Genetics and Molecular Biology, 37, 1 (suppl), 194-209 (2014)

www.sbg.org.br

Review Article

Human molecular cytogenetics: From cells to nucleotides

Mariluce Riegel[1,2]

[1]*Serviço de Genética Médica, Hospital de Clínicas, Porto Alegre, RS, Brazil.*
[2]*Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.*

Abstract

The field of cytogenetics has focused on studying the number, structure, function and origin of chromosomal abnormalities and the evolution of chromosomes. The development of fluorescent molecules that either directly or via an intermediate molecule bind to DNA has led to the development of fluorescent *in situ* hybridization (FISH), a technology linking cytogenetics to molecular genetics. This technique has a wide range of applications that increased the dimension of chromosome analysis. The field of cytogenetics is particularly important for medical diagnostics and research as well as for gene ordering and mapping. Furthermore, the increased application of molecular biology techniques, such as array-based technologies, has led to improved resolution, extending the recognized range of microdeletion/microduplication syndromes and genomic disorders. In adopting these newly expanded methods, cytogeneticists have used a range of technologies to study the association between visible chromosome rearrangements and defects at the single nucleotide level. Overall, molecular cytogenetic techniques offer a remarkable number of potential applications, ranging from physical mapping to clinical and evolutionary studies, making a powerful and informative complement to other molecular and genomic approaches. This manuscript does not present a detailed history of the development of molecular cytogenetics; however, references to historical reviews and experiments have been provided whenever possible. Herein, the basic principles of molecular cytogenetics, the technologies used to identify chromosomal rearrangements and copy number changes, and the applications for cytogenetics in biomedical diagnosis and research are presented and discussed.

Keywords: molecular cytogenetics, FISH, array-CGH, copy number variation, genomic disorders.

Introduction

Arnold (1879), Flemming (1882) and Hansemann (1890) reported the first microscopic observations of human mitotic chromosomes in the late 1800s. However, decades passed before the precise modal chromosome number in humans was determined. Until Eagle developed specific culture media in 1955, the cytogenetic analysis of chromosomes depended on spontaneously dividing cells. Tjio and Levan (1956), using cultured embryonic cells, were the first researchers to report the correct number of human chromosomes as 46. Moorhead *et al.* (1960) established an in vitro culture method for the accumulation of dividing cells using colchicine to arrest cells at metaphase. In the same year, Nowell (1960) discovered the mitogenic property of phytohemagglutinin, resulting in further technical improvements, particularly the use of peripheral blood cells. Both events significantly increased the number of metaphase spreads available for chromosome analysis. Steele and Breg Jr (1966) succeeded in culturing amniotic fluid cells and karyotyping fetal chromosomes. In the 1970s, an in vitro culture technique for chorionic villi was developed (Hahnemann, 1974), and Niazi *et al.* (1981) and Brambati and Simoni (1983) improved this culture technique several years later. Cytogenetics in hematology and oncology initially used peripheral blood as a specimen due to technical difficulties in processing and culturing solid tumor tissue. Because the development of newer techniques and more adequate methods has continued to increase the resolution of chromosomes, human cytogenetics has evolved from a more basic science into a valuable strategy for diagnosing prenatal, postnatal and acquired chromosomal abnormalities. The introduction and successful application of a variety of chromosome-staining techniques in previous years and molecular cytogenetic methods in recent years has tremendously improved the number of chromosomal abnormalities described. Since the first observation of an extra copy of chromosome 21 (Lejeune *et al.*, 1959) in patients with Down syndrome, many more chromosomal abnormalities, such as other trisomies, translocations, inversions, insertions, deletions, duplications and

Send correspondence to Mariluce Riegel. Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos 2350, 90035-003 Porto Alegre, RS, Brazil. E-mail: mriegel@hcpa.ufrgs.br.

complex chromosome rearrangements, have been described. Novel methods for investigating the mechanisms underlying copy number changes, characterizing gene interactions and analyzing genes within copy number variations (CNVs) are now being explored. Because the majority of techniques have been developed to study human genomes, man has been by far the most extensively studied organism in cytogenetics. An overview of the first years of human cytogenetics and descriptions of classical and molecular cytogenetic techniques applied to the study of chromosomal abnormalities and evaluate copy number changes are discussed in more detail below.

The Beginning of Human Cytogenetics

Human cytogenetics research began in 1879 with the observations of the German pathologist Arnold, who examined carcinoma and sarcoma cells because the voluminous nuclei of these cells facilitated analysis. Later, Flemming and Hansemann were the first to examine human mitotic chromosomes. In the late 19th century Waldeyer (1888) proposed the word "chromosome", which means, "colored body" (from the Greek *chroma* = color and *soma* = body). The use of colchicine for chromosome preparations was first implemented in plant cytogenetics in the 1930s (Blakeslee and Avery, 1937; Levan, 1938). This substance acts as a poison that inhibits spindle formation during mitosis, increasing the number of metaphase spreads available for analysis in a preparation. The treatment of cells with a hypotonic solution facilitated better chromosome spreading, leading to better definition for counting the chromosomes. Previous studies have shown that unspread and tangled chromosomes make it difficult to count the number of mammalian chromosomes in a preparation (Matthey, 1949). An improved hypotonic treatment technique (hypotonic shock) was then applied to examine lung fibroblasts in human embryos, thereby establishing the correct modal number of 46 chromosomes in human diploid cells (Tjio and Levan, 1956). In decades prior to this discovery, a human chromosome number of 48 had been described in a number of reports (see Gartler, 2006). This number was based on an examination of chromosome preparations of human spermatogonia, which suggested that humans had 48 chromosomes (Painter, 1923).

Although only a few chromosome details were known during the pre-banding era, the chromosomes themselves could be arranged in different groups based on their sizes and centromere positions. Following the determination of the correct modal chromosome number, the identification of the first inherited chromosomal abnormality (aneuploidy) leading to human diseases in man was identified. Lejeune *et al.* (1959) reported trisomy 21 in Down syndrome patients. Subsequently, the chromosomal abnormalities causing Klinefelter (47, XXY) and Turner (45, X) syndromes were identified (Ford *et al.*, 1959; Jacobs and Strong, 1959). During the same period, the first acquired chromosome anomaly (Philadelphia chromosome) was described in patients with chronic myeloid leukemia (Nowell, 1960). Subsequent technical improvements in cytogenetics included the use of phytohemagglutinin (a substance that stimulates the division of T lymphocytes in vitro) and the introduction of banding techniques at the end of the 1960s. Banding techniques use chemical treatments to produce differentially stained regions on chromosomes. The banding pattern is highly characteristic for each chromosome and facilitates the complete identification of the human karyotype.

Chromosome Banding Techniques

With the possibility of more specific identification and detailed analyses of human chromosomes, a new phase in cytogenetics began. The first method for the visualization of a pattern of bands on human chromosomes was Q-banding (Caspersson *et al.*, 1968). Subsequently, G-banding (Seabright 1971), a technique based on the application of trypsin (a proteolytic enzyme) using Giemsa staining, was developed, and this method is still the most widespread cytogenetic method routinely used in clinical settings. Classical cytogenetics became a traditional powerful diagnostic tool for detecting genomic aberrations, including both gains and losses of segments of the genome and rearrangements within and between chromosomes. However, the resolution of standard cytogenetics techniques remained limited, with a count of approximately 400-500 bands per haploid genome (Figure 1). The approaches described above facilitated the identification of structural chromosomal aberrations of at least 5-10 Mb in size. The average resolution depends on different elements, such as the optical characteristics of the microscope, the complex manner in which the DNA is packaged into chromosomes and the quality of the metaphase preparations. The resolution of the standard karyotype was improved after the introduction of high-resolution banding based on the use of synchronized lymphocyte cultures (Yunis, 1976). Using this technique, it was possible to increase the number of cells in the pro-metaphase or prophase stages. Detailed principles, protocols and potential applications for these cytogenetic banding techniques have been summarized elsewhere (Wegner, 1999).

Fluorescence *in situ* Hybridization (FISH) and Multiple Advances

The considerable gap between the limited resolution for observing chromosome structure through banding techniques (> 5 Mb, depending on the banding resolution applied) at the light microscopy and gene levels was bridged after the introduction and application of several molecular cytogenetic approaches. The first applications of molecular techniques to chromosome slide preparations, called in situ hybridization (ISH), were attempts to identify and locate

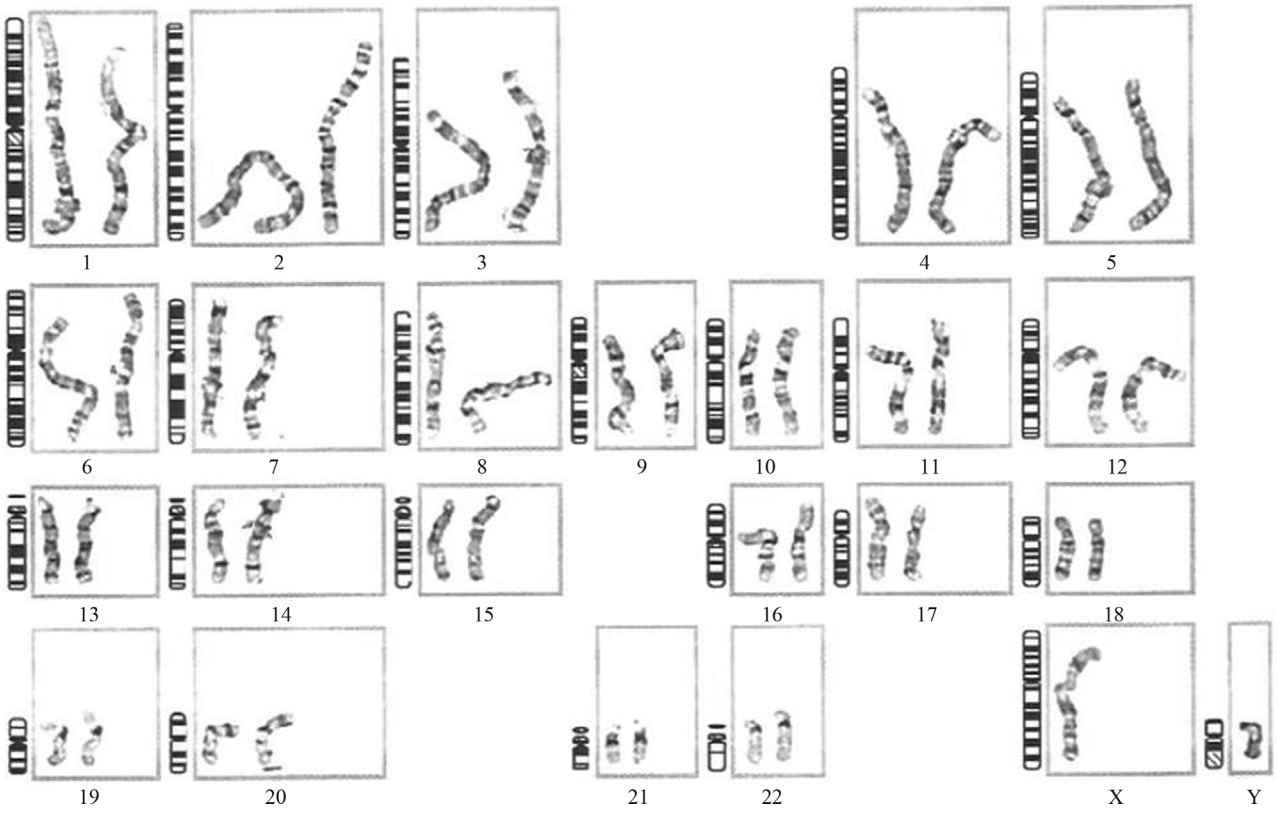

Figure 1 - Human Karyotype. GTG-banded male patient with a normal metaphase spread with approximately 550 bands.

specific nucleic acid sequences inside cells or on chromosomes (Gall and Pardue, 1969; John *et al.*, 1969). The ISH technique was based on the discovery that radioactively labeled ribosomal RNA hybridized to acrocentric chromosomes. The hybridization was visualized using autoradiography, which had been applied to human chromosomes since the early 1960s (German and Bearn, 1961). The use of ISH technology provided another dimension to the study of chromosomes, facilitating the visualization of DNA or complementary RNA sequences on chromosomes and in cells at the molecular level. However, the use of this method was limited due to the use of radioactive isotopes, highly repetitive DNA sequences and corresponding RNA in the satellite regions of chromosomes and centromeres (Pardue and Gall, 1970).

Subsequently, Langer *et al.* (1981) improved ISH with the development of a technique involving the use of a nonradioactive probe (such as biotin) for indirect labeling through nick translation. The hybridization (DNA probe and target sequence) could be visualized through avidin or streptavidin fluorescent labeling. The development of fluorescent molecules led to direct (combined with a fluorochrome) or indirect (through an intermediate molecule incorporated into a probe) binding to DNA bases, which eventually evolved into fluorescence in situ hybridization (FISH). FISH increased the resolution at which chromosome rearrangements could be identified at submicroscopic levels, making this technique applicable for both clinical diagnosis and research. FISH has been a driving force in the further development of cytogenetic techniques. The basic principle of FISH is that a target DNA in cells, nuclei or metaphase chromosomes is fixed and denatured on the surface of the slide. The probe DNA must be labeled with a nucleotide that is either conjugated to fluorescein (direct labeling) and/or a non-fluorescent hapten (indirect labeling), and the probe is first denatured and pre-hybridized with unlabeled repetitive DNA. Before hybridization, the metaphase chromosome suspension and/or interphase nuclei are enzymatically pretreated to enhance accessibility to the probe and reduce the amount of cytoplasm. The pretreated slide containing the target and probe DNA is heated to denature the DNA. The prepared probe is subsequently applied to the slide for ~16-48 h at 37°C for hybridization. The speed of the hybridization between the probe and the target DNA varies depending on the probe used. Post-hybridization washes remove unbound single-strand DNA and non-specifically bound DNA from the slide. When a non-fluorescent hapten is used (*e.g.*, biotin or digoxigenin), the detection occurs through a fluorescence-coupled anti-hapten. After washing, an anti-fade solution containing DAPI (4', 6-diamidino-2-phenylindole) is applied to the slide, and a coverslip must be added. DAPI is a fluorescent stain used extensively in fluorescence microscopy. FISH signals are typically observed using epifluorescence micro-

scopes with specific filters for identifying fluorochromes (Marcus, 1988; Reichman, 2000)), a charge-coupled device (CCD) camera captures the image and the fluorescent signals are subsequently quantified (Hiraoka *et al.*, 1987). The resulting images can be analyzed using commercially available systems.

Together with the development of standard FISH (Pinkel *et al.*, 1986a,b), more sensitive FISH-based techniques were gradually developed, and several digital imaging systems were introduced for FISH image acquisition, image pre-processing and digital image analysis. FISH provides the option for the simultaneous use of one or more DNA probes, and these probes can be distinguished after labeling with different colors or color combinations. The probes primarily determine the resolution of these molecular cytogenetic techniques and can be classified according to the pattern of detected DNA sequences. Many types of probes can be used for FISH (Figure 2). Currently, a range of commercial probes (*e.g.*, whole-chromosome painting probes, chromosome-arm painting probes, and repetitive centromeric, subtelomeric and locus-specific probes) is available for the detection of certain constitutional and acquired chromosomal abnormalities. Nevertheless, FISH probes can be generated through chromosome flow sorting (Pinkel *et al.*, 1988) or microdissection (Meltzer *et al.*, 1992) using universal degenerate oligonucleotide-primed PCR (DOP-PCR) (Telenius *et al.*, 1992).

FISH is a flexible technique that has driven the further development of other cytogenetic techniques. There are multiple approaches using FISH-based methods for different applications, *e.g.*, reverse-FISH (Carter *et al.*, 1992), fiber-FISH (Florijn *et al.*, 1995; Heiskanen *et al.*, 1995), (M-FISH multicolor FISH) (Speicher *et al.*, 1996), SKY (spectral karyotyping FISH) (Schröck *et al.*, 1996), flow-FISH (Rufer *et al.*, 1998), Q-FISH (quantitative FISH) (Martens *et al.*, 1998), COBRA-FISH (combined binary ratio labeling FISH) (Tanke *et al.*, 1999), cenM-FISH (centromere-specific M-FISH) (Nietzel *et al.*, 2001), pod-

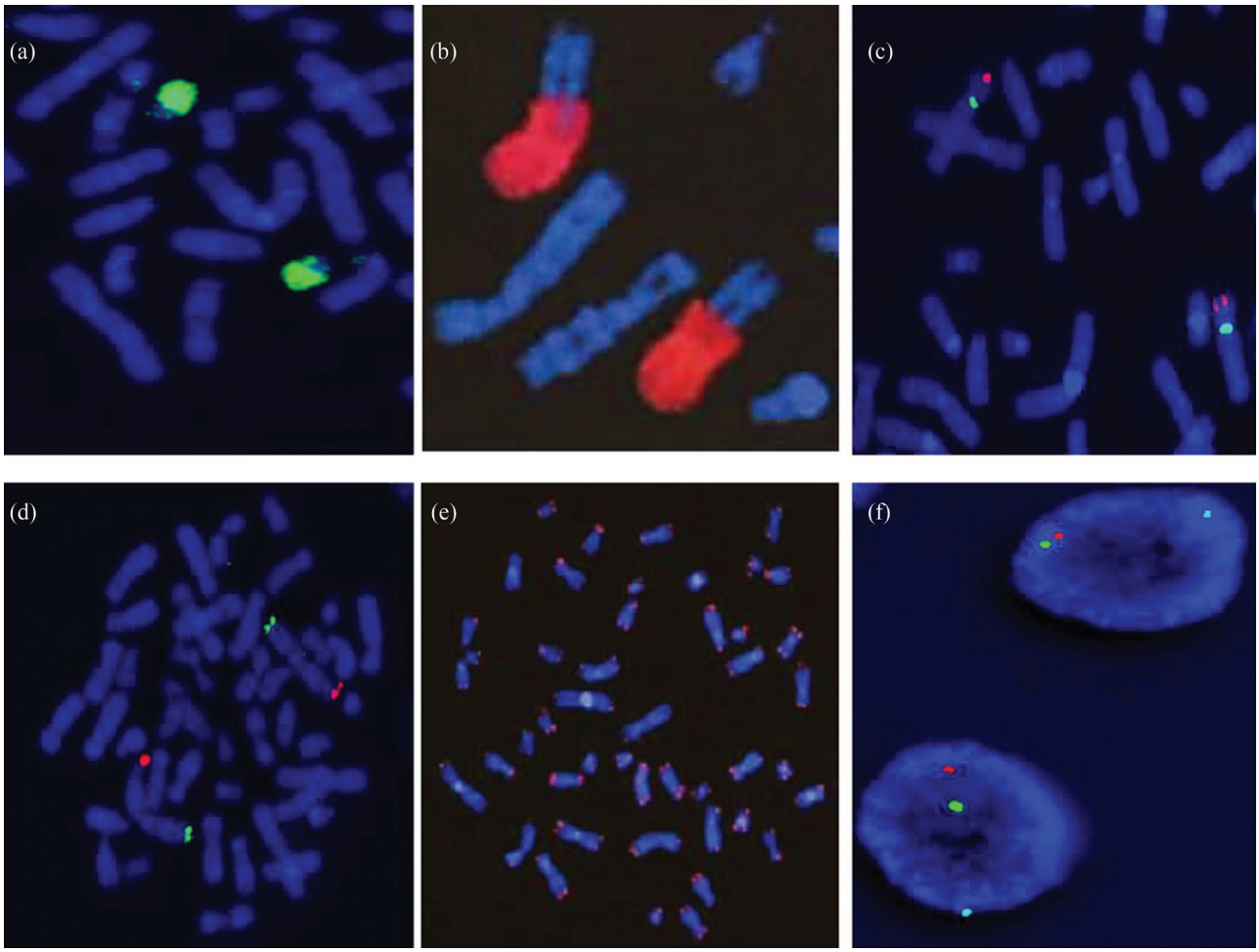

Figure 2 - FISH with different types of probes and partial metaphases. (a) Whole chromosome 21 painting; (b) partial chromosome painting probe for the long arm of chromosome 9; (c) locus-specific probe for chromosome 4p16.3 (red) and Alfa satellite probe 4p11-q11(green); (d) subtelomeric probe for the short arm (red) and long arm (green) of chromosome 1; (e) human telomeric probes; and (f) Interphase-FISH with locus-specific SRY (sex-determining region Y) probe located in Yp11.31(red) and control probes for the X centromere (DXZ1) (blue) and for the heterochromatic block at Yq12 (green).

FISH (parental origin determination FISH) (Weise *et al.*, 2008), (heterochromatin-M-FISH) (Bucksch *et al.*, 2012) and other modified FISH approaches. If modified, several FISH techniques can also be applied to interphase cells (interphase FISH) (Vorsanova *et al.*, 2010), which confers the advantages of FISH for the visualization of DNA probes in interphase nuclei (Cremer *et al.*, 1986). The limitation of standard FISH, however, is that it is not possible to simultaneously detect all of the chromosomes in the entire genome.

COBRA-FISH, M-FISH, and SKY are the most advanced FISH-based approaches, and these approaches facilitate the simultaneous visualization and detection of all human and non-human chromosomes through color karyotyping. The simultaneous staining of each of the 24 human chromosomes with a different color involves the use of whole-chromosome painting (WCP) probes, and all three of these FISH techniques use similar probe sets. Four to seven different fluorescence dyes can be used to label the WCP probes, and the chromosomes are counterstained with DAPI. The required 24 color combinations can be achieved through combinatorial or ratio labeling. The most important aspect of these techniques is the acquisition and measurement of the complete emission spectra between 400 and 800 nm, rendering a unique image that contains specific spectral information for each image point. The resulting chromosome classification is performed automatically using commercial software, and the DAPI image is also used to complement the analysis with chromosome banding information (Schröck *et al.*, 2006). A high-resolution molecular cytogenetic technique for the analysis of metaphase chromosomes, called multicolor banding (MCB), has been proposed, which involves the microdissection of chromosomal loci to obtain a set of probes that produce multicolor pseudo-G-banding (Liehr *et al.*, 2002).

For either standard or advanced FISH methods, the preparations should be analyzed using a well maintained and calibrated fluorescence microscope equipped with the optical filter sets appropriate for the fluorochromes used and an image-recording system. The development of numerous FISH protocols and multiple approaches is the result of the efforts of many diagnostic and research scientists from different research groups worldwide. These techniques have been continuously improved, and it is not possible to cover every modification of FISH in this manuscript. Detailed FISH protocols and applications are described elsewhere (Liehr, 2009).

Comparative Genomic Hybridization (CGH) and Array-based CGH

The comparative genomic hybridization (CGH) technique is an efficient approach to genome-wide screening for chromosomal copy number changes (gains/duplications and losses/deletions) within a single experiment, and this technique was initially introduced to study chromosomal abnormalities that occur in solid tumors and other malignancies (Kallioniemi *et al.*, 1992). Chromosomal CGH is based on quantitative two-color FISH and overcomes the problems of tissue culture failure and artifacts because this method is based on using tumor DNA extracted directly from either fresh or archival tumor tissue (Kallioniemi, 2008). The major advantage of CGH over standard FISH techniques is that only the DNA from the tumor cells is needed for analysis, avoiding the difficulties of obtaining metaphase chromosomes with good morphology and resolution for the analysis. In CGH, total genomic DNA obtained from control cells and test samples is differentially labeled using green (fluorescein isothiocyanate, FITC) and red (Texas red) fluorescent dyes, denatured, co-precipitated in the presence of blocking DNA to suppress repetitive sequences and subsequently co-hybridized to normal metaphase chromosomes. Due to the simultaneous hybridization to normal denatured metaphase chromosome spreads, there is competition for DNA hybridization to homologous sites. After hybridization and washing, the metaphase spreads are observed under a fluorescent microscope, and image analysis is performed using image analysis software. The resulting fluorescence intensities of the test and reference hybridizations are digitally quantified along the length of each chromosome. Chromosomal regions equally represented in both the test and reference samples appear yellow because of the presence of an identical amount of red and green dye, while regions with copy number loss are red and have a ratio below one (Figure 3a).

Although chromosomal CGH has increased the potential for identifying new chromosomal abnormalities, this technique is time consuming and does not significantly improve resolution (> 3 Mb) compared with routine G-banding chromosome analysis. More recently, the development of array-based CGH (array-CGH) approaches involving the substitution of metaphase chromosomes with DNA sequences adhered onto glass slides has increased the resolution for detecting copy number changes in the human genome, leading to more detailed information on genomic gains and losses (Figure 3b). Among all of the recent advances in techniques for examining chromosomes, array-CGH technology has been suggested as a technique that will gradually replace classical cytogenetics in clinical diagnosis. The fundamental principle of array-CGH is essentially the same as that in CGH. Indeed, the process involves comparative genomic hybridization using an array rather than a metaphase spread as the substrate (Solinas-Toldo *et al.*, 1997; Pinkel *et al.*, 1998).

The actual microarray comprises thousands of spots of reference DNA sequences applied in a precisely gridded manner on the slide. The initial arrayed DNA segments could be larger (~150 kb) human DNA segments inserted into a bacterial artificial chromosome (BAC clones) or bacterial/P1-derived artificial chromosomes (PAC clones)

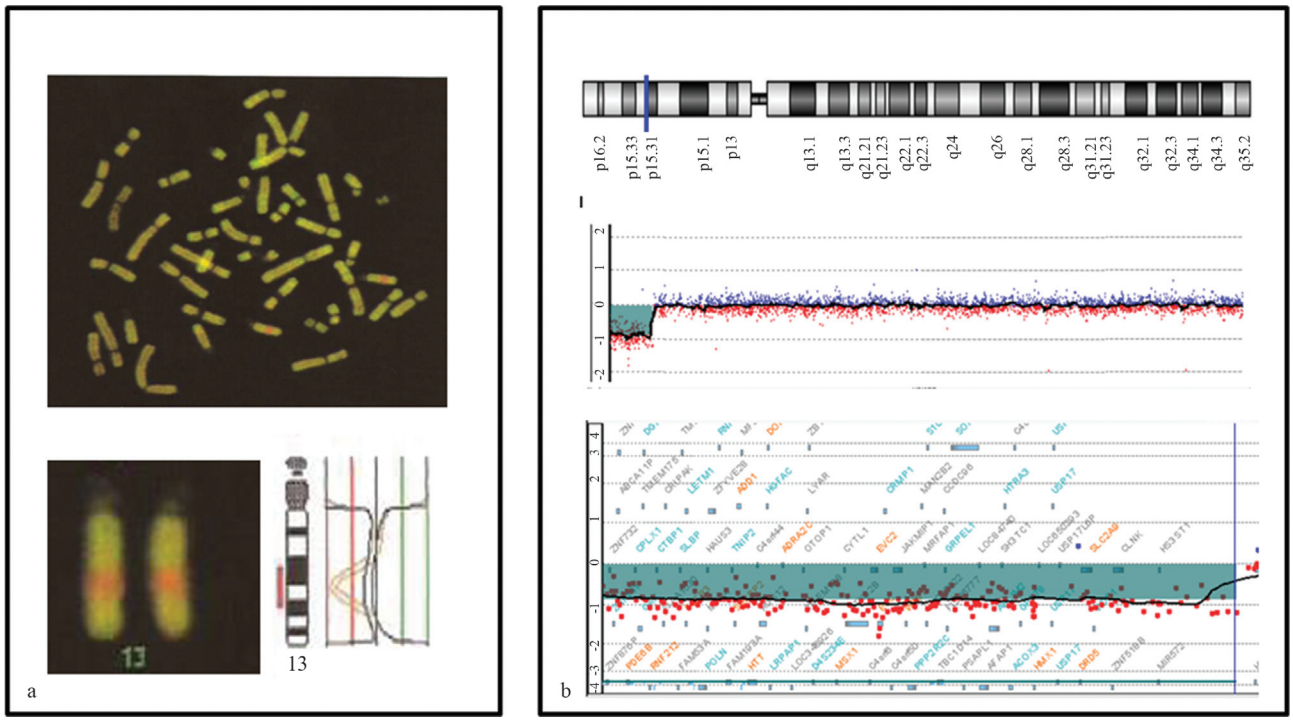

Figure 3 - Comparative Genomic Hybridization. (A) Conventional CGH analysis: a mixture of test DNA from a patient and a normal reference DNA labeled with different fluorochromes are hybridized to normal chromosome spreads (*top panel*). The left panel illustrates the hybridization pattern of chromosome 13. The interstitial segment of q-arm appears red, which indicates a loss of the region indicating rev ish dim (13q21q31). The right panel shows a graph of the ratio profiles of chromosome 13. The black line represents the balanced fluorescence intensities, and the red line is the threshold for loss, and the green line is the threshold for a gain of material. (B) Chromosome 4 array-CGH profile of a test DNA and a reference DNA. The figure shows a copy number loss corresponding to the segment of 4p16.3-p15.33 in a genomic segment with the median log2 ratio shifted to -1.0. The lower panel shows the 4p16.3-p15.33 region with the deletion segment and the genes present in this region.

(Snijders *et al.*, 2001; Fiegler *et al.*, 2003; Chung *et al.*, 2004; Ishkanian *et al.*, 2004). As the resolution of the array yields improves, shorter sequences have been used as targets, including smaller cDNA fragments (Pollack *et al.*, 1999), PCR products (Mantripragada *et al.*, 2004) and oligonucleotides (Rouillard *et al.*, 2002). Furthermore, array-CGH provides resolution at the nucleotide level. Single-nucleotide polymorphism arrays (SNP arrays) have the highest resolution (5-10 kb) of all of the available array-based platforms (see Le Scouarnec and Gribble, 2012). The co-hybridization of the test and reference DNAs is not required because the test DNA can hybridize directly to the SNP array. In addition to CNVs, the genotype information obtained from SNP arrays enables the detection of stretches of homozygosity and thus the identification of recessive disease genes, mosaic aneuploidy or uniparental disomy (UPD) (de Leeuw *et al.*, 2012). While only SNP arrays enable the detection of copy number-neutral regions in the absence of heterozygosity (AOH), these arrays have limited ability to detect single-exon copy CNVs due to the distribution of SNPs across the genome. Combining both array-CGH and SNP genotyping in a single platform optimizes the clinical diagnostic capability, offering the simultaneous detection of copy number neutral and small intragenic copy number changes (Wiszniewska *et al.*, 2014).

The number, size and distribution of the DNA segments on the glass slide determine the array resolution, but commonly, the higher the number of DNA fragments, the higher the resolution. According to Balliff *et al.* (2006) and Cheung *et al.* (2007), array-CGH also has increased the sensitivity for detecting cell lines with chromosomal abnormalities in peripheral blood, as chromosomal abnormalities are typically detected in only 5-7% of cells. Currently, there are several different commercially available diagnostic DNA microarray platforms comparing thousands of DNA sequences from a patient sample with reference (control) DNA samples or control datasets to detect chromosomal CNVs. A common limitation of SNP and CGH arrays is the inability to identify balanced translocations and inversions.

Recently, a modified array protocol, called translocation CGH (tCGH), was developed to address recurrent translocation breakpoints in hematological neoplasms. Prior to the hybridization step in the array procedure, a linear PCR amplification is performed across the known recurrent translocation breakpoints in hematological neoplasms. Thus, it is possible to detect copy number changes and known recurrent translocations near or at the breakpoints (Greisman *et al.*, 2011). Custom-made commercial arrays that use general standard protocols can also be or-

dered. Detailed information on the protocols and references is available elsewhere (Banerjee and Shah, 2013).

FISH Applications in Pre- and Postnatal Diagnostics and Research

Several decades ago, molecular methods were introduced into cytogenetic studies, facilitating the development of new applications, many of which were used diagnostically or as prognostic tools in medicine. Furthermore, molecular cytogenetic approaches have also become indispensable for a range of research purposes. The use of molecular techniques in cytogenetic studies is increasing, and the many variations, adaptations and specifications make it challenging to cover all of the possible applications. Since the introduction of FISH in the late 1980s, there has been a tremendous increase in the number of studies using molecular approaches in cytogenetics to detect chromosomal abnormalities and evaluate CNVs in the human genome. FISH offers numerous possibilities for studying either the whole genome or specific genomic loci (regions), and this technique has been widely used to detect aneuploidies and recurrent chromosomal abnormalities in preimplantation genetic, prenatal, and postnatal diagnoses and cancer cytogenetics. Moreover, the application of FISH has long been demonstrated as extremely valuable for studying chromosomal and genome organization, evolution and variations in health and disease (see Geurts and de Jong 2013; McNamara *et al.*, 2014; Pita *et al.*, 2014).

A significant advantage of FISH is that it can be applied in non-dividing cells, thereby facilitating the direct investigation of chromosomes in cytological preparations and tissue sections. Classical cytogenetic analysis depends on cells undergoing mitosis to obtain metaphase chromosome spreads. Therefore, cells must be cultured in vitro either as a short- or long-term culture. Thus, interphase FISH on uncultured amnion cells has become a useful method for the rapid and early diagnosis of the most common chromosome disorders (trisomies 21, 13, 18 and sex chromosome aneuploidies) in fetal cells (Eiben *et al.*, 1998). For prenatal aneuploidy screening using uncultured amniocytes, no time-consuming cell culture is required, and the results can be obtained within 24-48 hours. Three satellite centromeric probes for chromosomes X, Y and 18 and two locus-specific probes for the 13q14 and 21q22.13 regions are the most commonly applied. Interphase FISH in prenatal diagnosis is a quick, accurate, sensitive and relatively specific method to detect aneuploidies in samples of uncultured chorionic villus (Rosner *et al.*, 2013) and amniotic fluid cells (Stumm *et al.*, 2006).

Using site-specific DNA probes (YACs, BACs, PACs, and cosmids), FISH is typically applied for mapping chromosomal regions with located breakpoints (Liehr, 2009). In addition, using locus-specific probes, FISH has also been used to confirm clinical diagnoses of known microdeletion and microduplication syndromes (Riegel and coworkers, unpublished data). However, FISH has limitations in the detection of known microdeletion syndromes. Occasionally, patients with small and unusual deletions might escape detection, depending on the specificity of the fluorescent probe. Moreover, cases with gene or imprinting mutations, occurring in some microdeletion syndromes, *e.g.*, Angelman syndrome (AS), Prader-Willi syndrome (PWS), Sotos syndrome (SoS), Miller-Diecker syndrome (MDS), Smith-Magenis syndrome (SMS) and Rubinstein-Taybi syndrome (RTS), cannot be detected through FISH. The analysis of telomeres using FISH techniques has been conducted in cancer and aging research (telomere biology); however, due to the lack of specificity of the DNA probes (TTAGGG repetitive sequence motifs), this technique is poorly applicable for diagnosis (Aubert and Lansdorp, 2008). Multicolor FISH approaches have been most valuable for cancer cytogenetics, but these methods have also been applied to diagnose constitutional chromosomal abnormalities (Liehr *et al.*, 2004) and define translocations and marker chromosomes in complex karyotypes (Kearney, 2006).

Applications of CGH Analysis

Although CGH has primarily been applied to study solid tumors, this technique has also used to study leukemia and lymphoma (Kallioniemi *et al.*, 1992; Forozan *et al.*, 1997; Gebhart, 2004; Carless, 2009). However, given that CNVs are associated with many conditions, ranging from cancer to developmental abnormalities, CGH has also been applied to identify constitutional chromosomal abnormalities in clinical samples (Daniely *et al.*, 1998; Lestou *et al.*, 1999; Kirchhoff *et al.*, 2001; Ness *et al.*, 2002; Schou *et al.*, 2009). Several reports have demonstrated the use of either standard CGH or array-CGH to detect chromosomal abnormalities in single cells of pre-implantation embryos (Wells and Delhanty, 2000; Le Caignec *et al.*, 2006; Harton *et al.*, 2013).

Array-CGH was initially applied to identify chromosomal imbalances through the detection of CNVs in tumors to distinguish candidate genes involved in the pathogenesis of cancer (Cai *et al.*, 2002; Albertson and Pinkel, 2003). In clinical diagnostics, both oligonucleotide array-CGH and SNP genotyping have been demonstrated as powerful genomic technologies for evaluating idiopathic mental retardation (MR) (also referred to as developmental delay (DD), intellectual disability (ID) or learning difficulty), associated congenital abnormalities (MCA), autistic spectrum disorders (ASDs), schizophrenia and other neuropsychiatric disorders. Furthermore, the introduction of genome-wide array platforms facilitated the detection of chromosomal abnormalities consistent with genetic syndromes at earlier ages, when only a few clinical findings might be present.

CNVs are DNA segments that present a variable copy number compared with a reference genome, which has the typical copy number of N = 2 (Feuk *et al.*, 2006). In 2004, two studies employing array-based platforms revealed that CNVs exist in many large DNA genomic segments between normal human individuals, suggesting that these variations are fairly common and might represent polymorphic variations and a significant source of genetic variation (Iafrate *et al.*, 2004; Sebat *et al.*, 2004). Furthermore, the examination of the genomic content of CNVs revealed that these genomic regions include many functional genes involved in the regulation of cell growth and metabolism (Iafrate, 2004), implicating CNVs in human traits, disease and evolution. Since that time, many additional studies using a multitude of different high-resolution genome-analysis platforms have advanced our knowledge regarding CNVs.

Since Vissers *et al.* (2003) published the first report on detecting constitutional submicroscopic imbalances using array-based techniques in a series of patients with ID/MCA, the results of many more array-based studies have been published. Array-based genome investigations have been demonstrated to detect pathogenic imbalances in approximately 14-18% of consecutive ID/MCA cases referred for analysis. The rate differences might reflect differences in the resolutions of the array platforms used, the criteria for patient selection and the interpretation of the clinical relevance of the CNVs detected. Most of these CNVs are deletions and duplications that arise de novo, either as unique or recurrent events (Hochstenbach *et al.*, 2011). The increasing number of laboratories worldwide applying array-based methods for the diagnosis of patients with multiple congenital abnormalities has increased the detection of human genomic imbalances and led to the identification of a number of diseases caused by chromosomal microdeletions and microduplications. In recent years, common and newer microdeletion and microduplication syndromes associated with a variety of phenotypes have been revisited (Schinzel *et al.*, 2013; Riegel and coworkers, unpublished data;) and recognized (Deak *et al.*, 2011; Rafati *et al.*, 2012; Vissers and Stankiewicz, 2012; Weise *et al.*, 2012; Shimizu *et al.*, 2013).

The use of array-CGH as a genetic test in selected sporadic ASD patients has shown that non-syndromic, de novo CNVs occur in ~7.5% of boys and ~12% of girls. De novo deletions CNVs in female patients tend to be larger than in male patients and contain a higher number of protein-coding genes (Sanders *et al.*, 2011). According to Hochstenbach *et al.* (2011), these findings suggest that women are more resistant than men to developing ASD and are less likely to be diagnosed with ASD or both. In syndromic ASD cases, the chance of finding a causal CNV is nearly 25%. Based on recurrent microdeletions and microduplications identifiable on array-based platforms, a contributing CNV can be expected in approximately 5% of patients with schizophrenia. This rate only considers the currently known CNVs. Thus, it is likely that many more unique CNVs with major effects exist, similarly to ASD. In a small fraction of patients with schizophrenia, the alleles with CNVs are likely the strongest factors contributing to the pathogenesis of the disease (Stefansson *et al.*, 2014).

Recently, Nicholl *et al.* (2014) reported the frequency of pathogenic chromosomal microdeletions and microduplications in a large group of referred patients with developmental delay (DD), intellectual disability (ID) or autism spectrum disorders (ASD), and these authors provided a genetic diagnostic service. The first tier testing was applied using a standardized oligo-array CGH platform. The following detection rates, excluding the CNVs of uncertain significance, were observed: DD (13.0%), ID (15.6%), ASD (2.3%), ASD with DD (8.2%), ASD with ID (12.7%) and unexplained epilepsy with DD, ID and ASD (10.9%). Greater diagnostic sensitivity reflects the routine application of array CGH, compared with previously used conventional cytogenetics; according to Nicholl *et al.* (2014), the greater diagnostic sensitivity outweighs the interpretative issues arising from the detection of CNVs of uncertain significance.

Microarray approaches are increasingly used in prenatal settings in pregnancies with ultrasound anomalies and pregnancies referred for other reasons. However, challenges in interpreting the results, quality control and ethical issues have delayed the use of microarray approaches in prenatal care compared with postnatal diagnoses (Rickman *et al.*, 2005; Vetro *et al.*, 2012). Numerous case series and case reports have since been published on the application of array-CGH in prenatal settings (Brady and Vermeesch, 2012; Brady *et al.*, 2013; Evangelidou *et al.*, 2013). Array-CGH increases the diagnostic yield for detecting additional genomic imbalances 1-5% compared with normal karyotyping, depending on the reference source (ACOG Committee, 2009; Hillman *et al.*, 2011; Lichtenbelt *et al.*, 2011).

In hematologic and oncologic disorders, the implementation of array-based chromosome analysis has been critical. The complexity of cancer cells requires a sensitive technique that facilitates the detection of small genomic changes in a mixed cell population and segmental regions of homozygosity. However, recurrent balanced genomic aberrations with important prognostic value in cancer might be not detected through array-based analyses. Because array-CGH is based on the principle of CNV detection, this technique is limited by an inability to identify balanced translocations and inversions. Nevertheless, arrays have been previously demonstrated as clinically essential for identifying novel genomic abnormalities that escape detection using current diagnostic methodologies in a number of hematological diseases, such as chronic lymphocytic leukemia (CLL), myelodysplastic syndrome (MDS), multiple myeloma (MM), acute lymphoblastic leukemia (ALL),

acute myeloid leukemia (AML) and chronic myelomonocytic leukemia (CMML) (Shao *et al.*, 2010; Simons *et al.*, 2012). Moreover, the identification and accurate genomic mapping of genomic alterations in hematological malignances in a preclinical stage have shown that it is possible to refine the current risk stratification of patients, and this technique might eventually contribute to the development of enhanced treatment modalities (van der Veken and Buijs, 2011; Simons *et al.*, 2012).

The detection of common and rare CNVs using array-based platforms has generated questions concerning the origin and molecular mechanisms leading to recurrent and non-recurrent CNVs (Lupski and Stankiewicz 2005; Currall *et al.*, 2013; Dittwald *et al.*, 2013; Sun *et al.*, 2013) and the phenotypic effects of CNVs and recurrence risks (Girirajan *et al.*, 2012; Priest *et al.*, 2012; Boone *et al.*, 2013). Recombination-based mechanisms, *i.e.*, non-allelic homologous recombination (NAHR), non-homologous end joining (NHEJ) (Lupski and Stankiewicz, 2005) and retrotransposition (Kazazian Jr and Moran, 1998; Xing *et al.*, 2009), have been implicated in genomic rearrangements and the formation of CNVs. A replication-based mechanism, fork stalling and template switching (FoSTeS) might account for the complex genomic rearrangements that cannot be readily explained through NAHR, NHEJ or retrotransposition (Lee *et al.*, 2007; Perry *et al.*, 2008; Arlt *et al.*, 2012). CNVs represent an important component of genetic variation and have been described as a major contributor to phenotype diversity and disease (Girirajan and Eichler, 2010; Arlt *et al.*, 2011; Cooper *et al.*, 2011; Girirajan *et al.*, 2011; Girirajan, 2013).

Interpretation of CNVs

The widespread use of array-CGH has revealed that a large proportion of the human genome contains regions of copy number variability, and distinguishing between pathogenic and benign gains and losses has been challenging. Although array-CGH technology has been well developed and there are numerous algorithms available for estimating copy number (McDonnell *et al.*, 2013), the resolution of the array platforms used in molecular cytogenetics and our understanding of the clinical effects of CNVs are still improving. Recurrent CNVs can occur in both patients and healthy individuals, and frequently, more than one unique CNV is identified in a patient. A given copy number change with a high penetrance pathogenic might reduce or aggravate the clinical phenotype in the presence of other CNVs/SNPs. For example, Girirajan *et al.* (2010) demonstrated that the 16p11.2 microdeletion predisposes individuals to neuropsychiatric phenotypes as a single event and aggravates neurodevelopmental phenotypes in association with other large deletions or duplications within the genome of an individual.

The large quantity of clinical and cytogenetic data available in open access databases can help decipher which combinations of variants lead to varying degrees of pathogenicity. Factors that influence the pathogenicity of CNVs and an evidence-based classification for the clinical interpretation of CNVs have been discussed and proposed (Lee *et al.*, 2007; Hehir-Kwa *et al.*, 2010; Miller *et al.*, 2010; Gijsbers *et al.*, 2011; de Leeuw *et al.*, 2012; Riggs *et al.*, 2012; Liehr, 2014). Online resources and public databases have been developed and are utilized by the scientific and biomedical community, which has been encouraged to submit cases to the databases to provide data on the test results (Vulto-van Silfhout *et al.*, 2013).

Common strategies have been proposed to help interpret CNV findings, and no universal criteria have been established thus far. Most laboratories classify the various CNVs into different categories using some or all of the CNV classifications: benign CNV or normal genomic variant; benign CNV; CNV with uncertain clinical relevance or variants of uncertain significance (VOUS); and CNV with potential clinical relevance or pathogenic variants. When array-CGH was initially used, all identified CNVs were generally reported. In recent years, the trend towards standardizing the reporting among laboratories worldwide, and the current tendency is to report only potentially meaningful CNVs. Nevertheless, the array platform used and the reporting criteria might vary between individual laboratories. Different laboratories might also use different methods to confirm the array findings (*e.g.*, FISH, multiplex ligation-dependent probe amplification (MLPA), Quantitative Fluorescence Polymerase Chain Reaction (QF-PCR), and a second array-CGH).

When interpreting and classifying CNVs, it is essential to distinguish gains from losses because the potential clinical consequences might significantly differ. Furthermore, it is essential to compare gains with gains and losses with losses (Vermeesch *et al.*, 2007; Conrad *et al.*, 2010; Vermeesch *et al.*, 2012). de Leeuw *et al.* (2012) summarized the characteristics of the most commonly used Internet databases and resources and proposed a general interpretation strategy that can be used for comparative hybridization, comparative intensity and genotype-based array data. Some of the available online databases associated with chromosome abnormalities and variants are listed below (as of January 2014):

Centre for the Development and Evaluation of Complex Interventions for Public Health Improvement (DECIPHER) project: http://decipher.sanger.ac.uk.

The Chromosome Anomaly Collection: http://www.ngrl.org.uk/wessex/collection/.

Chromosomal Variation in Man Online Database: http://www.wiley.com/legacy/products/subject/life/borgaonkar/access.html.

Cytogenetic Data Analysis System (CyDAS): http://www.cydas.org/.

Database of genomic structural variation (bdVar): http://www.ncbi.nlm.nih.gov/dbvar/.

Ensembl: www.ensembl.org/.

European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations (ECARUCA): www.ecaruc.net.

The International Standards for Cytogenomic Arrays (ISCA) Consortium:https://www.iscaconsortium.org/index.php.

Small supernumerary marker chromosomes: http://ssmc-tl.com/sSMC.html.

Final Remarks

The methods described herein provide information on the human genome at different levels of resolution and have shown potential for diagnostic and research purposes. The resolution for studying chromosomes has improved from > 5 Mb (metaphase) to 50 kb-2 Mb (interphase) and 5-500 kb (DNA fibers) and ultimately, to a single nucleotide. Molecular cytogenetics and array-based technologies facilitate higher resolutions through genome-wide screening for submicroscopic genomic CNVs. However, to identify cytogenetically visible CNVs (*e.g.*, heterochromatin), low mosaicisms and balanced translocations, banding cytogenetics has been demonstrated as useful. Cytogenetic testing in developed countries primarily uses array-CGH technology to detect novel or rare microdeletions/microduplications and has become the first-line test in the diagnostic investigation of individuals with MCAs, DDs or unexplained IDs. Although the use of banding and FISH has gradually been replaced by array-based technologies in several laboratories, G-banding remains the most commonly used approach worldwide to study the human genome. Moreover, the comparison of chromosome and array-based chromosome analyses has demonstrated that chromosome analysis remains valuable for detecting mosaicisms and to delineate chromosomal structural rearrangements (Bi *et al.*, 2013). Evaluating the use of conventional karyotypes or molecular approaches will likely require continuous evaluation, as questions regarding how to achieve cost-effective diagnoses still remain in many clinical situations, *e.g.*, rare chromosome breakage syndromes and low-risk pregnancies (van Ravenswaaij-Arts, personal communication 2013).

As the number of recognized genetic syndromes and chromosomal abnormalities grows and as the clinical characteristics of those syndromes overlap, it will be more difficult to precisely infer which syndrome affects an individual based only on the clinical examination. Currently, the detection of large numbers of CNVs using molecular cytogenetic approaches in patients and healthy individuals has been considered a diagnostic pitfall due to interpretation difficulties. Most chromosomal abnormalities have clinical effects; however, the number of instances in which genomic changes are benign has increased, as the resolution of chromosome analysis has also increased. In clinical diagnosis, both array-CGH and SNP genotyping have been demonstrated as powerful genomic technologies to evaluate DD, MCAs and neuropsychiatric disorders. Differences in the ability to detect genomic changes between these arrays might constitute a challenge for laboratory managers, as the request to provide the best approach to detect underlying genetic causes of diseases is increasing. In most cases, imbalances that are cytogenetically visible in size (several Mb) lead to severe clinical consequences and are responsible for specific syndromes or clinical features (Schinzel, 2001). However, CNVs can be expected in every individual on a chromosomal or molecular genetic level (1000 Genomes Project Consortium *et al.*, 2012). Thus, it is expected that the identification of variants of unknown clinical significance will significantly increase, particularly as many individuals now have their entire genomes sequenced (Bale *et al.*, 2011; Palmer *et al.*, 2014). Segmental chromosome regions that might be present in variable copy numbers in the genome without phenotypic consequences are constantly being identified (Barber, 2005; Liehr, 2012).

To date, the critical point has been to distinguish similar-looking benign imbalances from pathological imbalances. To facilitate the interpretation and analysis of the information obtained using molecular cytogenetic approaches, widely available public databases have been developed and are constantly updated (*e.g.*, CyDAS, DECIPHER, ECARUCA, ISCA). Nevertheless, many genomic imbalances are novel or extremely rare, making interpretation problematic and uncertain. Thus, further molecular cytogenetic screenings of large patient cohorts with common phenotypic features contribute to the ongoing development of genotype-phenotype correlations, identifying CNVs in dosage-sensitivity genes and defining their locations in the human genome. The use of whole-genome sequencing and whole-exome sequencing platforms has been increasingly popular and powerful for genetic diagnosis (Bick and Dimmock, 2011; Greisman *et al.*, 2013; Johansen Taber *et al.*, 2013; Rabbani *et al.*, 2014). These methods might potentially be alternatives to the use of microarrays in molecular cytogenetic laboratories. The technologies applied to study genomic imbalances have been rapidly changing. Therefore, the comprehensive collection, organization and maintenance of the raw genotype-phenotype data obtained through different approaches are major challenges.

The implementation and updating of national, regional and international guidelines on the indications and interpretations of molecular cytogenetics results along with clinical management to improve expertise and experience in clinical and laboratory praxis are necessary to improve scientific knowledge and medical care. In addition, the reporting of molecular cytogenetic results is also another important issue (ISCN. An International System for Human Cytogenetic Nomenclature, 2013). As new techniques are implemented in cytogenetic laboratories for clinical use, additional provisions for reporting findings should be de-

veloped though international guidelines. The number of chromosomal abnormalities and potential genomic rearrangements in the human genome are likely unlimited. In the last decade, the importance of both high-quality cytogenetics and genome sequencing for detecting and understanding the molecular mechanisms that lead to these chromosomal changes has been clear. Regardless of the development of next-generation molecular techniques for identifying chromosomal imbalances and CNVs in the human genome, the essential purpose of cytogenetics will remain the same: to study genomic organization and the structure, function and evolution of chromosomes.

Acknowledgments

The author would like to apologize to those colleagues whose contributions to the field have been unwittingly omitted and to the authors of relevant papers who could not be cited because of space limitations. Unpublished results were generally not included except as personally observed by the author.

References

1000 Genomes Project Consortium, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, Kang HM, Marth GT and McVean GA (2012) An integrated map of genetic variation from 1,092 human genomes. Nature 491:56-65.

ACOG Committee (2009) Opinion No. 446: Array comparative genomic hybridization in prenatal diagnosis. Obstet Gynecol 114:1161-1163.

Albertson DG and Pinkel D (2003) Genomic microarrays in human genetic disease and cancer. Hum Mol Genet 2 (Spec No 2):R145-R152.

Arlt MF, Ozdemir AC, Birkeland SR, Lyons Jr RH, Glover TW and Wilson TE (2011) Comparison of constitutional and replication stress-induced genome structural variation by SNP array and mate-pair sequencing. Genetics 187:675-83.

Arlt MF, Wilson TE and Glover TW (2012) Replication stress and mechanisms of CNV formation. Curr Opin Genet Dev 22:204-210.

Arnold J (1879) Beobachtungen über Kernteilungen in den Zellen der Geschwülste. Virchows Arch Pathol Anat 78:279.

Aubert G and Lansdorp PM (2008) Telomeres and aging. Physiol Ver 88:557-579.

Bale S, Devisscher M, Van Criekinge W, Rehm HL, Decouttere F, Nussbaum R, Dunnen JT and Willems P (2011) MutaDATABASE: A centralized and standardized DNA variation database. Nat Biotechnol 29:117-1188.

Balliff BC, Rorem EA, Sundin K, Linicium M, Gaskin S, Coppinger J, Kashork CD, Shaffer LG and Beijani BA (2006) Detection of low-level mosaicism by array CGH in routine diagnostic specimens. Am J Med Genet A 140A:2757-2767.

Banerjee D and Shah SP (2013) Methods in Molecular Biology 973: Array Comparative Genomic Hybridization (Protocols and Applications). Humana Press, New Jersey, 382 pp.

Barber JC (2005) Directly transmitted unbalanced chromosome abnormalities and euchromatic variants. J Med Genet 42:609-629.

Bi W, Borgan C, Pursley AN, Hixson P, Shaw CA, Bacino CA, Lalani SR, Patel A, Stankiewicz P, Lupski JR, *et al.* (2013) Comparison of chromosome analysis and chromosomal microarray analysis: What is the value of chromosome analysis in today's genomic array era? Genet Med 15:450-457.

Bick D and Dimmock D (2011) Whole exome and whole genome sequencing. Curr Opin Pediatr 23:594-600.

Blakeslee AF and Avery AG (1937) Methods of inducing doubling of chromosomes in plants. J Hered 28:392-411.

Boone PM, Campbell IM, Baggett BC, Soens ZT, Rao MM, Hixson PM, Patel A, Bi W, Cheung SW, Lalani SR, *et al.* (2013) Deletions of recessive disease genes: CNV contribution to carrier states and disease-causing alleles. Genome Res 23:1383-1394.

Brady PD and Vermeesch JR (2012) Genomic microarrays: A technology overview. Prenat Diag 32:336-343.

Brady PD, Delle Chiaie B, Christenhusz G, Dierickx K, Van Den Bogaert K, Menten B, Janssens S, Defoort P, Roets E, Sleurs E, *et al.* (2013) A prospective study of the clinical utility of prenatal chromosomal microarray analysis in fetuses with ultrasound abnormalities and an exploration of a framework for reporting unclassified variants and risk factors. Genet Med 2013 [Epub ahead of print].

Brambati B and Simoni G (1983) Diagnosis of fetal trisomy 21 in first trimester. Lancet 1:586.

Bucksch M, Ziegler M, Kosayakova N, Mulatinho MV, Llerena Jr JC, Morlot S, Fischer W, Polityko AD, Kulpanovich AI, Petersen MB, *et al.* (2012) A new multicolor fluorescence in situ hybridization probe set directed against human heterochromatin: HCM-FISH. J Histochem Cytochem 60:530-536.

Cai WW, Mao JH, Chow CW, Damani S, Balmain A and Bradley A (2002) Genome-wide detection of chromosomal imbalances in tumors using BAC microarrays. Nat Biotechnol 20:393-396.

Carless M (2009) Analysis of genomic aberrations using comparative genomic hybridization of metaphase chromosomes. Methods Mol Biol 523:177-202.

Carter NP, Ferguson-Smith MA, Perryman MT, Telenius H, Pelmear AH, Leversha MA, Glancy MT, Wood SL, Cook K, Dyson HM, *et al.* (1992) Reverse chromosome painting: A method for the rapid analysis of aberrant chromosomes in clinical cytogenetics. J Med Genet 29:299-307.

Caspersson T, Farber S, Foley GE, Kudynowski J, Modest EJ, Simonsson E, Wagh U and Zech L (1968) Chemical differentiation along metaphase chromosomes. Exp Cell Res 49:219-222.

Cheung SW, Shaw CA, Scott DA, Patel A, Sahoo T, Bacino CA, Pursley A, Li J, Erickson R, Gropman AL, *et al.* (2007) Microarray-based CGH detects chromosomal mosaicism not revealed by conventional cytogenetics. Am J Med Genet A 143:1679-1686.

Chung YJ, Jonkers J, Kitson H, Fiegler H, Humphray S, Scott C, Hunt S, Yu Y, Nishijima I, Velds A, *et al.* (2004) A whole-genome mouse BAC microarray with 1-Mb resolution for analysis of DNA copy number changes by array comparative genomic hybridization. Genome Res 14:188-196.

Conrad DF, Pinto D, Redon R, Feuk L, Gokcumen O, Zhang Y, Aerts J, Andrews TD, Barnes C, Campbell P, *et al.* (2010) Origins and functional impact of copy number variation in the human genome. Nature 464:704-712.

Cooper GM, Coe BP, Girirajan S, Rosenfeld JA, Vu TH, Baker C, Williams C, Stalker H, Hamid R, Hannig V, *et al.* (2011) A copy number variation morbidity map of developmental delay. Nat Genet 43:838-846.

Cremer T, Landegent J, Brückner A, Scholl HP, Schardin M, Hager HD, Devilee P, Pearson P and van der Ploeg M (1986) Detection of chromosome aberrations in the human interphase nucleus by visualization of specific target DNAs with radioactive and non-radioactive in situ hybridization techniques: Diagnosis of trisomy 18 with probe L1.84. Hum Genet 74:346-352.

Currall BB, Chiang C, Talkowski ME and Morton CC (2013) Mechanisms for structural variation in the human genome. Curr Genet Med Rep 1:81-90.

Daniely M, Aviram-Goldring A, Barkai G and Goldman B (1998) Detection of chromosomal aberration in fetuses arising from recurrent spontaneous abortion by comparative genomic hybridization. Hum Reprod 13:805-809.

de Leeuw N, Dijkhuizen T, Hehir-Kwa JY, Carter NP, Feuk L, Firth HV, Kuhn RM, Ledbetter DH, Martin CL, van Ravenswaaij-Arts CM, *et al.* (2012) Diagnostic interpretation of array data using public databases and internet sources. Hum Mutat [Epub ahead of print].

Deak KL, Horn SR and Rehder CW (2011) The evolving picture of microdeletion/microduplication syndromes in the age of microarray analysis: Variable expressivity and genomic complexity. Clin Lab Med 4:543-564.

Dittwald P, Gambin T, Szafranski P, Li J, Amato S, Divon MY, Rodríguez Rojas LX, Elton LE, Scott DA, Schaaf CP, *et al.* (2013) P NAHR-mediated copy-number variants in a clinical population: Mechanistic insights into both genomic disorders and Mendelizing traits. Genome Res 23:1395-1409.

Eagle H (1955) Nutrition Needs of Mammalian Cells in Tissue Culture. Science 122:501-504.

Eiben B, Hammans W, Goebel R and Epplen JT (1998) Ein neuer Schnelltest (FISH) zur pränatalen Diagnostik der häufigsten Chromosomenaberrationen - welche Bedeutung hat er für die Praxis? Dtsch Med Wochenschr 123:55-57.

Evangelidou P, Alexandrou A, Moutafi M, Ioannides M, Antoniou P, Koumbaris G, Kallikas I, Velissariou V, Sismani C and Patsalis PC (2013) Implementation of high resolution whole genome array CGH in the prenatal clinical setting: Advantages, challenges, and review of the literature. Biomed Res Int 2013:e346762.

Feuk L, Carson AR and Scherer SW (2006) Structural variation in the human genome. Nat Rev Genet 7:85-97.

Fiegler H, Gribble SM, Burford DC, Carr P, Prigmore E, Porter KM, Clegg S, Crolla JA, Dennis NR, Jacobs P, *et al.* (2003) Array painting: A method for the rapid analysis of aberrant chromosomes using DNA microarrays. J Med Genet 40:664-670.

Flemming W (1882) Beiträge zur Kenntnis der Zelle und ihrer Lebenserscheinungen III. Arch Mikrosk Anat 20:1.

Florijn RJ, Bonden LA, Vrolijk H, Wiegant J, Vaandrager JW, Baas F, den Dunnen JT, Tanke HJ, van Ommen GJ and Raap AK (1995) High-resolution DNA Fiber-FISH for genomic DNA mapping and colour bar-coding of large genes. Hum Mol Genet 4:831-836.

Ford CE, Miller OJ, Polani PE, de Almeida JC and Briggs JH (1959) A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). Lancet 1:711-713.

Forozan F, Karhu R, Kononen J, Kallioniemi A and Kallioniemi OP (1997) Genome screening by comparative genomic hybridization. Trends Genet 13:405-409.

Gall JG and Pardue ML (1969) Formation and detection of RNA-DNA hybrid molecules in cytological preparations. Proc Natl Acad Sci USA 63:378-383.

Gartler SM (2006) The chromosome number in humans: A brief history. Nat Rev Genet 7:655-660.

Gebhart E (2004) Comparative genomic hybridization (CGH): Ten years of substantial progress in human solid tumor molecular cytogenetics. Cytogenet Genome Res 104:352-358.

German JL and Bearn AG (1961) Asynchronous thymidine uptake by human chromosomes. J Clin Invest 40:1041-1042.

Geurts R and de Jong H (2013) Fluorescent In Situ Hybridization (FISH) on pachytene chromosomes as a tool for genome characterization. Methods Mol Biol 1069:15-24.

Gijsbers AC, Schoumans J and Ruivenkamp CA (2011) Interpretation of array comparative genome hybridization data: A major challenge. Cytogenet Genome Res 135:222-227.

Girirajan S (2013) Genomic disorders: Complexity at multiple levels. Genome Med 29:5-43.

Girirajan S and Eichler EE (2010) Phenotypic variability and genetic susceptibility to genomic disorders. Hum Mol Genet 19(R2):R176-R87.

Girirajan S, Rosenfeld JA, Cooper GM, Antonacci F, Siswara P, Itsara A, Vives L, Walsh T, McCarthy SE, Baker C, *et al.* (2010) A recurrent 16p12.1 microdeletion supports a two-hit model for severe developmental delay. Nat Genet 42:203-209.

Girirajan S, Campbell CD and Eichler EE (2011) Human copy number variation and complex genetic disease. Annu Rev Genet 45:203-226.

Girirajan S, Rosenfeld JA, Coe BP, Parikh S, Friedman N, Goldstein A, Filipink RA, McConnell JS, Angle B, Meschino WS, *et al.* (2012) Phenotypic heterogeneity of genomic disorders and rare copy-number variants. N Engl J Med 367:1321-1331.

Greisman HA, Hoffman NG and Yi HS (2011) Rapid high-resolution mapping of balanced chromosomal rearrangements on tiling CGH arrays. J Mol Diagn 13:621-633.

Greisman HA, Hoffman NG, Yi HS. Grody WW, Thompson BH and Hudgins L (2013) Whole-exome/genome sequencing and genomics. Pediatrics 132(Suppl 3):S211-S215.

Hahnemann N (1974) Early prenatal diagnosis: A study of biopsy techniques and cell culturing from extraembryonic membranes. Clin Genet 6:294-306.

Hansemann D (1890) Über asymmetrische Zellteilung in Epithelkrebsen und deren biologische Bedeutung. Arch Pathol Anat 119:299-326.

Harton GL, Munné S, Surrey M, Grifo J, Kaplan B, McCulloh DH, Griffin DK, Wells D and PGD Practitioners Group (2013) Diminished effect of maternal age on implantation after preimplantation genetic diagnosis with array comparative genomic hybridization. Fertil Steril 100:1695-1703.

Hehir-Kwa JY, Wieskamp N, Webber C, Pfundt R, Brunner HG, Gilissen C, de Vries BB, Ponting CP and Veltman JA (2010) Accurate distinction of pathogenic from benign CNVs in mental retardation. PLoS Comput Biol 6:e1000752.

Heiskanen M, Hellsten E, Kallioniemi OP, Mäkelä TP, Alitalo K, Peltonen L and Palotie A (1995) Visual mapping by fiber-FISH. Genomics 30:31-36.

Hillman SC, Pretlove S, Coomarasamy A, McMullan DJ, Davison EV, Maher ER and Kilby MD (2011) Additional information from array comparative genomic hybridization technology over conventional karyotyping in prenatal diagnosis: A systematic review and meta-analysis. Ultrasound Obstet Gynecol 37:6-14.

Hiraoka Y, Sedat JW and Agard DA (1987) The use of a charge-coupled device for quantitative optical microscopy of biological structures. Science 238:36-41.

Hochstenbach R, Buizer-Voskamp JE, Vorstman JA and Ophoff RA (2011) Genome arrays for the detection of copy number variations in idiopathic mental retardation, idiopathic generalized epilepsy and neuropsychiatric disorders: Lessons for diagnostic workflow and research. Cytogenet Genome Res 135:174-202.

Iafrate AJ, Feuk L, Rivera MN, Listewnik ML, Donahoe PK, Qi Y, Scherer SW and Lee C (2004) Detection of large-scale variation in the human genome. Nat Genet 36:949-51.

ISCN (2013) An International System for Human Cytogenetic Nomenclature. Karger, Basel, 140 pp.

Ishkanian AS, Malloff CA, Watson SK, DeLeeuw RJ, Chi B, Coe BP, Snijders A, Albertson DG, Pinkel D, Marra MA, *et al.* (2004) A tiling resolution DNA microarray with complete coverage of the human genome. Nat Genet 36:299-303.

Jacobs PA and Strong JA (1959) A case of human intersexuality having a possible XXY sex-determining mechanism. Nature 183:302-303.

Johansen Taber KA, Dickinson BD and Wilson M (2013) The promise and challenges of next-generation genome sequencing for clinical care. JAMA Intern Med 174:275-280.

John HA, Birnstiel ML and Jones KW (1969) RNA-DNA hybrids at the cytological level. Nature 223:582-587.

Kallioniemi A (2008) CGH microarrays and cancer. Curr Opin Biotechnol 19:36-40.

Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F and Pinkel D (1992) Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 258:818-821.

Kazazian Jr HH and Moran JV (1998) The impact of L1 retrotransposons on the human genome. Nat Genet 19:19-24.

Kearney L (2006) Multiplex-FISH (M-FISH): Technique, developments and applications. Cytogenet Genome Res 114:189-198.

Kirchhoff M, Rose H and Lundsteen C (2001) High resolution comparative genomic hybridisation in clinical cytogenetics. J Med Genet 38:740-744.

Langer PR, Waldrop AA and Ward DC (1981) Enzymatic synthesis of biotin-labeled polynucleotides: Novel nucleic acid affinity probes. Proc Natl Acad Sci USA 78:6633-6637.

Le Caignec C, Spits C, Sermon K, De Rycke M, Thienpont B, Debrock S, Staessen C, Moreau Y, Fryns JP, Van Steirteghem A, *et al.* (2006) Single-cell chromosomal imbalances detection by array CGH. Nucleic Acids Res 34:e68.

Le Scouarnec S and Gribble SM (2012) Characterising chromosome rearrangements: Recent technical advances in molecular cytogenetics. Heredity (Edinb) 108:75-85.

Lee JA, Carvalho CM and Lupski JR (2007) A DNA replication mechanism for generating nonrecurrent rearrangements associated with genomic disorders. Cell 131:1235-1247.

Lejeune J, Gautier M and Turpin MR (1959) Etude des chromosomessomatiques de neuf enfants mongoliens. C R Acad Sci (Paris) 248:1721-1722.

Lestou VS, Lomax BL, Barrett IJ and Kalousek DK (1999) Screening of human placentas for chromosomal mosaicism using comparative genomic hybridization. Teratology 59:325-330.

Levan A (1938) The effect of colchicine on root mitosis in Allium. Heredity 24:471-486.

Lichtenbelt KD, Knoers NV and Schuring-Blom GH (2011) From karyotyping to array-CGH in prenatal diagnosis. Cytogenet Genome Res 135:241-250.

Liehr T (2009) Fluorescence In Situ Hybridization (FISH) Application Guide. Springer, Berlin, 452 pp.

Liehr T (2012) Small Supernumerary Marker Chromosomes (SSMC). A Guide for Human Geneticists and Clinicians. Springer, Heidelberg, 220 pp .

Liehr T (2014) Benign & Pathological Chromosomal Imbalances. Microscopic and Submicroscopic Copy Number Variations (CNVs) in Genetics and Counseling. Elsevier, London, 232 pp.

Liehr T, Heller A, Starke H, Rubtsov N, Trifonov V, Mrasek K, Weise A, Kuechler A and Claussen U (2002) Microdissection based high resolution multicolor banding for all 24 human chromosomes. Int J Mol Med 9:335-339.

Liehr T, Starke H, Weise A, Lehrer H and Claussen U (2004) Multicolor FISH probe sets and their applications. Histol Histopathol 19:229-237.

Lupski JR and Stankiewicz P (2005) Genomic disorders: Molecular mechanisms for rearrangements and conveyed phenotypes. PLoS Genet 1:e49.

Mantripragada KK, Tapia-Páez I, Blennow E, Nilsson P, Wedell A and Dumanski JP (2004) DNA copy-number analysis of the 22q11 deletion-syndrome region using array-CGH with genomic and PCR-based targets. Int J Mol Med 13:273-279.

Marcus DA (1988) High-performance optical filters for fluorescence analysis. Cell Motil Cytoskeleton 10:62-70.

Martens UM, Zijlmans JM, Poon SS, Dragowska W, Yui J, Chavez EA, Ward RK and Lansdorp PM (1998) Short telomeres on human chromosome 17p. Nat Genet 18:76-80.

Matthey R (1949) Les Chromosomes des Vertébrés. Librairie de I'Universite, F. Rouge-Lausanne, 356 pp.

McDonnell SK, Riska SM, Klee EW, Thorland EC, Kay NE, Thibodeau SN, Parker AS and Eckel-Passow JE (2013) Experimental designs for array comparative genomic hybridization technology. Cytogenet Genome Res 139:250-257.

McNamara LE, Dalby MJ and Tsimbouri MP (2014) The use of microarrays and fluorescence in situ hybridization for the study of mechanotransduction from topography. Methods Cell Biol 119:293-309.

Meltzer PS, Guan XY, Burgess A and Trent JM (1992) Rapid generation of region specific probes by chromosome microdissection and their application. Nat Genet 1:24-28.

Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, Carter NP, Church DM, Crolla JA, Eichler EE, Epstein CJ, *et al.* (2010) Consensus statement: Chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am J Hum Genet 86:749-764.

Moorhead PS, Nowell PC, Mellman WJ, Battips DM and Hungerford DA (1960) Chromosome preparations of leukocytes

cultured from human peripheral blood. Exp Cell Res 20:613-616.

Ness GO, Lybaek H and Houge G (2002) Usefulness of high-resolution comparative genomic hybridization (CGH) for detecting and characterizing constitutional chromosome abnormalities. Am J Med Genet 113:125-136.

Niazi M, Coleman DV and Loeffler FE (1981) Trophoblast sampling in early pregnancy. Culture of rapidly dividing cells from immature placental villi Br J Obstet Gynaecol 88:1081-1085.

Nicholl J, Waters W, Mulley JC, Suwalski S, Brown S, Hull Y, Barnett C, Haan E, Thompson EM, Liebelt J, *et al.* (2014) Cognitive deficit and autism spectrum disorders: Prospective diagnosis by array CGH. Pathology 46:41-45.

Nietzel A, Rocchi M, Starke H, Heller A, Fiedler W, Wlodarska I, Loncarevic IF, Beensen V, Claussen U and Liehr T (2001) A new multicolor-FISH approach for the characterization of marker chromosomes: Centromere-specific multicolor-FISH (cenM-FISH). Hum Genet 108:199-204.

Nowell PC (1960) Phytohemagglutinin: An initiator of mitosis in cultures of normal human leukocytes. Cancer Res 20:462-466.

Painter TS (1923) Studies in mammalian spermatogenesis II. The spermatogenesis of man. J Exp Zool 37:291-321.

Palmer E, Speirs H, Taylor PJ, Mullan G, Turner G, Einfeld S, Tonge B and Mowat D (2014) Changing interpretation of chromosomal microarray over time in a community cohort with intellectual disability. Am J Med Genet A 164:377-385.

Pardue ML and Gall JG (1970) Chromosomal localization of mouse satellite DNA. Science 168:1356-1358.

Perry GH, Ben-Dor A, Tsalenko A, Sampas N, Rodriguez-Revenga L, Tran CW, Scheffer A, Steinfeld I, Tsang P, Yamada NA, *et al.* (2008) The fine-scale and complex architecture of human copy-number variation. Am J Hum Genet 82:685-695.

Pinkel D, Gray JW, Trask B, van den Engh G, Fuscoe J and van Dekken H (1986a) Cytogenetic analysis by in situ hybridization with fluorescently labeled nucleic acid probes. Cold Spring Harbor Symp Quant Biol 51:151-157.

Pinkel D, Straume T and Gray JW (1986b) Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci USA 83:2934-2938.

Pinkel D, Landegent J, Collins C, Fuscoe J, Segraves R, Lucas J and Gray JW (1988) Fluorescence in situ hybridization with human chromosome-specifi c libraries: Detection of trisomy 21 and translocations of chromosome 4. Proc Natl Acad Sci USA 85:9138-9142.

Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, Chen C, Zhai Y, *et al.* (1998) High resolution analysis of DNA copy number variations using comparative genomic hybridization to microarrays. Nat Genet 20:207-211.

Pita M, Orellana J, Martínez-Rodríguez P, Martínez-Ramírez A, Fernández-Calvín B and Bella JL (2014) FISH methods in cytogenetic studies. Methods Mol Biol 1094:109-135.

Pollack JR, Perou CM, Alizadeh AA, Eisen MB, Pergamenschikov A, Williams CF, Jeffrey SS, Botstein D and Brown PO (1999) Genome-wide analysis of DNA copy-number changes using cDNA microarrays. Nat Genet 23:41-46.

Priest JR, Girirajan S, Vu TH, Olson A, Eichler EE and Portman MA (2012) Rare copy number variants in isolated sporadic and syndromic atrioventricular septal defects. Am J Med Genet 158A:1279-1284.

Rabbani B, Tekin M and Mahdieh N (2014) The promise of whole-exome sequencing in medical genetics. J Hum Genet 59:5-15.

Rafati M, Seyyedaboutorabi E, Ghadirzadeh MR, Heshmati Y, Adibi H, Keihanidoust Z, Eshraghian MR, Javadi GR, Dastan J, Mosavi-Jarrahi A, *et al.* (2012) "Familial" *vs.* "Sporadic" intellectual disability: Contribution of common microdeletion and microduplication syndromes. Mol Cytogenet 5:9.

Reichman J (2000) Handbook of optical filters for fluorescence microscopy. Chroma Technology, Brattleboro, 36 pp.

Rickman L, Fiegler H, Carter NP and Bobrow M (2005) Prenatal diagnosis by array-CGH. Eur J Med Genet 48:232-240.

Riggs ER, Church DM, Hanson K, Horner VL, Kaminsky EB, Kuhn RM, Wain KE, Williams ES, Aradhya S, Kearney HM, *et al.* (2012) Towards an evidence-based process for the clinical interpretation of copy number variation. Clin Genet 81:403-412.

Rosner M, Pergament E, Andriole S, Gebb J, Dar P and Evans MI (2013) Detection of genetic abnormalities by using CVS and FISH prior to fetal reduction in sonographically normal appearing fetuses. Prenat Diagn 33:940-944.

Rouillard JM, Herbert CJ and Zuker M (2002) OligoArray: Genome-scale oligonucleotide design for microarrays. Bioinformatics 18:486-487.

Rufer N, Dragowska W, Thornbury G, Roosnek E, Lansdorp PM (1998) Telomere length dynamics in human lymphocyte subpopulations measured by flow cytometry. Nat Biotechnol 16:743-747.

Sanders SJ, Ercan-Sencicek AG, Hus V, Luo R, Murtha MT, Moreno-De-Luca D, Chu SH, Moreau MP, Gupta AR, Thomson SA, *et al.* (2011) Multiple recurrent de novo CNVs, including duplications of the 7q11.23 Williams syndrome region, are strongly associated with autism. Neuron 70:863-885.

Schinzel A (2001) Catalogue of Unbalanced Chromosome Aberrations in Man. 2nd edition. Walter de Gruyter, Berlin, 966 pp.

Schinzel A, Riegel M, Baumer A, Superti-Furga A, Moreira LM, Santo LD, Schiper PP, Carvalho JH and Giedion A (2013) Long-term follow-up of four patients with Langer-Giedion syndrome: Clinical course and complications. Am J Med Genet A 161:2216-2225.

Schou KV, Kirchhoff M, Nygaard U, Jørgensen C and Sundberg K (2009) Increased nuchal translucency with normal karyotype: A follow-up study of 100 cases supplemented with CGH and MLPA analyses. Ultrasound Obstet Gynecol 34:618-622.

Schröck E, du Manoir S, Veldman T, Schoell B, Wienberg J, Ferguson-Smith MA, Ning Y, Ledbetter DH, Bar-Am I, Soenksen D, *et al.* (1996) Multicolor spectral karyotyping of human chromosomes. Science 273:494-497.

Schröck E, Zschieschang P, O'Brien P, Helmrich A, Hardt T, Matthaei A and Stout-Weider K (2006) Spectral karyotyping of human, mouse, rat and ape chromosomes - Applications for genetic diagnostics and research. Cytogenet Genome Res 114:199-221.

Seabright M (1971). A rapid banding technique for human chromosomes. Lancet 2:971-972.

Sebat J, Lakshmi B, Troge J, Alexander J, Young J, Lundin P, Mànér S, Massa H, Walker M, Chi M, *et al.* (2004) Large-scale copy number polymorphism in the human genome. Science 305:525-528.

Shao L, Kang SH, Li J, Hixson P, Taylor J, Yatsenko SA, Shaw CA, Milosavljevic A, Chang CC, Cheung SW, *et al.* (2010) Array comparative genomic hybridization detects chromosomal abnormalities in hematological cancers that are not detected by conventional cytogenetics. J Mol Diagn 12:670-679.

Shimizu K, Wakui K, Kosho T, Okamoto N, Mizuno S, Itomi K, Hattori S, Nishio K, Samura O, Kobayashi Y, *et al.* (2013) Microarray and FISH-based genotype-phenotype analysis of 22 Japanese patients with Wolf-Hirschhorn syndrome. Am J Med Genet A [Epub ahead of print].

Simons A, Sikkema-Raddatz B, de Leeuw N, Konrad NC, Hastings RJ and Schoumans J (2012) Genome-wide arrays in routine diagnostics of hematological malignancies. Hum Mutat Mutat 33:941-948.

Snijders AM, Nowak N, Segraves R, Blackwood S, Brown N, Conroy J, Hamilton G, Hindle AK, Huey B, Kimura K, *et al.* (2001) Assembly of microarrays for genome-wide measurement of DNA copy number. Nat Genet 29:263-264.

Solinas-Toldo S, Lampel S, Stilgenbauer S, Nickolenko J, Benner A, Döhner H, Cremer T and Lichter P (1997) Matrixbased comparative genomic hybridization: Biochips to screen for genomic imbalances. Genes Chromosomes Cancer 20:399-407.

Speicher MR, Ballard SG and Ward DC (1996) Karyotyping human chromosomes by combinatorial multi-fl uor FISH. Nat Genet 12:368-375.

Steele MW and Breg Jr WR (1966) Chromosome analysis of human amniotic-fluid cells. Lancet 1:383-385.

Stefansson H, Meyer-Lindenberg A, Steinberg S, Magnusdottir B, Morgen K, Arnarsdottir S, Bjornsdottir G, Walters GB, Jonsdottir GA, Doyle OM, *et al.* (2014) CNVs conferring risk of autism or schizophrenia affect cognition in controls. Nature 505:361-6.

Stumm M, Wegner RD, Bloechle M and Eckel H (2006) Interphase M-FISH applications using commercial probes in prenatal and PGD diagnostics. Cytogenet Genome Res 114:296-301.

Sun Z, Liu P, Jia X, Withers MA, Jin L, Lupski JR and Zhang F (2013) Replicative mechanisms of CNV formation preferentially occur as intrachromosomal events: Evidence from Potocki-Lupski duplication syndrome. Hum Mol Genet 22:749-756.

Tanke HJ, Wiegant J, van Gijlswijk RP, Bezrookove V, Pattenier H, Heetebrij RJ, Talman EG, Raap AK and Vrolijk J (1999) New strategy for multi-colour fluorescence in situ hybridisation: COBRA: COmbined Binary RAtio labelling. Eur J Hum Genet 7:2-11.

Telenius H, Carter NP, Bebb CE, Nordenskjöld M, Ponder BA and Tunnacliffe A (1992) Degenerate oligonucleotide-primed PCR: General amplification of target DNA by a single degenerate primer. Genomics 13:718-725.

Tijo JH and Levan A (1956) The chromosome number of man. Hereditas 42:1-6.

van der Veken LT and Buijs A (2011) Array CGH in human leukemia: From somatics to genetics. Cytogenet Genome Res 135:260-270.

Vermeesch JR, Fiegler H, de Leeuw N, Szuhai K, Schoumans J, Ciccone R, Speleman F, Rauch A, Clayton-Smith J, Van Ravenswaaij C, *et al.* (2007) Guidelines for molecular karyotyping in constitutional genetic diagnosis. Eur J Hum Genet 15:1105-1114.

Vermeesch JR, Brady PD, Sanlaville D, Kok K and Hastings RJ (2012) Genome-wide arrays: Quality criteria and platforms to be used in routine diagnostics. Hum Mutat 33:906-915.

Vetro A, Bouman K, Hastings R, McMullan DJ, Vermeesch JR, Miller K, Sikkema-Raddatz B, Ledbetter DH, Zuffardi O and van Ravenswaaij-Arts CM (2012) The introduction of arrays in prenatal diagnosis: A special challenge. Hum Mutat 33:923-929.

Vissers LE and Stankiewicz P (2012) Microdeletion and microduplication syndromes. Methods Mol Biol. 838:29-75.

Vissers LE, de Vries BB, Osoegawa K, Janssen IM, Feuth T, Choy CO, Straatman H, van der Vliet W, Huys EH, van Rijk A, *et al.* (2003) Array-based comparative genomic hybridization for the genomewide detection of submicroscopic chromosomal abnormalities. Am J Hum Genet 73:1261-1270.

Vorsanova SG, Yurov YB and Iourov IY (2010) Human interphase chromosomes: A review of available molecular cytogenetic technologies. Mol Cytogenet 3:1-15.

Vulto-van Silfhout AT, van Ravenswaaij CM, Hehir-Kwa JY, Verwiel ET, Dirks R, van Vooren S, Schinzel A, de Vries BB and de Leeuw N (2013) An update on ECARUCA, the European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations. Eur J Med Genet 56:471-474.

Waldeyer W (1888) Über Karykinese und ihre Beziehung zu den Befruchtungsvorgängen. Arch Mikrosk Anat 32:1-112.

Wegner DE (1999) Diagnostic Cytogenetics. Springer, Berlin, 460 pp.

Weise A, Gross M, Mrasek K, Mkrtchyan H, Horsthemke B, Jonsrud C, Von Eggeling F, Hinreiner S, Witthuhn V, Claussen U, *et al.* (2008) Parental-origin-determination fluorescence in situ hybridization distinguishes homologous human chromosomes on a single-cell level. Int J Mol Med 21:189-200.

Weise A, Mrasek K, Klein E, Mulatinho MV, Llerena Jr JC, Hardekopf D, Pekova S, Bhatt S, Kosyakova N and Liehr T (2012) Microdeletion and microduplication syndromes. J Histochem Cytochem 60:346-58.

Wellcome Trust Case Control Consortium, Craddock N, Hurles ME, Cardin N, Pearson RD, Plagnol V, Robson S, Vukcevic D, Barnes C, Conrad DF, *et al.* (2010) Genome-wide association study of CNVs in 16,000 cases of eight common diseases and 3,000 shared controls. Nature 464:713-720.

Wells D and Delhanty JD (2000) Comprehensive chromosomal analysis of human preimplantation embryos using whole genome amplification and single cell comparative genomic hybridization. Mol Hum Reprod 6:1055-1062.

Wiszniewska J, Bi W, Shaw C, Stankiewicz P, Kang SH, Pursley AN, Lalani S, Hixson P, Gambin T, Tsai CH, *et al.* (2014) Combined array CGH plus SNP genome analyses in a single assay for optimized clinical testing. Eur J Hum Genet 22:79-87.

Xing J, Zhang Y, Han K, Salem AH, Sen SK, Huff CD, Zhou Q, Kirkness EF, Levy S, Batzer MA and Jorde LB (2009) Mobile elements create structural variation: Analysis of a complete human genome. Genome Res 19:1516-1526.

Yunis JJ (1976) High resolution of human chromosomes. Science 191:1268-1270.

Internet Resources

Centre for the Development and Evaluation of Complex Interventions for Public Health Improvement (DECIPHER) project: http://decipher.sanger.ac.uk (2014-01-28).

The Chromosome Anomaly Collection: http://www.ngrl.org.uk/wessex/collection/ (2014-01-28).

Chromosomal Variation in Man Online Database: http://www.wiley.com/legacy/products/subject/life/borgaonkar/access.html (2014-01-28).

Cytogenetic Data Analysis System (CyDAS): http://www.cydas.org/ (2014-01-28).

Database of genomic structural variation (bdVar): http://www.ncbi.nlm.nih.gov/dbvar/ (2014-01-28).

Ensembl: www.ensembl.org/ (2014-01-28).

European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations (ECARUCA): www.ecaruc.net (2014-01-28).

The International Standards for Cytogenomic Arrays (ISCA) Consortium:https://www.iscaconsortium.org/index.php (2014-01-28).

Small supernumerary marker chromosomes: http://ssmc-tl.com/sSMC.html (2014-01-28).