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Noninvasive Prenatal Testing for Wilson Disease by Use of Circulating Single-Molecule Amplification and Resequencing Technology (cSMART)

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Guest:
Dr. Desheng Liang is Professor and Deputy Director of the State Laboratory of Medical Genetics of China at Central South University and Vice Chairman of the Chinese Society of Birth Defect, Prevention and Control.

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.

Prenatal testing using blood specimens rather than more invasive sampling has been successfully used for common chromosome disorders and for clinically significant copy number variations. However, detecting single gene disorders which are caused by mutations remains an analytical challenge.

In the January 2015 issue of *Clinical Chemistry*, a special issue devoted to molecular diagnostics, a group of researchers from the Laboratory of Medical Genetics and neighboring institutions in Changsha, China, described a noninvasive prenatal test for Wilson's disease using Circulating Single-Molecule Amplification and Resequencing Technology.

In this podcast we are joined by one of the authors of that paper, Dr. Desheng Liang. He is Professor and Deputy Director of the State Laboratory of Medical Genetics of China at Central South University in Changsha and Vice Chairman of the Chinese Society of Birth Defect, Prevention and Control.

Doctor, detecting single gene disorders such as Wilson's disease, which are caused by gene mutations remains challenging. Tell us why such testing is so difficult for laboratories?

Dr. Desheng Liang: Okay, the difficulty lies on the fact that the fetal DNA, which is circulating in the maternal plasma, is fragmented randomly into very small pieces. To detect fetal mutation or mutations, we must therefore enrich the target DNA.

This random fragmentation dramatically reduces the templates for traditional amplification methodologies such as

PCR. And as we know, maternally derived DNA fragments are on average larger in size than fetally derived fragments. The preferential PCR amplification of smaller over larger fragments could potentially skew the true allelic ratios.

Also, the dichotomy of size distribution between maternal and fetal fragments may promote variable amounts of linear versus exponential amplification in the two DNA populations, further skewing allelic ratios.

Together with our collaborator, Berry Genomics in Beijing, we developed a method named Circulating Single-Molecule Amplification and Resequencing Technology, or short for cSMART.

In cSMART, pre-amplified single allelic molecules with unique barcodes were targeted and sequenced, but only counted once, eliminating potential PCR size bias and allowing for more precise quantification of the mutant allele percentage in the original plasma sample.

That is to say, the cSMART technology smartly counts individual DNA mutation fragments in the maternal plasma, hence avoids such template loss and allelic ratio bias. The percentage of mutation in the maternal plasma can then be used to determine whether the fetus has inherited the single gene disease.

Bob Barrett: Doctor, how broadly can the cSMART technology be applied?

Dr. Desheng Liang: In theory, it is applicable to any genetic diseases, with a known causal gene. In the paper, we use Wilson's disease as an example, which is caused by mutations in the ATP7 gene.

That was our first case study. Since then we have successfully developed assays for many other diseases, including PKU, DMD, I mean Duchenne Muscular Dystrophy, et cetera. We are very excited about the progress and future possibilities. Of course, we are also very mindful of the ethical and legal consequences of developing such tests.

Bob Barrett: Very interesting! Are there any implications or applications to the general population?

Dr. Desheng Liang: Yes, we think that this is quite revolutionary. For the first time, we can determine accurately if the fetus carries certain genetic mutations by simply drawing a tube of maternal blood. For some genetic diseases, such as RhD, in which the blood transfusion between mother and fetus may cause problems, there are substantial risks associated with an invasive procedure. cSMART technology will be the choice.

In addition, like any other NIPT, because it is simple, scalable and cost-effective for severe genetic diseases, cSMART opens the way for future prenatal genetic screening of single gene disorders. That is to say cSMART is anticipated to apply in carrier testing and early gestation testing, as well as simultaneous screening for multiple monogenic disorders.

Bob Barrett: Doctor, does your laboratory make cSMART testing generally available or is it currently in the research or trial phase?

Dr. Desheng Liang: Now, in our labs, the cSMART technology is now available in my lab.

Bob Barrett: Well, besides genetic diseases, can cSMART be applied to the detection of other diseases?

Dr. Desheng Liang: Of course, no doubt about it, particularly for oncology. We all believe that cancer is caused by DNA alterations, around which somatic mutations play a major role. Oncology and pregnancy share a lot of similarities in this aspect. Like a fetal DNA in the maternal circulation, tumor DNA can be found in the host blood. This is so-called circulating tumor DNA or ctDNA.

The scientific and medical community has long been searching for a sensitive and specific method that can reliably detect somatic mutations using host blood samples, a notion we call liquid biopsy. We have accumulated a lot of evidences supporting that cSMART is the appropriate technology.

Our collaborator, Berry Genomics, is currently conducting clinical trials to demonstrate the sensitivity and accuracy. If things turn out to be as we hope for, we may soon be able to prospectively follow the mutation profile of a tumor through a tube of blood, which may tell us if cancer exists in the earlier stage, or a tumor is shrinking after treatment.

Bob Barrett: Dr. Desheng Liang is Professor and Deputy Director of the State Laboratory of Medical Genetics of China at Central South University and Vice Chairman of the Chinese Society of Birth Defect, Prevention and Control. He has been our guest in this podcast from *Clinical Chemistry* on molecular technologies to detect Wilson's disease. 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!