

Review article

The contribution of Chromosomal Microarray Analysis to the Identification of Microdeletion/Microduplication syndromes associated with Intellectual Disability

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Abstract

Chromosomal Microarray Analysis (CMA) is currently considered to be the first-tier clinical test for neurodevelopmental disorders, such as Intellectual Disability (ID)/ Developmental Delay (DD) and Autism Spectrum Disease (ASD) due to its ability to detect Copy Number Variants (CNVs). CNVs are defined as segments of DNA which might either be deleted or duplicated and represent the most prevalent type of structural variation in the human genome. Recently, they have been found to be the causative agent for approximately 15-20% of previously undiagnosed cases of neuro developmental disorders. The unceasingly growing number of microdeletion and microduplication syndromes (MMSs), identified through CMA, has significantly altered the diagnostic approach to disorders such as ID and ASD.

Keywords: Chromosomal Microarray Analysis, Intellectual Disability, Copy Number Variants, Microdeletion and Microduplication Syndromes

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Abbreviations:

CMA: Chromosomal Microarray Analysis

ACGH: array Comparative Genome Hybridization

SNP: Single-Nucleotide Polymorphism

CNV: Copy Number Variant

ID: Intellectual Disability

DD: Developmental Delay

ASD: Autism Spectrum Disorder

ADHD: Attention Deficit Hyperactivity Disorder

VUS: Variant of Unknown Significance

MMS: Microdeletion and Microduplication Syndromes

FISH: Fluorescent in situ Hybridization

BAC: Bacterial Artificial Chromosome

UPD: Uniparental Disomy

ACMG: American College of Medical Genetics

Introduction

The implementation of array Comparative Genome Hybridization (aCGH) and array of Single-Nucleotide Polymorphism (SNP) into the genome-wide detection of Copy Number Variants (CNVs) related to Intellectual Disability (ID) has revolutionized the field and has significantly broadened the diagnostic spectrum.

Chromosomal Microarray Analysis (CMA) is currently considered to be the first-line diagnostic test for Neurodevelopmental disorders, such as Intellectual Disability (ID)/developmental delay (DD), autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD) with a diagnostic yield of approximately 15-20% (Miller et al., 2010). Intellectual Disability is defined as the restricted intellectual and behavioral functioning of an individual which originates before the age of 18 years/ during the developmental period and can be further categorized into one of the following: mild, moderate, severe or profound (Birch et al., 1970; Lee et al., 2019). Nowadays, the classification criteria are based more on the adaptive functioning of the individual rather than his IQ (Bass and Skuse, 2018). ID occurs in 1-3 % of the general population but is found to have a higher prevalence among individuals with concurrent congenital deficits. In the majority of cases, especially when referring to a non-syndromic ID, the underlying cause cannot be defined. Copy number variants (CNVs), including microdeletions and microduplications, have recently been found to be highly implicated in the aetiology of ID and count for approximately 15-20 % of patients with previously unrecognized/undiagnosed neurodevelopmental disorders (Cooper, 2011). They can be inherited

or arise de novo and they range in size from 15 kb to 1 Mb in length. Furthermore, they can be classified as pathogenic, benign or variants of unknown significance (VUS) based on various criteria (**Table 2**).

The detection of numerical and balanced or unbalanced structural chromosomal abnormalities was traditionally achieved through the cytogenetic analysis of G-banded karyotype. This technique successfully contributed to the diagnosis of various genetic syndromes. However, due to its lack of sensitivity and a minimum resolution of 5-10 Mb, arose the need for a technique with an improved diagnostic resolution (Vickers and Gibson, 2018). The evolution of Fluorescent in situ hybridization (FISH) has managed to accomplish a minimum resolution of 40-250 kb and has significantly ameliorated the detection of submicroscopic chromosomal imbalances and rearrangements. This particular method uses sequence complementarity in order for fluorescently labeled DNA probes to be hybridized to specific genes of interest in interphase cells or metaphase chromosomes. Its application resulted in the identification of more than 500 syndromes which are currently well characterized. However, its greatest limitation is the need for prior knowledge of the chromosomal region(s) of interest and therefore can be strictly implemented in the detection of one or a few certain candidate chromosomal loci and cannot be utilized for genome-wide analysis (Shaffer, 2005; Beaudet, 2013). On the contrary, the application

of CMA is not only able to detect genome-wide submicroscopic deletions and duplications at the same time and in a single assay, but also achieves it with even higher resolution compared to G-banded karyotype and FISH analysis (Miller et al, 2010; Beaudet, 2013). CMA has increased the diagnostic yield up to 10%, especially for individuals with previously undetermined/unexplained developmental disabilities.

Chromosomal Microarray Analysis Technique

Microarray-based genomic copy-numbers analysis, commonly known as chromosomal microarray analysis or molecular karyotype, encompasses all types of area-based genomic analyses. Two types of CMA that are currently mostly utilized are array-based comparative genomic hybridization (aCGH) and single nucleotide polymorphism (SNP) arrays:

1) The aCGH technique is able to detect CNVs using bacterial artificial chromosome (BAC) or oligonucleotide probes that are approximately 60-70bp long. By this method, patient DNA and control DNA samples are fluorescently labelled with different colors and are united on microarrays, where hybridization takes place. Any variations in fluorescence intensities reveal gains or losses of genomic segments, and therefore may allow the identification of CNVs which account for the particular deletion or duplication (Theisen, 2008). The application of aCGH has permitted an etiologic diagnosis in 15-20% of individuals with previously undetermined/unexplained

developmental delay/intellectual disability (D'Arrigo et al, 2016).

2) The SNP array technique is based on the usage of two oligonucleotide probes that are approximately 25bp long and target even the slightest variations throughout the genome. Regarding to SNP arrays, only the patient's DNA is labelled and hybridized to the array. The signal intensity of the patient's sample is then compared to allele-specific probes, which correlate to approximately 1,500 SNPs and correspond to a specific genetic location (Ji et al, 2004). Compared to aCGH, microarray-based SNP analysis is able to spot cases of uniparental disomy (UPD) which is defined as the inheritance of two copies of a chromosome from the same parent, instead of inheriting a maternal and a paternal copy. Furthermore, SNP analysis enables the detection of low-level mosaicism (as low as 5%), a condition in which an individual consists of 2 or more genetically different sets of cells (Flore and Milunsky, 2012; Miller et al, 2010) (**Table 1**). Even though both array-based technologies are unable to identify balanced translocations such as Robertsonian or other reciprocal translocations, balanced inversions or insertions, a respectable amount of cytogenetic events that are ostensibly balanced, turn out to have a submicroscopic imbalance when analyzed with high resolution array technology (Feenstra et al, 2011; De Gregori et al, 2007).

Apart from distinct microdeletions or microduplications, copy number variants (CNVs) represent a different entity which have proven to be

pathogenic in many previously undiagnosed disorders. The above-mentioned techniques have contributed to their detection in different extent. SNP arrays seem to prevail in such

diagnoses, due to their higher probe density and coverage (Li and Olivier, 2013).

Table 1: CMA techniques comparison

aCGH	SNP array
-oligonucleotideprobelength: 60-70bp	-oligonucleotideprobelength: ~25bp
-patient DNA + control DNA samples	-hybridization of patient DNA only
-fluorescentlylabelled	-fluorescentlylabelled
-diagnosis based on fluorescence intensities' differences	-diagnosis based on comparison of patient's sample DNA to allele-specific probes
-no detection of UPD and mosaicism	-detection of UPD and mosaicism

Copy Number Variants (CNVs)

Copy number variants (CNVs) are defined as a type of structural alteration concerning the number of copies of specific regions of DNA, which can either be deleted or duplicated. They vastly contribute to the genetic variation found among individuals and can be inherited or arise de novo. CNVs have multiple functional effects; they usually encompass genes that are dosage sensitive such as the ones involved in the brain development. In fact, recent studies have demonstrated their major implication in the aetiology of certain neurodevelopmental disorders including, but not limited to, ID, DD, ASD and ADHD. Additionally, they can affect gene expression at a distance or insert into varying positions of the

genome. They can be characterized by incomplete penetrance and variable expressivity, furtherly complicating the interpretation of their contribution to certain diseases. Multiple CNVs are considered to be risk factors for various diseases such as schizophrenia. The American College of Medical Genetics and Genomics (ACMG) recently published the clinical classification and description of CNVs (**Table 2**) which is based on their size, genomic content, existing databases and parental analysis. According to the current ACMG clinical classification, CNVs can be pathogenic, likely pathogenic, Variants of unknown significance (VUS), likely benign and benign (Kearney, 2011).

Table 2: ACMG classification guidelines

CNV classification	Description
Pathogenic	Clinically significant in multiple peer-reviewed publications/overlapping CNV of known genomic syndrome. Overlapping CNV of patients reported in database (es. DECIPHER,DGV,ClinGen).
Likely Pathogenic	Described in a single case report with similar phenotype to the patient. Contains a gene relevant and specific to the reason for patient referral.
Variant of unknown significance (VUS)	Reported in contradictory publications and/or databases. Contains genes that are not known to be dosage sensitive.
Likely Benign	Large CNV that is gene poor/absence of regulatory elements. Not reported in databases as benign.
Benign	Common polymorphism/known benign variant in multiple peer-reviewed publications.

Ideally, parental analysis through FISH, CMA or qPCR should be performed in order to interpretate the pathogenicity of the identified CNVs. The presence of a CNV in an affected parent indicates its pathogenicity. However, the inheritance of a CNV by a phenotypically unaffected parent does not exclude its contribution to the disease; incomplete penetrance or variable expressivity may be the underlying causes of this phenomenon (Miller, 2010).

In a similar way, even though a CNV occurring de novo has a greater chance of being pathogenic compared to an inherited one, further investigation of the variant's correlation to the particular phenotype is necessary, before classifying it as pathogenic. The clinical interpretation of VUS poses a significant diagnostic dilemma for

clinicians and geneticists. According to some, such a finding should not be made known to the patient; if the particular variant is not responsible for the condition, it will only cause unnecessary frustration. On the other hand, it may be the causative agent in regard to the disease and omitting such valuable information might severely affect the future decision-making process (Hoffman-Andrews, 2017). The ACMG suggests that a VUS should not be utilized in the clinical determination; however, there should be a continuous attempt to resolve the classification of the variant to "pathogenic" or "benign" (Kearney, 2011). In the meantime, additional monitoring of the patient for the disorder in question must be considered by clinicians. Finally, genetic laboratories ought to be alert

for any possible changes in the literature/in databases concerning the transformation of VUS to likely pathogenic or likely benign.

Microdeletion and microduplication syndromes

The greatest contribution of CMA lies in the diagnosis and research of various genetic disorders that are caused by infrequent genomic rearrangements and could not until recently be discovered. The unceasingly growing number of microdeletion and microduplication syndromes (MMSs), identified through this method, has significantly altered the diagnostic approach to neurodevelopmental disorders such as ID and ASD (Weise et al., 2011; Nevado et al., 2014;). MMSs are caused by microscopic and submicroscopic gains or deletions of various genomic regions and frequently appear with a recognizable collection of clinical features. Certain gene segments can be both deleted and duplicated, a phenomenon known as a reciprocal duplication. It usually appears with similar but less severe clinical effects, compared to the ones deriving from the deletion (Watson et al., 2014; Conrad et al., 2010; Kiezun et al., 2013). Some of the best described microdeletion and reciprocal microduplication syndromes that are caused by CNVs and have been identified through CMA are presented below (Table 3).

1. 7q11.23 distal microdeletion syndrome

It is referred to the distal deletion of the Williams-Beuren region. Epilepsy and neurodevelopmental disorders commonly appear.

2. 7q11.23 microduplication syndrome

Speech disorders, ID, ASD, epilepsy and distinctive craniofacial features such as brachycephaly, broad nasal tip and straight eyebrows have been observed in patients.

3. 15q24 microdeletion syndrome/microduplication syndrome

Similar clinical features with varying severity are observed in both syndromes and include: failure to thrive, ID, distinctive facial characteristics (es. Long face, anterior hairline, hypertelorism and long philtrum). Hypotonia, behavioral abnormalities, hearing impairment and hernias have also been reported in various patients.

4. 16p11.2 microdeletion syndrome

Mild ID, autism, epilepsy, macrocephaly (apparent by 2 years of age), microphthalmia and obesity are frequently associated with this syndrome.

5. 16p11.2 microduplication syndrome

The reciprocal duplication usually appears with microcephaly, ID, ASD and schizophrenia.

6. 17p11.2 microdeletion (Smith-Magenis) syndrome

Neurodevelopmental and psychiatric disorders, sleep disturbance, craniofacial and skeletal anomalies are the most frequently observed findings in SMS.

7. 17p11.2 microduplication (Potocki-Lupski) syndrome

The reciprocal duplication presents similar findings including: hypotonia, poor feeding, growth delay, learning disabilities, mild to moderate ID, ASD and ADHD. Structural cardiovascular

anomalies (es dilated aortic root) and sleep disturbance (obstructive and central sleep apnea) are commonly reported in patients.

8. 17q21.31 microdeletion (Koolen-De Vries) syndrome

This condition is characterized by hypotonia, mild to moderate ID, epilepsy, macrocephaly, dysmorphic facial alterations, congenital heart anomalies (es dilation of the aortic root) and congenital renal/urologic anomalies.

9. 17q21.32 microduplication syndrome

Hypotonia, ASD and developmental disorders have been associated with this newly described condition.

(Goldenberg, 2018; Nevado, 2014; Weise, 2012; Watson, 2014).

Table 3: Microdeletion/ Microduplication Syndromes

1. <i>7q11.23</i> distal deletion / <i>7q11.23</i> duplication syndrome
2. <i>15q24</i> deletion / <i>15q24</i> duplication syndrome
3. <i>16p11.2</i> deletion / <i>16p11.2</i> duplication syndrome
4. <i>17p11.2</i> deletion (<i>Smith Magenis</i>) / <i>17p11.2</i> duplication (<i>Potocki-Lupski</i>) syndrome
5. <i>17q21.31</i> deletion (<i>Koolen-De Vries</i>) / <i>17q21.31</i> duplication syndrome

Conclusions

The clinical implementation of CMA in the discovery of ID-related CNVs has notably raised the number of recognizable MMSs and has revolutionized the diagnostic approach to intellectual disability. When applied as a first-tier clinical test for broadly defined neurodevelopmental disorders, CMA can detect pathogenic variants, including CNVs that were undetectable by other techniques such as karyotyping and FISH, in approximately 15% of individuals. However, specific clinical genetic training and cautiousness in regard to the interpretation of certain findings such as VUS, is needed, as well as additional counseling skills for the communication of the results to patients. Nowadays with all the

advances in technology, the continuous sharing of information across laboratories and clinicians is necessary so that uniformal interpretation of results can be achieved. Thus, the establishment of better diagnostic definitions may hopefully lead to the provision of personalized medical treatment in the near future.

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