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When a "Disease-Causing Mutation" Is Not a Pathogenic Variant

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Guest:
Dr. Yiping Shen is R&D Co-Director and Medical Director of Claritas Genomics, and an Assistant Professor at the Department of Pathology and Laboratory Medicine at the Harvard Medical School.

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

The clinical utility of molecular genetic testing relies on an accurate and comprehensive knowledge about the relationships between genes and variants, and diseases. Correctly interpreting the clinical significance of variants that may be detected continues to be a constant challenge for molecular diagnostic practice. This challenge has become substantially enlarged as next generation sequencing-based testing becomes rapidly integrated into routine clinical practice.

A perspective article in the June 2014 issue of *Clinical Chemistry* examined the situations where a disease causing mutation is not a pathogenic variant.

One of the authors of that article, Dr. Yiping Shen, joins us in this podcast. Dr. Shen is R&D Co-Director and Medical Director of Claritas Genomics, and an Assistant Professor at the Department of Pathology and Laboratory Medicine at the Harvard Medical School.

Dr. Shen, about two years ago you and your colleagues wrote a perspective for *Clinical Chemistry* talking about the coming of age of exome clinical testing, you called it a transformative practice in molecular diagnostics. How is this field moving forward since then?

Dr. Yiping Shen: This field is moving very fast since the proof of principle studies that I mentioned in my last perspective. Next-generation sequencing-based exome clinical testing has been adopted in some clinical laboratories as a new exploratory testing for patients with complicated clinical presentations.

The results are very encouraging and exciting. Exome sequencing revealed causal variant in disease genes in about 25% of patients. Certainly, this new practice is still at its infancy stage, many more laboratories are right now

actively validating and incrementing the assay for routine clinical utilization.

The promising future of this powerful and useful tool has hastened the birth of a new generation of molecular diagnostic laboratories, such as Claritas Genomics, which is positioned to best utilize the power of next-generation sequencing technologies, partnering with experts, specialists, and researchers in genomics and genetics to offer a front to end comprehensive service to a much broader patient population.

I must say, we have stepped into the future practice mode of medicine. Exome sequencing testing is a very complicated testing. In our last perspective article we discussed the technical challenges that we face in validating the assay at the clinical setting.

In the recent perspective we discussed a significant clinical issue regarding gene mutation and the data interpretation. These are a few examples of challenges that we face during our efforts in implementing clinical exome sequencing in CLIA certified laboratories.

But it is certain that exome clinical testing will soon take the center stage in the molecular diagnostic arena and it will be the main tool in the clinical laboratories for solving diagnostic mysteries for many patients.

Bob Barrett: So specifically doctor, how are you and your colleagues at Claritas dealing with the challenges that you discussed in assay validation for clinical exome sequencing?

Dr. Yiping Shen: Okay. Let me put my R&D Director’s hat on. Indeed, validating an exome sequencing assay using next-generation sequencing technology is a lot more complicated than validating single gene assay using traditional Sanger sequencing.

It is due to the dramatically increased complexity and heterogeneity of the targeted gene [*unintelligible*], which often leads to highly variable sequence results from one run to another and one region to another.

Incomplete coverage of some targeted regions with less than 100% of the sensitivity for in-built variants are a few of current limitations of today’s next-generation sequencing technology.

This problem will not be completely solved any time soon, but it is important for us to know these intrinsic problems. Having this in mind, we are now able to validate a platform

by using a standard reference sample recommended by the National Institute of Standards and Technology.

This referenced sample includes a set of true variants. We first define disease specific regions of interests or a broader disease group. Our capture method targets all coding exomes of the genes, intra-exome boundaries and the regulatory regions that are known to harbor mutation.

We evaluate the sequence performance for the defined region of interest, and we design experiments that can generate the data to assess sensitivity, specificity, and the reproducibility.

Given the fact that we are still at a very early stage of this new practice, the supporting technologies are imperfect, yet are quickly changing. Each technical platform has its own unique performance characteristics and we believe that the key to be successful in NGS based testing is to take advantage of strengths and avoid the weaknesses of different platform.

We believe a combined approach will enable us to better capture all the real variants, improve the test sensitivity, and at the same time minimize the false positive rate, that they improve the specificity and reduce the confirmation burden.

This means that we will be confident in the results that we issue to the healthcare professionals who order testing from Claritas, so that they will be confident in the information they are giving to the patient.

Bob Barrett: In your recent perspective entitled ‘When a Disease-Causing Mutation Is Not a Pathogenic Variant’ you discussed other important aspects of exome clinical testing. Could you please elaborate on your main points?

Dr. Yiping Shen: Sure! Now, I am taking off my R&D hat and I am putting on my Medical Director’s hat. Whole-exome sequencing interrogates all genes in the genome, about 20,000 of them in total, for a possible cause of patient's genetic disorder. Currently, one-fifth of those 20,000 genes in the human genome have been reported to be associated with human mutation disorders. But the causal relationship between the gene and the genetic disorders is a lot more complicated than a one-to-one relationship or a yes-no relationship.

In particular, in the recent perspective article we discussed the challenge in clinical interpretation of whole-exome sequencing due to the incomplete and sometimes incorrect knowledge about a gene disease relationship.

Based on the new allele frequency information from large population level sequencing projects, we are able to appreciate that a significant fraction of previously claimed gene disease relationships are not true. This incorrect information has significant consequences; patient might receive a wrong diagnosis if we did not recognize and fix the problem.

A wrong diagnosis not only denies patient’s opportunity for identifying the true, real causes, but misses the opportunity to see the right specialist and to get the right treatment, but also could impact this diagnosis and management of family members who test negative or positive for the false positive variants.

Bob Barrett: Well, finally Dr. Shen, could you please tell us what you are doing in the diagnostic lab to avoid problems related to that issue?

Dr. Yiping Shen: Yeah, this is a problem for the whole medical genetics and even the whole medical community. Currently, there is no single database that is rigorously curated to reach a clinical standard. Curating the whole medical exome demands an organized team effort by integrating the knowledge from experts of genetics, medicines, statistics, informatics, and functional biology. This is not an easy task, but it is extremely necessary.

Currently, there is no standard gene curating algorithm developed by any professional society. Before a consensus algorithm and our database became available, clinical laboratories like Claritas are developing gene curating algorithms and databases based on generally agreed upon principles.

For example, we integrate evidence from disease incidence, allele frequency, genetic studies, animal model, and other functional evidence, as well as informatics prediction. We started this effort on our disease group, where we have a commanding knowledge about and a strong interest in.

We believe the effort in gene curation is a key aspect of our work, because it helps to ensure we deliver the most accurate results for patients who have their samples tested at the Claritas.

The next-generation of diagnostic labs will be responsible for more than just taking the sample, doing the test and turning out results. Due to the testing possibilities and the mountain of results that are being generated, diagnostic labs of the future will be responsible for guiding, partnering with the healthcare providers through the genetic testing process.

The new wave of genetic diagnostic labs will position themselves in the operative medical genomics discipline and will take responsibility for helping the health provider, healthcare provider choose the right test, then interpret the results with the end goal of correctly diagnosing the patient, and then managing the next step in the patient’s care.

Bob Barrett: That was Dr. Yiping Shen, R&D Co-Director and Medical Director of Claritas Genomics and an Assistant Professor at the Department of Pathology and Laboratory Medicine at the Harvard Medical School. He has been our guest in this podcast from *Clinical Chemistry.*

I am Bob Barrett. Thanks for listening!