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Biological Insights from Cell-Free DNA Methylome Analysis in Preeclampsia

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Guest: Dr. Stephanie Yu is a Clinical Lecturer in the Department of Chemical Pathology 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. Preeclampsia is a relatively common condition affecting an estimated 2% to 10% of pregnancies worldwide, marked by new onset hypertension and proteinuria. With or without other organ dysfunction, preeclampsia is life threatening if left untreated. Complicating matters is that the only treatment is delivery of the placenta, which often puts physicians and their patients in a difficult position: induce delivery despite the known risks of preterm birth, or prolong pregnancy and increase the risk of adverse events for the pregnant woman. While effective treatments are lacking, low dose aspirin can help prevent preeclampsia, but is most effective when initiated before 16 weeks gestation, well before signs and symptoms of preeclampsia become apparent. To tackle this problem, we need a tool that reliably identifies asymptomatic women early in pregnancy who will develop preeclampsia weeks later, thereby helping to ensure that they receive prophylactic treatment. A new perspective article, appearing in the June 2024 issue of *Clinical Chemistry*, highlights efforts to better predict preeclampsia using a novel cell-free, DNA based approach.

In this podcast, we welcome the article's lead author. Dr. Stephanie Yu is a Clinical Lecturer in the Department of Chemical Pathology at the Chinese University of Hong Kong. Her research focuses on the biology and clinical applications of cell-free nucleic acid. So, Dr. Yu, first of all, what are the benefits of early prediction of preeclampsia and what tools do we currently have at our disposal for this purpose?

Stephanie Yu: Early prediction of preeclampsia allows for the prophylactic use of low dose aspirin, which several professional bodies recommend as a preventive intervention. Using low dose aspirin in pregnancies at risk for preeclampsia can lower the occurrence of early onset and preterm preeclampsia. The treatment with lower dose aspirin should be started between 12 to 28 weeks of gestation, but ideally before 16 weeks. Therefore, early prediction of preeclampsia enables timely initiation of aspirin prophylaxis. Early prediction of preeclampsia also allows for close monitoring of high risk

patients. If preeclampsia does develop, early prediction can facilitate timely and appropriate management, potentially preventing severe complications like eclampsia and multi-organ failure.

Currently, there are two main types of screening tests used in routine clinical practice to predict preeclampsia in early pregnancy. The first type relies solely on assessing maternal risk factors and this approach is recommended by professional bodies such as the American College of Obstetricians and Gynecologists. While the strategy is simple and cost effective, it only has a sensitivity of around 40% for preterm preeclampsia and performs even worse for all preeclampsia cases. The second type combines maternal characteristics with biophysical and biochemical markers. For example, the Fetal Medicine Foundation has developed an algorithm that combines maternal characteristics with mean arterial pressure, mean uterine artery pulsatility index, and serum level of placental growth factor for first trimester preeclampsia risk assessment. These tests have a sensitivity of around 80% for preterm preeclampsia and 40% for preeclampsia.

Bob Barrett: Can you briefly outline the approach used in the De Borre study to predict preeclampsia? How well did that perform and how does it compare to other existing approaches?

Stephanie Yu: Well, their method involves analyzing cell-free DNA in maternal plasma samples with targeted bisulfite sequencing. The study by De Borre and colleagues focused on predicting early onset preeclampsia. Therefore, they used around 200 first trimester cell-free DNA samples, with more than one third of the samples from women who later developed early onset preeclampsia to develop their prediction model. Their prediction model relies on detecting the differences in methylation patterns between samples from pregnant women who later developed preeclampsia and those who did not.

It's important to emphasize that the samples were collected about 20 weeks before the onset of preeclampsia, which highlights the potential of early prediction by this method. When using cell-free DNA methylation alone, the sensitivity was 38% with a specificity of 90%. However, when combining cell-free DNA methylation with maternal characteristics and medical history, the sensitivity increased to 57% while maintaining the same specificity of 90%. This combined screening test seems to perform better than existing tests that only look at clinical risk factors, but it didn't do as well as the screening test from the Fetal Medicine Foundation, which combines the maternal characteristics with biophysical and biochemical markers. Despite that, the De Borre combined screening test doesn't

need expertise to measure a sonographic markers, so it eliminates the variabilities that come with different operators. But of course, large scale prospective studies would be needed to validate the clinical performance of the De Borre method.

Bob Barrett: In the article, it's mentioned that fetal fraction is an important confounder in cfDNA methylome analysis in pregnancy. Can you elaborate more on this and suggest ways to address it?

Stephanie Yu: The fetal fraction is the percentage of cell-free DNA in a sample that comes from the fetus. In the mother's blood, the fetal DNA mainly comes from the placenta, while the maternal DNA comes from the mother's blood cells. Since the placenta has lower levels of DNA methylation compared to the maternal blood cells, a sample with higher fetal fraction will have lower cell-free DNA methylation levels. Therefore, when analyzing the methylation of cell-free DNA in maternal plasma, it is important to consider how the fetal fraction might affect the observed methylation patterns. Based on previous studies, there is a significant variation in fetal fraction among pregnant individuals. Any conditions that alter the placental or maternal contribution may affect the fetal fraction. For example, maternal obesity is often linked to a reduced fetal fraction. Additionally, previous studies have shown that patients who later develop preeclampsia tend to have a lower fetal fraction in the late first trimester before the onset of the disease. So, if we notice differences in the methylation patterns between preeclampsia and gestation matched controls, these differences could be due to variations in fetal fraction alone or a combination of differences in fetal fraction and tissue methylation levels, especially if the cases and controls were not matched for fetal fraction. Therefore, it is important to consider the confounding effect of fetal fraction on the observed methylation profile.

As discussed in the article, the first strategy employed by De Borre and colleagues is to match the fetal fraction of the cases and controls in the first trimester training cohort for identifying preeclampsia associated differentially methylated regions. The second strategy is to normalize the methylation level of individual region by the overall methylation level of the sample.

Bob Barrett: Dr. Yu, can you share any insights you might have gained from reading the De Borre manuscript?

Stephanie Yu: I think this study has revealed several important findings about the pathophysiology of preeclampsia. Firstly, it showed that methylation changes related to preeclampsia can be identified as early as the late first trimester, which is

around 20 weeks before the onset of clinical signs and symptoms. Secondly, it appears that these changes mainly originate from the placenta. Lastly, the preeclampsia associated methylation changes differs between the first trimester and the time of diagnosis. This reflects the dynamic nature of cell-free DNA methylation and its potential for monitoring placental development as pregnancy progresses.

Bob Barrett: Finally, Dr. Yu, how might cfDNA methylome analysis be used to address other clinical questions in pregnancy?

Stephanie Yu: There are a couple of things we could look into in the future. Firstly, we could see if analyzing cell-free DNA methylome can help predict late onset preeclampsia. Even though late onset preeclampsia leads to fewer adverse maternal and prenatal outcomes, it is more common than early onset preeclampsia. Secondly, we could explore if analyzing cell-free DNA methylome can help predict and monitor organ dysfunction in preeclampsia. For example, we could see if different cell-free DNA mutation profiles are linked to damage in different organs. We could also see if changes in cfDNA methylation can be detected before clinical and other biochemical manifestations of the disease appear. Lastly, we could explore if cell-free DNA methylation analysis can be applied to other pregnancy complications such as fetal growth restriction and preterm labor, as these conditions may also be associated with placental pathology.

Bob Barrett: That was Dr. Stephanie Yu from the Chinese University of Hong Kong. She is the lead author of a perspective article describing cell-free DNA methylome analysis for the prediction of preeclampsia in the June 2024 issue of *Clinical Chemistry*, and she's been our guest in this podcast on that topic. I'm Bob Barrett. Thanks for listening.