Next-Gen Sequencing: Expanded Genetic Testing in the Real World

Marshall L. Summar, MD, Brendan C. Lanpher, MD, Sean E. Hofherr, MD | September 15, 2014

Editor's Note: *Marshall L. Summar, MD, is Chief of the Division of Genetics and Metabolism and the Margaret O'Malley Chair of Molecular Genetics at Children's National Medical Center in Washington, DC. He is an internationally recognized expert in translational studies with an emphasis on developing clinical applications based on molecular genetics research. In an interview for Medscape, Dr. Summar spoke with Brendan C. Lanpher, MD, Clinical Director in the Division of Genetics and Metabolism, and Sean E. Hofherr, PhD, Director of Molecular Genetics at Children's National. Their discussion focuses on real-world practical aspects and possible pitfalls of expanded genetic testing in this environment of rapid technological advances.*

Expanding Horizons in Genetic Testing

Dr. Summar: I am here with Dr. Brendan Lanpher and Dr. Sean Hofherr. Dr. Hofherr is Director of the Molecular Genetics Program at Children's National Medical Center, and Dr. Lanpher is Clinical Director in the Division of Genetics and Metabolism at Children's National.

The topic today is next-generation testing, with some "dos and don'ts" and practical pointers. There has been a lot of talk, chatter, advertising, and hype over the past few years about next-generation expanded DNA testing, and I would like to discuss the practical aspects of these tests.

Brendan, what do you see as the current role of expanded genetic testing?

Dr. Lanpher: The current world of next-generation sequencing and expanded molecular genetic testing is very exciting, but also very daunting. It allows us to test for things that we have never been able to test for before. But it also allows us to find things that we are not looking for in more effective ways than we have ever had before.

Dr. Summar: Could you elaborate on that?

Dr. Lanpher: The most exciting new trend in genetic testing is that with the newer technology -- the next-generation sequencing -- you can test a large number of genes with one blood sample, up to and including whole-exome sequencing, in which you test nearly all of the currently known genes with a single test.

The problem is that we all have a huge amount of genetic variation. Knowing and determining whether a variation that we find is disease-causing or not gets more and more challenging with the increasing number of variations found in a single test. Correlating a test result with a patient's phenotype -- the patient's actual clinical situation -- is critical. Having a well-defined, well-thought-out, and well-described phenotype is very important for the laboratory to give you the best interpretation possible.

Dr. Summar: By "phenotype," do you mean a detailed description of the patient?

The Critical Importance of Preparation

Dr. Lanpher: Exactly. Another downside is that we find things that we didn't set out to find, which can be confusing and anxiety-provoking for both families and physicians. When you are testing for a large number of genes, you will inevitably find variations. Sometimes, these variations are in genes that can be medically important, such as susceptibility genes for cancer,

cardiac disease, or other later-onset diseases. Sorting out whether the patient is at risk can be very challenging.

Dr. Summar: That sounds very time-intensive from a clinical standpoint.

Dr. Lanpher: It is. It takes a lot of time to counsel patients and families both before and after the test. It's very important before you embark on next-generation genetic testing that the patient and family receive very detailed genetic counseling about the potential and the limitations of the test. There is no single test for all diseases. There is no perfect test.

Patients may not appreciate the complexity of these tests. It's very important for a family to have a good sense of that complexity before they go down that road. Our genetic counselors spend a lot of time with families before we do this kind of testing. We hope that families have a good understanding of the potential for complex results before they receive them. You can only prepare people so much, but it is very important that they have that time with us before and after the test results come back so that they are as prepared as possible.

Do Different Technologies Mean Different Results?

Dr. Summar: Let's shift to some of the practical aspects. Running a molecular laboratory, you stay up on the current techniques. Is it a bit of a misnomer to refer to "whole-exome sequencing"? What exactly are we getting when we order these tests?

Dr. Hofherr: Unlike the first generation of sequencing -- which is Sanger sequencing, when we were typically looking at one gene for one disorder -- next-generation sequencing is a collection of different technologies and different platforms. Each platform has benefits and disadvantages.

Each laboratory has a choice of which platform they will purchase and use. On top of the platform that generates the data and differs from lab to lab, available reagents and kits also differ. Whole-exome sequencing library prep kits are available from several different vendors, and they are completely different products. Therefore, you are going to have variation, depending on which preparation kit you use.

Furthermore, the amount of coverage that you get on a single base across the exome in general also varies, depending on how the laboratory sets it up. The more samples that you multiplex and put together in one run, the less coverage you will get per base. The less coverage you get per base, the worse the test's sensitivity.

Dr. Summar: Let's explore that. When you say "coverage," do you mean that on the same chip or whatever modality we are talking about, the same point of DNA is interrogated multiple times? Is there a certain number that you consider reliable or ideal?

Dr. Hofherr: The way next-generation sequencing works is that it sequences many different regions at the same time. A given base is sequenced many times -- anywhere from once to 10,000 times. The more times it is read, the better the sensitivity.

Ideally, with a clinical whole exome, we are looking at coverages of between 80 and 200 times per base on average across the genome. That does not mean that every single base is covered 80-200 times. Some regions of the genome are difficult to sequence, and some regions are unable to be sequenced. You end up with a large variation. Some regions are going to be left off because they are unable to be sequenced, and some regions are only going to have a couple of reads on that given sequence.

Dr. Summar: There is a misconception that "whole-exome sequencing" means that every known gene is sequenced -- all the exomes. Is that a misconception?

Dr. Hofherr: Yes. There is overlap from one technique to another and from one kit to another among all of the different capture and library preparation techniques. No single kit has coverage of the entire exome. Typically, you get approximately 80% of the gene-coding regions (exomes) of the genome, and there are clinically relevant regions in that other 20% that are not being sequenced.

Dr. Summar: So among the multiple coverages and the large number of fragments being looked at, there are huge

information processing issues?

Dr. Hofherr: There are. That is also where the variability from lab to lab comes in. Next-generation sequencing is very heavy in informatics and bioinformatics, so the way you look at the data is going to differ from laboratory to laboratory. Because we are looking at so many regions and so much data, we have to use filters so that we are not looking at all of the results at the same time. As you start using filters, sometimes you find that you are filtering out clinically relevant regions. It's impossible to have no filters, because it would be too much information to go through. Every lab sets up their bioinformatics differently.

Some third-party solutions are available, and more are coming to market, and they are getting better. Many of the large reference labs are using their own bioinformatics solutions. There have been multiple reports about sending samples from the same patient to several different clinical laboratories and receiving different results from different laboratories. It's a consequence of all of the differences we have talked about.

Dr. Summar: What you are describing sounds more like a screening test than a diagnostic test.

Dr. Hofherr: Whether it's a screening test or a diagnostic test, we have to be aware that with the current technology and the way that every single laboratory is performing this test, there are major limitations. It doesn't mean that it's a bad test, or that when you get a result, it's not diagnostic.

You have to realize that a negative result is difficult to interpret because when you have a negative result at the whole exome, you have to wonder whether it is negative because of the technology. Is it negative because of the state of knowledge at this particular time point? Or is it negative because the patient does not have a mutation in any of the genes? All of those factors have to be taken into account.

Whether we are going to be resequencing these patients at a later time is still up in the air. We have gone through the same process with such tests as chromosomal microarray. Some patients were tested on an earlier chromosomal microarray platform and reported out as negative, and when these patients came back to the clinic years later, clinicians wondered whether they should be retested with the newer version, in case regions were missed on the earlier test. The same thing might happen with next-generation sequencing.

Dr. Lanpher: This isn't a screening test per se. It's a very powerful and useful test for us in clinic. You have to be very careful in how you counsel families to understand the benefits and limitations of the test.

Who Should Be Using Next-Generation Sequencing?

Dr. Summar: Any time a new technology comes along, many people are interested in using it. Who should be using this test?

Dr. Lanpher: It's very important that people with expertise in genetic diagnosis and interpreting genetic results are ordering these tests and reporting these test results to patients and their families. This is not a first-line test. It is done after a very careful history, examination, and assessment for dysmorphic syndromes. It is useful when more targeted testing has been done and is unrevealing, or when such testing can't be done. We have used it on quite a few families in our clinic. We have made diagnoses with it. We have also had the experience of the test not providing a diagnosis.

Dr. Summar: What proportion of the time is it useful?

Dr. Lanpher: In our hands, about 10%-15% have led to a confirmed diagnosis.

Dr. Summar: Are those patients who have been evaluated by an experienced geneticist and typically referred from a primary care provider or another specialist?

Dr. Lanpher: That's right. These patients are on that diagnostic odyssey. They have been through many previous tests that haven't been revealing. We have had the situation in which patients have had abnormalities on earlier tests that explained part, but not all, of the phenotype, and we have gone on to whole-exome sequencing. Sometimes, those previously identified mutations have been seen, and sometimes they haven't been seen, which is partly what gives us pause about next-generation testing. It is an important tool in our repertoire, but it does not replace more targeted testing and a careful examination and

history.

Dr. Summar: Who should be using it?

Dr. Lanpher: Genetic professionals should be using it.

Dr. Summar: What about other fields? Obviously there is a lot of interest, and many people want to reach for this test. It's being marketed that way, too. Who should be cautious about using it?

Dr. Lanpher: Everybody should be cautious about using it. Even geneticists should be cautious about using it. The potential for physicians, families, or patients to misunderstand results is very high. These results are difficult and challenging to deal with and to understand. Even from the best labs, which write very nice reports, these results can be very challenging to interpret.

It Takes a Team

Dr. Lanpher: Access to genetics is variable across different regions. Patients should not be limited in terms of their options just because a geneticist isn't nearby. But make sure that you have someone who can help you understand these results -- a clinician, ideally -- and genetic counselors to help you and the family walk through these results.

Dr. Summar: It sounds like the counseling issues before the test is done are as important, if not more important, than those afterward.

Now, I will ask you both to speculate. Sean, what's next?

Dr. Hofherr: The problem is that for a long time, the technology -- whole-exome and next-generation sequencing -- has been isolated to large sequencing centers. Most of them were spun out of the whole-genome sequencing project. When physicians order a test, they fill out a sheet with the relevant clinical information on that particular patient to help guide the interpretation of results. You want the test to be performed and interpreted by those who have access to the physicians and the clinical information from the electronic medical record.

There has been a big disconnect between reference laboratories that are able to perform the test and the clinical centers that are ordering the test. That has presented a difficult situation, because variants are reported that are not clinically relevant to that patient. You could also be missing something that is important to that patient because it is, by necessity, filtered out.

Now that the technology is becoming much easier to use and the price is coming down significantly, a lot of the smaller, in-house molecular diagnostics laboratories that support genetics practices are going to be performing this test in a way that integrates the clinical group when they are looking through the results on any given patient.

Dr. Lanpher: That probably represents the best-case scenario.

Dr. Summar: Are you saying that we will see decentralization of the technology, going from when it was very expensive and performed in only a few sites to more of a cottage industry?

Dr. Lanpher: The integration between the molecular laboratory and the clinical team is what is necessary for the most useful, and most accurate, interpretation of the variants found.

Dr. Summar: You are describing a team made up of genetic counselors, geneticists, the molecular laboratory, and the diagnosticians -- people who can interpret and make sense of the sequence data.

Dr. Lanpher: It's an ongoing conversation. You are going back and forth between the molecular lab and the medical team to help build the story that integrates the molecular and clinical data. It's not as simple as just filling out a phenotype sheet.

Dr. Summar: This is very different from classic laboratory testing, and this is what people need to adapt to.

Dr. Hofherr: Correct. This totally changes the paradigm of how laboratory medicine is being performed right now and has been for the past couple of decades.

Dr. Summar: I would like to thank you both very much. We will revisit this topic in the future. Every six months, I suspect that we will find something new.

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