January 2016-Genetics Webinar

Female Speaker:
The topic today is next generation sequencing in the clinic, gene panel testing for inherited conditions. And I know you've all been, you know, listening to many NGS talks already, so I don't have any technical background, but I do have a couple of background slides just to state facts as a prelude to what I'm going to talk to you about. So, this is a very, very widely publicized slide showing the decline of costs per -- here for a genome caused by the advent of next generation sequencing. And just to refresh everybody's memory, it's been fairly recent that NGS, you know, started being available first, of course, in research, but that was about 2005. And very, very shortly after, it actually started being implemented in the clinics. So it was very -- a couple labs in 2009. My lab here implemented in 2011 and that was still considered early.

So, you can see that the cost declines more and more, and as a result, it is now increasingly implemented in many, many laboratories. The majority, to the best of my knowledge, focus on gene panels. However, implementation of exome genome sequencing is also quickly increasing, and I'll touch on that a little bit towards the end. And here is why NGS has been so successful and so interesting and attractive for many. So, what you see here is an example of how our detection rates for our diagnostic gene panels evolved over time. So, you see the percent of positive cases on the Y axis. And then, going across, different gene panels that our laboratory here was offering at different time points starting very early in 2006 or 2007 with just five genes, and then moving up to 10, 19, 24.

And NGS started happening over here with, at the beginning, 46 genes. And the point I'm trying to make here is that over this timeframe, we nearly quadrupled our detection rate. And this was for dilated cardiomyopathies. The scale is a little bit unfortunate because it doesn't look as much, but you go here from about 10 percent to nearly 40 percent. So, that's why everybody got so excited. It seems like a great thing to increase detection rates.

And now the obvious question is is that a good thing for every disease? When should one do this type of testing? Is it a generally applicable? So, I thought the best thing would be to use a real disease example. And of course, I chose the one that I'm most familiar with --

[laughter]

-- which is inherited cardiomyopathy. So, what you see here is just a, you know, a representation similar to common cardiomyopathies. We have hypertrophic dilated arrhythmogenic right ventricular and a couple of other rare ones. And collectively, their incident is about -- is greater than one in 500 individuals. They all are quite severe. They can lead to sudden cardiac death, and what they all have in common, in addition, is that they have a substantial genetic component. And all this makes up for a really high incentive for predictive testing -- diagnostic and predictive testing, I should say.

But why screen for mutations? And that should be obvious, but I think it's useful to review. So, for diseases like -- for all genetic testing, there is, in principle, two different incentives: One,

clinical management; and that is sort of the lesser of the two reasons for cardio myopathy, but there are some examples. For example, there's a cardiac variant of a rare disease that manifests only as hypertrophic cardiomyopathy. And now, if you don't test for this, you know, using your gene panel, you can actually pin point the cause to the fabry gene, and then, potentially give enzyme replacement therapy and cure this individual.

But by and large, clinical management is not quite yet -- quite as available for cardiomyopathy as it is for other disorders. Cost is another argument, though. So, current guidelines recommend clinical screening of first degree relatives, of affected first degree relatives. And here is something from a, you know, five year old paper from Carolyn Ho, but it's still relevant. But for the child of a patient with hypertrophic cardiomyopathy, this recommended clinical screening, it comes to $6,000 for puberty and $20,000 over their lifetime. And if you contrast that with genetic testing, you can see how that starts saving money because once you have a pathogenic variant, you no longer have to follow up every first degree relative. You can just reduce that to mutation positive family members.

And just to state the same thing with a more recent paper, this is from our laboratory as of this year reporting our experience with nearly 3,000 probands with hypertrophic cardiomyopathy. What we did here is to look at the impact of identifying positives and no longer having to screen negatives, affected negative individuals. And so, that came out to be about $1.7 million savings over that cohort. So anyway, that's just meant to sort of set the stage as to why genetic testing is believed to be useful for these disorders and showing a little bit the history of how it has evolved.

It's actually overall a very young discipline for cardio myopathy. It started in 1990 only, where the first gene was discovered. And you know, 13 years later, we had the first test, the very small test, as I showed you before. It was just a couple of genes and then moving up quickly to our next generation sequencing in 2011. And now, it is routine to screen even more than 51 genes for patients. It's rapidly expanding, as you probably know. So, what makes -- so, there are challenges. And what are those? That gets us a little closer as to what disorders really benefit from next generation sequencing. The cardiomyopathies all have locus heterogeneity, and what we mean by that there is one disease, but the mutation can reside in any one of several to many genes.

We also have allelic heterogeneity, meaning that there are usually many different disease causing variants in a given gene, to the extent that the majority can be private. We also don't quite understand the pathogenic variation spectrum yet because it's a young discipline and because there's so many new mutations that arise. So, typically, you know, what -- to sequence it is -- or for a very long time many, many thousand probands, you will eventually understand the spectrum of prevalent pathogenic variants. We're not quite there yet for cardiomyopathies.

And then finally, there is a fair deal of phenotypic clinical overlap, which can complicate the testing process. What I'll do in the subsequent slides is move through examples for each one of these [unintelligible]. So, there's a bunch of them --

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A photo or anything --

Female Speaker:
Is it possible to mute -- I get a lot of background noise. Would it be possible to mute? Thank you. Okay, so locus heterogeneity was the first problem and that's illustrated here for dilated cardiomyopathy on the left and hypertrophic on the right. And as you can appreciate, it's not terrible. For HDM, it is actually quite manageable. But still, I mean, you have two main genes and then a whole slew of other genes that can contribute. For DCM, it's already looking a little worse, you know? So, there's no single gene that contributes the most. We have one that is pretty strong, but then there is a lot of different other genes. So, you really want to test them all. So, you need an -- you need an assay that can actually interrogate that many in a single test in a given patient.

What about allelic heterogeneity? So, as I said, HDM, we have about 11 genes. And we have been testing them in our laboratory for about a decade now. And what you see here is an analysis of the result, and it's asking, "How many variants have been seen in just one proband?" Two, three, four and so forth, along the X-axis. And what's shown here is that two thirds of all variants have only been seen once. And there's a few outliers up here; those are prevalent but, you know, the vast majority is sort of once, twice, three times. So, that means one needs to really sequence the entire coding sequence of all these genes for maximum clinical sensitivity.

And that's not just true for cardiomyopathy. If you look at all diagnostic testing performed in our laboratory -- and this was shared with me by Heidi Raine [spelled phonetically] this slide -- we did about 15,000 probands, and the diseases we're offering are listed down here. It ranges from cardiomyopathy, hearing loss, RASopathy, and so on and so forth. It's actually quite the same, you know? Two thirds or more are seen once, and then it trails off.

And then, this is really the very tricky point -- clinical heterogeneity that has been largely underappreciated in the early days of genetic testing. And I'll show you a little bit of why that was and what the outcome is. So, traditional genetic testing is usually configured this way; one gene panel for each diagnosis. If you have HCM, you order an HCM test. If you have dilated cardiomyopathy, you go order a dilated, and so on and so forth. But here's a case, a real case example, that we received in our laboratory. This is the proband here with the arrow denoting an individual with a clinical diagnosis and a family history of dilated cardiomyopathy, strong family history. The physician ordered what was customary at the time; a DCM gene panel. And we did detect also what was quite frequent, a variant of uncertain significance.

Now, the variant ended up not segregating, so we tested all the affected individuals that were available. And it was not present in a few, so unfortunately, this variant was not the cause of disease, which is quite common. Now, the interesting thing was that about a year, later this patient was seen again by the physician. And at that point, he revised -- and that was Dr. Lakabel [spelled [phonetically] at the Brigham, by the way -- he actually revised the diagnosis to arrhythmogenic right ventricular cardiomyopathy.

That is not uncommon either, because ARVC has extensive clinical overlap with dilated cardiomyopathy. It's very difficult to diagnose. Really, the only true way to diagnose it with certainty is upon biopsy, which is usually not performed, of course. And then, he ordered a second panel on top of the DCM panel that has already been performed now for this disorder. And this one identified a likely pathogenic variance, which did segregate.

So, what I wanted to -- the point I wanted to make is that this traditional, disease centric testing does not make sense for disorders with this type of clinical and genetic overlaps. It's causing a lot of -- it's costly. It's very time consuming, because between the first time the first test was ordered and this result I'm showing here, we're over a year. So, that was not good for the family and, you know, and costly. And here is a summary of this concept again. The reason why these older tests were configured for one disorder only, usually, is that these disorders were typically defined quite narrowly, based on morphological criteria. And also, these represented the most severe cases, which is typically how a disorder gets recognized. It's first, you know, recognized by severe cases, and then over time, the true spectrum of associative phenotypes is more appreciated. So, this is "the tip of the iceberg" phenomenon. Perfectly understandable why we all configured our tests this way, but today we do know this overlap with ARVC, but also overlap with HCM.

So, really what we're doing now is offering -- and it's not just our laboratory. I think the community has moved to multi-disease gene panel testing because of this overlap. And, you know, we know now that what I showed you in this isolated case example is true for about three percent of patients with dilated cardiomyopathy. They come in with a diagnosis of DCM. We end up finding a pathogenic variant in a gene known to be associated with ARVC. All right.

And one final example of this from a different type of disorder, which is now the RASopathies, which are Noonan spectrum disorders; if you look at Gene Review, this is what was written in 2012. I actually don't know if it's been updated now. It wasn't updated as of a year ago, but anyway, at that time, it says that 80-90 percent of patients with Costello syndrome, one of the RASopathies, carry a mutation in the HRAS gene. So, that sounds great. So, as a physician reading this, you would go ahead and order HRAS first, and then, maybe, if negative, you know, do something else. But here's what we thought. So, this is the abroad referral populations that we received in our laboratory. And you can see that the minority of patients actually had a variant in HRAS. The majority had mutations elsewhere in related pathway genes. So, adhering to this traditional paradigm would have caused a negative report. And it is unclear how many physicians would have reflexed to an additional test because again, that is costly.

So, to summarize this, these multi-disease gene panels do often improve a clinical diagnosis. And the reasons are -- just to summarize this phenomenon of phenotypic expansion -- as I told you, the original clinical definition was naturally based on the more severe cases, but then, as a consequence, it ended up being too narrow as the full range of clinical variability emerged over time. Phenotypic overlap -- you know, that is not uncommon. So, here the disorders present the same and that can lead to a diagnostic "error." It's not really an error, but how is the physician going to be able to be accurate here?

It happens more often, though, as genetic testing is moving out of specialty clinics to more general genetics care which, you know, just almost invariably leads to a decrease in detection rate. We're seeing this more and more because the cases aren't precisely diagnosed. And this is now widely recognized by the clinical and diagnostic community. I've had several conversations with physicians here at Partners Healthcare who are saying, "Yes, this makes so much sense." We're actually now beginning to change our workflow, and this is shown here. So, this is what next gen sequencing has caused, which is now beginning to be called this sequence first then diagnose workflow.

And, you know, not to go through all these, but this shows the full clinical workload from the patient over diagnosis, and then, ordering a genetic test and receiving a report. What's happening is that the up front work -- establishing a clinical diagnosis, and then, using that to order a test -- is replaced by sequencing first, and then, putting the diagnosis at the end; taking the clinical as well as molecular data into account. And that's quite exciting, actually. It's very rewarding to be part of this, and we do solve cases more than we did before.

And as a last background slide, there is definitely a trend towards genome wide testing, but it doesn't stop at just sequencing more and more genes. And this is a little off topic, but I thought I'd throw it in because it is just breathtaking what's happening right now. So, we've already seen this trend towards genome-wide testing in other disciplines. You know, in cytogenetics, it was a very, very early already that that field transitioned to genome-wide copy number arrays; where in the old days, they were single gene or single analyzed tests like a FISH, a Southern, and so on. The same thing happened for genotyping tests, where in the old days, you would look at a mutation, maybe some, and, you know, if you were lucky, a lot, but not -- never so many. And it quickly moved to genome wide SNP chips and arrays.

And now, we have the same thing happening for NGS -- next-gen sequencing. And the reason I'm throwing this all in one slide is that NGS actualy has the potential of replacing all these tests, because it is beginning to be for sure possible to genotype, because, you know, you don't have to interrogate every position in the sequencing test. You could chose to just look at your SNPs. But it's also beginning to be feasible to call copy number alterations, which is a really great argument for using an NGS test. And so, I would say it's fully expected by most doctors in the community that this technology will eventually consolidate most genetic testing, when appropriate. Of course, this isn't going to be the case for every disorder.

So, I want to move on to talking -- and talk a little bit about which genes should be on the panel. And that gets us to assessing the clinical validity of variants and genes. The two are connected, so you really can't assess the clinical validity of a gene without looking at the variants that have been published in that gene. And so I want to mention, up front, that this is a very hot topic in the community, and there are various bodies now that have geared up to develop standards for assessing clinical validity.

And you're probably very familiar with some of that. The ACNG and AMP have come out this year -- sorry, last year -- with a new guideline for clinical grade variant assessments for Mendelian disorders. And there is also the fairly young consortium called ClinGen, the clinical

genome resource, which is really aimed at uniting medical genetists and developing approaches to really curate and centralize and share all that data. And its focusing not just on variants, but also genes; testing which genes are clinically valid in terms of their published evidence.

So, let me dive a little deeper into that. Like I said, it is impossible to figure out the clinical validity of a gene without understanding the published variants, whether or not they are pathogenic. So, a few words on that; when we assess variants clinically -- I mean, it should actually be the same in research, but in a clinic, we have a very structured process. We ask, basically, whether the variant affects the protein or gene function first and then, we ask whether that causes disease. And those two aren't always linked. And then we classify the variants based on the available evidence into one of five categories.

Five is -- these categories that you see here as the ones recommended by the College of Medical Genetics and EMP. But -- and most laboratories begin to adhere to this. And at the end, we ask one more question. We then take that variant and ask whether this variant also causes this patient's disease, because it may be pathogenic, but it may not be responsible for the patient that I have in front of me. Maybe it's an adult onset variant, but the patient I see is a child, so it's questionable whether this variant is really causing that. So, there is layers in clinical variant assessment. And then, we string together what we see. So, to summarize this again, we go through the results, annotate and classify these variants into these five categories, and the end result is that a patient gets the report. And there's three flavors: positive, negative, and in between -- inconclusive. So, what is classified as likely pathogenic or pathogenic ends up being a positive report, meaning that we believe that we found the cause of disease, definitively or likely.

So, I want to show you this slide again and now focus on a different aspect that you might have already picked up on when I showed it the first time and I just glossed over it. There is this nasty surge in inconclusive test reports. And we thought, you know, yes, we have a quadruplication of positive cases, but an even steeper increase in inconclusive. Now this is not good, but it's worth diving a little bit deeper to understanding what the causes are, and if all of it is really bad. So, why more of these inconclusives? There are two main reasons: one is that we can have a novel variance that has no published evidence, and the variant type on top of it is of unclear impact. So, this is true for many novel missense variants, even when the gene is very well established.

But the second category is the one that I want to dive into a little deeper later. It could be a novel variance of any kind, really, in a gene whose role in disease is not definitively established. And this is really -- this is -- this is very prevalent in our community still. But let me just quickly, you know, go over the first reason: novel variant with no published evidence, and the variant is of unclear impact. So, this is unavoidable. As soon as we start sequencing a gene that is even definitively and very strongly established with a disease, and we sequence it in its entirety because there is so much allelic heterogeneity and so many private mutations, with the good improved diagnosis sensitivity comes some inevitable bad.

So, how bad is the bad? That depends on many factors, actually, and here's where the physician comes in. It is entirely influenced by the patient's ability to deal with uncertainty. It's also, as I

showed you, important to see whether there's a family history, because one can turn a variant of uncertain significance into a pathogenic variant by family studies. And also, the world is moving closer together now and with many, many large databases being established centralized and interconnected, we've had -- we've seen an increased ability to solve cases by connecting patients around the globe. So, it's not as easy to condemn these BUS' as you might think. And so my personal opinion on this is, here, for those disorders with a high degree of allelic heterogeneity, there simply would never be any progress if one only tested what is already known. You'd be stuck with just five or six common pathogenic variants, and that's just it. And here's just an example, you know, underlining how you can, you know use family testing -- I showed you that before -- to make variants -- to move this out of an uncertain significance category.

Now, much more important is this: the novel variant in a gene whose role is not definitely established. And that is a relatively young discipline, to go into the assessment of gene disease relationships. So, what's happening is that if a gene -- if the role of a gene is not well understood, you will never be able to interpret a variant if you don't understand the role of the gene, for the most part. Traditionally, that's not been a problem because the old tests were limited to just a few genes so naturally one would choose those that are really well established. There was no doubt.

But that barrier is gone with NGS. So, all of a sudden there was this possibility of adding more and more genes. And naturally we all added as many as we could, only to realize what I just described to you; that "Hey, that isn't a great thing always because, we should have actually read the publication a little more deeper and assessed its validity critically." Luckily, we've all caught on to that, and now, there is this discipline called gene assessment. And the sad truth is that many published claims for a gene disease relationship just do not withstand the rigor of clinical grade curation.

And it's not easy to point fingers because there is the publication pressure everybody has. Journals don't like to take negative papers. Everybody is trying to hype up their findings. This is all very normal, but it does hurt you when you use these genes clinically. So, now we actually do this. The Clinical Genome Resource -- and I didn't mention it; a large NIH funded consortium of many centers -- has established guidelines, and is about to publish them, establishing evidence levels ranging from definitive over strong, moderate, limited, and so on to none. And then, it has established a rule-based framework of what evidence is required to make a gene definitively or strongly associated with disease.

So, the pillars of evidence that are used are, you know, the number of clearly pathogenic variants I reported -- and here's where you need to understand variant assessments -- the number of studies available, the number of probands with a variant, statistical evidence, other type, case control boards [phonetic], and then, functional data. All of these things are actually tricky because one needs to establish rules for what is a valid piece of functional data. But that's underway.

And I wanted to show you an example that I've personally lived through. So, this gene xylene is on our -- is on most laboratory cardiomyopathy panels. And here are the original -- sorry --

publications. We have one for dilated and one publication for hypertrophic. And you see the titles, you know? Xylene mutations lead to dilated cardiomyopathy. Mutations in this gene are associated with HCM. So, that is the statement, but if you look more closely, in black is what's in the paper, and then, red is, you know, what you see. And this is a couple of years ago, when you really look. So, there was good evidence as per the paper, but when we looked at the variant that they based their claim on -- they found a particular variant -- and we found these variants in .3 percent of the population -- sorry, in .7 percent of the population. This was the exome sequencing project.

So, no matter what the evidence is there, this is a red flag. And we're not saying it doesn't cause disease, but it's not really a slam dunk gene. So, we approached it a little bit more cautiously today. And even worse for the HCM paper, the two missing variants that were found by these authors. One of them we've already down-classified as likely benign based on the frequency. And this was pre -- this was before The Broden [spelled phonetically] had released their exact database. I actually don't know; they might have moved on to benign now. So, this is what we do clinically.

And at the end of the day, the goal is to develop guidance as to what type of evidence is right for what type of tests. And what you see here is a pyramid with the different levels of evidence. And naturally, most genes actually live in this bucket down here -- limited or no evidence -- then moving up to moderate, strong, and definitive. And there is no expert -- there's no clear consensus yet, but most of us include moderate, strong, and definitive in diagnostic panels. But when you go to predictive testing, you do want to be a little more selective and only use definitive, potentially strongly associated.

But this is, right now, where a lot of activity happens in the community to actually form expert panels and adjudicate these genes, and say, "Okay, of the 50 published hypertrophic cardiomyopathy genes, these are the ones that meet criteria to be included in the diagnostic panel." And that's precisely what is happening under the ClinGen umbrella. Just one example: So, I'm co-chairing a group for cardiovascular domain -- cardiovascular disorders -- and we do tackle both. We try to really nail down a framework for variant curation for this disease. And even more important, we are trying to establish a recommendation for cardiomyopathy panel testing doing those [unintelligible].

So, now the question is -- I mean, no, I would say -- I would like to start by saying in my mind, the utility of these multi gene and multi disease panels is quite recognized. I don't think anybody's debating that. But yes, there's a higher risk of detecting the BUSes, and the only negative -- that is a negative, but it can be minimized with rigorous gene selection, as I just explained. How on Earth, though, are we going to keep up with the increasing rate of gene disease discovery? Disease gene discovery, sorry. As a laboratory director, it's quite hard to constantly redevelop, revalidate, and update these gene panels. It simply isn't sustainable, and in some disease orders, the knowledge is virtually exploding.

So, we need to find a way to keep up. How do we do that? So, this is a big debate in our community right now. Are we -- what's better? A gene panel or an exome? Why not just -- if

more genes are better for some disorders, why not just do all of them? So, this just, like, summarizes the current landscape. Gene panels are the predominate next generation sequencing test really focusing, usually, on tens to hundreds of genes. And they come with a lot of perks, you know? We have high coverage. We can usually return a credible result for every single base in the test, and they're by and large used for clinically very well-defined cases. But, there is a big push to go here, because exome is getting better and, you know, it's been living in this reach of being used for complex phenotypes or diagnostic odysseys. But there really isn't -- there's less and less difference between an exome and a gene panel, and I'll show you why.

So, a lot of laboratories are trying to figure out if and when it is appropriate to even move over here. The incentives are very obvious. A large fraction of the gene panels we are offering are negative. The accuracy rate, at best is often 50 percent and, you know, that's just less than optimal. And I've shown you before, there's a growing appreciation of phenotypic expansions. There's always been an argument for the hypothesis retesting. How sure are you that you have the right phenotypes? Well, you don't, often. And additional tests simply can end up being more expensive in the end. If you count up all the tests that you end up ordering, if you do it sequentially, you're quickly more expensive than an exome.

And of course, you have to always be up to date, dah, dah, dah, and it is also operationally easier to maintain for labs. Barriers -- yeah, there are barriers. Cost is one still, though the gap is quickly closing, so I'd almost disregard this. Incomplete coverage is another frequently cited barrier, and that is true. Exomes are still not quite as good as the targeted panels, but a lot of it is a design flaw. The vendors that are offering these have not done a good job, and so, the community is stepping up to help them with that. There is also an additional risk, and that is a little more difficult to deal with, which is as we're rapidly expanding into more and more genes we're losing our intimate or any prior knowledge on these tested genes. In the old days -- and I've launched many genes over the years -- you knew this gene inside and out down to, like, "Oh, here is an exome that is repetitive or something." We totally lose that ability now that it's possible to overnight test so many genes.

But these barriers are really fast disappearing. And I said before, small tests -- many small tests can quickly end up being more expensive. When we look into this a little bit here, in our ecosystem, this is one example that is representative. It's a real example; the order test will [unintelligible] to a sequencing, and that's blanked out the laboratory. The test consists of 23 genes and included copy number analysis. The sensitivity, clinical sensitivity was 65 percent. Now we ask the question, if this physician had ordered an exome, you know, how would it have looked then? And so, in this case, one wouldn't have needed additional deltope [spelled phonetically] testing because the exome doesn't really do this well. But we factor that in.

We would have had 10 more genes at our disposal because since this laboratory developed their tests, 10 more credible genes game out. And we also looked at our exome quality. It was quite well-covered, so it was legitimate. And the clinical sensitivity would have been 10 percent, actually 15 percent, higher. And the exome turned out to be cheaper, despite the fact that we wouldn't have added separate deltope testing. So, this is really how it often goes.

The next barrier that is almost gone is the last of completeness. And this is a slide that I use to show -- shown many times, but I think I need to stop doing it, because it's no longer a big issue -- showing you what happens to our 51 cardiomyopathy genes that we had in our cardiomyopathy panel a few years back. If we look at our targeted captured assays, or the gene panel by itself, it looked like this. In blue, you see everything that is fully and adequately covered, and there's a small slice that we would say does not reach the required coverage; less than one percent. And what we do in our clinical, and many labs do that, is we use Sanger sequencing to then fill in this slice providing 100 percent coverage. And that, you can see on an exome; the same 51 genes on am exome look much worse. So, only 85 percent are fully covered, 15 percent are not, and that is something that is not possible to fill in by Sanger sequencing.

Now, you know, we got together in the community and helped the vendors develop a better test, which is shown here -- an enhanced exome. And you can see the same kind of analysis. And now, the exome derived data was almost the same as the targeted capture data. So, that's no longer really an argument to not do the exome. The most severe barrier, I think, is the educational gap today. We're still living up here in this quadrant -- exome sequencing is ordered by experts. If you have, like, a low, medium, high scale for testing labs and physicians, this is where we are right now. Highly educated, genetics study physicians order, and highly capable laboratories do the tests. So, we're doing this now. And it's just really difficult for a laboratories to keep up, and also physicians. So, we need to do a lot to educate, but that's a separate topic really.

So, in my mind, we need to actually redefine the question we're asking. So, assuming adequate coverage and assay class -- and I've shown you that you think is likely no longer going to be an issue in the near term future -- exome and genome sequencing can be -- one has to remember that the way we're using exome and genome sequencing can be, can be different. So, everybody thinks sequencing everything means you have to analyze everything, and that's not true. So, what we can do is we can genotype. We can run the exome, but we can only look at known pathogenic positions. We can sequence. We can do panel testing, and if we know the well-established genes, we can also do all the genes when there's clinical diagnosis is not clear, but the family history suggested genetic etiology. And that's sort of the way exome genome sequencing is used currently.

But price and coverage is really the only factor that's gating our ability to use it like a genotyping test or a smaller scale sequencing test. So, the critical question, really, is how specific is the patients' phenotype? That will dictate which set of genes we look at first, and maybe stop there, you know? And how deep the analysis needs to be. And this is now, you know, really -- this is almost what we're building right now, right. This is a test we're about to launch, and we're not the only ones in the community that are trying to marry these two worlds, you know. Can we use an exome? But can we make it behave entirely like a targeted panel? And that's exactly what we're trying to do.

So, a traditional disease-focused panel looks like this. And I said this before; we have 100 percent coverage using Sanger sequencing to fill in of just, you know, a small number of genes. We typically report deep, like pathogenic down to variants of uncertain significance, or even

likely benign variants. On the other hand, we have exome genome sequencing, where we also often start with an indication driven gene list. So, if the patient has something that looks like cardiomyopathy, many laboratories will look specifically at cardiomyopathy genes.

But here, typically, the coverage is variable and, you know, fill-in sequencing is often not done. And reporting is restricted often to pathogenic and likely pathogenic variants only simply because the scope of the exome is bigger, particularly when you go down to the next layer, where you start looking at all the genes and ask, "Is this useful when -- you know, to factor in potential phenotypic uncertainty? Is it something that I didn't expect or, you know?" And then, there's also the possibility of finding things incidentally.

What we would like to do is really this: have a targeted panel that's doing everything that we're doing here. So, we're guaranteeing 100 percent coverage, and we're reporting very deep, but we retain the ability and the nice things of an exome if and when we need it. If this is negative there is -- it's easy to then say, "Well, let's go look at the rest. You know, maybe the diagnosis wasn't accurate. You know, maybe it is worth just looking in related disorders." So, that started a new thing. It's meant to bridge the gap between exome and panel testing.

So, here is a couple of final words on the importance of standardizing structured gene evaluation. This is also a real example from our laboratory. The goal was to define -- to define the contents of a new indication-driven gene panel, and it happens to be inherited renal disorders. We did a survey of databases. So, it was using ontology driven data base tools to create a draft list, and it was 279 genes. And then two things: We worked with a clinical expert and sort of ran this by this expert and said, "Which genes do you think one should do?" And we then used a ClinGen matrix I showed you before, which is not rocket science, but forces us to do a very structured clinical validity assessment. And here's the result of the 279 genes; the expert-driven opinion yielded this. So, you know 126 genes were deemed mission critical, and 22 were nice to have and the rest were, you know, neither; was unimportant, essentially.

When we looked at this with the ClinGen matrix, we found that not all of them -- about a third, actually, a little bit more than a third only met definitive evidence criteria for a gene disease association. And also, in the rest of the genes that were not deemed important, we found some that met these criteria. And also some of these genes that were deemed to be important didn't meet evidence levels at all. But it just goes to show you that it's very important to do this in a structured, rigorous way.

And with that, I wanted to summarize. I hope that you can appreciate that multi gene and multi disease testing can be useful for disorders with clinical and genetic heterogeneity. A genome will soon be cheap enough to be the first line test for all genetic disorders. And how soon is soon? I don't know. But that's where we're going. And understanding the clinical scenario is key. The test really becomes an informatics exercise. You can do anything from analyzing just a few sites.

And here, I'm listing an example. You know, provided the genome sequencing is incredibly cheap, you could just totally run it and ask only, you know, what is present at the two positions

that you need to analyze for achondroplasia. You can do a single gene, if need be. You know, there's an example -- Birt-Hogg-Dubé syndrome -- 90 percent all variants are filament C, so it would make sense to start there first. You can analyze a set of genes. And I took you through HTM or XLM. And curating the validity of gene disease relationships is probably the most important thing we have to do over the next few years. And with that I'm going to acknowledge just so many people that have contributed to all these things, and thank you for your patience. And I'm happy to take questions.

[end of transcript]