"Presence of Fetal DNA in Maternal Plasma and Serum" (1997), by Dennis Lo, et al. [1]

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In the late 1990s researchers Yuk Ming Dennis Lo and his colleagues isolated fetal DNA extracted from pregnant woman's blood. The technique enabled for more efficient and less invasive diagnoses of genetic abnormalities in fetuses, such as having too many copies of chromosomes. Lo's team published their results in their 1997 article "Presence of Fetal DNA in Maternal Plasma and Serum." The results led to developments of clinical tests that can access fetal genetic information and detect genetic abnormalities before birth without the risks associated with invasive genetic testing techniques of potentially harming the fetus [2].

In 1997 Lo worked with a team of researchers on the project at the Chinese University of Hong Kong [3] in Hong Kong, China. Noemi Corbetta worked at the Istituto di Medicina Interna e Fisiopatologia Medica in Milan, Italy. Paul Chamberlain, Vik Rai, Ian Sargent, and Christopher Redman worked at the Nuffield Department of Obstetrics and Gynecology at the University of Oxford [4] in Oxford, England, and James Wainscoat worked in at John Radcliffe Hospital at the University of Oxford [4]. Lo and the research team investigated how to access fetal DNA to assess genetic abnormalities *in utero*. Prior to that, obstetricians used two potentially harmful methods to collect DNA from placental tissue. Lo and team's research aimed to develop a noninvasive technique to obtain the same DNA information with a decreased risk to pregnant women and fetuses.

In 1996 Xu Qi Chen and a team of researchers at the Laboratory of Plant Biochemistry and Physiology in Geneva, Switzerland, had found free-floating DNA outside of the individual cells from tumors circulating in patients' bloodstreams. The ability to detect tumor DNA helped doctors to identify patients with cancer without needing to first find the cancerous tissue. The results were published in September of 1996. Stuart Emanuel and Sidney Pestka at the University of Medicine and Dentistry of New Jersey in Piscataway, New Jersey, further conducted research to facilitate analysis of the free-floating DNA found in circulation using a method called polymerase chain reaction (PCR) in the late 1990s. The researchers used PCR to replicate the small fragmented pieces of DNA found in the blood samples into an amount large enough to sequence and test. PCR is a process used to amplify a small sequence of DNA in order to produce more copies of it for further study.

Lo and his team researched whether or not a fetus's DNA could be collected from a pregnant woman's blood stream in the same way that tumor DNA could be collected from a cancer patient's blood. They conducted their research at the John Radcliffe Hospital in Oxford, England. They worked with forty-three women who were between twelve and forty weeks pregnant and were already receiving care at the hospital. The researchers extracted plasma, the liquid component of blood, from each of the forty-three women as well as from ten women who were not pregnant. The ten non-pregnant women comprised the control group for the experiment to ensure that the free-floating DNA isolated from the pregnant women was fetal DNA, not adult DNA. The researchers tested specifically for male fetuses, which would have had a Y-chromosome that the pregnant woman lacked, allowing for a simpler method to differentiate between the DNA of the pregnant women and that of their fetuses' DNA.

The researchers collected DNA samples and replicated the small fragments to analyze them for abnormalities. They centrifuged the blood samples, using a machine that spins solutions at a high velocity so that the different substances in the sample are separated by density. By centrifuging the samples, Lo and the researchers extracted the plasma and serum from the original samples, as both the plasma and serum had significantly more free-floating DNA than the other cellular components of blood. They centrifuged the samples again to remove any further red blood cells as well as the buffy coat, a portion of the blood that contains most of the white blood cells and platelets, to further purify the sample of interest. The purified serum and plasma that contained the free floating DNA was then chilled to preserve the sample.

Lo and the researchers began another round of purification to further isolate possible fetal DNA out of the serum and plasma. They heated the samples to disassociate some of the proteins. They spun the heated solution in a microcentrifuge, a stronger centrifuge used to separate layers with very small differences in densities. The researchers collected the solid material, like the DNA that collects at the bottom of the tube during centrifuging, and removed the rest of the sample. The researchers studied the final purified liquid containing the small pieces of free floating DNA.

After the researchers had centrifuged and purified the samples, they had small amounts of DNA and created more copies of the DNA. They first heated the sample tubes to high temperatures to prepare the DNA. After the samples were heated, they added

primers, or small proteins that have matching sequences to a particular part of the DNA. The primers used for this experiment were all Y-chromosome specific, in order to allow for easy distinction between maternal DNA, which lacks a Y chromosome, and the fetal DNA, if the fetus [2] was a male. The researchers then added enzymes used to synthesize new DNA to produce new DNA that is the exact complement of the original. The process was repeated 60 times to make enough copies of DNA for analysis.

The purification and PCR procedure did not clear out all free-floating maternal DNA and cell fragments. To clear out the remaining maternal DNA and cell fragments, the researchers tagged the DNA with a fluorescent chemical and ran the sample through a gel that separates the PCR products (DNA and some cell fragments), by size, charge, and length. That procedure enabled the researchers to identify the maternal DNA sequences for investigation as the DNA with florescent tags group together on the gel and fluoresce.

Lo and his team also tried to isolate fetal DNA from the cellular component of whole blood, rather than the serum or plasma, as did the researchers who'd worked on tumor DNA. They detected Y-specific fetal DNA in only five of the thirty women pregnant with male fetuses when they tested the cellular component. The results demonstrated that the component of blood with the highest likelihood of fetal DNA detection was in the plasma and serum.

Lo and his colleagues identified thirty of the forty-three women as pregnant with males. The researchers could not detect any fetal DNA in the women pregnant with female fetuses because they have no Y-chromosomes in their bodies. The researchers also did not find any fetal DNA in the non-pregnant women used as controls, validating the test. In almost all of the thirty cases of women pregnant with male fetuses, the researchers detected fetal DNA in the plasma samples by looking for Y chromosome specific genes [5]. The researchers found that there was a higher probability of finding fetal DNA in plasma samples than in serum samples. The research method designed by Lo and his team had restrictions due to its reliance on the Y chromosome signal as the differentiation [6] factor between fetal and maternal DNA. Because they used a Y-chromosome specific method, the researchers could only isolate and test the fetal DNA of male fetuses.

Although the detection rates were fairly high in plasma and serum, Lo and his team determined that extracting more maternal blood could increase the detection rate. Also, the researchers found a correlation between the length of gestation [7] and the concentration of fetal DNA in the maternal blood stream. The longer a woman is pregnant, the more fetal DNA accumulates in her blood stream and the easier it is to isolate the fetal DNA. This same pattern was found in several women whose plasma samples tested positive, but their serum samples tested negative. All of them were tested before twenty-three weeks of gestation [7]. That was important for the researchers to also understand the probability of fetal DNA isolation increased as pregnancies progressed as more accumulates in the maternal blood.

Lo and his team established a new opportunity to access fetal DNA without the invasive techniques previously employed by obstetricians. Lo later furthered his research by creating particular tests to identify genetic diseases such as aneuploidies like Down syndrome [8] with a simple blood draw. Lo and the researchers' work led to prenatal genetic testing and diagnosis of genetic disorders in fetuses with smaller risks to the pregnant woman and fetus [2] compared to invasive screening processes.

Sources

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Subject

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