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Challenges in Translating Clinical Metabolomics Data Sets from the Bench to the Bedside

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Guest: Dr. Ravinder Singh is Director of the Endocrinology Laboratory and professor of Laboratory Medicine and Pathology at Mayo Clinic in Rochester, Minnesota. Dr. Candace Ullmer is a Clinical Chemist at the Centers for Disease Control and Prevention in Atlanta as Project Lead in the Clinical Chemistry branch.

Bob Barrett: This is a podcast from *Clinical Chemistry*, sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I'm Bob Barrett. While the term metabolome was coined in 1998, and the metabolics field was considered as emerging until recently metabolic profiles have been studied in biological fluids well into the last century. A perspective article appearing in the December 2021 issue of *Clinical Chemistry*, explores the Challenges in Translating Clinical Metabolomics Data Sets from the Bench to the Bedside.

The senior author for that paper is Dr. Ravinder Singh, Director of the Endocrinology Laboratory and professor of Laboratory Medicine and Pathology at Mayo Clinic in Rochester, Minnesota. He is joined in this podcast by Dr. Candice Ulmer, a Clinical Chemist at the Centers for Disease Control and Prevention in Atlanta as Project Lead in the Clinical Chemistry branch.

And Dr. Singh, let's start with you. In a review article published already 15 years ago, Yadav found that there were over 800 topics with the suffix -omics. And that list must now be in the thousands. With your considerable experience in the area of clinical chemistry testing, what is your view on all of the -omics that are being tossed around; such as genomics, proteomics, and the focus of your paper metabolomics?

Ravinder Singh: So Bob, you know being in a clinical chemistry environment, so for the last 21 years, I live in a department where we do perform millions of chemistry testing and the technologies we use are radioimmunoassay, chemiluminescence assays, some of which are highly automated, and some are manual. But it was realized that all these reagents antibodies we used are not very specific.

So to get this specificity, to get the better signal-to-noise then we have to use alternative technologies, which is chromatography which could be HPLC, which could be gas chromatography, with and without a mass spect, but then the latest one is LC tandem mass spectrometry.

So this was our chemistry world, but with the human genome being solved, and with the next gen sequencing, which is the pretty popular term in general public now, from the number of testing we do by genetic testing as you know, the basic foundation is PCR has been exponentially increasing. The COVID testing was one of those examples that is the gold standard.

So now there is going to be integration between all of these technologies in order to discover something better for other non-COVID kind of diseases.

Candice Ulmer: Bob, I think that summed up really nicely. The only thing I would add is that each one of the -omics fields is giving us some different information. And so when we think about, especially the central dogma, and the flow that genetic information, we start off with the genomics field and with the genomics we are getting some analysis of DNA. We are seeing essentially what's possible on biochemical level, in a cellular level. And as we kind of work our way from genomics to the transcriptomics field, now we are beginning to analyze a little bit of RNA, gene expression profiling of mRNA and we're getting an idea of what's happening biochemically.

And so when we transition from transcriptomics into proteomics, now what we're seeing essentially is the study of those proteins, right. So essentially what's making all of those biological events happen, what's facilitating in catalyzing some of those chemical reactions. And so when we end specifically with the field in which our paper was written, the metabolomics fields, what we're looking at now is the end product of all of those cellular processes, right. So we're getting that upstream interactive view of these metabolites and we are seeing essentially a biochemical snapshot of what's actually happening on that cellular level.

So we see that all of these omics fields are very much so interrelated, but we're getting different information from each one of them.

Bob Barrett: Well let's get into the meat of your paper, what is targeted clinical metabolomics and how does that differ from untargeted metabolomics?

Ravinder Singh: Bob, I think again, I will just add what we were talking before was, one of the disease which most of the Americans and internationally known is called Phenylketonuria. But kids unfortunately, who are born with the disease, they can't metabolize a common metabolite or substrate you can say, phenylalanine to tyrosine.

So this conversion is done by an enzyme, which is protein, phenylalanine hydroxylase. So this protein is present but it's

not present in the native form, which is present in the general healthy public. So there's a gene present PAH gene which is making a protein but because both parents were affected, the child got a mutations for mom and dad and then the child didn't have this enzyme. So then this child cannot metabolize phenylalanine and can't convert into tyrosine and phenylalanine being a toxic metabolite or compound amino acid is very toxic to the brain and causes lot of damage.

So this is an example of where the targeted is there to our best of our knowledge we think for Phenylketonuria disease, we know the target isn't one gene, but as we knew and more and more, it could be different types of diseases, different variants, and that is where one biomarker, one gene, one protein, one metabolite, may not work it out in the long run. That is where the untargeted metabolomics and untargeted proteomics and we can see the same thing for untargeted genomics is going to open the field and as the Artificial Intelligence is becoming more and more powerful, it will help us integrating the information we get from untargeted, and eventually, then we will assume one day that now we know what we didn't know before and it's all targeted in coming decades.

Candice Ulmer: I think it was a great synopsis of the differentiation between targeted and untargeted metabolomics. The only thing that I would add is that with targeted metabolomics, we are quantitating some predefined selection of metabolites. And with that predefined amount, we can use a lot of the clinical guidelines that are present for quality assurance, quality control for those assays.

But with the untargeted metabolomics space, it's very much so in its infancy stages and as a result, we don't have a lot of those same protocols and guidelines and harmonized workflows that we do see in the targeted clinical metabolomics space, but that just means that there's a lot of untapped potential in this space. As Dr. Singh mentioned, we have applications in the untargeted space that range anywhere from biomarker discovery to screen to personalized medicine.

Bob Barrett: The increasing role of mass spectrometric based analyses in the Clinical Laboratory is apparent just about everyone, though there are issues with those methods which are often thought of as the gold standard techniques. A paper that you cite in your perspective article found that ion suppression has led to the inaccurate classification of potential biomarkers. What is Ion suppression and how do you overcome it Dr. Ulmer?

Candice Ulmer: Thank you for that question. So when we talk about ion suppression is essentially a result of the electrospray

ionization. That's Incorporated in many of our mass spectrometric assays. And so ion suppression is going to result from the presence of those more abundant colluding biomolecules that are present during that electrospray ionization process and often times those biomolecules that I'm referring to, they are introduced in your blood matrix or matrix that you're incorporating and oftentimes those biomolecules look like proteins and phospholipids.

And so, basically, when is it happening is that we see this alter patient of the droplet formation and some altering in the formation of those gaseous ions, which is going to ultimately affect our instrument sensitivity as well as our data quality.

And so, with that being in mind, there are some ways to mitigate some of the effects of ion suppression. And we see these incorporated substantially in the targeted metabolomic assays. And so for example, we see sample purification protocols being implemented. We see the use of colluding isotopic internal standards, being able to alter that chromatography, the grade after incorporating to separate out that interfering biomolecule from your analyte of interest, as well as just kind of altering some of those electrospray ion source conditions.

Ravinder Singh: So I will agree what Candice said and then in addition is that, in mass spec area we had never anticipated this as a huge problem as it turned out to be. We thought we were moving from amino acids because the antibody didn't have the specificity, but now we are finding is if you go don't do some of the layers which Candice just said, the cleanup of the sample suppression, if you don't do chromatography, then there are chances even in a gold standard methodology like mass spect, these additional Matrix can show up as an interference. So it's a double whammy that not only, it can cause an interference, but in addition, it is press your sensitivity as well.

And when you looking for a target, which is finding a needle in the haystack, that means you are against the noise of the matrix. So that means most suppression will happen.

So this is where I think of our folks, our colleagues in Clinical Chemistry would have to accept this as a reality and being with it. There's no shortcut here Bob, we would have to make sure this is a real stuff.

But then in the same thing, which we called in targeted as a matrix which can interfere, but can also be a very informative in the untargeted analysis. And that was the whole focus of the paper based upon our paper was written where Dr. Boselin was trying to find new novel biomarkers. So all those matrix can be identified as a novel biomarkers, but we have

to be very vigilant that what are we looking for, how we are annotating those signals and we have to make sure we have a way of confirming before we make it a novel biomarker or novel clinical test.

Bob Barrett: Well, finally, Dr. Ravinder, given all of the potential benefits of untargeted metabolomics data, why has it been so challenging to translate these mass spectrometrically derived biomarkers from the bench to the bedside?

Ravinder Singh: Yes Bob, I think again, a million-dollar question here is that this is a very expensive technology. Lot of money is being invested into a wonderful work, a lot of researchers in untargeted area. It's just that, so because we are trying to look for a novel biomarker which is already not known means you don't have the standard for it, you don't have the retention time for it, you don't have the internal standard, it really puts a burden on the researcher. That means not only you have to be good at analytical, sensitive, and specific, but you have to make sure this novel biomarker really correlates with the disease.

So the amount of investment and political coalition required is making this progress little bit slow than what we all going to expect. But I think we have the right tools and right talent available like Dr. Candice Ulmer, which will address all these problems and there are societies also which are regressing but the progress is going to be slow Bob, unfortunately.

Bob Barrett: And Dr. Ulmer, any closing thoughts on that?

Candice Ulmer: Sure. And I think so just summate everything that we said is that, there's a lot of untapped potential in metabolomics field and it's not until we begin kind of looking at that biomarker discovery workflow for untargeted metabolomics that we can tap into its full potential.

And so when we talk about harmonized workflows for metabolomics we are talking about the sample collection, the sample preparation, the data acquisition, data processing and biochemical interpretation. But as Dr. Singh implied, there are organizations that are very much so present and are fervently working to address many of those issues and just a couple of them include, you know, the Metabolomics Quality Assurance and Quality Control Consortium mQACC, the International Lipidomics Society, as well as the International Metabolomic Society. And so we are going to see within the next couple of years a lot of these consensus based standards that we see in targeted space, shifting, over to the untargeted space to help us address a lot of these issues like ion suppression that we see pop up in the untargeted metabolomics workflow.

Bob Barrett: Dr. Candice Ulmer is a Clinical Chemist at the Centers for Disease Control and Prevention in Atlanta, Georgia. Dr. Ravinder Singh is Director of the Endocrinology Laboratory and Professor of Laboratory Medicine and Pathology at Mayo Clinic in Rochester Minnesota. They have been our guests in this podcast on Translating Clinical Metabolomics Data Sets from the Bench to the Bedside.

I am Bob Barrett. Thanks for listening.