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Clinical Exome Performance for Reporting Secondary Genetic Findings

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Guest:
Dr. Jason Park is Director of the Advanced Diagnostics Laboratory at the Children's Medical Center in Dallas, an Assistant Professor with the University of Texas Southwestern Medical Center in the Department of Pathology and the Eugene McDermott Center for Human Growth and Development.

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.

In the last year, multiple clinical studies have shown the diagnostic power of testing a patient's exome. Clinical exome testing is now available at over a dozen clinical laboratories in the US, and has been performed for thousands of patients.

In the January 2015 issue of *Clinical Chemistry*, a special issue devoted to molecular diagnostics, an article titled 'Clinical Exome Performance for Reporting Secondary Genetic Findings' examines the analytical performance of exomes for testing specific genes.

One of the authors of this article is Dr. Jason Park; Director of the Advanced Diagnostics Laboratory at the Children's Medical Center Dallas, an Assistant Professor with the University of Texas Southwestern Medical Center in the Department of Pathology, and the Eugene McDermott Center for Human Growth and Development.

Dr. Park is our guest in this podcast. And doctor, first tell us just what is an Exome Test?

Dr. Jason Park: An exome test is designed to sequence the patient's DNA which is the blueprint or code for protein production. Similar types of test include whole genome sequencing, genome analysis by microarray and gene sequencing panels. Whole genome sequencing examines both the exome as well as additional DNA sequence that does not code for protein but that may be important in turning genes on or off. In contrast, genomic microarray tests look for large deletions or duplications of DNA sequence across the patient's genome. Microarray testing is good for finding changes affecting thousands to millions of bases of DNA but it's

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typically not the test designed to examine all of the individual DNA bases.

Finally, gene panels include multiple genes which are all related to the same clinical syndrome or disease. Gene panels may include dozens to hundreds of genes and are ordered in the clinical setting of a well-defined genetic disease.

For perspective, Exome Tests are designed to examine approximately 30 million bases of DNA from approximately 23,000 genes from a patient. A human genome has 3 billion bases of DNA.

Bob Barrett: So when did laboratories start using exomes as a clinical test?

Dr. Jason Park: Clinical exome testing was enabled by advances in high-throughput DNA sequencing. This technology, which is also referred to as next-generation sequencing, analyzes millions of fragments of DNA in parallel to generate billions of bases of DNA information in a single instrument run.

In contract, Sanger Sequencing, which is the more traditional technology that was used for instance by the Human Genome Project, can at best examine hundreds of DNA fragments in parallel to generate only millions of bases of DNA information in a single instrument run.

In 2011, clinical exome sequencing was first offered by two laboratories in the United States. Currently, I am aware of approximately 20 US laboratories providing clinical exome sequencing as a service. Based on recently published reports on clinical exome tests, I will estimate that there are probably more than 10,000 patients in the United States that have had a clinical exome test.

Bob Barrett: Okay, so how useful are exome tests?

Dr. Jason Park: There's a definite clinical role for exome tests. A good scenario for using this test is when a patient presents with an unusual disease that defies classification, but has a strong family history consistent with a genetic etiology. In this clinical presentation, there may not be a rational approach to examining a single gene or even a handful of genes; instead, the best approach may be to perform a comprehensive exome test.

Another scenario that deserves consideration is when a patient has a genetic syndrome that could be attributed to dozens or hundreds of genes. In this scenario a targeted

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panel of these genes that we are concerned about or an exome maybe the best test.

Over the past two years multiple publications have shown that the diagnostic yield of exome testing ranges from 20% to 60% depending on the patient's clinical presentation. The caveat to exome testing occurs when the test result is negative.

Bob Barrett: Well why should a clinician or a patient be worried if a clinical exome is negative?

Dr. Jason Park: Clinicians and patients should not assume that a negative exome test means that there is no genetic basis for disease. There are multiple reasons for a negative exome test. First, the disease may have a genetic basis but the pathogenic change may not be in the regions of DNA that encode protein. Exome Tests are only designed to test DNA that encodes protein and most exome test do not examine deletions, duplications or translocations which are other DNA changes that can lead to disease.

Second, the disease may have a genetic basis but the pathogenic change may occur in a gene that has never been previously associated with human disease. Of the approximately 23,000 genes in the human genome less than 5,000 are well studied in the relationship to human disease. Exome tests are only informative for genes that have been previously studied.

Finally, the disease may have a genetic basis or change in a well-known gene but that gene may not have adequate sequencing data due to analytical problems with the test. This final reason was the focus of my recent study published in *Clinical Chemistry*.

Bob Barrett: Dr. Park, how did you become interested in the analytical problems of exome testing?

Dr. Jason Park: My clinical laboratory began to develop next-generation sequencing panels in 2012. At that time we examined a variety of test methods, which can also be referred to as capture or enrichment technology, to sequence hundreds of genes. These test methods are the same ones used for exome testing. What we found was that no method could return complete data on all of the genes that we were interested in.

Furthermore, as we spoke to commercial manufacturers of the technology as well as genome research groups, this problem of incomplete capture or incomplete coverage of all genes of interest was a well understood problem with next-generation sequencing.

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There are methods to mitigate the incompleteness of gene sequencing by this technology, but there are no methods that return 100% coverage of all genes in panels or exomes. Even whole genome sequencing which does not use capture technology does not return 100% coverage of all genes.

Bob Barrett: Do clinical exome laboratories report this possibility of incomplete coverage?

Dr. Jason Park: Most clinical exome labs report the overall incompleteness of coverage and several labs go further to describe the specific genes that are incompletely sequenced. However, at the time we conducted our study, no laboratory had focused on the problems with using exomes to evaluate panels of genes in the clinical setting. Everything had been reported up to that point as averages across multiple samples or multiple genes.

Bob Barrett: So what are the potential problems of using averages of multiple samples or genes?

Dr. Jason Park: Well, let's consider that there are approximately 30 million bases in an exome, most clinical exome laboratories report on 90 to 95% of bases which encode protein. This means that 1.5 to 3 million bases that you're trying to find an answer on do not have informative data. These millions of bases are distributed over only 23,000 genes.

So 5-10% of coding bases without informative data can result in a much greater percentage of genes with incomplete coverage. In one prior study 5-10% of coding bases without informative data could result in incomplete analysis of approximately 50% of the genes of interest.

Thus, when we hear a number of 90-95% coverage this may appeal to our academic sense of an A grade, but it is actually hiding a potential for an F grade with regard to complete coverage of genes.

Bob Barrett: So how did you examine this potential problem in your study?

Dr. Jason Park: Well, my laboratory is clinically focused, so I worked with a number of collaborators from multiple institutions with backgrounds in bioinformatics and genomics. Dr. Paolo Fortina was the senior author on the study; he directs a clinical exome laboratory at Thomas Jefferson University. Bioinformatics expertise was provided by Dr. Peter Clark from the Children's Hospital, Philadelphia and Dr. Eric Londin from Thomas Jefferson University. Dr. Marialuisa Sponziello from Sapienza University in Rome and Larry Kricka from the

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University of Pennsylvania provided expertise on the experimental design and the interpretation of results.

We centered our study on a set of 56 genes which the American College of Medical Genetics and Genomics, the ACMG had identified in 2013 as critically important in genomic analysis. Indeed these 56 genes were recommended for analysis in all exome and genome sequencing tests in a guideline by ACMG on incidental findings. Because the ACMG guideline did not require laboratory validation of these 56 genes prior to implementation, my collaborators and I believed that we should determine the possibility of inadequacy of coverage of these genes by various exome sequencing methods.

We examined datasets from the Clinical Exome Laboratory of Thomas Jefferson University as well as the research core facility at the University of Texas Southwestern Medical Center.

Bob Barrett: Okay, let's get into it: what were your findings?

Dr. Jason Park: We examined 57 exome datasets performed by three different methods, 12 of the datasets were from clinical exomes, the remainder was performed as research samples. The overall performance statistics of the datasets were comparable to previous reports on clinical exomes. For the 56 genes, we identified in a database 18,336 nucleotide locations that were known to be associated with pathogenic changes, so associated with human disease.

We examined whether the 57 exome datasets had adequate sequencing data to identify potential pathogenic changes that could occur at these 18,336 locations. What we found was that a high number of variant locations were not covered in these datasets.

Furthermore, the variant locations with poor data differed between each exome method. Although the clinical exome method had the best performance, it still had 7 genes of the 56 genes where greater than 50% of the pathogenic variant locations were inadequately covered.

Bob Barrett: Did anything in these findings surprise you?

Dr. Jason Park: Well, we knew going into the study that there would be genes that have poor or incomplete coverage; however, we did not know that this would be a common occurrence for most genes. Indeed in our study only three of the 56 genes had all pathogenic variant locations with complete coverage. The remaining 53 genes were incomplete in terms of sequencing data in almost all methods.

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Bob Barrett: Do you think the results of your study were representative of exome tests from other clinical laboratories?

Dr. Jason Park: I believe that our findings on incomplete coverage of genes are inherent to current exome methods, and apply to all laboratories performing exome testing, both in research as well as clinical settings.

Bob Barrett: So is there a way to improve the incomplete coverage issue in exome tests?

Dr. Jason Park: In the past year, there have been several reports of improving exome methods for this coverage issue. These improvements include increasing capture probe concentrations at regions that are inadequately covered. Improved exome methods have been reported to result in greater than 99% coverage of clinically relevant genes. This is a vast improvement over the previously reported exome nucleotide coverages of 90-95%.

However, even at 99% nucleotide coverage, 10-15% of genes can still be inadequately covered. Clinical laboratories need to recognize coverage as a significant problem and continue to improve this issue with clinical tests. There is a lot of room for innovation.

Bob Barrett: Are there any other quality issues that were not addressed in your study?

Dr. Jason Park: Bob, this is a very good question! Our study only examined the most basic issue of having enough data for completely covering each gene of interest. There are other critical quality issues that still need to be addressed; these issues include informatics problems such as the accuracy of the data at each base position, and the correctness of clinical interpretation of the sequencing data.

Bob Barrett: Well, finally, let's look ahead, what do you think will be the clinical role of exome testing over the next, say 2-3 years?

Dr. Jason Park: Clinical exome test currently have a role in rare genetic disease testing. In order for exome testing to become a more mainstream genetic diagnostic tool, the quality of these tests need to be improved to match if not exceed the quality of traditional DNA sequencing tests. If the quality and analytical performance of exome tests can be improved then I think they may replace most conventional DNA sequencing tests.

Bob Barrett: Dr. Jason Park is Director of the Advanced Diagnostics Laboratory at the Children's Medical Center in Dallas, an Assistant Professor with the University of Texas Southwestern Medical Center in the Department of

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Pathology and the Eugene McDermott Center for Human Growth and Development. He has been our guest in this podcast from *Clinical Chemistry* looking at exome analysis in the clinical laboratory. His paper appeared in the January 2015 issue of *Clinical Chemistry*, a special issue devoted to molecular diagnostics.

I am Bob Barrett. Thanks for listening!

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