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New approach of non-invasive prenatal screening using droplet digital PCR

Seung Yong Hwang[1,2]

[1]Department of Molecular and Life Science & Department of Bio-Nanotechnology, Hanyang University, Sangnok-gu, Ansan, Gyeonggi-do, Korea
[2]BioCore Co. Ltd., Guro-gu, Seoul, Korea

The probability of fetal hereditary disease has rapidly increased over the last decade because of an aging marriage population and reproductive aging. Hereditary diseases of fetus due to abnormality in number of chromosomes can be relieved of pain for mothers and children if they are found at an early stage and preparations can be made for their efforts, so the importance of early diagnosis is emphasized. However, invasive prenatal test methods for early diagnosis caused side effect such as, fetal injury, premature rupture of membranes, abortion. A non-invasive prenatal screening method for predicting inherited diseases of the fetus using only the maternal blood without these side effects has been developed and actively studied.
Digital PCR is a method for overcoming the limit of quantitative analysis of conventional PCR method and enables accurate quantitative analysis and highly sensitive detection of target nucleic acid molecule as compared with existing real time PCR. Digital PCR has many advantages such as analysis of a very small amount of samples, simultaneous processing of many samples, and analysis of large volume samples, inspection of various samples and various inspection items at once. This method is utilizing the cell free fetal DNA (cffDNA) of the cells contained in the mother's blood. It is actively studied and applied to the field of diagnosis of chromosome number such as Down syndrome (trisomy 21) occupying the most frequent. Non-invasive prenatal screening method using Digital PCR is a technique to achieve the objective of early diagnosis of fetal genetic diseases.
We used a method to amplify only the specific sites of the control group and the target group by the digital PCR method and analyze the fluorescence value extracted from each amplification section. To prevent inaccurate results from very small amounts of cffDNA, several samples were selected to verify the feasibility of digital PCR and establishment of cut-off value. To confirm the feasibility of digital PCR, experiments were conducted to ensure the theoretically possible results using various kinds of samples. After conformation of feasibility, we set a cut-off value distinguishable from negative and positive risk for Down syndrome using 156 samples of various types. Finally we experimented 877 plasma samples. 50 positive samples were all identified as high-risk group of Down syndrome and 824 samples among 827 negative samples were identified as low-risk of Down syndrome. The specificity of these results is 99.63%, which can be seen as the result that noninvasive prenatal screening using digital PCR is effectively applied to clinical samples. Through these results, non-invasive prenatal diagnostic screening using Digital PCR can be utilized as an auxiliary diagnostic screening method that can be useful for diagnosis with high clinical utility.

References

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