Clinical Chemistry

# Increased Clinical Sensitivity and Specificity of Plasma Protein N-Glycan Profiling for Diagnosing Congenital Disorders of Glycosylation by Use of Flow Injection–Electrospray Ionization–Quadrupole Time-of-Flight Mass Spectrometry

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**Guest:** Dr. Miao He is Assistant Professor in the Department of Pathology and the Laboratory Medicine at the University of Pennsylvania, and Co-director at the Michael J. Palmieri Laboratory for Metabolic Diseases at Children's Hospital Philadelphia.

Bob Barrett: This is a podcast from *Clinical Chemistry,* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I am Bob Barrett.

Inborn errors of metabolism are rare diseases in which a single gene defect causes a clinically significant block in a metabolic pathway resulting either in an accumulation of substrate behind the block or a lack or deficiency of the product. Profiling metabolites in the pathway could allow for accurate and timely identification of patients who have these diseases and help physicians to devise effective treatment.

Congenital disorders of glycosylation represent one of the largest groups of such metabolic disorders. In the May 2019 issue of *Clinical Chemistry*, Dr. Miao He and her colleagues published the study on the development and validation of a plasma protein N-glycan assay using a flow injection-electrospray ionization-quadrupole time-of-flight mass spectrometry.

This new method showed increased clinical sensitivity and specificity for diagnosing congenital disorders of glycosylation over current procedures. Dr. He is currently assistant in professor in the Department of Pathology and the Laboratory Medicine at the University of Pennsylvania, and also serves as co-director at the Michael J. Palmieri Laboratory for Metabolic Diseases at Children's Hospital Philadelphia. She joins us today to talk about this new technique for metabolic profiling of the protein glycosylation pathway, one of the longest pathways in human metabolism.

So, first, doctor, let's start off with the basics. What are congenital disorders of glycosylation and why are they so important?

Dr. Miao He: Congenital disorders of glycosylation, also known as CDG, is one of the largest groups of inborn errors of metabolism, with more than 140 subtypes. Basically, these are genetic

 

defects that lead to deficiencies in making normal glycans in humans. For each CDG subtype, it is a very rare disease, but if we look at the combined frequency of all CDGs, it is not as rare. In certain populations, the prevalence of CDG could be as high as 1 in 20,000 individuals, very close to the most common metabolic disease PKU. As we know, PKU is screened in every baby born in the United States.

Why is CDG important? Because firstly, glycosylation is extremely important. Not a single cell on earth is not covered with glycans. It is one of the most important post-translational modification of proteins. For example, the common ABO blood types are decided by glycans. Secondly, almost everything that we know about protein glycosylation in humans was learned from patients with CDG.

For example, certain CDG patients are resistant to the majority of the viral infections which we learned why that is in our patients. We were be able to develop new anti-viral drugs for the general population.

Bob Barrett: How are congenital disorders of glycosylation currently diagnosed, and how does the laboratory contribute to that diagnosis?

Dr. Miao He: Currently, most genetic clinics use carbohydrate deficient transferrin test to diagnose CDG. The carbohydrate deficient transferrin test is also known as CDT test, which is a test generally used for detecting chronic alcohol abuse. So, you can imagine CDT is not a specific test for diagnosing CDG. The first CDG case was diagnosed by CDT in 1997.

More than a decade after that, we had found 35 CDG subtypes. That is on average about three discoveries a year, which is pretty impressive. But in the recent two years, by whole-exome sequencing, on average, 24 new CDG subtypes are published every year. That is one discovery every two weeks.

So, clearly, CDT is not adequate for diagnosing CDG. The whole-exome sequencing and the whole-genome sequencing are probably the way to go. But we'll have to be aware that genetic variances do not define diseases. Because of that, we developed a new semi-quantitative CDG test by metabolic profiling the plasma n-glycans. In that way, we can identify a specific blockage in the pathway and diagnose CDG at the functional level.

In our *Clinical Chemistry* paper, we found that as expected, this new test has better clinical sensitivity and specificity than the CDT test. More importantly, with these

measurements, now it is possible for clinicians to use this test to monitor the disease progression and guide the treatment for CDG.

Bob Barrett: It's interesting you mentioned treatment. Are congenital disorders of glycosylation treatable?

Dr. Miao He: Yes. A growing number of CDG subtypes are treatable by monosaccharide supplementation. Many subtypes of CDG are related to deficiencies in transporting or activating one particular monosaccharide. If we give patients the monosaccharide that is deficient in them, they can get better. For example, one exciting strategy is the galactose treatment for CDG because it is newly discovered that human cells can make almost all the monosaccharides from glucose if there is enough galactose available.

Therefore, the theory is that if we can deliver enough galactose to different tissues such as the brain, then most CDG patients may benefit from the treatment. In order to develop and optimize such therapies, we need biomarkers to guide our treatment, the n-glycan measurement could potentially serve for this purpose.

Bob Barrett: Now, that's pretty exciting news. For the past 50 years, newborn screenings have been shown to be a successful way of detecting and, now where possible, treating genetic conditions. If we diagnose and start treating a genetic disease when a baby is only a few days old, the affected baby could have a much healthier life and sometimes a completely normal life. Is it possible to use your new method in newborn screening programs?

Dr. Miao He: Technically, the short answer is yes. This method is high throughput and very robust. It is compatible with the existing techniques used in the newborn screening laboratories across the country. One important improvement of this new method comparing to the old method that I published six years ago is the simplicity of the sample processing. The old method requires two days to prepare a batch of samples. With the new method, one lab technician can process 96 samples within an hour. The variation between the batches is very small. Further, the method is also highly sensitive and it only requires 100 nanoliters of the plasma.

In theory, one drop of blood from a fingertip is enough to run this new test 400 times; therefore, it meets all the technical requirements for a newborn screening test. So, it is now doable and possible to have newborn screening for CDG.

Bob Barrett: That was Dr. Miao He. She is assistant professor in the Department of Pathology and Laboratory Medicine at the University of Pennsylvania, and co-director of the Laboratory for Metabolic Diseases at Children's Hospital at Philadelphia. She was our guest in this podcast about a new metabolic profiling technique for protein glycosylation. Her paper appears in the May 2019 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening!