



# The array CGH and its clinical applications

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Array comparative genomic hybridization (aCGH) is a technique enabling high-resolution, genome-wide screening of segmental genomic copy number variations (CNVs). It is becoming an essential and a routine clinical diagnostic tool and is gradually replacing cytogenetic methods. Most of the clinically available aCGH platforms are designed to detect aneuploidies, well-characterized microdeletion/microduplication syndromes and subtelomeric or other unbalanced chromosomal rearrangements. In addition, aCGH can uncover numerous CNVs of unclear significance scattered throughout the human genome. But this technology is not able to identify balanced chromosomal imbalances such as translocations and inversions and some ploidies. aCGH increased the ability to detect segmental genomic CNVs in patients with global developmental delay, mental retardation, autism, multiple congenital anomalies and dysmorphism, and is becoming a powerful tool in disease gene discovery and prenatal diagnostics. This tool is also showing promising data in cancer research and in the diagnosis, classification and prognostification of different malignancies.

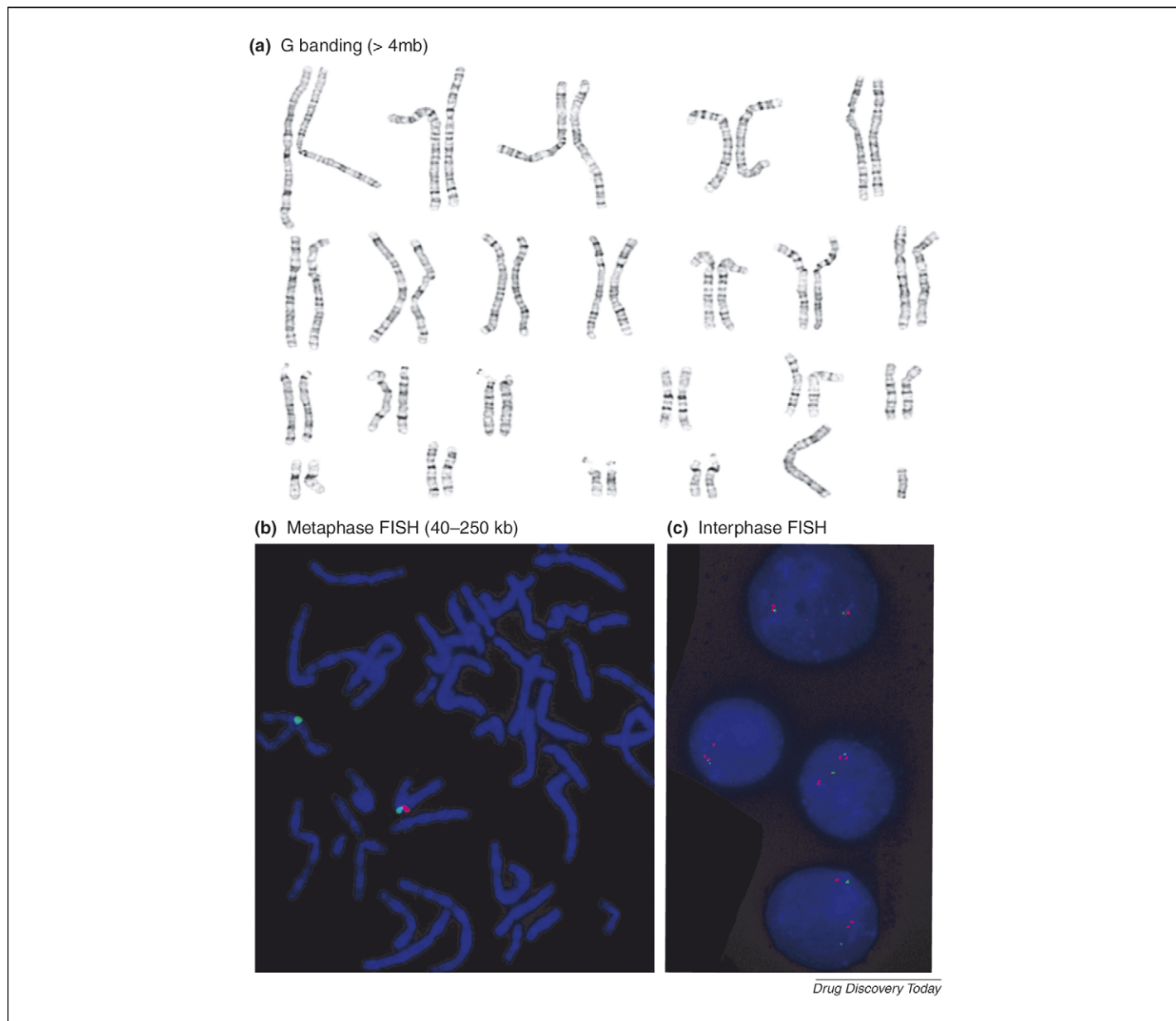
## Introduction

Array comparative genomic hybridization (aCGH), also called molecular karyotyping, is a technique that was developed for high-resolution, genome-wide screening of segmental genomic copy number variations (CNVs) [1,2]. It allows for comprehensive interrogation of hundreds of discrete genomic loci for DNA copy number gains and losses. The development and the clinical applications of aCGH in the past few years have revolutionized the diagnostic work-up of patients and facilitated enormously the identification of the molecular basis of many genetic diseases. After being first developed as a research tool for the investigation of genomic imbalances in cancer, aCGH has become an essential and a routine diagnostic tool and is gradually replacing cytogenetic methods in an increasing number of genetic laboratories [3–5].

Chromosomal aneuploidies (extra or missing chromosomes) and structural aberrations (deletions, duplications, translocations, inversions and marker chromosomes) are an underlying cause of congenital anomalies, dysmorphism, global developmental delay (GDD), autism, miscarriages and several other genetic syndromes.

Traditionally, cytogenetic analysis of Giemsa-stained metaphase chromosomes was applied to ascertain these abnormalities (Fig. 1a). This technique identifies balanced and unbalanced structural and numerical chromosomal abnormalities. However, routine karyotype analysis is not sensitive enough to detect subtle chromosome rearrangements (less than ~4 Mb). The introduction of fluorescent *in situ* hybridization (FISH) improved the diagnostic resolution and, until recently, had been considered the method of choice for detecting chromosomal imbalances and rearrangements. In FISH analysis, fluorescently labeled DNA probes are hybridized to interphase cells or metaphase chromosome preparation to determine the presence, location and number of specific genomic segments being interrogated (Fig. 1b and c). FISH analysis is, however, a time-consuming, targeted method that requires prior knowledge of the chromosomal region(s) of interest and therefore interrogates one (e.g. microdeletion syndrome) or more candidate chromosomal loci (e.g. subtelomeric regions) at a time. It does not provide a genome-wide screen for unexpected imbalances and returns only the result that is asked for on the basis of the clinical assessment of the phenotype by the clinician.

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**FIGURE 1**

Resolution of cytogenetic and molecular methods. **(a)** Representation of G-banded karyotype at 600-band level, which resolves chromosomal rearrangements greater than 4 Mb. **(b)** Representation of metaphase FISH analysis, which typically uses fluorescently labeled genomic DNA as probes of approximately 40–250 kb. This example showing a single red signal of the test probe as compared to two green signals of the control probe indicating a microdeletion on one chromosome 7 (Williams syndrome). **(c)** An interphase FISH using the PMP22 probe specific for the Charcot-Marie-Tooth disease type 1A (CMT1A) critical region. Note the presence of three red signals in the four interphase nuclei confirming the duplication on chromosome 17, consistent with the clinical diagnosis of CMT. The control probe (green) is showing the expected two signals.

Metaphase CGH was developed initially as a molecular tool in tumor cytogenetics [6]. In this technique, patient and reference whole-genomic DNA are differentially labeled and cohybridized to normal metaphase spread on glass slides. Unbalanced chromosomal rearrangements at a resolution of ~3–10 Mb across the whole genome can be detected by differential hybridization signals [7]. Although metaphase CGH was shown to be a useful diagnostic tool, providing an explanation for approximately 10% of previously undiagnosed cases of developmental delay and congenital anomalies [8], the low resolution of the metaphase chromosomes and technical challenge limited the widespread application of this technology.

Substitution of the metaphase chromosomes with target DNAs robotically spotted and immobilized onto glass microscope slides using split metal pins or glass capillaries has significantly enhanced the resolution and simplified the analysis procedure.

**aCGH methodology**

In aCGH, equal amounts of labeled genomic DNA from a test and a reference sample are cohybridized to an array containing the DNA targets. Some laboratories use pooled male and pooled female DNA as controls, and some use individual male and female controls. In addition, same-sex or opposite-sex controls are used in different laboratories. Genomic DNA of the patient and control are differen-

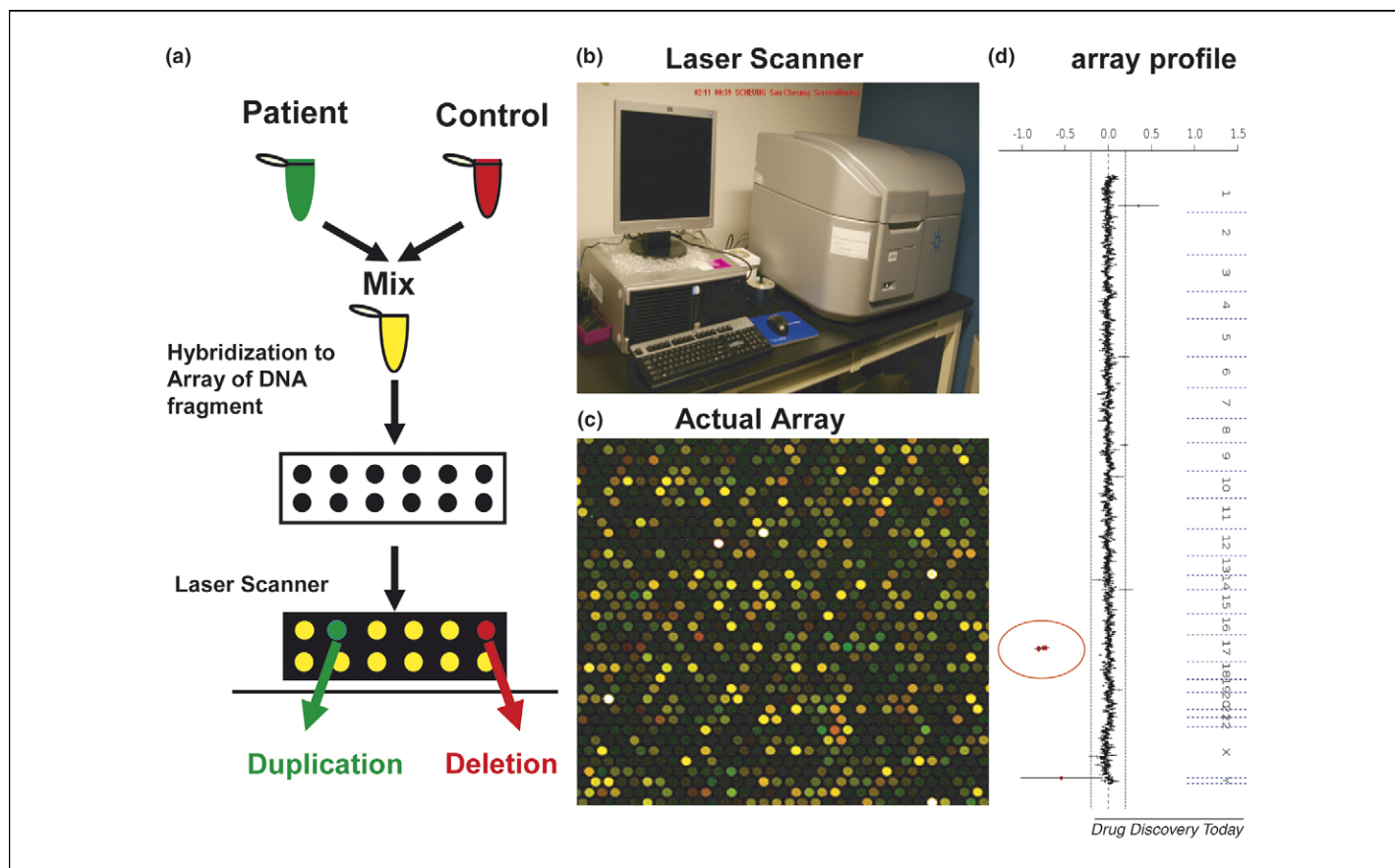
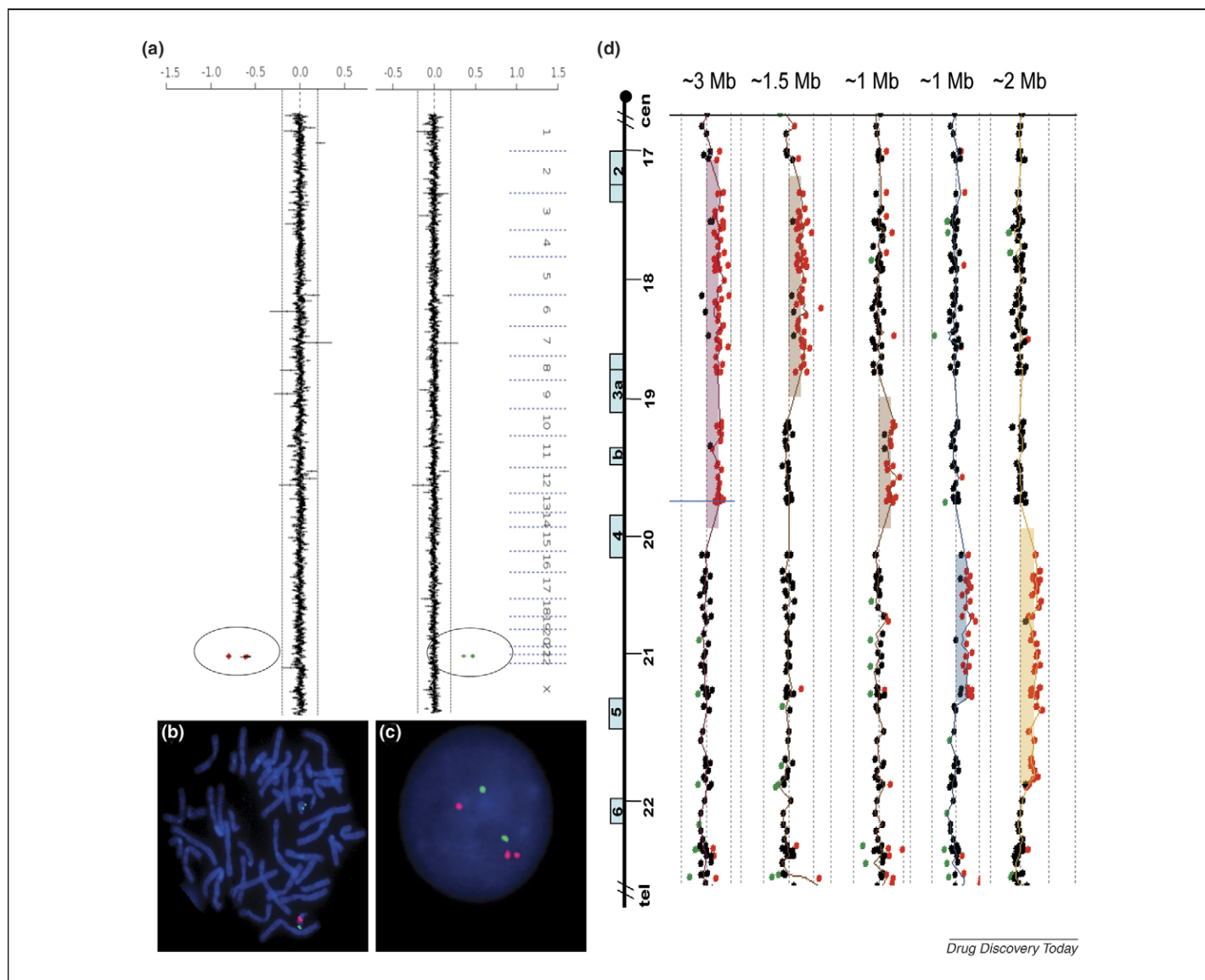


FIGURE 2

Principles of the aCGH technology. (a) DNA from the sample to be tested (e.g. blood or amniotic fluid) is labeled with a green fluorescence dye (Cy3) and a reference DNA is labeled with red (Cy5). The two samples are mixed and competitively cohybridized to an array containing genomic DNA targets that have been spotted on a glass slide. The resulting ratio of the fluorescence intensities is proportional to the ratio of the copy numbers of DNA sequences in the test and reference genomes. The areas on the slide that appear green indicate extra chromosomal material (duplication) in the test sample at that particular region. Areas on the slide that appear red indicate relatively less test DNA (deletion) in the sample at that specific spot. (b) The slides are scanned into image files using a specific microarray scanner. Shown here is the Agilent G2565BA microarray scanner in (c). An output of scanning depicts hundreds of spots with different ratios of the fluorescence intensities in (d). Microarray image files are quantified using Agilent Feature Extraction Software (V9.0), and text file outputs from the quantitation analysis are converted to BAC-level emulation data by combining oligo data corresponding to regions encompassed by BAC clones ('emulated BAC clone'). Shown here is a typical output of chromosomal microarray data analysis using our in-house software package for copy number analysis. The results depict a copy number loss of three clones encompassing the neurofibromatosis type 1 (NF1) critical region on 17p11.2.

tially labeled with Cyanine 3 (Cy3) and Cyanine 5 (Cy5) (Fig. 2a). Hybridization of the repetitive sequences can be blocked by the addition of Cot-1 DNA. The slides are scanned into image files using a microarray scanner (Fig. 2b). The spot intensities are measured (Fig. 2c) and the image files are quantified using feature extraction software, and text file outputs from the quantitative analyses are imported into software programs for copy number analysis (Fig. 2d) [4,9]. The resulting ratio of the fluorescence intensities is proportional to the ratio of the copy numbers of DNA sequences in the test and reference genomes. If the intensities of the fluorescent dyes are equal on one probe, this region of the patient's genome is interpreted as having equal quantity of DNA in the test and reference samples; if there is an altered Cy3: Cy5 ratio this indicates a loss or a gain of the patient DNA at that specific genomic region. These genomic imbalances are validated with other cytogenetic and molecular methods that include metaphase or interphase FISH analyses, long-range and quantitative PCR methods, customized multiplex ligation-dependent probe amplification (MLPA) assays and other aCGH platforms with higher resolution [10].

The interrogating probes (targets) are pieces of human genomic DNA in the form of bacterial artificial chromosomes (BACs) or P1 (PAC) clones (size of 75–200 kb), smaller insert clones such as cosmids (size of 30–40 kb) and fosmids (size of 40–50 kb), or oligonucleotides (25–85 mers). The genomic resolution of the different aCGH platforms is determined by spacing and length of the DNA probes. Most of the clinically available aCGH platforms are targeted microarrays that have been designed to detect aneuploidies, well-characterized microdeletion or microduplication syndromes and subtelomeric or other unbalanced chromosomal rearrangements. There are also whole-genome aCGH platforms in which the targets are equally spaced with coverage of approximately one clone per megabase to one clone per 100 kb [11]. The coverage of currently commercially available, whole-genome oligonucleotide arrays ranges from one probe per 6 kb to one probe per 70 kb. A detailed review and comparison of the different commercially available oligonucleotide aCGH platforms is available [12]. Some clinically available aCGHs encompass oligonucleotides that were designed to emulate the BAC or to target random

**FIGURE 3**

Examples of aCGH data. **(a)** A typical output of chromosomal microarray analysis (see legend for Fig. 2d) showing a 3 Mb loss (left) and its reciprocal gain (right) in 22q11.2 region in patients with DiGeorge syndrome or 22q11.2 microduplication, respectively. **(b)** The deletion in (a) was confirmed by FISH analysis showing a single red signal in a metaphase cell as compared to two green signals of the control probe. **(c)** The duplication in (a) was confirmed by interphase FISH showing three red signals of the reference probe as compared to the two control signals. **(d)** A summary of variable 22q11.2 microduplications in five patients identified by aCGH. Schematic representation of LCRs (blue boxes) and the sequence position in megabase (UCSC genome browser) are shown in the left [(d), courtesy of Dr Zhishuo Ou].

genomic sequences (Fig. 3a) [13]. The advantages of using focused or whole-genome oligonucleotide arrays include the ability to examine changes smaller than the average BAC size, higher resolution and enhanced dynamic range (signal to noise ratio) [12–14].

### The advantages and limitations of diagnostic aCGH

The higher resolution and throughput with possibilities for automation, robustness, simplicity, high reproducibility and precise mapping of aberrations are the most significant advantages of aCGH over cytogenetic methods. In addition, there is no need for cell culture, making the turn around time shorter than in cytogenetic methods. Most clinical aCGH platforms require only a few micrograms of genomic DNA, and whole-genome amplification procedures enable further reduction of the amount needed for analysis. In some cases, aCGH reveals additional and clinically

unsuspected genomic imbalances, emphasizing the advantage of the whole-genome approach as compared to focused locus-specific methods, which can only provide information on the interrogated loci. In addition, aCGH detects genomic duplications that cannot be identified by metaphase or even interphase FISH analyses. It has been proved that aCGH detects chromosomal mosaicism that would be missed by cytogenetic analysis [15,16]. We recently reported five patients with trisomy 14 mosaicism, in three of them the initial chromosome analysis was normal [17].

As with other clinical diagnostic methods, there are limitations in aCGH technology. aCGH is not able to identify balanced rearrangements such as translocations and inversions. This technology is only able to detect copy number imbalances relative to other DNA regions within the same sample and therefore aCGH is unable to detect the polyploidy. Furthermore, platforms that cover

the entire genome at very high resolution are more expensive and are likely to detect genomic imbalances of unclear significance.

### Copy number variations/polymorphisms

aCGH can uncover numerous variations in the number of DNA copies scattered throughout the human genome. Analyzing the DNA of 270 individuals from the HapMap project collection using aCGH and single nucleotide polymorphism (SNP), genotyping arrays detected 1447 submicroscopic copy variable regions (12% of the genome) [18]. The sizes of these regions are in the order of several kilobases and, therefore, with increasing resolution, aCGH platforms will be detecting more variations. Some of these aberrations are apparently benign CNVs and are usually inherited from a parent [10]. If identical alterations are found either in one of the unaffected parents, or in independent normal controls, they most probably have no direct phenotypic consequences; however, low penetrance and variable expressivity of the phenotype may complicate the analysis and genetic counseling. Currently, the publicly available CNV databases assist in making decisions about the clinical significance of imbalances detected by microarrays. Examples of such databases are the Database of Genomic Variants (<http://www.projects.tcag.ca/variation/>, <http://www.genome.ucsc.edu/> and <http://www.sanger.ac.uk/PostGenomics/decipher/>). Investigations of the parents and additional family members may often be necessary to interpret and clarify these results. The elimination of such regions from the new generations of microarray can improve the specificity and subsequently facilitate the genetic counseling.

When determined as *de novo* in origin genomic imbalances are considered pathogenic. This can be further supported if the implicated region contains gene(s) with functions compatible with the abnormal clinical findings or previously described patients with a similar genomic imbalance and a similar phenotype. The *de novo* occurrence of copy number alteration is, however, not an absolute evidence of its pathogenicity and caution must be exercised for possible nonpaternity.

### Evaluation of mental retardation/developmental delays by aCGH

The term 'developmental delay' (DD) is usually reserved for younger children (typically <5 years), and 'mental retardation' (MR) is usually applied to older children when IQ testing is valid and reliable. MR occurs in 1–3% of the general population and its cause is unknown in more than one-half of the cases [19]. The yield of the diagnostic evaluation of those children has varied widely in different studies, reflecting population differences and different diagnostic tools used for evaluation.

The diagnostic yield of banded chromosome analysis in children with GDD is approximately 3.7% [20]. The identification of submicroscopic subtelomeric imbalances by the application of FISH analysis in ~2.5–7% of patients with idiopathic MR [21,22] suggested that applying higher resolution, whole-genome screening techniques will potentially increase the diagnostic yield in a significant proportion of cases.

Initial studies using a genome-wide aCGH to investigate cytogenetically normal patients with idiopathic MR associated with dysmorphism showed a potential diagnostic yield of ~15% [23,3]. Additional studies with larger numbers of GDD/MR patients reported detection rates of clinically significant *de novo* alterations

between 10% and 16% [24–28]. Recently, a meta-analysis of previously reported aCGH studies and the analysis of 140 additional patients with idiopathic MR (total of 432 patients) showed that 20% of patients have genomic imbalances, 11% have subtelomeric rearrangements [29]. Interestingly, the recurrence rate of deletions or duplications was very low in these studies emphasizing the genetic and genomic heterogeneity of MR/GDD, which can be tested only by applying whole-genome methods such as aCGH. The diagnostic yield of aCGH is dependent on the genomic coverage and, therefore, it is expected that the next generation of aCGH will significantly increase the detection rate.

### aCGH and autism

Autism spectrum disorders (ASDs) are common heritable, clinically heterogeneous, neurodevelopmental conditions characterized by impairment in social interaction, accompanied by a delay or lack of language and stereotyped behavior and movements. Until recently, a definitive etiology could be identified in approximately 10% of individuals with ASD [30]. These etiologies include chromosomal abnormalities visible with cytogenetic methods (e.g. sex chromosome abnormalities, duplications involving chromosome 15q11q12) [31], single gene disorders (e.g. fragile X syndrome, Rett syndrome, tuberous sclerosis or mutations in the *SHANK3*, *NLGN3*, *NLGN4* genes), or metabolic conditions. But in the majority (~90%) of patients with 'idiopathic autism' an intensive search has not revealed a definitive etiology [30]. These individuals can be subgrouped further into 'complex autism' in the presence of dysmorphic features (DFs), microcephaly and/or structural brain malformations and 'essential autism' in the absence of these findings [32].

It has been shown over the past few years that *de novo* deletions and duplications play a significant part in the etiology of autism. Jacquemont *et al.* applied aCGH with large insert clones spaced at approximately 1 Mb intervals and identified clinically relevant rearrangements in 8 out of 27 patients with 'syndromic' autism [33]. This study suggested that the aCGH would be especially effective in the diagnosis of autism associated with DFs. But even after excluding cases of syndromic autism, a whole-genome custom-designed oligonucleotide array with 35 kb resolution detected *de novo* CNVs in 10% of individuals with autism [34]. Likewise, a genome-wide analysis in 427 ASD patients discovered *de novo* CNVs in ~7% of patients with idiopathic autism. The molecular information of this study and previous karyotypic data were integrated to build The Autism Chromosome Rearrangement Database: <http://www.projects.tcag.ca/autism/> [35]. Most recently, a *de novo* recurrent 593 kb microdeletion and a *de novo* or inherited reciprocal microduplication on 16p11.2 were found in 1% of patients with autism and in 1.5% of patients with DD [36]. As in many other microdeletion abnormalities, the flanking 147 kb segmental duplications most probably mediate this chromosomal rearrangement through unequal crossing over during meiosis [37]. The wider application of aCGH will improve the detection yield in patients with ASD and pave the way for the identification of new autism genes.

### aCGH and congenital anomalies

Cytogenetic abnormalities are a major cause of multiple congenital anomalies (MCA), especially when they are associated with growth or developmental delay, malformations affecting a second

organ or DFs [38]. It has been shown that the sensitivity of clinical aCGH is the highest among patients with DF, MCA or the combination of both [10]. This study showed that 25 out of 299 (8.4%) patients with MCA/DF have clinically relevant chromosomal imbalances as compared, for example, to 3.9% of patients with DD/MR [9]. Likewise, the application of aCGH in patients with congenital heart defects and DF provided an etiological diagnosis in a large proportion of cases. In one study, 30% of patients with congenital heart defects associated with other malformations, DF or DD and in whom the karyotype analysis was normal, carried pathogenically significant genomic imbalances [39].

Approximately 6% of carriers with apparently 'balanced' reciprocal translocation and about one quarter of patients with 'balanced' complex chromosome rearrangement (involving >2 chromosomes or >2 breakpoints) have abnormal phenotypes [40,41], mainly MCA and/or MR. The abnormal phenotype suggests that chromosomal imbalances, causing disruption of dosage-sensitive genes or separation of *cis* regulatory elements, are common in these patients. The application of high-resolution aCGH revealed that 40% (11 of 27) of phenotypically abnormal patients with *de novo* reciprocal translocations and 'balanced' karyotypes by conventional cytogenetics had, in fact, unbalanced chromosomes, and 18% (5 of 27) of them had complex rearrangements with >3 breakpoints instead [42]. Furthermore, the vast majority of patients with apparently balanced complex translocation were found in the same study to have unbalanced rearrangements. These data emphasize the need to conduct aCGH in any phenotypically abnormal child who has a 'balanced' chromosomal rearrangement by conventional cytogenetic methods.

## Identification of new syndromes by aCGH

Deletion and duplication syndromes represent recurrent chromosomal abnormalities that are associated with distinct phenotypes. These microdeletions/microduplications often occur between low copy repeats (LCRs) and are commonly because of nonallelic homologous recombination (NAHR) events [37]. The detection of a *de novo* genomic imbalance in a single patient does not prove pathogenicity. Only the identification of similar genomic imbalances with a recognizable phenotype can help clarify the role of these genomic changes in causing the specific clinical features and will ultimately define a genetic syndrome. The scarcity of these conditions emphasizes the need for collaborative international efforts to collect results systematically from genomic microarrays together with clinical information by using databases such as the Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (DECIPHER) (<http://www.sanger.ac.uk/Software/analysis/decipher/database.shtml>) and the European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations (ECARUCA) (<http://www.ECARUCA.net>). These databases facilitate the careful phenotypic and microarray data collection and improve clinical interpretation of genomic aCGH results from individuals with rare genetic disorders, leading to rapid characterization of new genomic syndromes.

The list of novel syndromes that are being discovered by aCGH is continuously growing (Table 1) [43–47]. The number of recurrent microdeletion syndromes will undoubtedly increase in the future because of the wider clinical application of aCGH.

TABLE 1

### Clinical applications of array CGH

Condition	Array	Size	Clinical features or main findings	Refs
<b>Novel deletion syndromes</b>				
<b>16p11.2–p12.12</b>	Clinical aCGH (SignatureChip); NimbleGen; Affymetrix 250 K SNP	7.1–8.7 Mb	DD and DF (flat facies, downslanting palpebral fissures, low-set and malformed ears and eye anomalies)	[43]
<b>15q24</b>	Targeted NimbleGen (147 bp <sup>a</sup> )	1.7–3.9 Mb	FTT, microcephally, digital abnormalities, hypospadias and loose connective tissue	[44]
<b>17q21.31</b>	Tiling WG: 32,477 BAC clones	600 kb	Moderate MR, hypotonia and DF (ptosis, blepharophimosis, abnormal ears, tubular nose, long columella and a broad chin)	[45]
<b>15q13.3</b>	WG, targeted to segmental dup: 2007 BACs	1.5 Mb	Mild dysmorphic features, MR and seizures	[46]
<b>21q22.12</b>	Clinical aCGH (Baylor, V.5, 6.3); Agilent 244 K	Minimal overlapping del.: 0.7 Mb	Syndromic thrombocytopenia, acute myelogenous leukemia, FTT, DD	[47]
<b>Microduplication syndromes</b>				
<b>17p11.2</b>	A custom 17p array (83 BAC/PAC clones)	3.7 Mb nonrecurrent: 1.3–15.2 Mb	Infantile hypotonia, failure to thrive, MR, congenital anomalies and autistic features	[48]
<b>7q11.23</b>	Clinical aCGH (Baylor, V.5)	1.55 Mb	Variable DD, prominent language delay, autistic features	[49,50]
<b>Xq28</b>	Clinical aCGH (Baylor, V.4&5)	0.2–2.2 Mb	X-linked GDD/MR and recurrent infections in males	[51,52]
<b>22q11.2</b>	Clinical aCGH (Baylor, V.4&5) & WG 44 K Oligo (Agilent)	3-Mb-common, 1.5-Mb-nested, ~1 to ~2-Mb – atypical	A variety of dup. observed. The phenotypes were generally mild and highly variable; familial transmission was frequently observed	[53]

TABLE 1 (Continued)

Condition	Array	Size	Clinical features or main findings	Refs
<b>Delineation of known syndromes</b>				
'Atypical' 22q11.2 del	Clinical aCGH (Baylor, V.4&5); Agilent 244 K	1.4–2.1 Mb	Distinct from classic del: DF, DD, growth delay, skeletal abnormalities	[54]
1p36 del	Clinical aCGH (Baylor, V.5); Agilent 44B	2.97–14.69 Mb	Atypical: FTT, DD, DF, feeding difficulties, seizures, cardiovascular and limb anomalies, microcephaly	[55]
4p-syndrome	BAC 1-Mb <sup>a</sup> & chr.4 tiling BAC	1.9–30 Mb	Genotype–phenotype correlation. 1.4 Mb del. in a patient with mild phenotype. Mapping the genes causing different physical findings	[56]
GCPS	Custom 44K Agilent for 10 Mb around <i>GLI3</i> gene (730 bp <sup>a</sup> )	59 kb–10.4 Mb	GCPS can be caused by <i>GLI3</i> deletions or duplications of widely varying sizes. Deletion size correlates with disease severity	[57]
Angelman syndrome	Custom: BAC of 15q (>1 Mb <sup>a</sup> ) & Subtel	5–6 Mb	Class I deletions had more autism, lower IQ, and lower expressive language	[58]
PWS	Clinical aCGH (Baylor, V.6); Custom 15q-specific oligo-array	175 Kb	The data provide a conclusive evidence that deficiency of HBI-85 snoRNAs causes the key characteristics of the PWS phenotype	[59]
<b>Gene identification</b>				
CHARGE syndrome	WG tiling 1-Mb <sup>a</sup> & Chr.8 tiling (918 BACs)	5 Mb	~1.23 Mb <i>de novo</i> overlapping del in two patients led to the discovery of <i>CHD7</i> as the disease-causing gene	[60]
Peters Plus syndrome	WG 1-Mb <sup>a</sup>	1.5 Mb	A ~1.5 Mb del on 13q12.3q13.1 in two affected brothers. Several genes sequenced and mutation found in <i>B3GALTL</i>	[61]
Goltz syndrome (FDH)	244 K Agilent, targeted tiling NimbleGen	219 kb; 80 kb	Two teams detected deletions in Xp11.23 in four patients and sequencing of candidate genes identified <i>PORCN</i> as the gene mutated in FDH	[62,63]
STAR syndrome	244 K Agilent (~12 kb <sup>a</sup> ) and customized 105 K Agilent enriched for Xq28	37.9–50.7 kb	A del. at Xq28 in one patient removed exons 1 & 2 of <i>FAM58A</i> . Heterozygous point mutations found in other cases	[64]
EIEE	WG (4219 BACs)-0.7 Mb <sup>a</sup>	2 Mb	A del at 9q33.3–q34.11 detected in one affected girl. <i>STXBP1</i> mutations were found in other affected individuals	[65]

BAC, bacterial artificial chromosome; Chr., chromosome; DD, developmental delay; Del, deletion; DF, dysmorphic features; Dup, duplication; EIEE, early infantile epileptic encephalopathy; FDH, focal dermal hypoplasia; FTT, failure to thrive; GCPS, Greig cephalopolysyndactyly syndrome; Mb, megabase; PWS, Prader-Willi syndrome; STAR, syndactyly, telecanthus and anogenital and renal malformations; Subtel, subtelomeric; WG, whole genome.

<sup>a</sup>Resolution of aCGH.

## Microduplication syndromes

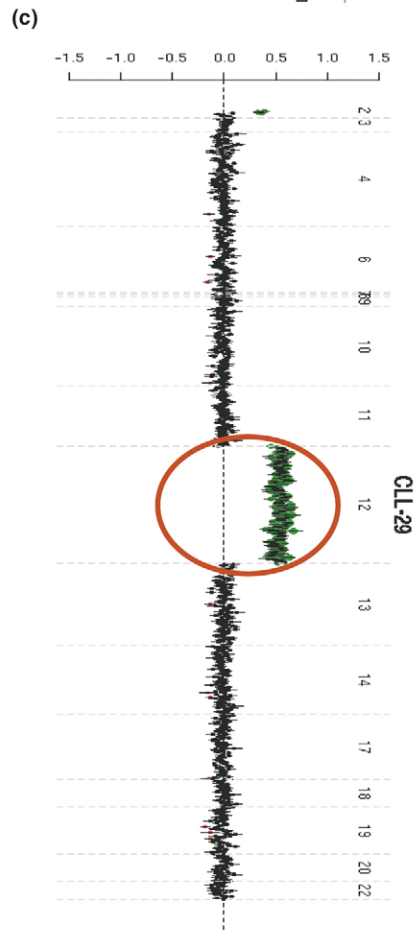
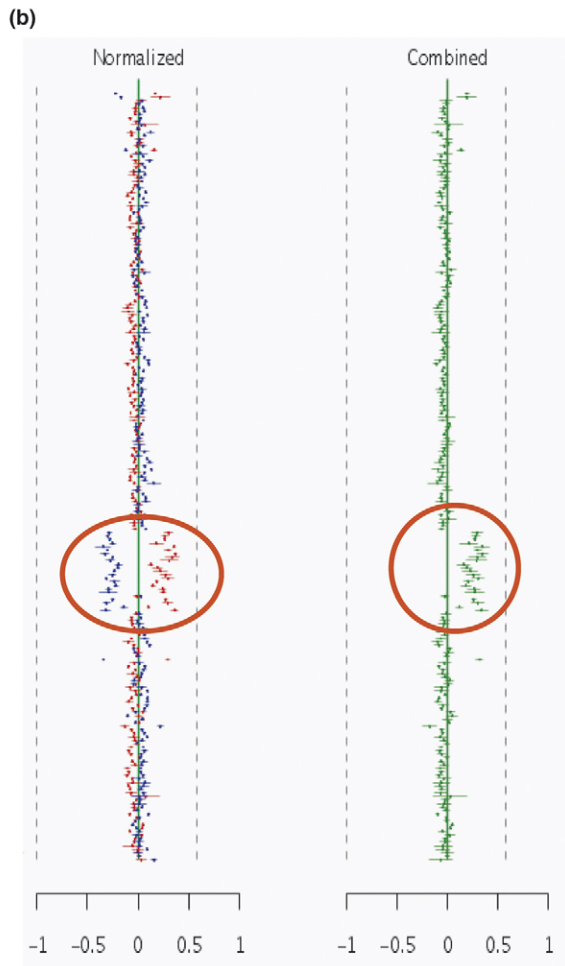
Segmental duplications mediate genomic rearrangements that are responsible for many of the well-known microdeletion syndromes [37]. The clinical phenotypes associated with the reciprocal microduplications of the same genomic regions are, however, less well characterized. In addition, there are difficulties in detecting microduplications by FISH examination of metaphase cells suggesting

that the prevalence of these rearrangements could be higher than previously expected.

The wide application of aCGH in individuals with nonspecific phenotypes, such as GDD, identified several microduplications that are associated with a recognizable phenotype (Table 1) [48–53]. Examples of various reciprocal 22q11.2 microduplications are shown in Fig. 3. Although the sensitivity of detecting these micro-

FIGURE 4

(a) Chromosome regions represented on the custom-design targeted CLL oligoarray generated by Agilent software: ideogram on the left and the covered region on the right. (b) An output BAC-based aCGH in CLL patient representing two hybridizations performed simultaneously with dye reversal using a reference DNA. In the 'normalized' plots, data from two hybridizations are shown independently. All clones representing chromosome 12 show displacements to the left (in blue) and to the right (in the dye reversal), both indicating gain of chromosome 12 material in the patient versus the reference DNA as shown in the red circle. In the 'combined' column, the sign of one of the two reversed hybridizations is changed and data are averaged with gains showing to the right and losses to the left. The gain of the chromosome 12 is indicated in the red circle. (c) An output of OLIGO-based aCGH of same patient as in (b) with similar coverage by Oligo-60mer for each probe distributed along the entire chromosome 12 in a single hybridization. The same gain in chromosome 12 is shown (red circle). Notice that the dynamic range of the signal to background log ratios in the OLIGO-based array are much higher than the BAC-based array indicating an increased resolution that allows for detection of copy number changes with greater confidence [courtesy of Dr Ankita Patel].



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duplications has increased, in all aCGH reports the number of deletions is greater than the number of duplications potentially reflecting an ascertainment bias caused by a milder phenotype in duplication syndromes.

### Delineation of known syndromes

High-resolution aCGH has been used successfully to refine breakpoints of genomic imbalances in known microdeletion/duplication syndromes. The results are used to construct a deletion map and to correlate the different elements of the phenotype with the genes within the imbalanced genomic region. It is usually expected that the extent of the deletions in contiguous gene deletion/duplication syndromes correlates with the severity of the phenotype [54–56]. We provide a few examples in Table 1 and Fig. 3 to illustrate our points, but the phenotype–genotype correlation was described for many other contiguous gene syndromes. Similarly, aCGH designed for specific chromosomes can be utilized to identify small deletions and accurately map the breakpoints of genomic imbalances of specific syndromes [57,58].

### Use of aCGH in disease gene discovery

The ability of aCGH to detect small deletions encompassing single or few dosage-sensitive genes suggests that it can also serve as an effective and powerful tool to localize disease-causing genes and to uncover the molecular basis of genetic syndromes. Occasionally, the delineation of disrupted dosage-sensitive genes at translocation breakpoints may pave the way for disease gene discovery. In fact, the identification of a chromosomal aberration in specific patients has proved to be a successful way to identify the implicated genes and to gain insight in the pathogenesis of different genetic conditions (Table 1) [60–65]. The widespread use of high-resolution aCGH will enhance our abilities of mapping of genes underlying several genetic conditions.

It is important to note that, in addition to causing autosomal dominant phenotypes, the deletion of genes can occasionally unmask a mutation in the second allele resulting in an autosomal recessive phenotype or could cause an imprinting disorder because of the deletion of imprinted genes [66].

### Prenatal diagnosis

There are many limitations of conventional G-banding analysis for the detection of fetal chromosomal abnormalities. These include failure to culture the cells, time required for culturing process resulting in a delay to report test results, low resolution and poor quality of chromosome morphology. To avoid these obstacles, genomic DNA (with or without whole-genome amplification) from cultured or uncultured amniotic or chorionic villous cells can be used as the test material in aCGH [67]. aCGH used for analyzing products of conception samples was shown to be sensitive and valid in detecting all abnormalities discovered by G-banding and in revealing previously undetected submicroscopic rearrangements [68]. The higher sensitivity of aCGH over cytogenetic methods was demonstrated in one study where the prenatal aCGH detected genomic imbalances in 16% of normally karyotyped fetuses with multiple malformations – at least half of these rearrangements were causative genomic imbalances [69]. Due to the complexity in interpreting CNVs, ‘targeted’ arrays containing genomic clones for tel-

omeres and known microdeletion/microduplication regions are being applied (see discussion on CNVs) [67,70].

DNA isolated from as little as 1 ml of uncultured amniotic fluid was shown to be capable of detecting genomic imbalances in 29/30 samples, the exception was a triploidy case [69]. Likewise, the aCGH on cell-free fetal DNA extracted from the routinely discarded supernatant of amniotic fluid has been shown to be effective in identifying human chromosome abnormalities [71].

These studies demonstrate the potential for aCGH to replace cytogenetics in the great majority of prenatal diagnosis cases. This technology is, however, unable to detect balanced translocations or polyploidy. It has been suggested that cytogenetic techniques should be employed on samples tested because of abnormal fetal ultrasound and for which the aCGH yielded normal results [72].

### aCGH and cancer

Somatic chromosomal dosage-alterations and rearrangements occur frequently in cancer and contribute to its pathogenesis. Detecting these aberrations by aCGH provides information on the locations of important cancer genes and can have clinical use in diagnosis, cancer classification and prognostification. Technical considerations related to aCGH analysis of tumor cells have been reviewed [73].

Cancer gene identification by high-resolution, genome-wide aCGH is assisted by the discovery of recurrent, amplified chromosomal segments, small homozygous deletions of tumor suppressor genes and deletions or rearrangements of dosage-sensitive proto-oncogenes [74,75]. aCGH also has the potential to be used for tumor classification [76] and to predict tumor progression and prognosis [77,78]. But the application of this technology for prognostic purposes is relatively limited because of the limited ability to detect balanced translocations. There is a wealth of the literature accumulated over the past two decades on various gene fusions on cancer causation resulting from balanced translocation [79]. In this review, we specifically discuss chronic lymphocytic leukemia (CLL) as an example. CLL is the most common form of leukemia in the western world and accounts for 25% of all leukemia in the USA. CLL is unique among leukemia, in that copy number changes are commonly seen rather than translocations, and certain genomic alterations are associated with prognostic significance [80]. The current standard practice in CLL is to use a panel of FISH probes on interphase cells from the patient to diagnose the condition and provide prognostification. The 13q14 deletion, the most common abnormality in CLL detected by FISH analysis correlated, in the absence of other abnormalities, with favorable prognosis. In a recent pilot study, various sizes of deletions, some of them undetected by conventional cytogenetic methods, were identified simultaneously when using a custom-designed aCGH (Fig. 4) demonstrating the robustness, high sensitivity and high specificity of this technique [81].

### Conclusions

The introduction of aCGH as a more efficient and comprehensive diagnostic tool remarkably improved the detection of segmental DNA CNVs. It has revolutionized the diagnostic work-up of patients with GDD/MR, MCA, autism and dysmorphism, and is increasingly becoming a powerful tool in disease gene discovery and in deciphering the genomic basis of many novel microdeletion and microduplication syndromes. In addition, aCGH is shedding light on the abundance of CNVs of unclear significance that are scattered throu-

ghout the human genome, though more intensive research is needed to understand their involvement in human diseases. Targeted aCGH enables the detection of all clinically relevant genomic imbalances but has limitations in the detection of polyploidy and balanced translocations. Whole-genome higher density arrays significantly increase the sensitivity of the method and are important for the

discovery of new genomic syndromes, but complicate the clinical interpretation of copy number variants of unclear significance.

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