JIG Journal of Interdisciplinary Genomics

https://doi.org/10.22742/JIG.2023.5.2.24
Journal of Interdisciplinary Genomics 2023;5(2):24-28
eISSN 2671-6771

# Clinical Application of Chromosomal Microarray for Germline Disorders

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Chromosomal microarray (CMA) is primarily recommended for detecting clinically significant copy number variants (CNVs) in the genetic diagnosis of developmental delay, intellectual disability, autism, and congenital malformations. Prenatal CMA is recommended when a fetus has major congenital malformations. The main principles of CMA can be divided into array comparative genomic hybridization and single-nucleotide polymorphism arrays. In the current CMA platforms, these two principles are combined, and detection of genetic abnormalities including CNVs and absence of heterozygosity is facilitated. In this review, I described practical assessment of CMA testing regarding to laboratory management of CMA, interpretation of CNVs, and special considerations for comprehensive genetic counseling.

Key words: DNA microarray, Hereditary disease, Genetic testing

REVIEW ARTICLE

Received: August 25, 2023
Revised: September 25, 2023
Accepted: October 13, 2023

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## INTRODUCTION

Karyotyping has limitations in detecting copy number variants (CNVs) less than 5–10 Mb in size, and may not identify the origin of CNVs corresponding to marker chromosomes or unbalanced translocations. Fluorescence in situ hybridization (FISH) can only identify a few CNVs in specific genomic regions because of the limited application of FISH probes. Chromosomal microarray (CMA) analysis overcomes the limitations of karyotyping and FISH (Table 1) [1].

Array comparative genomic hybridization (aCGH) is a method used for detecting CNVs in a patient by attaching fluorescent probes to the patient and control samples, hybridizing the two samples to a DNA array, and measuring the relative fluorescence to call the CNVs. A single nucleotide polymorphism (SNP) array is used to measure the genotype signals in SNPs using allele-specific oligonucleotide probes complementary to a wild-type sequence or a SNP, in which genotype information such as B-allele frequency (a normalized allelic intensity ratio) can be obtained. CNV as well as the absence of heterozygosity (AOH) can be detected using SNP arrays [2]. Currently, a platform combining array CGH and SNP array is widely used in clinical laboratories [3], and also CNV detection has become possible through a relative comparison between patient data and control data from hundreds of healthy subjects eliminating the need for a control sample which was necessary in conventional aCGH [4,5].

Clinical indications for postnatal CMA testing include developmental delay, intellectual disability, autism, and multiple congenital malformations [6]. Prenatal CMA is recommended when one or more major fetal malformations are observed on prenatal ultrasound [7]. The diagnostic yield of postnatal CMA is 15%–20% and in prenatal CMA, 6% of the cases with abnormal prenatal ultrasonography

Table 1. Comparison of abnormality detection by cytogenetic tests

| Abnormality | Karyo-typing | FISH | CMA |
|---|---|---|---|
| Aneuploidy | + | + | + |
| Balanced rearrangement | + | + | - |
| Copy number variation (>10 Mb) | + | + | + |
| Copy number variation (<5–10 Mb) | - | + | + |
| Mosaicism | >20% | >5% | >20% |
| Marker chromosomes | + | + | + |
| Target | Whole genome | Limited | Whole genome |

FISH, fluorescence in situ hybridization; CMA, chromosomal microarray.

and normal karyotype results show clinically significant CNVs [6,8].

## VALIDATION, VERIFICATION AND QUALITY CONTROL OF CMA

According to the standards and guidelines for constitutional CMA presented by the American College of Medical Genetics (ACMG), using appropriate positive samples, evaluation of accuracy, precision, analytical sensitivity/specificity, and reportable ranges is recommended for validation, and evaluating accuracy, precision, and reportable range is recommended for verification with FDA-approved CMA platforms [9].

In terms of quality control, the values of quality control metrics (QC metrics) presented by manufacturers need to be managed. In the wet bench process, DNA concentration, OD 260/280, and the size of PCR and fragmentation products are important. Further, in the bioinformatics process, statistical values such as MAPD (median absolute pairwise difference) and SNPQC (SNP QC metric) need to be within the allowable ranges and managing data by batch is necessary. Retesting can be considered if QC metric values are outside the allowable range. However, in some cases, retesting may not be necessary if no significant deviation from routine results is determined after checking the raw data, which is both applicable to prenatal and postnatal CMA according to the policy of the laboratory [10]. Notably, a retest may be necessary even if the QC metrics are within the allowable range, if the noise is severe or if there are many false CNV calls [10].

## INTERPRETATION OF CMA RESULTS

When CMA testing is completed and QC metrics are within the allowable range, it is important to check whether the detected CNV and AOH values are true values using the analysis software provided by the manufacturer. For true CNV and AOH values identified in this manner, the clinical significance of variants is assessed using existing laboratory data (in-house data), CNVs reported in open databases, and literature review; the CNV classification is then determined as P (pathogenic), LP (likely pathogenic), VUS (variant of uncertain significance), LB (likely benign), and B (benign) according to the CNV interpretation guidelines presented by the ACMG [11,12]. In the recently proposed CNV interpretation guidelines, CNV classification is facilitated by scoring the evidence with digitization; in particular, quantification according to the number of genes in the CNV is possible, and there are differences between copy number loss and gain as follows.

Loss: 0–24 genes (0 points)/25–34 genes (0.45 points)/ 35 or more genes (0.9 points)

Gain: 0–34 genes (0 points)/35–49 genes (0.45 points)/ 50 or more genes (0.9 points)

Further, the CNV guidelines state that uncoupling between patient clinical information and CNV classification is important, which means that the same CNV should not be classified differently for each patient [12].

When recommending a CNV public database or useful website for CNV interpretation, firstly checking the ClinGen Dosage Sensitivity map information is important; genes or regions of haploinsufficiency or triplosensitivity are reviewed by dosage sensitivity curation working group in ClinGen. Then additional checking of the information in OMIM or ClinVar may be required for the genes which were not curated in ClinGen. The public databases recommended for CNV interpretation are as follows [13,14].

ClinGen Dosage Sensitivity map: https://dosage.clinicalgenome.org/

Database of Genomic Variants (DGV): http://dgv.tcag.ca/dgv/app/home

Decipher: https://decipher.sanger.ac.uk/

UCSC Genome Browser: https://genome.ucsc.edu/

Orphanet: https://www.orpha.net/

ClinGen Dosage Sensitivity map has curated information of genes or genomic regions in which haploinsufficiency or triplosensitivity is associated with genetic diseases. DGV shows structural variants in healthy control samples. Decipher has cases with pathogenic CNVs and phenotype information. In UCSC Genome Browser, comprehensive analysis of CNV using combined other databases including DGV, ClinVar, OMIM, ClinGen, and Decipher is possible. Orphanet provides general

information according to cytogenetic abnormalities such as microdeletion/duplication syndromes.

## SPECIAL CONSIDERATIONS

### Secondary findings

Among the genes included in a CNV, it is taken into consideration when genes that are not related to the current phenotype or those associated with late onset disease are included. A laboratory policy should be established on whether to report the detection of CNV associated with late-onset diseases such as PMP22 duplication [12]. Additionally, referring to the list of 81 genes reported in the ACMG secondary findings to confirming dosage sensitivity is necessary [15].

### Carrier status

In general, heterozygous deletions of genes associated with autosomal recessive genetic disorders are not considered for reporting. However, CNVs that have genes associated with recessive genetic diseases may be considered for reporting, if the allele frequency is high and the loss of function is a well-known mechanism for pathogenesis, or if the CNVs appear to be closely related to patient clinical information. In particular, if dosage-sensitive genes are included in CNVs on the X chromosome, reporting of female carriers may be considered [12].

### Duplication of part of a gene

In most cases, duplications that contain only a part of the gene (not intragenic) (>80%) are direct-oriented tandem duplications, and no effect on gene function is expected. However, the possibility of other duplication types such as inverted duplications or insertions cannot be ruled out [16]. Therefore, in rare cases of duplications involving only a subset of genes whose haploinsufficiency is associated with disease pathogenesis, VUS reporting should be considered.

### Consanguinity and uniparental disomy

If consanguinity is suspected because of an increased AOH ratio throughout autosomal chromosomes, the laboratory should establish its own policy regarding reporting methods. Additionally, AOH observed at a uniparental disomy (UPD) disorder-related site can be reported [3]. To confirm heterodisomy, a trio analysis of the CMA test can be performed, and the genotype signal patterns can be compared and confirmed. However, the CMA cannot cover all UPD disorders. In Prader-Willi syndrome, up to 90% of the cases can be diagnosed by CMA, but the rest can only be diagnosed by performing methylation analysis [17].

### Prenatal CMA

The American College of Obstetricians and Gynecologists recommends CMA testing when one or more major structural abnormalities are observed in the fetus on prenatal ultrasound and an invasive prenatal examination is performed [7]. In prenatal CMA, selecting an appropriate platform is important considering the resolution and cost, and caution is required when reporting the VUS. CNVs often have a low genotype-phenotype correlation [18]. In the case of prenatal CMA, CNVs showing a low correlation with malformation are often detected; therefore, patients may suffer from toxic knowledge when reporting unnecessary VUS. The Society of Obstetricians and Gynecologists of the Canadian College of Medical Geneticists states that VUS with deletions of 500 kb or less, or duplications of 1 Mb or less, should not be reported. These can only be reported when there is sufficient evidence for the pathogenic possibility of the relevant part. Further, comprehensive genetic counseling considering various possible situations, such as secondary findings and the possibility of consanguinity is essential [19].

The possibility of maternal cell contamination is also considered. Contamination of maternal cells in prenatal specimens can affect the results of cytogenetic analysis. In general, the ACMG guidelines recommend that prenatal genetic testing should include a check for maternal cell contamination [9]. However, in practice, for prenatal CMA, the need for checking maternal cell contamination should be considered carefully because the test price is increased when an additional STR analysis is performed. Further, the usefulness of information obtained from the confirmation of maternal cell contamination in interpreting the test results should be considered. In general, slight contamination by maternal cells in the prenatal CMA test (<20%), does not significantly affect the test results. Further, increased maternal cell contamination can be checked by CMA itself because QC metric values such as SNPQC fall outside the allowable ranges. Thus, whether to implement a confirmation test for maternal cell contamination considering various factors and test characteristics needs to be determined [20].

### Internal database management

Establishing and managing a laboratory database of detected CNV information is necessary to provide a reference for the frequency and content of previous reports on detected CNVs. Currently, a database needs to be built by considering the range

of coverage and overlapping percentage for each CNV (an appropriate algorithm design is required) [21].

## CONCLUSION

CMA is a state-of-the-art molecular cytogenetic test that can easily detect CNVs among structural variants in the genome and check for information on AOH, compensating for the shortcomings of conventional karyotyping and FISH tests. Its clinical utility has been proven sufficient as a primary test for congenital disorders after childbirth; notably, it can supplement the limitations of conventional tests even in prenatal testing. In clinical laboratories, management of QC metrics is important for quality control, and comprehensive confirmation of clinical information, open databases, and literature reviews are required for accurate interpretation of results. An improved understanding of the characteristics of CMA testing can help diagnose various genetic diseases and increase its clinical value when used in conjunction with other genetic tests.

## CONFLICTS OF INTEREST

I declare that I do not have any conflicts of interests.

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