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Noninvasive Prenatal Diagnosis of Monogenic Diseases by Targeted Massively Parallel Sequencing of Maternal Plasma: Application to β-Thalassemia

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Guest:
Dr. Dennis Lo is the Director of the Li Ka Shing Institute of Health Sciences, and Professor of Medicine and Chemical Pathology at The Chinese University of Hong Kong.

Bob Barrett: This is the podcast from *Clinical Chemistry*. I am Bob Barrett.

The discovery of cell-free fetal DNA in the maternal plasma during pregnancy has opened up many new possibilities for noninvasive prenatal diagnosis. In the October 2012 issue of *Clinical Chemistry*, Dr. Dennis Lo and his colleagues in Hong Kong have applied their massively parallel sequencing approach to β-thalassemia. Dr. Lo is our guest in this podcast.

Dr., what background work was necessary that led to this recent paper in *Clinical Chemistry* on thalassemia?

Dr. Dennis Lo: We have previously shown that fetal DNA is present in the plasma of pregnant women. In 2010, we showed that a genomewide genomic map of fetus can be deduced by deep sequencing of the DNA molecules in the mother's plasma. And these results have recently been confirmed by two other studies.

But however, whole genome fetal sequencing is expensive. And so for clinical application, one important point is to see if we can specifically target selected genomic regions in the genome which are involved in common genetic diseases.

And so the current work basically focuses on trying to see if we can indeed achieve such a targeted approach.

Bob Barrett: What is the most important advance reported in your paper?

Dr. Dennis Lo: The most important advance reported in this paper is that this selected targeted sequencing approach actually, indeed, works. To achieve this, basically we have to elucidate what the fetus has inherited from its parent separately.

So first, we have to elucidate what the fetus has inherited from its father, and then, to separately elucidate what it has inherited from its mother.

So, for the former approach, basically we have to look at sequences which is only present in father's genome, but absent in mother's genome, and then we try to look for these sequences in the mother's plasma. So, this is essentially a qualitative diagnosis.

The second part is to elucidate what the fetus has inherited from its mother. And this is more difficult because the fetal DNA is surrounded by an excess of the mother's DNA in the mother's plasma. So, in effect what basically we need to do is to try to measure the quantitative difference, different sequences which are present in mother's plasma.

So this second part of diagnosis is a quantitative diagnosis. So, what we have achieved here is that even with such targeted sequencing of the DNA in the mother's blood, we can achieve this precise qualitative and quantitative analysis.

Bob Barrett: Dr. Lo, can you tell us about some of the other new findings in your paper?

Dr. Dennis Lo: Okay. So apart from what we discussed just now, another important new finding is that we show for the first time that even if the father and mother are very similar genetically in the region which we analyze, we can still apply this approach.

And this is important because sometimes for certain genetic diseases, some families, the father and mother might be related to each other like in consanguineous marriages or in genetic diseases in which there is a very strong family effect.

So, the ability to carry out the analysis even if father and mother are very similar genetically in the analyzed region is important and we show that that is possible.

Bob Barrett: During your work, did you encounter any difficulties?

Dr. Dennis Lo: So one very important component of this work is that we have to elucidate what the fetus has inherited from its mother. And to do this, we have to analyze the haplotype structure of the mother. So, haplotype means to elucidate the interrelationship of different polymorphisms which are present on the same chromosome.

And so, in this work, to elucidate the haplotype structure of the mother, we have to do single-molecule PCR followed by

sequencing, and this is actually quite tedious. And so this is the part which has taken us the most time in the current work. But luckily, on the horizon, there are a number of approaches which allow us to do genomewide haplotyping much more easily. So hopefully, this approach would become easier as time goes on.

Bob Barrett: So doctor, what was the main clinical implication for your work?

Dr. Dennis Lo: The main clinical implication of this work is that we can now do targeted sequencing of selected part of the human genome to allow us to carry out noninvasive prenatal diagnosis of selected single gene disorders. And in this work, we have shown that you can do this with β-thalassemia, which is a common genetic form of anemia which affects many parts of the world. And if we can do this for β-thalassemia, then theoretically, we can extend this to virtually all monogenic diseases.

Bob Barrett: Well finally doctor, let's look ahead. What follow-up work are you planning to do?

Dr. Dennis Lo: So, the follow-up work is that we're trying to show that what our theoretical prediction, that the approach can be applied to virtually all monogenic disease, actually holds true. So we're now testing a number of single gene disorders by using this approach.

And also, we're trying to see if we can further simplify the protocol to make it faster, easier, and more cost-effective.

Bob Barrett: Dr. Dennis Lo is the Director of the Li Ka Shing Institute of Health Sciences, and Professor of Medicine and Chemical Pathology at The Chinese University of Hong Kong. He has been our guest in this podcast from *Clinical Chemistry*.

I am Bob Barrett. Thanks for listening!