes. Biotin metabolism primarily consists of two steps- 1) loading the free biotin into an apocarboxylase to form the active form of the enzyme, called holocarboylases and 2) recycling biocytin back to lysine and free biotin after protein degradation. The enzyme responsible for loading free biotin into new enzymes is holocarboxylase synthetase. Loss of function of this enzyme can cause clinical features similar to biotinidase deficiency, typically with an earlier age of onset and greater severity. Biotinidase deficiency results in failure to recycle biocytin back to free biotin for re-incorporation into a new apoenzyme.

Slide 4: Clinical Symptoms and Therapy

Classical clinical symptoms associated with biotinidase deficiency include: alopecia, eczema, hearing and/or vision loss, and acidosis. During acute illness, hyperammonemia, seizures, and coma can also manifest. Symptoms in an untreated patient typically appear between 2 and 5 months of age. Adult on-set cases have been described with varying combinations of symptoms. There are also reports of asymptomatic adults with profound enzyme deficiency, who were only identified because newborn screening detected low activity in their children.

Typically, treatment entails a daily dose of oral biotin in the range of 5-20 mg/day; often 10 mg/day is sufficient. Therapy must be the free biotin form and early implementation can prevent all clinical symptoms. If a patient is diagnosed later in life, after the onset of hearing and vision loss, these symptoms typically do not improve with biotin therapy, but the progressive sensory loss can be stopped.

Slide 5: Inheritance and Disease Variation

Biotinidase deficiency is a genetically inherited enzymatic defect that follows autosomal recessive inheritance. The gene is encoded on chromosome 3, so both copies must carry pathogenic alterations to cause disease. The estimated incidence is 1:30,000-80,000 depending on the population. In the state of Washington, we have approximately 85,000 births per year and typically observe 1 true positive case per year. Other countries may experience much higher incidence depending on their populations. Brazil and several middle eastern countries have reported estimates as high as 1:9,000.

Classic or profound biotinidase deficiency is defined as having <10% of mean normal serum activity. Partial biotinidase deficiency is defined as enzyme activity between 10 and 30% of mean normal. Some patients who fall within this range develop symptoms while others remain asymptomatic. There is on-going debate among physicians who treat these patients about the importance or need for long-term care and oral biotin therapy for partial enzyme deficiencies.

Slide 6: Metabolic Role

We can’t have a biochemistry talk without a couple of chemical pathways. As I mentioned in the introduction, biotin is a cofactor for four enzymes essential for three different areas of basic metabolism- amino acid catabolism, lipid metabolism, and gluconeogenesis. Poor

function of these carboxylases leads to biochemical abnormalities that can be detected in blood and urine.

Within amino acid catabolism, the enzyme 3-methylcrotonyl-CoA carboxylase is responsible for converting 3-methylcrotonyl-CoA to 3-methylglutaconyl-CoA as part of leucine breakdown. Propionyl-CoA carboxylase is part of both amino acid and lipid metabolism by converting propionyl-CoA into methylmalonyl-CoA and finally succinic acid to feed the citric acid cycle. Pyruvate carboxylase is also part of lipid metabolism and converts pyruvate into oxaloacetate, again feeding carbon units into the citric acid cycle. The final carboxylase is acetyl-CoA carboxylase that is part of gluconeogenesis and reacts with acetyl-CoA to form malonyl-CoA ultimately leading to glucose synthesis. Defects in these enzymes can manifest as abnormal biochemistry with accumulation of organic acids causing acidosis and hyperammonemia.

Slide 7: Clinical testing

Testing for biotinidase deficiency is considered high complexity testing and typically goes to biochemical genetics laboratories. All of the tests that can be used to detect abnormalities associated with biotinidase deficiency are considered laboratory developed tests (LDTs). The most diagnostic assay is measuring biotinidase activity in plasma. An abnormal enzyme result typically leads to DNA sequencing for confirmation. DNA analysis can be helpful for partially reduced activities, particularly if there is a question about sample handling. There is a common variant, where an aspartic acid is changed to histidine and causes 50% loss of activity for that allele. If we revisit our model pedigree, now one parent carries the common variant, aspartic acid to histidine, and the other carries a classic null mutation. Here the total measurable enzymatic activity varies with the individual’s genotype. Our D444H carrier would have about 75% of normal activity, with full activity from the orange allele and half of normal activity from the red allele. The children would have a mixture of activities ranging from 25 to 100%. DNA sequencing can clarify the reduced enzyme activity and provide additional information for counseling the family.

Slide 8: Untargeted Evaluation

For patients not covered by a newborn screening program, presenting symptoms can be nonspecific and lead to a broader metabolic workup. Such evaluation often includes urine

organic acid analysis and plasma acylcarnitines. While these are not typically diagnostic for biotinidase deficiency, these results can be suggestive and trigger more specific enzyme analysis. On this slide, the yellow and blue highlights indicate which test would detect each compound. As mentioned earlier, the carboxylases in amino acid catabolism can lead to increased excretion of organic acid intermediates: 3-hydroxyisovaleric acid, 3-methylcrotonylglycine, propionylglycine, or 3-hydroxypropionic acid. Plasma acylcarnitine analysis can have increased C5-OH; this is 3-hydroxyisovalerylcarnitine. Note, the carnitine conjugate is a metabolite included in many newborn screening programs. Significant elevations of C5-OH with normal biotinidase activity by newborn screening may be indicative of holocarboxylase deficiency.

Slide 9: Enzyme Activity

Biotinidase enzyme activity is the most specific clinical testing for biotinidase deficiency. A UV absorption based colorimetric assay is the gold standard. Patient plasma is incubated with an artificial substrate linked to 4-amidobenzoic acid. Following incubation at 37°C, biotin is cleaved and 4-amidobenzoic acid undergoes a second reaction with excess nitrite and N-1-naphthyl-ethylene-diamine dihydrochloride to generate a mauve-colored product. The amount of product formed can be quantified based on the absorbance at 546 nm and a rate calculated from the total incubation time. Absolute values differ between labs and requires local validation of the assay to establish normal and affected ranges. Percentage of mean normal activity may be a more consistent way of comparing results between laboratories. Note, measured enzyme activity in general depends on what tissue the enzyme is expressed in. Many enzymes measured in the biochemical genetics laboratory (such as for storage diseases) require intact cells. Biotinidase is fairly robust and can be readily assayed from serum, plasma, or dried blood spots, facilitating its early incorporation into newborn screening programs. For the newborn screening, a multiplexed fluorescence-based assay was developed, and FDA approved. Diagnostic testing methods vary between laboratories and as of 2019, there is not yet commercial proficiency testing available.

Slide 10: Assay Controls

Despite the absence of official proficiency testing, there are good practices expected of all laboratories who perform this analysis. An enzyme assay should always include normal and

"deficient" controls, as well as patient blanks to test for endogenous interference. In the absence of genuine affected patients, a normal plasma sample can be heat inactivated to create a "deficient" specimen. Depending on the situation, additional controls may be helpful such as collecting a second individual at the same time as the patient for shipping and handling control. Measuring the activity of a second enzyme on the same sample can also serve as sample quality assurance. Labs rely on repeat analysis, correlation with DNA results and/or sample exchange with other biochemical genetics labs to establish and maintain testing proficiency.

Slide 11: Interference Effects

A variety of causes can lead to assay interference, manifesting as either reduced or inappropriately normal results. Some common causes of reduced activity include sample handling (excess heat or humidity), incomplete drying of the blood spot card, repeat freeze/thaw cycles, or delayed sample processing of the plasma. Clinical therapy that can cause artificially increased activity include blood transfusions or treatment with sulfa drugs. The fluorescence-based newborn screening kits are prone to different interferences including: ampicillin, bilirubin, hemoglobin, and glutathione.

Secondary biotin deficiency with normal enzyme activity has been reported in individuals who consume excessive amounts of raw eggs. The avidin protein readily binds biotin, as we know from the common use of streptavidin beads in many clinical immunoassays. On a related note, high dose biotin supplementation can cause interference with many of these bead-based assays. Patients on therapy should inform the laboratory when having other testing performed.

Slide 12: Biotinidase Deficiency is Ideal for Newborn Screening

The first guidelines for population health screening were proposed by Wilson and Jungner in 1968 and these have served as guidance for a variety of programs around the world. Newborn screening programs in the United States have used this framework to evaluate metabolic disorders for inclusion. Biotinidase deficiency is well described and easily diagnosed by enzyme analysis. Therapy is cheap and effective. Patients typically present between 2 and 5 months of age, so diagnosis in the pre-symptomatic period can prevent all clinical symptoms. The screen itself is cheap and easy to perform on a dried blood spot. We have infrastructure to test babies and connect affected individuals with health care resources to manage the disease. Multiple studies have estimated the cost-effectiveness of biotinidase screening and supported screening

of this disease. Finally, Americans have supported newborn screening and in recent years advocated for expansion of the program to include even more diseases. For all of these reasons, biotinidase deficiency screening has expanded throughout the world. All 50 states in the United States and more than 30 other countries include biotinidase enzyme activity evaluation as part of neonatal screening to facilitate early diagnosis and therapeutic intervention.

Slide 13: Main Points

Here are three main points that I hope you take away from this presentation:

1. Biotinidase deficiency is a systemic enzyme defect that results in a variety of clinical features.
2. The disease is completely treatable with oral biotin (vitamin B7) supplementation.
3. Measuring enzyme activity in plasma is the most rapid and specific means of diagnosing a patient.

Slide 14: References

1. Strovel ET, Cowan TM, Scott AI, Wolf B. Laboratory diagnosis of biotinidase deficiency, 2017 update: a technical standard and guideline of the American College of Medical Genetics and Genomics. Genet Med 2017; online only.
2. Wolf B. Biotinidase deficiency. GeneReviews. https://www.ncbi.nlm.nih.gov/books/NBK1322/ (updated June 2016).
3. Colon PJ and Greene DN. Biotin Interference in Clinical Immunoassays. J Appl Lab Med 2018;6:941-951.
4. Dobrow MJ, Hagens V, Chafe R, Sullivan T, Rabeneck L. Consolidate principles for screening based on a systematic review and consensus process. CMAJ 2018;190:E422-E429.

Slide 15: Disclosures

I work for a biochemical genetics laboratory that offers biotinidase testing as fee-for-service testing.

Slide 16: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "**Biotinidase Deficiency**."