Clinical Chemistry

# Exome and Whole-Genome Sequencing as Clinical Tests: A Transformative Practice in Molecular Diagnostics

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**Guest:**
Dr. Yiping Shen is Medical Director of the Genetic Diagnostic Laboratory at Boston Children's Hospital and an Assistant Professor at the Department of Pathology, Harvard Medical School.

Bob Barrett: This is the podcast from *Clinical Chemistry*. I am Bob Barrett. Next-generation sequencing has revolutionized the way researchers interrogate the genetic causes of rare single gene disorders. More recently, next-gen sequencing has been rapidly moving into the clinical diagnostic arena and transforming the practice of molecular diagnostic testing.

In a Perspective article published in the November 2012 issue of *Clinical Chemistry*, Dr. Yiping Shen and his colleagues in Boston and Shanghai explored a whole exome and whole genome sequencing as clinical tests.

Dr. Shen is Medical Director of the Genetic Diagnostic Laboratory at Boston Children's Hospital and an Assistant Professor at the Department of Pathology, Harvard Medical School. He is our guest on this podcast.

Dr. Shen, in your Perspective article you discuss the transformative effect of next-gen sequencing-based genetic testing, particularly exome and whole genome-based test on the molecular diagnostic practice.

Could you please elaborate on what are the fundamental differences between conventional Sanger sequencing-based test and next-gen sequencing-based test?

Dr. Yiping Shen: Sure. What I mean by the transformative effect of next-gen sequencing in diagnostic laboratory is that next-gen sequencing has not just brought us a powerful new way of sequencing genes, most importantly, it has brought new approach on how we access patients, or the other way around, how patients can access the diagnostic testing, and how we perform tests and how we interpret the results.

Many patients can undergo DNA testing now with next-gen sequencing while they were not appropriate for conventional sequencing, because doctors did not have a reasonable clinical diagnosis for them and we did not know which gene to test for for Sanger sequencing.

So next-gen can now offer answers for patients without a clear clinical diagnosis, and that has opened doors for more patients that were previously not suitable for DNA test sequencing.

Next-gen sequencing has also brought many changes in the way we do assay validation in the lab, the way we do data analysis, data interpretation, and the way we report the finding to the referring physicians.

Next-gen has significantly changed the infrastructure and the personal composition of molecular diagnostic laboratories. For instance, bioinformatics became an essential key component of the laboratory and large data storage space and computing power, also essential in order to perform next-gen based testing.

In terms of clinical utility, next-gen sequencing will significantly improve the positive detection rate of tests, because many genes are tested simultaneously, as in the case of gene panel-based test, or all genes are interrogated in the case of whole exome or whole genome sequencing.

The paper that I mentioned in this Perspective reported 20-40% of positive detection rate for a diverse group of diseases, which is quite remarkable.

After initial investment on sequencing machine and assay validation, the per gene cost of a next-gen sequencing test is much lower than Sanger-based single gene test, and we also anticipate faster turnaround time, because much of the process can be automated.

The most significant difference though between next-gen and conventional test is that for exome and whole genome sequencing-based test have a very strong discovery component; it has the possibility of detecting causal variants in genes that are not previously known to be associated with the disease.

So, many changes brought by next-gen sequencing are transforming the molecular diagnostic practice.

Bob Barrett: Now Dr., you mentioned there were panels of test and whole exome and whole genome-based next-gen sequencing tests. What are the important differences among these?

Dr. Yiping Shen: Currently many next-gen based tests are being developed to test for a particular group of diseases that share similar clinical features, or a disease with very complicated underlying genetic causes that involve many different genes. For example, there are more than 200 different genetic loci responsible for hearing loss and currently more than 80 genes are known to cause hearing loss.

In this case a gene panel-based test that includes all known hearing loss genes will be very helpful and appropriate to test for a hearing loss patient.

If a patient was tested negative using such a panel, it is likely that he or she may be carrying an unknown gene, in this case whole exome, whole genome is more appropriate given that there is strong evidence to suggest a genetic cause for the patient's disorder.

Gene panel test is considered as targeted test. It requires relative certain clinical diagnosis. Whole exome, whole genome are designed to test for all known genes, although clinical diagnosis and detailed clinical information are very useful for a variation, but not absolutely required to start for testing.

Currently, gene panel test covers every base of the targeted genes in the panel. It is not possible for whole exome or whole genome sequencing to cover every base of all genes. These are their differences.

Bob Barrett: What are the major benefits of doing whole exome, whole genome sequencing in comparison to gene panel or single gene-based tests?

Dr. Yiping Shen: So, conventional molecular testing starts with a clinical diagnosis of a patient and then a possible responsible gene was suspected. When the result of molecular test identified a causal mutation, the clinical diagnosis is confirmed. If the test does not confirm the clinical diagnosis, the ordering physician will often continue to predict another possible responsible gene.

Now, with whole exome, whole-genome sequencing, we can start testing the patients without a clinical diagnosis in a possible responsible gene as long as there is a strong evidence to suggest that that patient's condition is genetic.

Most excitingly, whole exome sequencing, whole-genome sequencing are possible to discover unexpected cause of the disease or the patient condition. Or the result of a whole exome, whole genome can lead to correct the initial clinical diagnosis and subsequently change patients' treatment plan.

This is the most exciting aspect of next-gen sequencing-based test and this aspect makes the test extremely valuable for patient care.

Bob Barrett: Dr., what are the main challenges of developing and implementing whole exome, whole-genome sequencing-based test in the clinical laboratory?

Dr. Yiping Shen: Yes, there are several major challenges in developing and implementing next-gen-based test in diagnostic laboratory.

First, validating a next-gen-based test is much more complicated than validating a single gene Sanger-based test. There are a lot of more variables to control for and more quality control metrics to consider.

Second, the next-gen sequencing generates huge data. It requires much strong computing power and informatics infrastructure and know-how.

Third, we are dealing with several magnitudes more numbers of variants. It is very challenging to interpret their clinical significance for every one of them. It is challenging to know their significance individually and it is even more challenging to know them as a whole or in combination what their significance is. I think we have a long way to go in that front.

Bob Barrett: Well, since next-gen sequencing procedures are so powerful and the cost of performing next-gen-based tests will certainly continue to drop, what do you think the utility of Sanger sequencing will be in the diagnostic lab of the future?

Dr. Yiping Shen: Indeed, next-gen sequencing will become the main workhorse soon for many diagnostic laboratories, just as Sanger sequencing is now for most of the current laboratories. Since Sanger sequencing is the most widely used and the best established, best accepted sequencing technology, it is being used as the gold standard for detecting small sequence variance, for validating new platform. For that reason I think we will also use Sanger sequencing for confirming variants detected by next-gen sequencing in the laboratory.

In addition, Sanger sequencing will continue to be useful for targeted sequencing testing, such as for specific mutation tests for family members where there is no need for large-scale data generation in order to see that variant. I am sure Sanger sequencing will stay with us in the laboratory for quite some time.

Bob Barrett: Well, let's continue to look ahead. Since the whole genome

 

sequencing will be able to detect more genomic changes, including copy number variations, what do you think of the future of array-based test in the molecular diagnostic labs?

Dr. Yiping Shen: So currently exome sequencing data can be used to detect gene-level copy number change. It is believed that whole genome sequencing will be able to detect intragenic copy number change, as well as other kind of structural arrangements, such as inversing and the translocation.

This is very exciting, and for that reason, it is very possible that next-gen sequencing will replace array-based technology. It has happened for gene expression profiling analysis, where RNA-Seq, which is based on next-gene is replacing expression array.

This could also happen to array for copy number detection, but currently the cost of doing array for copy number is much lower than using next-gen sequencing. Array-based test will be used in the diagnostic laboratory for some time until next-gen sequencing-based approach is well established and the cost for doing whole genome sequencing become comparable to array test.

Bob Barrett: Well, finally, Dr., when do you believe that whole exome, whole genome next-gen-based test will be commonplace in medical laboratories?

Dr. Yiping Shen: I think this is happening already and it is a gradual process, and we are actively developing whole genome, whole exome sequencing. And the society, the whole medical community, will need time to prepare for this and to absorb, to take this as a routine practice, but I think this momentum is huge and it's coming quite rapidly.

Bob Barrett: Dr. Yiping Shen is Medical Director of the Genetic Diagnostic Laboratory at Boston Children's Hospital and an Assistant Professor at the Department of Pathology, Harvard Medical School. He has been our guest in this podcast from *Clinical Chemistry.*

I am Bob Barrett. Thanks for listening!