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REVIEW

What's new in karyotyping? The move towards array comparative genomic hybridisation (CGH)

Thomy J. L. de Ravel · Koen Devriendt · Jean-Pierre Fryns · Joris R. Vermeesch

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Abstract Molecular karyotyping by array comparative genomic hybridisation (array CGH) has doubled the detection rate of pathogenic chromosomal imbalances in patients. This has been possible by increasing the resolution level from the 5 Mb obtained using the conventional karyotype to as low as 100 kb by array technology. Moreover, the technology revealed that over 12% of the human genome includes sub-microscopic benign copy number variable regions. These new findings have implications in genetic counselling and patient management.

Keywords Molecular karyotyping · Array CGH · Comparative genomic hybridisation

Abbreviations

Array CGH	array comparative genomic hybridisation
BAC	bacterial artificial chromosome
Cy	cyanide dye
dCTP	deoxycytidine triphosphate
FISH	fluorescence in situ hybridisation
kb	kilobases
Mb	megabases
MCA	multiple congenital anomalies
MR	mental retardation
PAC	p1 artificial chromosome
SNP	single nucleotide polymorphism

T. J. L. de Ravel (✉) · K. Devriendt · J.-P. Fryns · J. R. Vermeesch
Centre for Human Genetics,
University Hospitals Leuven,
Herestraat 49, 3000 Leuven, Belgium
e-mail: Thomy.deRavel@uz.kuleuven.ac.be

Introduction

Routine chromosome analysis, or karyotyping, has successfully been used for the last 50 years in investigating the cause in patients with mental retardation, specific organ malformations and dysmorphism, whether or not they are part of a syndrome. This has also led to the discovery of genes responsible for various conditions. Standard karyotyping is, however, constrained by the limits of resolution possible by using a microscope. The advent of molecular karyotyping, whereby sub-microscopic copy number changes across the whole genome are evaluated in a single analysis, has greatly increased the detection of pathogenic chromosome imbalances. Whereas standard karyotyping and fluorescence in situ hybridisation (FISH) studies detected chromosome imbalances in 10% of patients with mental retardation, molecular karyotyping has added an additional 10% of detection. This is having a great impact on the understanding of pathologies, the counselling of families and patient management [25].

Background

The human chromosome number of 46 was discovered as recently as 1956 [23], and aneuploidy in 1959 when Lejeune et al. [8] reported trisomy 21 (OMIM 190685) to be the common underlying cause in individuals with the characteristic mental retardation syndrome, also known as Down syndrome. The development of better culture and slide preparations, and the introduction of banding techniques in the 1970s, facilitated the identification of each individual chromosome, and soon, chromosome aberrations were reported in a number of patients with syndromes, as well as in aborted and stillborn foetuses. The occurrence of

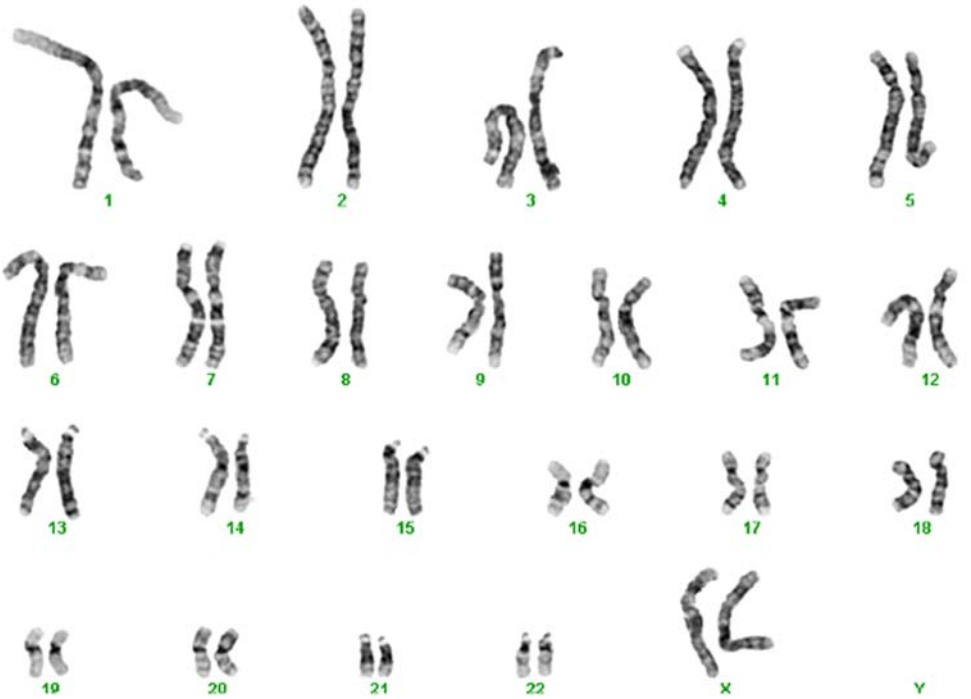

Fig. 1 Standard Giemsa-banded karyotype of a female patient

non-pathogenic variations (polymorphisms) was also noted. Present-day standard karyotyping, carried out on dividing cells and using a 10,000× magnification, detects numerical as well as structural chromosome aberrations, such as deletions, duplications, inversions and translocations, as long as they involve at least 5 to 10 million base pairs of DNA (5–10 Mb resolution) (Fig. 1).

Further refinement in the form of fluorescence in situ hybridisation (FISH) uses labelled DNA probes to detect the presence, number and location of small (sub-microscopic) regions of chromosomes, each probe hybridising to a specific already-known DNA sequence. This can confirm the clinical suspicion of known microdeletion syndromes, such as the velocardiofacial (VCFS, 22q11 deletion, OMIM 192430, Fig. 2), William's (7q11.23 deletion, OMIM 194050) and Prader-Willi (15q11.2–13 deletion, OMIM 176270) syndromes. FISH also detects deletions in the gene-rich subtelomeres which are involved in mental retardation and a number of syndromes, such as the Wolf-Hirschhorn (deletion 4p, OMIM 194190) and chromosome 1p36 deletion (OMIM 607872) syndromes. Using 27 different fluorescent DNA probes simultaneously, "whole chromosome painting" shows re-arrangements at a 1 to 5 Mb resolution but not deletions, duplications and inversions [21].

Tumour tissue was long-known to have chromosome re-arrangements, especially deletions or duplications (amplifications). In order to detect these regions (and eventually relate them to prognosis), differentially labelled patient DNA and normal reference DNA were simultaneously hybridised to normal metaphase spreads. Regions of loss

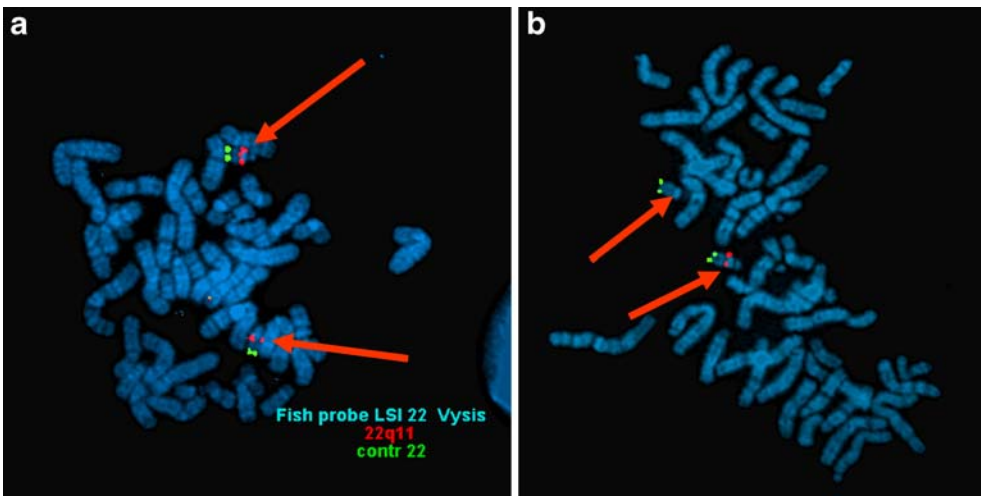

Fig. 2 Fluorescence in situ hybridisation (FISH) analysis using probe 22q11 (red colour) to demonstrate a normal individual (**a**) and an individual with a deletion of the chromosome 22q11 region (**b**). The green fluorescent probe is a control probe on chromosome 22

(deletions) and gain (duplications) were seen as changes in the intensities of the two fluorochromes along the chromosomes. This map of DNA sequence copy number as a function of chromosome location was termed comparative genomic hybridisation (CGH) [6]. As chromosomes are used as the template, the resolution is still only 3–5 Mb nowadays. Solinas-Toldo et al. [20] and Pinkel et al. [12] developed array CGH, whereby the hybridisation of the patient DNA takes place on an array of mapped DNA clones instead of metaphase chromosomes. Although chromosomes are no longer visualised under a microscope, the term "molecular karyotyping" is used for this group of techniques since, in analogy to conventional karyotyping, the purpose is the identification of chromosomal imbalances [27].

The array CGH technology

Genomic DNA of the patient is extracted from peripheral blood lymphocytes, skin fibroblasts or other available tissue and labelled with one fluorescent dye (usually Cy3-labelled dCTPs). The labelled patient DNA, together with an equal amount of control DNA labelled with another fluorescent dye (usually Cy5-labelled dCTPs), are co-hybridised to a selected set of pre-spotted genomic fragments [26]. The spot intensities are measured at 532 nm (Cy3) and 635 nm (Cy5). If the amount of Cy3 and Cy5 fluorescent intensities are equal in one spot, this region of the patient DNA is interpreted as being normal/balanced; if a threshold of increased ratio of Cy3 to Cy5 is detected, a duplication of the patient DNA is suspected, and inversely if a deletion is present (Fig. 3). In the last three years, studies with BAC/PAC arrays having a 1-Mb resolution have been the most frequently performed (Table 1). Thus, imbalances of 1 Mb and more can be detected with this technique. Following these initial studies, several technical platforms at increasingly higher resolution have been developed. These include the full tiling BAC arrays, cDNA arrays, oligonucleotide and the SNP arrays, with theoretical resolutions of up to 6 kb. In addition to the information obtained using BAC arrays, a special type of oligonucleotide array can distinguish SNPs, allowing recognition of the parental origin of each DNA copy and enabling the detection of uniparental disomy (UPD); this is important in UPD disorders and cancers. A number of targeted microarrays (e.g. only for the analysis of subtelomeric and microdeletion syndrome regions) are also offered in various centres (reviewed in [25]).

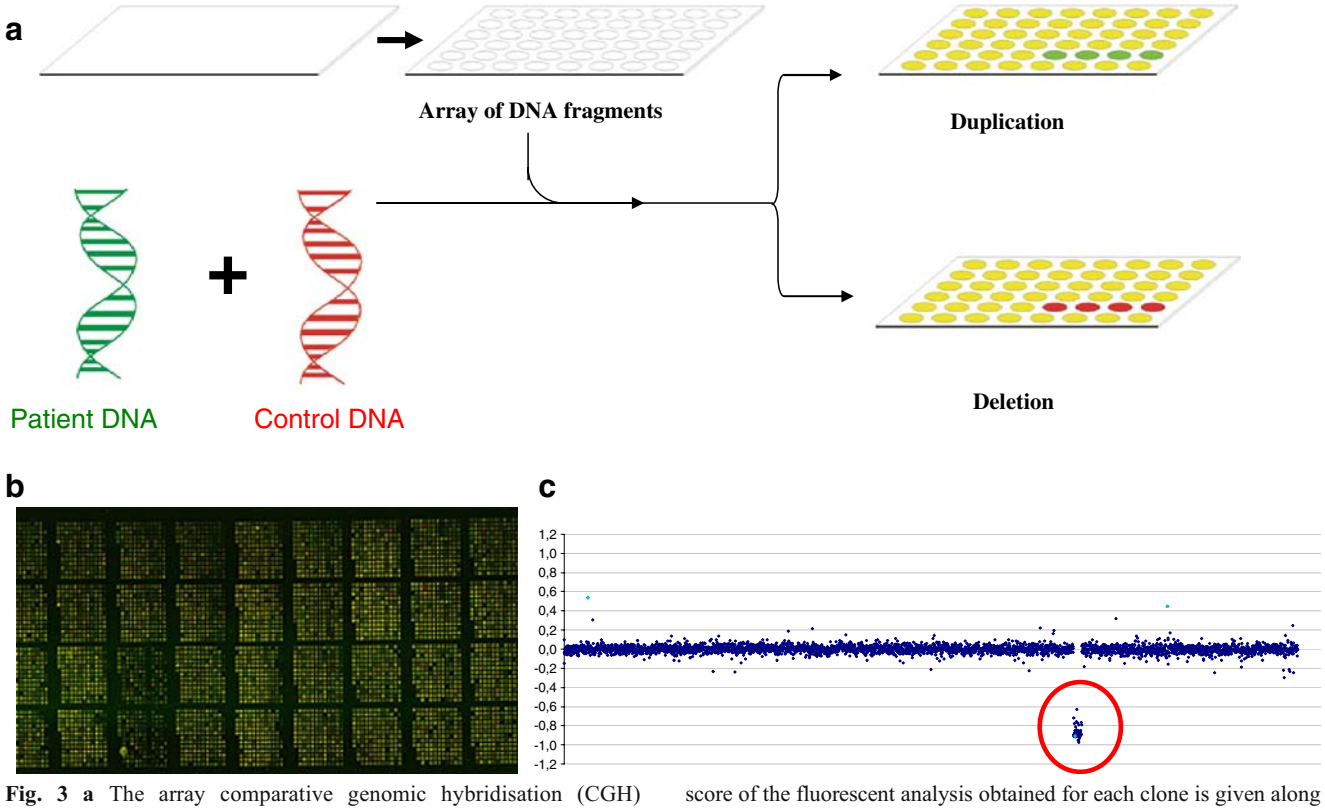

Fig. 3 **a** The array comparative genomic hybridisation (CGH) technique. **b** An example of the spotted plate. **c** Schematic representation of the results of the DNA of a patient with a chromosome 13 deletion co-hybridised against the DNA of a normal control. The DNA clones of chromosomes 1 to 22 and the sex chromosomes are aligned along the X axis (left to right), whilst the log score of the fluorescent analysis obtained for each clone is given along the Y axis. The deletion (seen in red on the spotted plate in **a** and **b**) is represented by negative log scores whilst a duplication (green on spotted plate in **a** and **b**) is seen as positive log scores. Thus, the chromosome 13 deletion in this patient is represented by negative log scores of the corresponding clones (shown within the red circle)

Table 1 Selected studies using molecular karyotyping, the selection criteria and results

Reference	Selection criteria	Resolution	Positive/total pts	Findings
Vissers et al. [28]	MR/dysmorphism	1 Mb	5/20	3 del, 1 pat
		3,500 BACs	25	2 dup, 1 pat
Shaw-Smith et al. [18]	Learning disability/dysmorphism	1 Mb	12/50	7 del, 1 pat
		3,431 BACs	24	5 dup, 1 pat, 3 mat
Menten et al. [11]	MR/MCA	1 Mb	28/140	23 del, 2 pat, 2 mat
		3,431 BACs	20	7 dup, 2 pat, 1 mat
Rosenberg et al. [16]	MR/MCA	1 Mb	20/81	14 del, 1 pat, 1 mat
		3,431 BACs	25	8 dup, 1 pat, 3 mat
Schoumans et al. [17]	MR/dysmorphism	1 Mb	4/41	4 del, *de novo*
		2,600 BACs	10	
Friedman et al. [4]	MR	100 kb	10/100	8 del
			10	2 dup
de Vries et al. [3]	MR	100 kb	10/100	7 del, de novo
		32,447 BACs	10	3 dup, de novo
Thienpont et al. [22]	Heart anomaly/dysmorphism	1 Mb	18/60	6 del
		3,431 BACs	30	8 dup
				3 del/dup; 1 mosaic

Array CGH was carried out except for Friedman et al. [3], where array-based SNP genotyping was performed. The percentage of positive imbalances does not represent the percentage pathogenic results

Clinical applications of array CGH

Mental retardation and dysmorphism

Eight studies screening individuals with mental retardation, multiple dysmorphic features and normal traditional karyotypes have demonstrated a high diagnostic yield in MCA/MR patients (Table 1). In summary, at the 1-Mb resolution, 20% to 25% of selected individuals have deletions or duplications or a combination of both. About half of these are conclusively causal for the disorder. The few studies at 100-kb resolution have also detected about 10% of pathogenic interstitial aberrations. The chromosome imbalances occur throughout the genome and are often single cases. In order to correlate the aberrant genotypes with the phenotypes worldwide, collaborative databases of results have been set up, e.g. Decipher (http://www.sanger.ac.uk/PostGenomics/decipher/) and Ecaruca (http://www.ECARUCA.net). Two new microdeletion syndromes have, thus far, been delineated using array CGH. An interstitial microdeletion at chromosome 9q22.3 is associated with a syndrome comprising of macrocephaly, overgrowth, psychomotor retardation and facial dysmorphism [13]. Similarly, a chromosome 17q21.31 microdeletion associated with a common inversion polymorphism results in a new syndrome comprising of moderate mental retardation, marked hypotonia, a long facies, blepharophimosis, ptosis, pear-shaped nose with a broad tip, long columella, large ears and a broad chin [7, 19].

Translocations

Disruption of dosage-sensitive genes at the translocation breakpoints has long been suspected in patients with mental retardation and/or dysmorphic features. Using array CGH and sequence analyses, both affected patients and normal apparently balanced translocation carrier-parents were shown to have not only insertions and duplications but also disrupted genes at the site of the translocations. More so, with the aid of array CGH, a significant proportion of translocation patients were found to have complex chromosome re-arrangements both in the chromosomes involved in the translocation as well as in other chromosomes [1, 5]. The non-translocation-related chromosome imbalance is, in some cases, responsible for the phenotype.

Prenatal investigation

Array CGH can be carried out on the DNA of single cells, of chorionic villus biopsies and of amniocytes. Due to the complexity in interpreting a complete array (see below), it is foreseen that "targeted arrays" to indicate aneuploidy and known microdeletion/duplication syndromes may be an option in the future [15].

Investigation of cancer

Both numerical and structural imbalances occur in (pre-) malignant cells and more and more of these are being associated with various prognostic factors. Array CGH has

brought a greater number of these to light and is changing the nature of their diagnoses. For example, using CGH, consistent genetic alterations were shown to be associated with primary cutaneous B-cell lymphomas [10]. However, the investigation of cancers is outside the scope of this review.

Research applications of array CGH

Gene identification

Traditional chromosome analysis has led to the identification of disease genes after one or more cases with a specific pathology were found to have the same chromosome translocation-breakpoint or deletion. Since molecular karyotyping now enables the rapid detection of small chromosomal imbalances, gene identification has dramatically increased. Dosage-sensitive genes are now detected on screening numerous patients with a specific pathology and the detection of a patient with a microdeletion/duplication locates the region of the genes involved in the pathology. Using this method, for example, the *CHD7* gene responsible for autosomal dominantly inherited (with many de novo cases) CHARGE syndrome was identified (reviewed in [29]). Likewise, the *B3GALTL* gene was found to be mutated in patients with the autosomal recessively inherited Peters Plus syndrome [9]. It is likely that the function of many more genes will be identified in this way.

Genotype–phenotype correlations

Array CGH is also used at the "full-tiling path" level, where the selected clones overlap, and so, the exact breakpoints of the aberrations can be determined. This is used in the known microdeletion/duplication syndromes which involve multiple genes. The purpose here is to correlate the various components of the phenotype with the loci/genes within the affected chromosomal region. For example, array CGH and FISH analysis permitted the delineation of the 2q32.2q33 syndrome in four patients, which were then compared to a further nine patients. All of these cases shared a minimal deleted chromosomal region and striking phenotypic similarities. As all patients had a cleft or high palate, it was speculated that hemizygosity of the *SATB2* gene within this region may be the underlying cause [24].

Challenges in interpretation

Copy number variation/polymorphisms

Numerous regions with non-pathogenic variations in the number of DNA copies (more or less than two copies) are scattered throughout the human genome. Using both array CGH and single nucleotide polymorphism (SNP) genotyping arrays on the 270 individuals of the HapMap collection from ancestry in Europe, Africa and Asia, 1,447 sub-microscopic copy variable regions in the human genome were found [14]. This involves at least 12% of the genome and includes hundreds of genes in deletions, duplications, insertions and complex multi-site variants. Interestingly, population-specific copy number variations have been detected, which needs to be considered when analysing the results of patients. This is facilitated by access to the Database of Genomic Variants (http://projects.tcag.ca/variation/) and also the results of the HapMap collection mentioned above [14], both of which are visualised in Ensembl (http://www.ensembl.org/).

In the clinical/diagnostic setting, the relevance of these copy number variations is challenging. The finding of an imbalance does not automatically indicate pathogenicity. At present, the following reasoning is followed in the analysis of array CGH results:

- If the imbalance is familial and not a known benign copy number variation, the phenotypic relationship is difficult to interpret
- If the aberration in a patient involves a known microdeletion/duplication syndrome, the imbalance is considered as pathogenic
- If the imbalance has occurred de novo in the patient, and especially if it contains genes with effects compatible with the clinical findings of the patient, this is in support of its pathogenicity but is not absolute proof

In conclusion, the pathogenicity of a chromosomal imbalance in a patient needs to be proved in order to be of use in the management of the patient and in counselling the family as to the implications [2]. To this end, the collaborative efforts through international databases such as Decipher and Ecaruca will, hopefully, with time, permit the detection of similar cases and the determination of the pathogenicity of the individual aberrations.

Thus, when investigating a patient using molecular karyotyping, investigation of the parents and additional family members may often be necessary in order to interpret the results. Without the availability of DNA from parents, molecular karyotyping at the higher (100-kb) resolution is not possible, as the hundreds of polymorphisms may be difficult to interpret.

Concluding remarks

The introduction of molecular karyotyping has doubled the detection rate of chromosomal imbalances in patients with

mental retardation and multiple congenital anomalies or dysmorphism and is, therefore, rapidly being introduced as a routine diagnostic technique in genetic diagnostic centres. It is, however, important to carefully select patients to undergo this investigation, as mutations in single-gene disorders will not be detected. Also, the technology is advancing the gene detection rate at a faster pace. The understanding of copy number variations in the human genome is now better understood than ever and its implications in diagnosis and the implications in genetic counselling are being rapidly uncovered. This is challenging the sector into re-thinking the indications for traditional chromosome analysis as opposed to molecular karyotyping.

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