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Noninvasive Prenatal Methylomic Analysis by Genomewide Bisulfite Sequencing of Maternal Plasma DNA

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Guest:
Dr. Rossa Chiu is a Professor of Medicine and Chemical Pathology at the Chinese University of Hong Kong, and a Researcher at the Li Ka Shing Institute of Health Sciences.

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

Prenatal development involves a series of highly-organized genetic and epigenetic events. Abnormalities in the epigenetic control of developmental processes have been implicated in infertility, spontaneous abortion, intra-uterine growth abnormalities, and numerous post-natal consequences.

A recent study published in *Clinical Chemistry* by researchers in Hong Kong used fetal DNA in the maternal plasma during pregnancy to study prenatal DNA methylation on a genome-wide basis. That paper is available online now and will appear in the November 2013 print edition of the journal.

Rossa Chiu was one of the lead authors of this paper. She is a Professor of Medicine and Chemical Pathology at the Chinese University of Hong Kong, and a Researcher at the Li Ka Shing Institute of Health Sciences. She is our guest in this podcast.

Dr. Chiu, what exactly is DNA methylation and why is it of interest?

Rossa W.K. Chiu: So most commonly, DNA methylation is actually called CpG methylation. What it refers to is half of the genome when a cytosine nucleotide is followed by a guanine nucleotide, and we call that a CpG site, and the cytosine residues in such site, the five-prime carbon of the cytosine can have a methyl group. So if that happens then we call that a cytosine methylation, so that is the most commonly studied DNA methylation feature.

This is biologically interesting because it is a DNA feature that affects DNA function, and yet, it is not related to a change in a nucleotide sequence. So what we mean by that, is that, it's not related to the order of the ACTG nucleotide, but instead, it's due to the presence of a methyl group on the cytosine. And how it affects DNA function is actually quite wide ranging, so for example, if we know that, mammalian cells are not viable without DNA methylation.

Also, it is known that the active and non-active parts of the genome have differences in amount of methylation. So for example, the relatively inactive part of the genome is called heterochromatin that is full of DNA methylation.

DNA methylation also acts to control gene transcription, so for example the path of the genome where they have a particular richness in CpG sites, we call them CpG Islands, and very often these are the beginnings of genes, and if these CpG islands are methylated, the genes tend to be switched off. And also now it's known that DNA methylation is participating, it has no genesis. So there is actually many reasons why DNA methylation is biologically interesting.

Bob Barrett: What is the importance of studying the methylation profile of the fetus or of the placenta?

Rossa W.K. Chiu: To think about it, the development of a pregnancy is a physiologically amazing process. Imagine, everybody starts from a fertilized egg and eventually becomes a developed fetus. So this process involves highly-coordinated and orchestrated embryological events, and because just now we mentioned that DNA methylation is very important in controlling DNA function, so of course, DNA methylation would have an important role to play in regulating development process.

So researchers have had a longstanding interest in trying to understand what is the role of DNA methylation in development. And in fact, the placenta actually has a lot of unusual features that are related to DNA methylation.

So for example, placenta as a tissue, in general, is more hypermethylated than many other tissues in the body, and also there are some genes that exhibit a phenomenon that we call tissue-specific methylation which means that it is methylated mainly in the placenta but not in other tissues, so it becomes a signature of the placenta.

And also another very interesting phenomenon is called gene imprinting, which reversed the phenomenon where one of the allele of the gene can be methylated or not methylated, and whether an allele is methylated or not methylated depends on whether you have inherited that allele from your father or your mother. Those imprinted genes play a very important role in fetal development and also in placenta function.

And of course, there are fetal and placental diseases that are known to be associated with abnormal DNA methylation. So there is an interest to study DNA methylation in a fetus and in a placenta, not just to understand its role in the physiological development and also in its role in relation to the fetus or other pathologies.

Bob Barrett: Dr. Chiu, your paper describes new techniques in this area. How were studies conducted in the past and did they have any limitations?

Rossa W.K. Chiu: DNA methylation is biologically interesting, but it’s technically challenging to study. Although there has been a lot of interest in studying DNA methylations, but basically researchers still believe that we have very little understanding of DNA methylation, and one reason is because it is not easy to study it.

The reason because, as I explained at the beginning, DNA methylation is a feature of DNA that doesn’t involve a sequence change. So if, unlike studying a mutation, where you just look at the sequence change and then you realize that there is a mutation. So other methods are needed to detect the presence or absence of cytosine methylation. And not only that -- not only we want to know the presence or absence of methylation in a particular CpG site, we are also interested in knowing the relationship of this particular CpG site with its neighboring CpG sites, so whether they are all methylated, or all unmethylated, or it varies.

In addition, we are interested in studying the differences in DNA methylation across different tissue types, because different tissue and different cell types, they carry out different DNA functions, and therefore the DNA methylation pattern will be different.

So in order to fully study a DNA methylation profile of the tissue, we also want to know even for the same locus, what is the proportion of cells that are methylated or unmethylated; and also to be greedy, we would like to know this information across as big a part of the genome as possible.

So to sum all that up basically what we want to know is we want to know the qualitative pattern, the pattern of a methylation profile. We also want to get quantitative data that is the proportion of molecules that are methylated, and we also want to study it in as global a manner as possible. But unfortunately a lot of the methods that were previously available to researchers, they have limitations.

So for example, some methods that are based on methylation sensitive restriction enzyme, they only recognize certain patterns of methylation and so our study limits those patterns of methylation.

Other methods, in fact the gold standard method is based on the chemical that actually converts unmethylated cytosine to cymene, so convert this signal into a sequence change to allow us to sequence, to distinguish, methylated and unmethylated DNA by the sequence change. But that method to get qualitative data, one would need to do chromed direct-sequencing, and it's a very labor-intensive method, so one might not be able to carry it out on a large scale.

And other methods, such as, microarray-based methods, will provide information restricted to genomic sites that are represented on the microarray.

So the various methods that have been available to date had very different types of limitation. And on top of all that, because we are talking about we are wanting to study the -- let's say, the methylation of placenta, and we are interested to know how the placenta develops or how is that normal in this process. And because the placenta is part of a pregnancy, one cannot readily obtain placenta tissue, unless there is a clinical need to biopsy it or the pregnancy has completed. And so there is actually not a way that had been available for researchers to actually monitor the dynamic changes of the placenta DNA methylation.

Bob Barrett: What has your team achieved in this study that overcame those previous limitations?

Rossa W.K. Chiu: Okay, so first in terms of technique, we make use of a recently available technique that allow, one, to study DNA methylation in both a qualitative, quantitative manner, and on a genome-wide scale, and that is the coupling of bisulfite conversion, that is a chemical conversion that I have talked about, together with next generation sequencing.

So this method is probably as close to the methods that researchers have been waiting for, that would allow us to study the methylation profile authentication. So that's one aspect.

But, the other key aspect, is that, we used whole-genome bisulfite sequencing, and instead of applying that to placental tissue that we obtained from biopsy or at the end of a pregnancy, we actually apply that to analyze cell-free DNA molecules that have been found to circulate in plasma samples of pregnant women.

So in 1997 our group was the first to report that during pregnancy the fetus actually releases small amount of its DNA, and predominately it is from the placenta, into the blood plasma of its mother.

So in this research we actually analyzed the methylation signal of these placenta-derived fetal DNA molecules in maternal plasma, and so that allowed us to develop a noninvasive way to monitor the changes in the placental methylation profile during a pregnancy.

So in fact, in this study, for one particular pregnancy we actually collected a maternal blood sample during the first trimester, and another blood sample during the third trimester, as well as a blood sample after the pregnancy. And so we were able to noninvasively, through the maternal blood sample, study the placental methylation profile from the beginning to the end of the pregnancy.

There are actually more tricks to this. The reason because we obtain maternal blood samples, and so the majority of DNA in the maternal blood sample is actually from the mother's blood cells and not from the placenta, only the minority of the DNA is from the placenta.

So there were additional algorithms that we developed to allow us to assume if the fetal or the placental were derived DNA molecules that were within the mother's blood sample.

So we actually developed two approaches, one approach is that we took advantage of the polymorphic differences between the mother and the fetus and particularly analyzed DNA molecules that carried a polymorphic allele that only came from the fetus and not from the mother.

So those molecules were definitively from the fetus, so by studying those DNA molecules we had conclusive evidence to show that we were picking up the methylation signal originating from fetal molecules but in the maternal blood sample.

But because the parts of the genome that was different between the mother and the fetus were relatively limited, so we also developed an additional method to allow us to potentially study the placental methylation profile from the DNA molecules in the maternal blood sample, but not restricted to DNA fragments that contain that fetal polymorphic marker.

So basically what we did was, let's imagine if a woman was not pregnant, we studied the baseline methylation profile of the maternal blood cells, so that will be representative of the methylation profile if she was not pregnant.

But now all of a sudden if she is pregnant, and the methylation profile changes, in her blood changes, we also know that because of placental methylation is more hypermethylated compared to the methylation profile of blood cells. And if we know what percentage of the DNA molecule in that plasma sample is from the pregnancy then by observing the change of the methylation profile, or particular site from the original blood cell baseline, then we can deduce what is the methylation profile of the placenta for that particular locus.

And for this particular algorithm we theoretically could apply it to the whole genome, and so through this other method we were able to study quite a large part of the placental methylone.

Bob Barrett: Just how much data have you generated in this study and how deeply have you analyzed the maternal plasma methylone?

Rossa W.K. Chiu: Actually we generated quite a lot of data, so because as I have just mentioned, we collected three blood samples from this one pregnant case -- we also -- because we wanted to confirm that the different DNA molecules that we were analyzing actually have a resemblance to the blood cell methylation profile as well as the placental methylation profile, so we also actually analyzed the placental tissue and a chorionic villus biopsy on the blood cell sample of this pregnant woman.

We also analyzed samples from non-pregnant individuals and so for this particular study we analyzed a total of 9.5 billion DNA fragments and for each DNA fragment we used Next-Generation Sequencing to analyze each end of the DNA fragments. So actually we obtained data from 9.5 billion pairs of DNA fragments in this study.

And for example, if we just talk about the first trimester maternal plasma sample that we got from this case, we analyzed so many DNA fragments that were equivalent to – as if we were analyzing 50 human genomes. And the reason why we have to generate so much data for each maternal blood sample, is because the majority of a DNA in a mother's blood sample is actually from a mother.

But because our primary interest is to study the fetal DNA molecules in that sample, so we need to obtain or analyze, many DNA fragments in the sample in order to make sure that we get a sizable coverage of the minor population, namely the fetal DNA.

So we believe that we have analyzed these samples and generated quite a lot of data.

Bob Barrett: Doctor, what are the major biological insights learned in this study?

Rossa W.K. Chiu: So because we were able to bisect the fetal-derived, and the non-fetal-derived DNA molecules from the mother's blood sample and then we studied the differences in the methylation profile of these two groups of DNA molecules that covered much of the entire genome.

We generated genome level proof of data that the fetal DNA molecules that are presented in a mother's blood sample, actually has a high resemblance in terms of the methylation profile to the placental tissue biopsies that were obtained from the same pregnancy.

So in other words we provided genome level proof that the majority of the fetal DNA in a mother's blood sample is indeed derived from the placenta. So this is one important biological insight that we obtained from the study.

And the other new biological insight that we obtained was that we made a observation that besides of the DNA molecules that are present in the mother's plasma sample actually had a correlation with the methylation level of that particular DNA fragment.

So it has been known by researches that DNA molecules in plasma are broken up into short fragments and it's generally less than 200 base pair, but in this study we found out that more unmethylated molecules tend to have shorter DNA size. And this is interesting, this suggests that maybe the unmethylated DNA molecules are less stable and they are broken up more readily. So this is another piece of biological insight that we have generated from this study.

Bob Barrett: Well finally, doctor, what are the long-term implications of having achieved a non-invasive means to study methylomic analysis of the fetus and of placenta, and what sort of future studies can you imagine being performed on the basis of your work?

Rossa W.K. Chiu: So first in our paper we show that many of the studies that were carried out in past to determine placental methylation features we were able to do them and apply them non-invasively by analyzing the mother's blood sample.

So for example in a study, using the mother's blood sample we were able to look for DNA loci that had the difference in methylation between the placental tissue and the maternal blood cells. We were able to observe gestational age variation of changes in the methylation state of particular gene loci. We were able to study imprinted genes of the placenta from the mother's blood samples.

So first we could carry out the analysis of various placenta-related methylation features non-invasively. So in the long-term this method may open up to the possibility to conduct safe, non-invasive, serial analysis of placental methylations.

Also as I said at the beginning, other researchers are interested in studying the methylation profile placenta of the fetus from the perspective of trying to understand the development of diseases, pathologies, or to diagnose them, so this method also potentially opens up the possibility to non-invasively study these pathological processes, and perhaps one day one may be able to develop biomarkers that could be detected in the mother's blood sample to monitor or assess such pathology.

And in fact in study just as a demonstration of one possible use of the non-invasive analysis, we showed that women who were carrying fetuses with Down syndrome, because of the babies having a third copy of Chromosome 21 in the genome. So because the Chromosome 21 DNA molecules that originated from the placenta then released into the mother's blood sample, and because the placenta is generally hypermethylated compared to the mother's blood cell, we were able to show that because of the additional amounts of Chromosome 21 DNA, that originated from the placenta, that ends up in a mother's blood sample, they will lead to a hypermethylation of Chromosome 21 DNA molecules in blood samples of women carrying Down syndrome fetuses compared with those not carrying Down syndrome fetuses.

So, in summary, we believe that this method will open up the possibility for a lot of placental methylation analysis to be done non-invasively through a mother's blood sample.

Bob Barrett: Rossa Chiu is a Professor of Medicine and Chemical Pathology at the Chinese University of Hong Kong and a researcher at the Li Ka Shing Institute of Health Sciences.

She has been our guest in this podcast from *Clinical Chemistry.*

I am Bob Barrett. Thanks for listening!