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Carol J Saunders, Luca Brunelli, Michael J Deem, Emily G Farrow, Madhuri Hegde, and Zornitza Stark.
More Than a Decade of Rapid Genomic Sequencing: Where Are We Now?
Clin Chem 2024; 70(4): 577–83. https://doi.org/10.1093/clinchem/hvae025

Guests: Drs. Carol Saunders and Emily Farrow from the Clinical Genetics and Genomics Laboratory at Children's Mercy Hospital in Kansas City, Missouri.

Bob Barrett: This is a podcast from *Clinical Chemistry*, a production of the Association for Diagnostics & Laboratory Medicine. I'm Bob Barrett. An estimated one in four patients in neonatal intensive care units has a rare genetic disease. As the initial signs and symptoms are often non-specific, it's difficult to accurately predict the pathogenic variant and conclusively identify the underlying condition by testing a single gene. Instead, casting a broad net by performing genome sequencing is often the shortest route to a definitive diagnosis.

Unfortunately, genome sequencing typically takes months, much too long to guide clinical decisions for patients in an acute care setting. To address this limitation, rapid genome sequencing has greatly reduced the time to result but many questions remain unanswered. The testing is expensive. Can clinicians and laboratorians use it selectively by identifying patients who are most likely to benefit? Are there alternative testing approaches that could accomplish the same goal at a lower cost? Are there ethical or moral questions that must be considered?

A Q&A article appearing in the April 2024 issue of *Clinical Chemistry* describes the benefits and limitations of rapid genome sequencing, discusses alternative testing options, and provides a glimpse of where the field is moving next. In this podcast, we are excited to welcome the moderator of that Q&A session and one of the expert panelists. Dr. Carol Saunders is the Division Director for the Clinical Genetics and Genomics Laboratory at Children's Mercy Hospital. She has extensive experience in interpretation and reporting of genomic data, including rapid exomes and genomes.

Dr. Emily Farrow is an Assistant Clinical Laboratory Director in the Clinical Genetics and Genomics Laboratory at Children's Mercy Hospital. She works closely with the laboratory, particularly in assay development and validation. Dr. Saunders, we'll start with you. Since this testing is still expensive, how do you determine which patients undergo rapid testing and how does your institution balance resources?

Carol Saunders: So, as we know, there are some patients for whom expedited turnaround times are necessary to impact their clinical care, and we definitely don't have the resources to offer this to everyone. So, we limit it to the ones most likely to be impacted by a quicker result. So at Children's Mercy, we were the first ones to run a rapid genome back in 2012, which was on a research basis. So, for a few years, we had a research program going. It was an NIH-funded research program where the inclusion criteria for having rapid genome sequencing was that the patient had to be in intensive care and suspected to have a monogenic disease.

Now, our current indications for considering rapid sequencing in our patients include those with abnormal newborn screening test results, in patients under the age of 60 days, those with arrhythmia or experiencing unexpected rapid decline, and other things on a case-by-case basis that the clinicians bring up. So, as far as how we balance the resources, since it is still expensive to run a rapid genome sequencing program, the implementation requires increased staffing above what we would normally need for the test volume that we see in order to meet our turnaround times.

So, it takes quite a bit of capital investment for equipment, which, this may not be feasible for small non-commercial laboratories. So, in order to balance a growing demand for clinical testing with these faster turnaround times within our own institutional constraints in 2019, we started offering an expedited option for clinical exome testing, as well as panels and single genes that we informatically carved out from an exome background. So, the test includes mitochondrial genome sequencing and copy number variation, and our average turnaround time is about 19 days.

We've been running exomes instead of genomes for several reasons. One is cost. A rapid genome is about three times the cost of an exome, depending on the batching and our configuration. So, this has recently changed for us. We have some newer instrumentation and the sequencing cost is definitely coming down. The other side of the coin is reimbursements. So for genome sequencing, reimbursement has not been great in our experience. We've been running clinical genomes since 2015 and we find that the government payers are reimbursing at about 13% in our great state of Missouri, and many large private payers still consider it experimental. So, in contrast, reimbursement is actually really good for exome sequencing.

Bob Barrett: Dr. Farrow, from the laboratory perspective, what are some of the operational considerations for performing rapid genome sequencing?

Emily Farrow: So, rapid genome sequencing requires increased staffing above testing levels to meet those quick turnaround times. In addition, the capital investments for equipment, that again may not be feasible for smaller laboratories. If you want to implement turnaround times of 24 to 48 hours, that requires another significant increase in laboratory and analyst staffing, as samples must be processed and analyzed immediately, in addition to a significant increase in sequencing cost to the inability to batch those samples together.

In contrast, our approach of implementing expedited testing with again, an average turnaround time of about 19 days, it's allowing us for the balance of a faster test for that smaller number of patients within the overall larger patient population, and this decreased turnaround time can be addressed through sample prioritization, flexible sample batching, and sequencing configurations, which has allowed us to eliminate the need for increased staffing bubbles. Really, in most cases, a two- to three-week turnaround time is sufficient to impact the clinical decision-making for our patients and their providers.

Bob Barrett: So, how can laboratories determine whether to perform rapid genome versus exome sequencing?

Carol Saunders: On the laboratory side, it's kind of intuitive, but genomes are actually less labor- and time-intensive to prepare for sequencing because there is no enrichment step. So, it's lighter on the staffing and it just takes less time. The data also gives you much more even coverage and you end up sequencing intronic regions that may harbor pathogenic variants that are not covered by a typical exome. So, as the cost of the sequencing continues to decrease, and this makes genomes more affordable, although your informatics and your storage costs are not going to decrease. So, all that just needs to be balanced out with your needs.

Bob Barrett: There are certain types of variants that short-read sequencing can't detect. Can you share the reasons why and describe newer methods that could be used instead?

Carol Saunders: Yeah. So, short-read sequencing struggles with any kind of homologous region because it's just not possible to know where it's aligning in the genome. The same goes for repeat expansions and structural variation. So, for certain disorders, short-read sequencing misses some really important variation. So, if you think of one of the most common reasons for referral for rapid genome sequencing in the NICU, hypotonia is on the top of the list. If you think of the most common genetic diseases that are associated with neonatal hypotonia, none of these are detectable by a short-read sequencing.

So, your top three, in my mind, are probably Angelman syndrome, which is an imprinting disorder that is caused by abnormal methylation, which is not detectable by a short-read sequencing. There is myotonic dystrophy, which is due to a repeat expansion, and spinal muscular atrophy, which is due to deletions of a gene called *SMN1,* which is indistinguishable by a short-read sequencing from its pseudo gene which is called *SMN2*. So, for cases like these, a newer technique called long-read sequencing is a really attractive option.

Bob Barrett: Well, several co-authors in the article mention long-read sequencing as a future direction. How does the throughput compare to short-read instruments and does long-read sequencing present unique challenges that laboratorians should consider?

Emily Farrow: So, third-generation sequencing, or long-read sequencing, has really dramatically changed over the past few years, with decreasing costs of sequencing and increasing throughput, allowing for this technology really to be utilized clinically. Our laboratory has chosen to use PacBio HiFi sequencing, which has an average read length of over 13,000 basepairs. In comparison, when we talk about short-read sequencing, it's typically 150 basepairs at a time. These longer read lengths allow for the accurate sequencing alignment in homologous regions over those repeat expansion and/or structural variants.

All the areas that we just mentioned that short-read sequencing struggle. Further, an exciting aspect of HiFi sequencing is that it also detects methylation. So, if we go back to that case of neonatal hypotonia as an example, this means that we can detect all of those common molecular mechanisms in a single test. So, our institution was the first to launch clinical HiFi genomes in October of 2023 last year for acutely ill in-patients, and thus far, with our long-read sequencing and our in-patient population, we are averaging a diagnostic rate of about 52% with an average turnaround time of about 25 days.

But there are still some caveats to long-read sequencing. The throughput although getting faster is still significantly lower than short-read sequencing, and while the costs have decreased, they still remain higher than their short-read counterpart. So, given these considerations, it would be difficult to transition all sequencing to long-read sequencing today. However, much as we have seen with the short-read world, we anticipate that the throughput will continue to increase while costs decrease.

As far as implementation on the laboratory side, there are additional caveats that may not be obvious at first. One

consideration is we don't often talk about sample requirements. So, current long-read sequencing requires high-quality, high-molecular weight DNA, which can limit the types of samples you can use. So, buccal examples are, for example, are not currently amenable to long-read sequencing. Sample processing also can require instrumentation that most laboratories may not have on hand to accurately size that longer DNA, and lastly, the bioinformatic resources for long-read sequencing are not as developed as they are for short-read sequencing.

Bob Barrett: Well, finally, and I think this should probably be my last question of every podcast from now on, how will AI change this field?

Emily Farrow: So, AI in genomics today, is limited and maybe not overly impressive at the moment. But in the future, I think it will change. I think mining the medical record for phenotypic terms, which can then be used in variant prioritization and interpretation, will be very helpful. Nearly every commercial software solution currently offers their own version of variant interpretation or prioritization. While this can be helpful in some cases, they today clearly are not a substitute for an experienced analyst.

My hope for the future is that AI can be leveraged more efficiently to help us gather information on specific genes and variants, which would result in an immense time savings per case. I think one could also imagine how AI could be leveraged at the clinical level to help clinicians that are in the NICU, or in our in-patient units, identify which patients would benefit the most from the rapid sequencing.

Bob Barrett: That was Dr. Emily Farrow and Dr. Carol Saunders from Children's Mercy Hospital in Kansas City, Missouri. They participated in a Q&A article in the April 2024 issue of *Clinical Chemistry* describing the benefits and limitations of rapid genome sequencing in the neonatal intensive care unit. I'm Bob Barrett. Thanks for listening.