Using Digital PCR to Detect Fetal Chromosomal Aneuploidy in Maternal Blood (2007) [1]

By: Zhu, Meilin Keywords: Digital PCR [2]

In 2007, Dennis Lo and his colleagues used digital polymerase chain reaction or PCR to detect trisomy 21 in maternal blood, validating the method as a means to detect fetal chromosomal aneuploidies, or an abnormal number of chromosomes in a cell. The team conducted their research at the Chinese University of Hong Kong [3] in Hong Kong, Hong Kong, and at the Boston University [4] in Boston, Massachusetts. Because small amounts of fetal DNA appear in maternal blood during pregnancy [5], Lo and his team hypothesized that they could detect fetal chromosomal aneuploidy [6] trisomy 21, or Down's syndrome, in a sample of maternal blood. The group diagnosed Down's syndrome in unborn fetuses by first taking a maternal blood sample, then amplifying the small amounts of fetal DNA in the maternal blood using digital PCR, and applying two genetic methods to that sample. Lo and his colleagues' experiment demonstrated the accuracy of a novel, noninvasive method for fetal chromosomal aneuploidy [6] testing that can enable people to make informed decisions about their pregnancies.

An individual has a chromosomal aneuploidy [6] when their cells contain an abnormal number of chromosomes. Individuals with trisomy 21, a chromosomal aneuploidy [6], have cells with three copies of chromosome 21 instead of two. Trisomy 21, commonly called Down's syndrome, causes lifelong developmental delays and physical disabilities. Prenatal genetic tests enable people to make informed decisions about pregnancies with fetuses affected by Down's syndrome.

Before the development of noninvasive prenatal testing for genetic or chromosomal anomalies, physicians used amniocentesis and chorionic villus sampling, or CVS, to detect fetal chromosomal aneuploidies. During traditional testing with amniocentesis, a physician inserts a syringe through a pregnant woman's abdomen to collect a sample of amniotic fluid, which surrounds a fetus [7] in the womb [8]. When performing chorionic villus sampling, a physician inserts a syringe through the pregnant woman's vagina [9] or abdominal wall to collect a sample of chorionic membrane surrounding the fetus [7]. Both amniotic fluid and chorionic membrane contain fetal DNA, which a physician uses to run genetic tests on the fetus [7]. The two testing methods, often considered invasive, pose the risk of sudden miscarriage [10] and may hurt the pregnant woman or fetus [7].

The presence of fetal DNA in maternal plasma offers the possibility of noninvasive genetic and chromosomal prenatal tests, where an instrument would not need to be inserted into the womb [8]. In 1997, researcher Dennis Lo discovered that fetal DNA outside of a cell, called cell-free fetal DNA, circulated in maternal blood plasma at the Chinese University of Hong Kong [3]. Lo discovered fetal DNA in maternal plasma by detecting DNA sequences specific to the Y chromosome, or male sex chromosome, in pregnant women. Women's plasma would not normally contain Y chromosomes as women do not have Y chromosomes. Therefore, Lo concluded that the Y chromosomes belonged to the male fetuses of the pregnant women he tested and that he had detected fetal DNA in maternal plasma.

Prior to Lo and his team's experiment, scientists detected fetal DNA in maternal plasma by targeting placenta [11]-specific DNA, which is unique to the fetus [7]. Scientists could use the fetal DNA to diagnose trisomy 21 in the fetus [7] by finding the ratio of chromosome 21 in the sample, called the single nucleotide polymorphism, or SNP method. To do that, researchers relied on differences between chromosomes. All individuals have two chromosomes, and thus two copies of each gene, called alleles. In an individual with two different alleles, or a heterozygote, the ratio between those two alleles is equal 1:1 in the blood. Individuals with two of the same allele, or homozygotes, will only have one allele present in their blood. Individuals with trisomy 21 have three copies of chromosome 21, or three alleles of each gene on chromosome 21. In heterozygotic individuals, two of those alleles are the same, and one is different. If the ratio of those alleles in the blood is 2:1, that ratio indicates that the fetus [7] has trisomy 21. In that way, researchers could prenatally diagnose heterozygotic fetuses with trisomy 21.

However, the technique did not enable researchers to diagnose homozygotic fetuses with trisomy 21. Homozygotic fetuses have three copies of the same allele, so only that allele is present in blood. Because there is not a ratio of alleles to measure, researchers could not detect the presence of an extra chromosome 21 and therefore could not diagnose the fetus [7] with trisomy 21. That meant that a significant number of fetuses with trisomy 21 could not be diagnosed prenatally.

Prior to Lo's team's experiment in 2007, other researchers set up a method for the diagnosis of trisomy 21 in heterozygous fetuses. In 2002, Bernhard Zimmermann, Wolfgang Holzgreve, Friedel Wenzel, and Sinuhe Hahn in Basel, Switzerland, used conventional PCR to amplify fetal DNA outside of maternal blood to detect trisomy 21 at the University of Basel [12] in Basel, Switzerland. However, Dennis Lo and his colleagues aimed to demonstrate a more sensitive detection of trisomy 21 in fetal DNA in maternal plasma using digital PCR, according to their paper published in *Proceedings of the National Academy of Sciences* "Digital PCR for the molecular detection of fetal chromosomal aneuploidy".

In 2007, Dennis Lo, Fiona Lun, Allen Chan, Nancy Tsui, Ka Chong, Tze Lau, Tak Leung, Benny Zee, Charles Cantor, and Rossa Chiu hypothesized a method to improve prenatal detection of trisomy 21. Lo and his team proposed to use digital PCR and two methods for detecting chromosomal abnormalities. The first method was the previously used single nucleotide polymorphism method, which relied on the differences in alleles. That method could not detect trisomy 21 in homozygotic individuals. The second method, a new method called relative chromosome dosage, or RCD analysis, did not rely on allelic differences and could diagnose trisomy 21 in any individuals.

Before genetically analyzing fetal DNA, the researchers used digital PCR to separate and amplify the fetal DNA from maternal DNA found in maternal blood. Lo and his team hypothesized that digital PCR could more precisely detect trisomy 21 in fetal DNA than conventional PCR. Digital PCR is more sensitive than conventional PCR to very low dilutions in which there is very little of the target DNA in the sample, like in the case of fetal DNA in maternal plasma. Conventional PCR amplifies any DNA present in a sample and produces many copies of any DNA present. Therefore, if researchers used conventional PCR on maternal plasma samples, the resulting DNA may be maternal or fetal DNA. That makes the detection of rare genetic abnormalities harder. Digital PCR is better able to detect rare genetic abnormalities than conventional PCR. When using digital PCR, researchers isolate each strand of DNA present in the sample. Those isolated pieces of DNA are then amplified, or multiplied to produce many copies of the piece of DNA. By isolating and then amplifying every strand of DNA, researchers can separate the fetal DNA from the maternal DNA in a sample of maternal plasma. That ensures that researchers can more easily and accurately detect fetal abnormalities.

After amplifying the DNA using digital PCR, Lo and his colleagues determined whether they could detect trisomy 21 in the resulting isolated fetal DNA. Lo and his team first used the SNP method, which relies on differences in alleles between chromosomes. Because they first used that method, the research team only used heterozygote fetuses for that portion of the experiment. The researchers measured the ratio of alleles in fetal messenger RNA in maternal plasma. Messenger RNA is a nucleic acid that conveys the genetic information of DNA to the rest of the cell. In a fetus [7] without trisomy 21, the researchers expected a 1:1 ratio of alleles. In a fetus [7] with trisomy 21, they expected an overrepresented allele indicating an extra chromosome 21. The researchers used a probability test, the sequential probability ratio test or SPRT to determine the degree of overrepresentation expected in a fetus [7] with trisomy 21 case. The probability test gave the researchers the minimum expected ratio for overrepresentation. That minimum expected ratio served as the baseline to determine whether the fetus [7] had trisomy 21. Although the test relied on differences in alleles and therefore only worked in heterozygotic individuals, Lo and his team established that digital PCR could more sensitively detect rare genetic abnormalities in samples than conventional PCR.

Next, the researchers evaluated a new method to detect fetal chromosomal aneuploidies in maternal plasma without depending on allele differences. Relying on allele differences meant that prenatal tests only worked in heterozygotic individuals. Lo and his team used a method that worked for both heterozygotic and homozygotic individuals, thus acting as a more versatile test than the ones previously utilized. The team tested a method called relative chromosome dosage, or RCD analysis. When researchers use RCD, they find the ratio of chromosome 21 and chromosome 1 present in the blood. Humans have twenty-three pairs of chromosomes in every cell of their body, identified as chromosome 1 through chromosome 23. In individuals without chromosomal abnormalities, chromosomes are present in equal amounts. However, individuals with trisomy 21 have one extra copy of chromosome 21. Because chromosome 1 and chromosome 21 should be present in equal amounts in fetuses without trisomy 21, samples with a relatively higher amount of chromosome 21 indicated that the fetus [7] had trisomy 21. Lo and his team found that at a concentration of twenty-five percent maternal plasma they could detect trisomy 21 in maternal plasma with an accuracy of ninety-seven percent.

With their experiment, Lo and his team demonstrated the feasibility of the application of digital PCR to test fetal DNA in maternal plasma for fetal chromosomal aneuploidies. The team showed that digital PCR could be used as a noninvasive method to diagnose trisomy 21 or any other chromosomal aneuploidy [6] in fetuses prenatally. The same group completed another experiment one year later, in 2008, showing more improvements in the method to achieve a more sensitive test for trisomy 21 in cell free fetal DNA in maternal plasma.

Sources

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Subject

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