Clinical Chemistry

Genetic Investigation of X-Linked Defects with Optimized Targeted Long-Read Sequencing

Article:
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Genetic Investigation of X-Linked Defects with Optimized Targeted Long-Read Sequencing.
Clin Chem 2026; 72(11): 1145–1160. https://doi.org/10.1093/clinchem/hvag088

Guest: Dr. Zirui Elvis Dong is an Assistant Professor and an Assistant Director of Bioinformatics of the Prenatal Diagnostic Laboratory in the Department of Obstetrics and Gynaecology at the Chinese University of Hong Kong.

Bob Barrett: This is a podcast from *Clinical Chemistry*, a production of the Association for Diagnostics & Laboratory Medicine. I'm Bob Barrett. Many inherited conditions are caused by genetic variants in the X-chromosome, including structural rearrangement, single nucleotide variants, insertions/deletions, and trinucleotide expansion. We generally think of these conditions affecting males, but females can also be impacted particularly if genomic variants lead to skewed X-chromosome inactivation and preferential silencing of one copy. Traditional next-generation sequencing can identify some of these variants, but it struggles with repetitive regions, and the short-read format makes it impossible to assess skewed X chromosome inactivation. Long-read sequencing can help address both these limitations, but it needs large DNA fragments to work. This presents a problem because DNA samples routinely available in the clinical laboratory are fragmented into small pieces that aren't compatible with current long-read sequencing protocols. A research article in the November 2026 issue of *Clinical Chemistry* addresses this problem with an adapted long-read sequencing protocol and demonstrates its effectiveness in detecting X-linked variants that were missed by traditional test methods.

Today, we welcome the article's senior author, Dr. Zirui Elvis Dong is an Assistant Professor and an Assistant Director of Bioinformatics of the Prenatal Diagnostic Laboratory in the Department of Obstetrics and Gynaecology at the Chinese University of Hong Kong. And Dr. Dong, your new test sounds like a major step forward for genetic diagnostics. What are the broader public health implications of being able to read these complex regions of the X chromosomes so much more clearly?

Zirui Dong: Yeah, this is a good question. The biggest public health impact is about accessibility and accuracy of a genetic diagnosis for the family. Like X-linked diseases, like the fragile X syndrome, it can be incredible severe. So

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particularly for the women, they can sometime as a carrier or can be an affected patient. The disease causing mechanism are complex as they are involving not only the DNA sequence change, but also the methylation alteration. So methylation, you can easily understood as a switch to turn on or turn off the gene expression. In current clinical setting, the patient need to undergo a series of tests to get the answer, but sometimes they will got a failed answer. So previously for the advanced long-read sequencing technology, they are not quite easily accessible for many patients because it requires sequencing the entire genome for the patient, which is causally, but it also asks for a high quality of the DNA as input, which is not practical in clinical setting.

So, after our optimization of this target sequencing based approach, a one-stop solution is likely off the table for the patient suspected with X-chromosomal related genetic disorder.

Bob Barrett: What is the bottom line here for families affected by rare or inherited disorders? Should patients with suspected X-linked conditions skip older genetic tests entirely and go straight to this new long-read sequencing method?

Zirui Dong: I guess you are referring to the patient who are suitable for this test. It is applicable for patient that their prior genetic investigation or their phenotypic presentation indicate that they might suffer X-linked genetic disorder, which warrants further confirmation. Therefore, for these kinds of patient, they might go directly for this test to get a diagnostic answer instead of going through a series of standard workups.

Bob Barrett: Can you tell us why standard next generation sequencing struggles with the X chromosome and, and why your adaptive sampling approach is needed to see the full picture?

Zirui Dong: Think about standard next generation sequencing [NGS] method like a small puzzle, and we need to try our best to put them back as a full picture. So if the puzzle have a lots of identical repeating patterns, you will completely lost. That's where standard tests fail on some diseases related to the X chromosome. In addition, X inactivation is another issue that standard NGS is not able to review. Routinely, we need to perform enzyme digestion with the whole family to consider whether one of the chromosome X was with skewed inactivation. But it is not feasible when the DNA changes are de novo, which means neither inherited from the father or the mother.

Long re-sequencing method leaves the puzzle in a much larger pieces and simultaneously provide methylation changes, which conventional target sequencing is not able to offer. So conventional target sequencing relies on PCR to

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amplify the target, which cannot reveal the methylation information. While our adaptive sampling act as a smart digital future to enrich the target region in a real-time manner. Most importantly, it can also provide the methylation information in a per gene level. But the standard approach is not optimal. Therefore, we tried to optimize this protocol. After optimization, it give us an over 15-fold enrichment for improving the sequencing clarity without the extra cost. And it also demonstrates the capability of delineating genomic changes with methylation information provided at the same time.

Bob Barrett: Doctor, your studies show that this technique actually changed the genetic diagnosis or risk category for 15% of the families involved. How does this clearer look at X chromosome inactivation change practical medical advice or family planning counseling?

Zirui Dong: Well, this is another good question. So it mostly helped the patient and the family to refine their planning and the peace of mind. As we know, women have two chromosome X, and in nature setting, it will randomly turn one on and turn one off. This is a process called X chromosomal inactivation. If a genetic defect is on the silence chromosome X, the woman might not be affected and will be presented perfectly healthy, and you can pass this defect to the next generation. But in contrast, she will be affected. Standard test can tell you a defect exists, but they can't tell you the layout sometimes. And usually I'm not able to tell you if the affected chromosome is active or not, particularly not the gene-based level. Our test looks at the DNA structure and the methylation signal at the same time. For example, in our study, we saw one female fetus having the same chromosome Xq28 deletion inherited from the healthy mother. Protein X inactivation assay told us that both the mother and the fetus have skewed X inactivation, but we're not able to know whether the skewed chromosome X in the fetus is the same as that in the mother because there are some signal from the routine X inactivation test indicating crossing over occur during the mother was producing a gamete.

In addition, as the breakpoint likely involve a protein coding gene, which has a pseudogene, which also making us worry that whether the fetus have the same breakpoint as the mother, because next generation sequencing was not able to delineate the breakpoint. But luckily, by our adaptive sampling approach, we not only confirm the same deletion in the fetus as that in the mother, but also indicate a skew inactivated chromosome X in the fetus was the same as that in the mother. So knowing this allowed the family to have precise life-changing reproductive counseling for her current and future pregnancy.

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Bob Barrett: Well, finally, Dr. Dong, can you give us a glimpse of where your research in this field is going? Since you successfully optimized this test for routine clinical DNA samples, is this technology ready for each lab to use or are there still hurdles to clear?

Zirui Dong: So, we are incredibly excited because we're making successfully to use the routine clinical DNA. It did bring us much closer to the clinical floor. However, before it becomes a standard test in every lab, we have a few hurdles. Currently, the process requires a relatively large amount of starting DNA material, which are not able to be accessible in some of the application, particularly in prenatal diagnosis. Our next step is to refine the protocol to reduce the amount of starting material and to validate it in a much larger patient cohort, which can prove whether or not this method can safely become a routine reality.

And I'm more than happy to share you that because of this pilot study, we just got a funding support from the Hong Kong Health Bureau to carry out a large-scale clinical validation. Last but not least, this optimized protocol is not just applicable for X-linked genetic disorder, but with the potential extension to the disease locus across the other genomic region. Thank you.

Bob Barrett: That was Dr. Zirui Elvis Dong from the Chinese University of Hong Kong. He wrote a research article in the November 2026 issue of *Clinical Chemistry*, describing a new long-read sequencing protocol for X-linked disorders. He's been our guest in this podcast on that topic. I'm Bob Barrett. Thanks for listening.

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