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Universal Haplotype-Based Noninvasive Prenatal Testing for Single Gene Diseases

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Guest: Dr. Rossa Chiu is the Choh-Ming Li Professor of Chemical Pathology and Assistant Dean of Research at the Faculty of Medicine the Chinese University of Hong Kong.

Bob Barrett: This is a podcast from *Clinical Chemistry* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I'm Bob Barrett.

Noninvasive prenatal testing relies on the detection and analysis of circulating free fetal nucleic acids found in the blood of pregnant women. This technology has revolutionized prenatal detection for some fetal chromosomal anomalies like trisomies 21, 13, and 18, because protocols for these applications have been relatively easy to implement in clinical care. Noninvasive prenatal testing for single-gene diseases has been more limited in part because labs needs to design a new assay for each mutation they want to investigate. Testing may also be restricted to those with an affected family member as their DNA is required for some protocols.

A more streamlined approach is needed to expand the utility of the noninvasive prenatal testing to include single gene disease like cystic fibrosis or beta thalassemia. Researchers at the Chinese University of Hong Kong and their collaborators believe they have done just that. They have developed a universal method for noninvasive prenatal testing for single-gene diseases that does not require knowledge of specific disease-causing mutations or DNA from an affected family member. The article describing their approach appears in the February 2017 issue of *Clinical Chemistry.*

For this podcast, we're joined by the first author, Dr. Rossa Chiu. She is the Choh-Ming Li Professor of Chemical Pathology and Assistant Dean of Research at the Faculty of Medicine, The Chinese University of Hong Kong. So Dr. Chiu, what are single-gene diseases?

Dr. Rossa Chiu: Single-gene diseases are diseases where the manifestation is a result of mutations that could be traceable to a gene in the genome. These are what we traditionally call the inherited diseases. For example, they include autosomal recessive disorders, autosomal dominant disorders, or sex linked disorders. Single-gene diseases, except some

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diseases which are relatively prevalent in certain ethnic groups such as cystic fibrosis, sickle cell anemia, beta thalassemia, most single-gene diseases are considered to have a rare occurrence. However, they are clinically very important because collectively, that is if we sum up every one of the single-gene diseases known to date, the World Health Organization has estimated that the disease burden can be as high as affecting 1 in 100 pregnancies.

Bob Barrett: Talk about this study, what's this about and what are the advances provided by this study?

Dr. Rossa Chiu: This study is about the development of an approach to perform noninvasive prenatal testing for single-gene diseases. And the approach is based on analyzing the small amounts of baby's DNA that one can find in the circulation of pregnant women. And so the advantage of noninvasive approaches is that one could avoid relying on invasive approaches such as amniocentesis to obtain the baby's DNA for analysis. And so the testing process would be safer for the fetus.

And in terms of this particular study, the advances that were brought about is that we have previously developed other approaches that would allow one to test for single gene diseases noninvasively during the prenatal period. But our previous approaches, most of them, are dependent on the development of different assays to target different mutations.

You can imagine, it would be quite complex to implement in that when we have a couple whose pregnancy is known to be at risk for certain single gene disease, then we might need to define a specific assay tailored for that particular family. So this makes the noninvasive prenatal testing quite difficult for laboratories to set up. And also there is pressure for the laboratory to optimize assays that they might not already have developed, in a short period of time so that the analysis could be completed in good time during the pregnancy.

And the advance that we have brought about in this particular study is that we have developed an approach that would allow laboratories to use a fairly similar set of reagents to apply for the noninvasive prenatal testing of most single-gene diseases.

So in other words, we see that with the approach described in this study, one could now genuinely develop a protocol that could be ready in the laboratory for the assessment of pregnancies who might be at risk for a certain single-gene diseases and for various different mutations.

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In other words, we believe that it would allow the clinical implementation of noninvasive approaches for single-gene diseases to become more of a reality.

Bob Barrett: So what are the key advantages provided by this newly reported approach?

Dr. Rossa Chiu: Well, there are several advantages. Actually in the past, there were several approaches developed for the noninvasive prenatal detection of single-gene diseases. And the latest approach that we have reported in this particular study, it has overcome most of the limitations of the previous approaches.

So for example in the past, there were a group of approaches that were dependent on the use of specific assays for specific mutations, and as I have mentioned this approach is a bit cumbersome to practice. So with the new approach we overcome this limitation. And actually in the past we have also attempted to develop methods that are more generic and actually we have developed a haplotype-based approach. Mainly in that, instead of trying to detect the specific mutations in maternal plasma, we try to detect the polymorphic alleles that might be linked to the mutant or the non-mutant alleles of the father or the mother, and try to detect these polymorphic alleles in the maternal plasma in order to make the interpretation of whether the baby has inherited the parents' mutations or not and hence provide a diagnosis.

The latest approach that we have reported in this study is based on this haplotype-based approach. But unlike the previous haplotype approach, in that previously--it's actually very difficult to obtain haplotype information on any person's DNA, one would either need to rely on a very labor-intensive technique or in one of the studies that we have performed in the past--we actually used information from another affected member of the family in order to obtain the haplotype information.

But the disadvantage of needing to use DNA from another affected member of the family is that it means that the noninvasive testing approach is only applicable to pregnancies where there is a prior affected member in the family. And also one would need to have access to DNA of that affected family. But in this study that we have conducted, we actually make use of a technique called "link resequencing." It allows us to perform direct haplotyping.

So, it's so streamlined that we show that all we need is a sample from the father which allows us to obtain the parental haplotype information, and then we also need a blood sample from the mother, and we could use the

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mother's blood cell DNA to obtain her haplotype information. And then we would use the maternal plasma portion, analyze the DNA there which contains the baby's DNA. And then after we have analyzed the maternal plasma DNA, then we interpret the maternal plasma data using the haplotype information from the parents.

Based on this algorithm we show that we could noninvasively assess if the fetus has inherited mutations from the parents' known conditions including beta thalassemia, congenital adrenal hyperplasia in the form of 21-hydroxylase deficiency, and hemophilia. So we demonstrated that we were able to achieve and arrive at the diagnosis with the use of only DNA from the parents. And so the advantage of this approach is that it has overcome the limitations of some of the previous approaches.

Bob Barrett: Finally Dr. Chiu, in your opinion what are the next steps needed before the approach could be applied clinically?

Dr. Rossa Chiu: As with any studies related to medical tests, we do need to perform larger scale analysis and clinical validation to better understand the sensitivities and the specificities of the test that we have developed. And so this is exactly the next phase that is needed for the approach that we have just reported.

And as I have mentioned that some of the single-gene diseases, the prevalence or the incidence is actually quite infrequent, so I foresee that it might take some time to recruit adequate numbers of various disease cases in order to test the approach and to better understand the performance before one could make a decision of whether it is useful enough in the clinical setting.

Bob Barrett: Dr. Rossa Chiu is the Choh-Ming Li Professor of Chemical Pathology and Assistant Dean of Research at the Faculty of Medicine, the Chinese University of Hong Kong. She is been our guest in this podcast on detection of single-gene diseases from *Clinical Chemistry*.

I'm Bob Barrett. Thanks for listening.

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