Review Article

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Clinical application of prenatal chromosomal microarray

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A prenatal chromosomal microarray (CMA) is generally recommended when a major anomaly is suspected on prenatal ultrasonography. As it can overcome the limitations of conventional karyotyping, it is expected that the number of prenatal CMA test requests will gradually increase. However, given the specificity of prenatal diagnosis, there are practical considerations compared to postnatal testing, such as the validation of prenatal specimens, maternal cell contamination, precautions when reporting variants of uncertain significance, and the need for comprehensive genetic counseling considering secondary findings. The purpose of this article is to provide necessary information to health care providers in consideration of these issues and to provide appropriate genetic counseling to patients.

Key words: DNA Microarray, Prenatal diagnosis, Practice guideline, Genetic counseling.

Introduction

A chromosomal microarray (CMA) does not require cell culture of a sample; it can test DNA extracted from stored or non-cultivable tissues and can detect submicroscopic copy number variants (CNV) that cannot be detected by karyotyping. It is also possible to confirm the objective data of the discovered CNV. Additionally, the absence of heterozygosity (AOH) can be detected using a single nucleotide polymorphism (SNP) marker, and a related uniparental disomy disease diagnosis is available, making it possible to check whether parental consanguinity is present [1,2]. Prenatal CMA can additionally detect clinically significant deletions and duplications in 6% of cases with abnormalities observed on prenatal ultrasound and in 1-2% of cases with normal prenatal ultrasound results when karyotyping is normal [3,4]. Furthermore, considering the general advantages of CMA tests, it is expected that the diagnostic yield of prenatal diagnosis can be increased, and more objective and accurate information can be obtained compared with karyotyping [5].

A limitation of CMA is that it cannot detect genetic abnormalities that do not affect CNV, such as balanced chromosomal rearrangements, and CNV may not be detected when present at a low level of mosaicism (<20%). Additionally, levels of tetraploidy or other polyploidy may be undetectable or difficult to detect, and depending on the platform, CNV in unmarked genomic regions may not be detected. However, if there are SNP markers in the CMA platform, triploidy can be detected, and in some cases, there is a possibility of confirming additional information from other polyploidy [1,6].

Guidelines for CMA

1. In United States

The American College of Obstetricians and Gynecologists (ACOG) and the Society for Maternal-Fetal Medicine (SMFM) presented the Committee Opinion in 2016 (reconfirmed in

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2020). The main recommendations were as follows: if one or more major structural abnormalities are observed in the fetus on prenatal ultrasound and invasive prenatal testing is performed, CMA testing is recommended. Major structural anomalies of the fetus include heart malformations, brain malformations, cleft lip and palate, and several congenital malformations. The CMA test is recommended in cases of fetal death in the uterus or stillbirth because it is more likely to produce results than karyotyping and can even detect potential CNV because it does not require cell culture of the specimen. Additionally, since submicroscopic CNVs are not associated with increased maternal age, CMA can be considered in all women undergoing prenatal testing, regardless of age. Comprehensive genetic counseling from an obstetrician, gynecologist, or medical staff with expertise in genetics before and after the test is important and should include information related to the benefits, limitations, and interpretation of the results. Moreover, prior to testing, informed consent must be obtained, which includes information about the result of uncertain significance, non-paternity, consanguinity, and the discovery of mutations related to adult-onset diseases [7-9].

2. In Canada

The Society of Obstetricians and Gynecologists of Canada (SOGC) and the Canadian College of Medical Geneticists (CCMG) jointly presented the following conclusions in the 2018 Prenatal CMA Guidelines: CMA is recommended when multiple fetal malformations are suspected and the rapid aneuploidy screen is normal or when the fetal nuchal translucency (NT) is greater than 3.5 mm. Before testing, informed consent must be obtained based on genetic counselling, including test method limitations and secondary findings, and genetic counselling after testing must also be provided. The resolution of prenatal CMA should be similar to that of the postnatal CMA platform. To minimize the reporting of variants of unknown significance (VUS), deletions of less than 500 kb or duplications of less than 1 Mb should not be reported. Additionally, a larger VUS may be reported only if there is sufficient evidence that the site is pathogenic. Secondary findings should be reported for childhood-onset diseases that can be treated medically and for adult-onset diseases, only at the request of parents or when serious harm to family members occurs if not reported [10].

Recommendations after the Positive Non-Invasive Prenatal test (NIPT)

Recently, NIPTs for chromosomal aneuploidy, such as those for Down syndrome, Edwards syndrome, and Patau syndrome, have been performed as screening tests in many clinical laboratories. When the result of NIPT is positive but karyotyping is normal or when a small CNV is suspected in NIPT, prenatal CMA is recommended as a confirmatory test [11].

Selection of the Platform for Prenatal CMA

Prenatal CMA is currently available as a research use only (RUO) platform in Korea, and representative examples include the CytoScan HD Suite (2011), CytoScan 750 K Suite (2012), and CytoScan Optima Suite (2015) (ThermoFisher Scientific, Waltham, MA, USA). The CytoScan HD Suite consists of 2.7 million markers, the CytoScan 750 K Suite is made by selecting 750,000 markers, and the CytoScan Optima Suite consists of a total of 166,000 markers. SNP markers were included in all platforms. Each platform differs in terms of the resolution and reagent cost. In prenatal CMA, there is a higher possibility of obtaining VUS results with higher resolution but with higher cost. Therefore, the resolution and cost should be considered when selecting a platform. Considering the noncoding regions, the resolution of the CytoScan HD suite is 43 kb, and the resolution of the CytoScan 750 K suite is 153 kb. The CytoScan Optima Suite was developed for prenatal specimens, such as amniotic fluid (AF), chorionic villus sampling (CVS), and fetal tissue. The general resolution of CytoScan Optima is 1 Mb for deletion and 2 Mb for duplication. However, it exhibits a 100 kb resolution for approximately 400 genes associated with prenatal diagnosis [12-15].

Assessment of Quality Control (QC) Metrics and CNV Identification

Even on platforms previously used for postnatal CMA testing, testing of new sample types, such as prenatal samples, requires an evaluation of the impact of new sample types on data quality. A review of QC metrics related to the DNA extraction process and QC metrics related to the entire testing process should be included in the validation plan, and QC metrics related to the bioinformatics analysis process should also be reviewed. In particular, it is necessary to review whether there are any changes in the QC metrics because of the need for sample preprocessing,

such as in CVS samples. In the evaluation of QC metrics of a new sample type, it is important to ensure that the QC metrics are within the established acceptable range [1,16].

In general, a retest can be considered if QC metrics do not meet the manufacturer's standards. However, when the QC metrics do not pass the standard, if the data are at a level that can be interpreted (suboptimal), it may be possible to describe the limitations and report the test results. However, even if the QC metrics pass the standard, the data may be impossible to interpret if there are many false positive CNVs due to severe background noise. In this case, DNA reextraction and retesting may be considered.

Furthermore, for the detected CNV, it is recommended to directly check the log2ratio value of the marker in the analysis software and compare it with the data of other patients. Depending on the CMA platform, there may be some differences in the resolution of the same CNV, limits to the accuracy of the analysis software in classifying breakpoints, and some overestimation or underestimation of the CNV. Even if a true CNV is observed, it is necessary to check and adjust the breakpoint of the CNV directly using the correction function of the analysis software, if necessary. Therefore, a process for accurate CNV confirmation is necessary.

Interpretation of Prenatal CMA Results and Genetic Counseling

In the interpretation of the test results, there is generally no difference between prenatal and postnatal CMA. Recently, guidelines for the interpretation of CNV results have been published by the American Academy of Medical Genomics (ACMG) and ClinGen. The main change is that scoring is possible considering the presence or absence of genomic content, number of protein-coding genes, and haploinsufficiency or triplosensitivity information reviewed in ClinGen. Five tier classes of P, likely pathogenic (LP), VUS, likely benign (LB), and benign (B) are available for reporting CNVs [17,18]. However, when reporting results, there are special considerations in prenatal testing. First, caution should be exercised when VUS findings are reported. In particular, CNVs with a small size and a small number of genes are not recommended. This is because reporting a VUS that is close to benign may cause the patient to suffer unnecessary worry [7,10]. The second is the genotype-phenotype correlation problem. When an abnormal finding on ultrasonography is suspected, prenatal CMA is frequently requested. Even if a pathogenic CNV is detected, a CNV that is not highly related to the clinical information may be detected. In particular, many pathogenic CNVs that are highly related to developmental disorders often have incomplete penetrance and variable expressivity, and there are cases in which the phenotype is almost normal with pathogenic CNVs [19-22]. Additionally, secondary findings include CNVs related to childhood or adult-onset diseases, CNV carriers related to X chromosome recessive diseases, and AOH information that can identify consanguinity between biological parents [23,24]. Therefore, comprehensive genetic counseling that considers these various situations is crucial. Cooperative communication between the laboratory and the clinic is necessary to solve the problems related to these cases.

Validation of Prenatal Specimens and Back-Up Culture

Experience with postnatal CMA is important in the assessment and interpretation of prenatal CMA results. Validation depends on whether the CMA platform has been previously validated for postnatal use or is new to the laboratory and whether both cultured and uncultured cells are used. Both cultured and uncultured AF and CVS cells should be included in test validation [1].

The analysis of DNA derived from uncultured amniotic fluid or CVS cells is primarily recommended for DNA from cultured amniotic fluid or CVS cells. With CMA analysis of uncultured amniotic fluid and CVS cells, most results can be confirmed within one week, avoiding the possibility of culture artifacts. Maternal cell contamination (MCC) may occur when uncultured AF or CVS cells are tested. CVS samples require manual removal of the maternal decidua before DNA extraction. Confined placental mosaicism (CPM) is a concern when examining uncultured CVS cells; however, only a low frequency of CPM has been reported in the CMA analysis of uncultured CVS cells [25]. This may be due to the evidence that the processed villi are composed mostly of a mesenchymal core representing the fetal genome. Moreover, it is necessary to establish and maintain backup cultures for all prenatal specimens undergoing CMA analysis because of the possibility of CMA assay failure using uncultured specimens. Additional karyotyping or FISH can be recommended to evaluate the level of mosaicism and identify structural abnormalities associated with CNVs [26].

If prenatal CMA is performed on an array platform newly introduced to the laboratory, the CMA validation process must be performed, and the validation of at least 30 prenatal samples is recommended. Because of the difficulty in obtaining abnormal prenatal specimens, the collection of 30 samples is likely to in-

clude cases previously classified as normal [1].

MCC and Mosaicism

It is recommended that prenatal specimens should be evaluated for MCC. MCC can be present in direct samples of amniocytes containing maternal blood, in CVS samples in which the maternal decidual membrane has not been adequately washed, and in cell cultures after extensive passages with maternal cell expansion. MCC can affect the detection and interpretation of CNVs, including CNV types and CNV sizes [27]. Low-level mosaicism in the fetus is likely to be missed because of the significant levels of MCC. MCC can be detected using a variety of methods, including short tandem repeats (STR) analysis and SNP-based CMA platforms. In male fetuses, changes in the sex chromosome plot that mimic mosaicism may indicate MCC. Each laboratory must validate its MCC detection methods to identify acceptable MCC levels for a particular CMA platform. Determination of acceptable MCC levels in uncultured samples can help evaluate the best time for cultured cells to obtain a successful CMA assay [27-29].

In general, the frequency of MCC is 0.5% for AF samples and 1-2% for CVS samples [30,31]. The ACMG generally recommends a confirmation test for MCC in the case of prenatal genetic testing [1]. However, whether an additional test such as STR analysis is needed to confirm MCC in prenatal CMA must be determined by considering the characteristics of the CMA platform, cost, and efficiency. In the case of the CytoScan product line (Thermo Fisher Scientific), there are SNP markers that can identify MCC. Additionally, it is considered that there is no problem in detecting CNV if there are sufficient fetal-derived cells compared to MCC. Due to limitations of the test, the possibility of MCC cannot be completely ruled out only by the test results, and the possibility of an effect on the result value cannot be ruled out either.

Mosaicism detected by CMA should be investigated to confirm its presence and level and to indicate a culture artifact (pseudo-mosaicism), true fetal mosaicism, or CPM in the case of CVS. Coverslip colony cultures can be used to investigate mosaicism. Depending on the chromosomes involved and the type of abnormality, additional testing using other samples (e.g., AF in CVS samples suspected of having CPM) may be considered to confirm or rule out mosaicism. FISH analysis can be used to investigate the presence and the level of mosaicism [32].

Conclusion

Prenatal CMA is primarily recommended when there are abnormal findings on prenatal ultrasonography and may also be recommended as a confirmatory test after NIPT. Resolution and cost-effectiveness should be considered when selecting a platform for prenatal CMA. Owing to the nature of prenatal testing, unnecessary VUS results should not be reported. Comprehensive genetic counseling is important because even for pathogenic CNVs, there are cases where the genotype-phenotype correlation is low, and secondary findings may occur. Furthermore, a validation process considering the characteristics of prenatal specimens is required, and a backup culture considering the possibility of test failure and the need for additional karyotyping may be necessary. Owing to the nature of prenatal testing, MCC and mosaicism should be considered. Currently, the CMA platform often includes SNP markers, and it is possible to detect MCC without additional testing. If mosaicism is suspected, the level of mosaicism can be confirmed using additional tests.

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