Clinical Chemistry

Clinical Utility of Transcriptome Sequencing: Toward a Better Diagnosis for Mendelian Disorders

Article:
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Clinical Utility of Transcriptome Sequencing:Toward a Better Diagnosis for Mendelian Disorders.
Clin Chem 2018;64:882-4.
http://clinchem.aaccjnls.org/content/64/6/882

Guest: Dr. Madhuri Hegde is VP and Chief Scientific Officer of Global Laboratory Services and an adjunct professor of genetics and pediatrics at Emory University and at Georgia Tech.

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.

The June 2018 issue of *Clinical Chemistry* published a Perspective article titled, "Clinical Utility of Transcriptome Sequencing: Towards a Better Diagnosis for Mendelian Disorders." In it, the authors Drs. Sumant Chakravorty and Madhuri Hegde discussed the impact of findings by Cummings and colleagues, who have shown for the first time in 2017 the clinical utility of next generation sequencing based transcriptome sequencing to increase molecular diagnostic yield by sequencing RNA.

Dr. Madhuri Hegde is the Vice President and Chief Scientific Officer of Global Laboratory Services and also an adjunct professor of genetics and pediatrics at Emory University and Georgia Tech. She is our guest in this podcast. So, doctor, can you provide of an overview of how RNA sequencing is now facilitating the next generation DNA sequencing boom in clinical diagnostics for genetics disease, or how might it in the future?

Madhuri Hegde: So, DNA sequencing really has come a long way. We started doing Sanger sequencing in the 1990s and then came along the next generation sequencing revolution. Because of that, we have been able to sequence a significant amount of genes in clinical diagnostic laboratories in a resourced setting, not only at a rapid phase, but as a substantially less cost.

But with that, what has happened really is that we have increased the burden of variants of unknown significance. And by that, I mean variants which we simply cannot interpret today. We do not know whether these are changes which do not cause disease, or do they cause disease? And this is where RNA sequencing comes into play. We need to go to the next level now to understand the effect of that variation on the gene.

So, the goal here really is to reduce the burden of variants of unknown significance that have been identified through this large amount of sequencing or the next generation sequencing we have done. So, in the Cummings et al paper, essentially what they have done is they have done RNA-seq to resolve the splicing defects due to intronic variation or variation that is in between the two exons or the flanking boundaries of an exon and an intron.

The other thing to think about here is that not just we want to resolve the variants of unknown significance in a clinical setting, but also to establish a better variant and a gene association at a functional level. And what do I mean by that? Many of these genes now have been identified just recently. We really don't know the true malrotation spectrum of these genes. We have a lot to learn about these genes.

So, doing DNA and RNA-seq is going to help us better establish the evidence of these genes and their ability to cause disease or that particular syndrome they have been published to be associated with.

Bob Barrett: Besides splicing, do you think RNA sequencing has the ability to resolve cases in the clinic?

Madhuri Hegde: Absolutely. So, the way I look at RNA sequencing is really a tiered approach. The understanding of the structure of the DNA and then looking at the transcript which we use in the laboratory, and then looking at what defect we are trying to look at. So, it could be a splicing defect, it could be a deep intronic change. It could be a change within the exon as well.

So, what we are proposing is a tier approach, beyond splicing, we can also look at the exon usage level, the patterns of the expression of the different isoform for the different transcripts based on tissue-based expression, gene expression, and then the global transcriptome profile, which can really give us a deep insight into the variant we are trying to resolve, the gene, and also the disease.

Bob Barrett: Along with RNA sequencing, are there other functional "-omics" platforms coming along to advance the clinical diagnostic field?

Madhuri Hegde: So, it's really amazing with what is going on in clinical diagnostics especially in molecular clinical diagnostics. We are now seeing next generation sequencing having a huge impact, both at the DNA level and hopefully soon at the RNA level. But there are many other technologies or -omics platforms, as we call it, that are going to come into the clinical world, epigenomics, proteomics, metabolomics. So,

let's just take the example of metabolomics because that has already made its way into clinical diagnostics.

There are now facilities which are using mass spec-based approaches to look at the metabolomics patterns for different diseases especially where diseases that there could be some unique signatures which we could look at. So, definitely, we are now moving from not just doing RNA-seq to a more functional or mixed platform which is very exciting.

Bob Barrett: Dr. Hegde, do you see RNA sequencing platforms becoming major tools in the clinic for faster and more accurate diagnosis of both genetic and non-genetic disorders when muscle biopsies, such as for neuromuscular disorders, are not available?

Madhuri Hegde: So, when we are talking of genetic diseases, we are really talking of a variety of genetic diseases which are operating in all systems in our body. In the Cummings et al paper and then in our editorial, we have really talked about the neuromuscular disorders. Neuromuscular disorders at the RNA-seq level are a little bit more easier. And by that, I actually mean that the target tissue can be made available that the individual undergoes muscle biopsy and it can be sent to the laboratory for RNA-seq because that is the target tissue we should be using for doing RNA-seq.

Now, it does not so much apply for some of the brain disorders where you cannot just get a brain biopsy for that. So, if we start looking at how can we apply RNA-seq to a variety of other genetic diseases, we have to start thinking of other ways of doing it.

So, for example, when cord blood storage, in many cases now, cord blood storage is attempted. And in those cases, we can use the mesenchymal stem cells which can be reprogrammed into any tissue type. So, the idea really is that, can we look at a variety of different approaches to use for RNA-seq?

Now, some genes which cause brain disorders could be expressed in blood and we can use a straightforward blood-driven approach, where we take a new blood sample and do the RNA-seq. But in some other cases, we might have to use some other approaches such as using the mesenchymal stem cells.

Bob Barrett: How do you see RNA sequencing data from research settings getting merged with clinical diagnostics for a better diagnosis of patients?

Madhuri Hegde: So, this is really exciting. I think as I see out to the future and the vision and there we are actually going with these combinatorial approaches of using DNA, RNA, and then when I said, the metabolomics. What can we do to bring these all together? So, the bottom line here is that, we have to develop strategies where we can use these three data sets together to help the patient and especially those individuals who have an undiagnosed disease.

So, we could be looking at specific biomarkers to sort of create a signature. This could also help us do gene discovery, but also develop gene therapy approaches using the RNA-seq evidence. And what do I mean by that? Because we are just doing the diagnostic testing, how is that going to help in gene therapy? Lately, we talk a lot about the CRISPR/Cas9 system which can be used to target a specific mutation.

So, really, what we are trying to get to is not just a mutation at the DNA level but understanding its effect at the RNA level and then add the protein level as well. There are significant RNA-seq consortia efforts that are going out right now to create reference datasets, such as the GTEx effort or the other personalized databases.

So, the idea really is that we take this RNA-seq data and it's again a piece of data that needs to be now collaborated and merged will all the other datasets to help the patients. And I think that the potential here is tremendous. We are on that path right now. It's going to take some time where we have a system which we all can use in diagnostic laboratories but we are definitely on that path to create that system.

Bob Barrett: Well, finally, doctor, based on what we now know, what is your strategy pipeline in the clinic for identifying patients' disease causing variants and their effects for a complete and efficient molecular diagnosis?

Madhuri Hegde: So, I have run a clinical diagnostic laboratory for over 20 years now, and just seeing the transition as we have come from just doing basic genotyping assays to starting to sequence the genes fully to doing gene panels, then whole exome sequencing, and now whole genome sequencing. We already have harnessed the power of the next generation technology. What we really want to do is now go to that next level and create a pipeline which can be complimentary for each other.

And that's where RNA-seq comes in. My own research interests are in neuromuscular disorders and the Cummings et al paper also talks about using RNA-seq. If we can combine these two approaches, which are really

complimentary to each other, there's a whole exome or whole genome sequencing with the RNA-seq, I think we can create a very powerful pipeline for identifying not just the defect in the gene, but also creating a complete map of what happens when that mutation is identified in the gene at the DNA level to the gene expression level with the RNA-seq data and then looking at the protein as well.

So, these are all complimentary approaches and we can combine them together to sort of make a more complete and efficient molecular diagnostic strategy.

Bob Barrett: Dr. Madhuri Hegde is the Vice President and Chief Scientific Officer of Global Laboratory Services and an adjunct professor of genetics and pediatrics at Emory University and at Georgia Tech. She has been our guest in this podcast from *Clinical Chemistry* on the clinical utility of transcriptome sequencing. I'm Bob Barrett. Thanks for listening.