Clinical Chemistry

Single Molecule Sequencing of Free DNA from Maternal Plasma for Noninvasive Trisomy 21 Detection

Bob Barrett: This is the podcast from *'Clinical Chemistry'*. I am Bob Barrett. The discovery of cell-free fetal DNA and RNA in maternal plasma in 1997 has opened many possibilities for Noninvasive Prenatal Diagnosis. In some cases prenatal diagnosis can now be performed without any invasive procedures, which were associated with a risk of miscarriage.

In 2008, Noninvasive Down Syndrome or Trisomy 21 detection by Next Generation Sequencing was introduced, opening a whole new way of analysis.

Our guest in this podcast today is Dr. Elles Boon who is a Clinical Molecular Geneticist working in the laboratory for Diagnostic Genome Analysis in Leiden, the Netherlands. She is the Principal Investigator for developing and implementing Noninvasive Prenatal Tests in diagnostics and her paper on a Noninvasive Assay for Trisomy 21 appeared in the April 2012 issue of *'Clinical Chemistry'.*

Doctor, Noninvasive Detection of Down Syndrome using Next Generation Sequencing has been published previously, so what's new about the method you describe in your article?

Dr. Elles Boon: Well, in this study we will use Single Molecule Sequencing, which differs from the technique used in previous publications. The Next Generation Sequencing platforms previously tested use the PCR step during sample preparation, which results in amplification bias and thus experimental noise, and since we aim to have a reliable detection of fetal trisomies like Down's syndrome in pregnant women, we used an alternative sequencing platform where single molecules can directly be sequenced without any amplification step, and therefore, without any PCR bias.

Bob Barrett: So your data would give a better representation of the fetal DNA that's present in maternal blood?

Dr. Elles Boon: Yes, the data generated using this method directly show the presence of fetal trisomies without any experimental bias. We could demonstrate this in this study by directly comparing Noninvasive Down's syndrome detection using single molecule sequencing to the previously described PCR-based Illumina Platform. Using the Illumina Platform we could clearly detect the bias in GC-rich areas of the human genome, which was not present in the data from single molecule sequencing.

Bob Barrett: Did this also influence the detection of fetuses with Down's syndrome in pregnant women?

Dr. Elles Boon: Well, normal ways of detection of trisomy 21 can be performed on both platforms; however, we did see a better distinction between fetuses with or without a trisomy 21 when using single molecule sequencing.

Bob Barrett: If we would use this method in clinical practice, is the blood sampling any different from what was previously described?

Dr. Elles Boon: No, the blood sampling remains the same. So in this study blood samples were taken from women wanting to participate during the first trimester of pregnancy. We isolated free DNA which contains fetal DNAs from maternal plasma and used this as input for the sample preparation for Next Generation Sequencing. The sample preparation for single molecule sequencing is however different from the PCR-based Next Generation Sequencing Platform. You only need low amounts of input DNA, which is important if you want to perform this test early in pregnancy and not a lot of fetal DNA is present in maternal plasma.

Also, the sample preparation in the lab is less laborious and faster, but the blood sampling itself remains the same.

Bob Barrett: In your study this method was shown to be successful for noninvasive detection of trisomy 21. How adaptable is this technique for the finding of other trisomies?

Dr. Elles Boon: We believe that this method will also have a better detection rate for trisomies 13 and 18. These trisomies can now also be detected using the current invasive procedures, but are more difficult to detect non-invasively using the previously described PCR-based Next Generation Sequencing Platforms. That is because chromosomes 13 and 18 has a different GC content compared to chromosomes 21, resulting in more skewed data when using a PCR step. This could be overcome by using Single Molecule Sequencing. So yes, we believe that it is adaptable for the detection of more than only trisomy 21.

Bob Barrett: Dr. Elles Boon is a Clinical Molecular Geneticist working in the laboratory for Diagnostic Genome Analysis in Leiden, the Netherlands. And she has been our guest in this podcast from *'Clinical Chemistry'*.

I am Bob Barrett. Thanks for listening!

Total Duration: 5 Minutes