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## Array CGH in Fetal Medicine Diagnosis

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### 1. Introduction

About 2% to 5% of live births have at least one identifiable congenital anomaly at birth (Kalter and Warkany, 1983), ranging from mild to severe abnormalities that compromise the survival. Congenital malformations have been showing increasing importance as a cause of suffering and harm to health of the population, accounting for a large percentage of perinatal morbidity and mortality (De Galan-Roosen et al., 1998, Rosano et al., 2000, Cornel, 2000).

The introduction of ultrasonography in prenatal care allowed the identification of the congenital malformations even in the intrauterine environment. The identification of malformations occurs directly by the detection of morphological changes or indirectly through signs such as fetal growth retardation and changes in amniotic fluid volume. Technological advances in ultrasound, growing experience for specialized services and easier access to ultrasound services have contributed to a significant increase in the detection of fetuses with congenital malformations in low risk populations, becoming a routine part of prenatal care. The ultrasound to identify fetuses with chromosomal abnormalities showed a method with high negative predictive value, which is to say that in the absence of defects detected, the possibility that the fetus has a chromosomal abnormality is low (Nicolaidis et al., 1992, Boue et al., 1988, Wladimiroff et al., 1988, Gonen et al., 1995). Adding to this evidence to the fact that the majority of affected fetuses are generated by young women with no identified risk factor (Nicolaidis et al., 1992, Zeitune et al., 1991), ultrasonography with emphasis on the pursuit of fetal malformations that are related to chromosomal abnormalities is recommended in all pregnant women in more than one occasion during pregnancy.

The genetic causes, alone or in conjunction with environmental causes, are involved in at least one third of congenital malformations. Among the genetic causes, numerical or structural chromosomal abnormalities are present in about 0.9% of unselected group of newborn (Jacobs et al., 1992) and in more than 10% of stillbirths (Jackson, 2002). The overall frequency of cytogenetic abnormalities in malformed fetuses is approximately 10% to 15% (Nelson and Holmes, 1989). Of these, about 80% are trisomies of chromosomes 13, 18 or 21. The rest involves numerical changes in the sex chromosomes and structural chromosomal rearrangements (Rickman et al., 2006). It is justifiable, therefore, the conduct to indicate the chromosomal analysis of all stillbirths and neonatal deaths, with or without dysmorphic

phenotypes, and all fetuses with malformations, especially when more than one anomaly is present or when there is family history of congenital malformations.

Since the description of the number of human chromosomes 50 years ago, there is a scientific effort to define the association between chromosomal abnormalities, genetic diseases and congenital malformations. The development of modern molecular techniques has allowed the analysis of the human genome in high resolution, especially useful in identifying new genomic changes, not previously identified by routine chromosome analysis.

Faced with a malformed fetus that has normal chromosomal study through G-banded karyotype, one must be very careful and avoid any rash attempts of syndromic diagnosis. Even after birth, with a complete physical exam and various laboratory tests, diagnostic centers specializing in dysmorphic syndrome cannot establish definitive diagnosis in about half of the patients (RE, 1993). For this group, molecular techniques involving genomic level studies allow the identification of new chromosomal microarray which might be responsible for abnormal phenotype, contributing to the molecular characterization and establishment of a more accurate diagnosis, a most appropriate perinatal approach and a more detailed genetic counseling.

## **2. Prenatal diagnosis of chromosomal abnormalities**

Microscopic analysis of chromosomes has been the gold standard for diagnosis of chromosomal abnormalities since the development of G-banding technique in the late 60's (Caspersson et al., 1968). In a Fetal Medicine Unit, it is common to obtain a karyotype from umbilical cord blood, amniotic fluid and chorionic villi, depending on gestational age. However, theoretically, it is possible to obtain the karyotype from any tissue that has preserved its viability and can be subjected to cell culture to obtain metaphase (Cabral et al., 2001). The time to obtain the results vary depending on the material studied the viability of cultured cells, the technical laboratory, the reagents and sample preparation, among other factors. On average, this period is 7 to 15 days for the culture of chorionic villi or amniotic fluid and from 3 to 7 days for fetal blood (Nussbaum et al., 2008). The direct preparation of chorionic villi, without cell culture, allows us to obtain results within 24 hours, but the low resolution of the chromosome limits the application, making it impossible sometimes to exclude structural abnormalities.

Although very reliable and still considered the gold standard for the investigation of abnormalities related to chromosomes, the conventional cytogenetic analysis has some limitations, such as failure of cell culture and the need for experienced professional to read and reliably interpret results. In prenatal diagnosis, other important limitations are related to the low quality of chromosome preparations, obtained in a significant portion of the time, and which prevents the detection of structural anomalies and chromosomal microarray smaller than 5Mb to 10Mb. In addition, the need for long-term cell culture ultimately significantly impact the relatively long time for the release of results.

In recent decades, clinical cytogenetics has seen extraordinary advances in molecular biology techniques. The confluence of molecular and cytogenetic approaches - molecular cytogenetics - revolutionized the possibilities and diagnostic accuracy. The sequencing of the human genome has contributed to this effect, allowing a higher tracking resolution for

chromosomal abnormalities. These genomic changes are about 15% of all mutations that involve single-gene diseases in humans (Vissers et al., 2005). The limitations of conventional banding resolution can be largely overcome by new molecular techniques, with obvious clinical applications in the diagnosis of microdeletion, subtelomeric rearrangements, marker chromosomes and derivatives, and gene rearrangements (Carpenter, 2001).

The most widespread molecular technique in prenatal diagnosis is fluorescence in situ hybridization (FISH), by assessing the presence or absence of a specific DNA sequence or chromosomal region. Therefore, the FISH technique is locus-specific and requires knowing the DNA sequence of interest to the appropriate choice of the probe to be used. It is used for early detection of trisomy in uncultured cells, simultaneously analyzing five probes for chromosomes frequently involved in aneuploidy (13, 18, 21, X and Y) and to confirm microduplications or microdeletions syndromes. The advantage over conventional techniques is the short time to obtain the results; it is possible in just 2 hours. Several other techniques have emerged based on the original method of FISH. Using the techniques of multicolor FISH (M-FISH) and spectral karyotyping (Spectral karyotyping - SKY), each chromosome takes a "signature" spectrum, enabling the identification of complex rearrangements involving more than two chromosomes, as well as the source and content of marker chromosomes (Haddad et al., 1998, Fan et al., 2000), with a resolution of 1Mb to 5Mb for the M-FISH (Speicher et al., 1996) and 1Mb to 2 Mb for SKY (Schröck et al., 1997).

The conventional cytogenetic techniques approach associated with the study of molecular dysmorphology have allowed a greater correlation between genotype and phenotype, with the diagnosis of an increasing number of "microarray syndromes" or "genomic instability". The term "genomic instability" has been widely used to describe a phenomenon that results in the accumulation of multiple changes that lead to conversion of a genome of a normal cell to an unstable genome (Smith et al., 2003). These unbalanced chromosomal rearrangements account for 1% to 2% of the abnormalities in prenatal samples, and can lead to severe phenotypic consequences (Ryall et al., 2001). The major limitation of molecular techniques described so far is that these techniques do not detect these "genomic instability".

### 3. Comparative genomic hybridization (CGH)

The development of microarray technology in the early 1990's at Stanford University (Schena et al., 1995) drew heavily from six major disciplines: Biology, Chemistry, Physics, Engineering, Mathematics and Computer Science. Besides no other technology has ever involved so much technological complexity, combining expertise from so many different disciplines, it provided a quantitative and systematic view of a biological system.

This revolutionary new science uses microscopic glass arrays (microarrays) for quantitative analysis of genes (or part of them) and gene products. It has its root in advances made between the discovery of DNA in 1950's and the Human Genome Project in 1990's.

The first hybridization experiments on glass were performed in the late 1980's and early 1990's (Khrapko et al., 1989, Fodor et al., 1991, Maskos and Southern, 1992, Lamture et al., 1994, Guo et al., 1994). These early experiments established the feasibility of glass-based hybridization, combinatorial oligonucleotide synthesis, linker and face chemistry; contact printing based on capillary action and early detection technologies. Many of these principles were used to develop the first microarray assays (Schena et al., 1995)

The comparative genomic hybridization (CGH) was developed as a method of comprehensive genome scan, in an attempt to identify imbalances in the number of copies of DNA, ie genomic instability. Developed in 1992, the CGH technique is the competitive hybridization of test DNA and normal DNA, marked with different fluorochromes (Kallioniemi et al., 1992). The original technique used normal metaphases in fixed blade and is known as metaphasic, chromosomal or conventional CGH.

The metaphasic CGH technique has its main applicability in the field of cancer genetics (Albertson and Pinkel, 2003, Weiss et al., 2003a, Weiss et al., 2003b). Other clinical applications as in dysmorphology, mental disorders and learning were also tested, showing an increase in the diagnosis of deletions or duplications not identified by G-banding (Kirchhoff et al., 2001, Ness et al., 2002). For prenatal diagnosis, the technique has been validated retrospectively studying fetuses known to be carriers or partial aneuploidy (Bryndorf et al., 1995, Yu et al., 1997, Lapierre et al., 1998, Thein et al., 2001), being equivalent to high-resolution cytogenetic techniques, but with the advantage of not requiring cell culture. A study demonstrated its utility as a complementary tool to conventional karyotype in a group of fetuses with abdominal wall defects (Heinrich et al., 2007). However, the delineation of specific regions and bands in conventional CGH technique is limited by the resolution of metaphase chromosomes and is estimated to be from 3Mb to 10Mb, and it is the most limiting (Kallioniemi et al., 1992, Bryndorf et al., 1995, Kirchhoff et al., 1999).

Keeping the same principle of comparing DNA test (sample) and normal DNA (reference) and coupling to the microarray technology (microarrays or arrays) (Solinas-Toldo et al., 1997, Pinkel et al., 1998), a new technique of comparative genomic analysis has been developed and it is now based in microarrays, known as array CGH. Basically, the technique uses multiple pre-selected fragments of DNA attached, so a locus-specific glass surface (microscope slide). The attached DNA can be cloned DNA fragments from bacteria (BAC - Bacterial Artificial Chromosomes) or P1 (PAC - P1 Artificial Chromosomes) (Telenius et al., 1992), cDNA (Pollack et al., 1999), synthetic oligonucleotides (Lucito et al., 2003) or PCR fragments (Mantripragada et al., 2004).

The type of DNA used and the amount of the surveyed areas vary between different protocols or platforms available. The choice of regions included in the survey defines their classification into two types: array CGH representative of the entire genome and targeted to specific regions, usually involved in chromosome arrangements already described. The resolution obtained by different types of array CGH depends on the number and size of clones studied and the distance between consecutive clones. Arrays constructed from BAC clones have the resolution of 50kb to 150kb (Ishkanian et al., 2004, Coe et al., 2009) and oligonucleotide arrays can come in the 25pb 85pb resolution (Shaikh, 2007).

Technically, identical amounts of DNA test and reference DNA are labeled with fluorochromes (cyanine), and after being co-hybridized, the difference of the intensities emitted by the cyanine for each area surveyed is measured. When the fluorescence intensity is lower in the sample tested against the reference sample, we infer that there is a loss in the respective genomic region ("deletion" or "loss") and in the opposite situation; it is inferred genomic gain ("double" or "gain").

The array CGH technique offers important advantages over other methods of cytogenetic diagnosis. First, because it is not necessary to obtain metaphases and allow the analysis of

DNA extracted from different tissues, even tissues embedded in paraffin. Other advantages include the ability to search thousands of chromosomal regions in a single analysis in a short period of time and with high resolution, overcoming the limitations of conventional karyotyping and metaphase CGH. Other advantages of this molecular method is that it does not require prior knowledge of the genomic region involved and the ability to study cases where only DNA is available and no chromosomes can be obtained. The method was reproducible in a clinical standpoint, with reliable results within 48 hours.

Its inherent limitation, based on the principles of the technique, lies in not being able to identify chromosomal abnormalities that do not lead to change in the total number of copies of a segment of DNA within the genome, such as balanced translocations and inversions. Also, the normalization of the doubled fluorescence intensity generated in euploidy can difficult their identification. The technique also finds limited use in cases of mosaicism. This last limitation has been overcome with increased experience in interpreting the results, and is believed to be possible to detect chromosomal rearrangements present in at least 20% (Vermeesch et al., 2005) or 50% of the cells (Shaw and Lupski, 2004). There is recent evidence that the technique of array CGH is able to diagnose cases of mosaicism that were not detected by conventional karyotyping (Ballif et al., 2006, Cheung et al., 2007, Shinawi et al., 2008, Wood et al., 2008).

Microarray-based CGH is a powerful method to detect and analyze genomic imbalances that are well below the level of detection on high resolution banded karyotype analysis, providing a better opportunity for genotype/phenotype correlations in other similarly affected individuals. The clinical application of array CGH as a diagnostic tool in patients with mental disorders and learning associated with dimorphic features has been extensively studied (Bejjani et al., 2005, Cheung et al., 2005 Jul-Aug, Shaffer et al., 2006, Lu et al., 2007, Stankiewicz and Beaudet, 2007), and has proven useful and reproducible for diagnosis and molecular characterization of this group (Vissers et al., 2003, Shaw-Smith et al., 2004, Gribble et al., 2005, Schoumans et al., 2005, Rosenberg et al., 2006, Menten et al., 2006, Aradhya et al., 2007, Pickering et al., 2008, Brunetti-Pierri et al., 2008). These studies have shown that array CGH technique can increase the rate of diagnosis in relation to conventional cytogenetic study in 20% to 30%, culminating in its inclusion in the flowcharts of genetic research of this group of patients (Sharkey et al., 2005). Microarray-based genomic copy-number analysis is now a commonly ordered clinical genetic test for patients with diagnoses including unexplained developmental delay/intellectual disability, autism spectrum disorders, and multiple congenital anomalies. It is offered under various names, such as “chromosomal microarray” (CMA) and “molecular karyotyping”.

The increase in coverage of genomic regions in the array CGH platforms representative of the entire genome is responsible for an additional fee of 5% in the detection of chromosomal microarray compared to array CGH platforms targeted to specific regions (target arrays) (Baldwin et al., 2008). However, while the high-resolution analysis can identify pathological abnormalities, they incur the problem of identification of genomic gains and losses in regions with unknown clinical significance. So as you increase the resolution of the platform, simultaneously increases the degree of uncertainty. Here comes the delicate balance between resolution of the method, the potential for clinical diagnosis and the ability

to interpret the results. This is the key issue to determine the use of CGH microarray for diagnostic use in clinical practice.

The CNV may be difficult to evaluate and interpret. A variety of methodologies used in the generation of information that form the research databases available electronically CNV makes it difficult to discern the exact extent of each CNV likely found. This lack of methodological uniformity may confuse the correct interpretation of finding abnormal chromosome present in a phenotypically abnormal individual, but overlapping all or part of a region with CNV described. Another problem is the finding of a gain in a region where it is described as a deletion CNV and vice versa. In these situations, we can not infer that the rearrangement is also a benign finding.

The identification of CNVs may also vary according to the CGH array platform used. A recent study showed that the number of CNVs found using the technique of array CGH containing BAC clones was higher when compared with those found when applied oligonucleotide array CGH (Aradhya et al., 2007). This discrepancy can be explained by the fact that the clones generated from bacteria (BAC) is that the larger fragments oligonucleotide probes, with the consequent overlapping regions. In addition, BAC clones can encompass regions of repetitive DNA, known to be associated with variations in the number of copies (Sharp et al., 2005).

However, at this point in time, genome-wide arrays will detect many copy number variants of unknown clinical significance. Growing clinical experience with genome-wide arrays and the development of copy number variants databases of both healthy and affected individuals will reduce the number of copy number variants of unknown clinical significance and will make genome-wide arrays more useful in clinical practice (ACOG, 2009).

#### **4. Array CGH in prenatal diagnosis**

Using the technique of array CGH in Maternal-Fetal Medicine has recently been tested. In 2004, Schaeffer et al. (Schaeffer et al., 2004) observed an increase in detection rate of chromosomal abnormalities not identified by the conventional technique in 9.8% of 41 cases of spontaneous abortions. In frozen lung tissue of 49 fetuses with multiple malformations and normal karyotyping that developed spontaneous abortion or elective termination of pregnancy, Le Caignec et al. (Le Caignec et al., 2005), identified increased detection rate of 16.3% using the technique of array CGH. Another retrospective study (Rickman et al., 2006) has validated two array CGH platforms in 30 samples of amniotic fluid and chorionic villi.

After initial studies that introduced retrospective validation of the technique of array CGH for prenatal diagnosis, the first prospective studies have been emerged. In recent publications, Van den Veyver et al. (Van den Veyver et al., 2009) found 4.8% abnormal findings in 84 fetuses with malformations of the ultrasound, and Kleeman et al. (Kleeman et al., 2009) found the percentage of 8% considering only those fetuses with normal conventional karyotype, showing that the technique of array CGH is a promising tool in prenatal diagnosis. Several reports have now shown the potential utility of array CGH in prenatal diagnosis (Le Caignec et al., 2005, Sahoo et al., 2006, Shaffer et al., 2008, Kleeman et al., 2009, Vialard et al., 2009, Van den Veyver et al., 2009, Machado et al., 2010a).

In the first prospective study in prenatal diagnosis, Sahoo et al. (Sahoo et al., 2006) reported a detection rate of chromosomal abnormalities of 43% of the 98 studied fetuses. Subsequent studies could not found the same rate. In an unselected group of 50 malformed fetuses with normal karyotype, Kleeman et al. (Kleeman et al., 2009) found 4 fetuses (8%) with abnormal array results. Studying a comparable number of cases, by including only fetuses with at least three anomalies and a target array of 287 clones, Le Caignec et al. (Le Caignec et al., 2005) found array abnormalities in 16.3% of the 49 investigated fetuses. Using the same target array CGH to study retrospectively 37 malformed fetuses with at least two anomalies and normal karyotype, Vialard et al. (Vialard et al., 2009) found abnormal results in 4 fetuses corresponding to 10.8%. In Machado et al.(Machado et al., 2010a), although the number of fetuses with copy number changes was much higher than other reports, the number of fetuses with described CNVs among the detected genomic imbalances failed to show such a difference (Table 1).

Study	N of included fetuses	Fetuses with chromosomal imbalances	Fetuses with CNV
Le Caignec et al. (2005)	49	8 (16%)	NL
Sahoo et al. (2006)	98	42 (43%)	30 (71%)
Shaffer et al. (2008)	151*	15 (10%)	12 (80%)
Kleeman et al. (2009)	50	4 ( 8%)	3 (75%)
Vialard et al. (2009)	37**	4 (10%)	NL
Van den Veyver et al. (2009)	300	58 (19%)	40 (69%)
Machado et al. (2010)	48	45 (94%)	39 (87%)

\* Considering only prenatal specimens  
\*\* Considering only fetuses with normal karyotype  
NL = not listed

Table 1. Number of fetuses with copy number abnormalities and copy number variation (CNV) in recent prenatal array CGH studies

Regardless of array CGH techniques have a theoretical principle and common molecular approaches, published studies have different methodologies in the selection of fetuses, the study design, array CGH platforms, bioinformatics analysis, and interpretation of results, making the comparison between the different works not entirely free from errors. It can explain the difference in detection rates observed in the different studies.

A difference with significant impact on the comparison of results is presented in the selection of fetuses, ranging from fetuses that died with more than three different indicated malformations in prenatal diagnosis, such as maternal anxiety. One study included 367 different indications for molecular analysis, including 20 cases where the indication was not specified. In Machado et. al.(Machado et al., 2010a), the fetuses' selection included only congenital malformations with a strong genetic background, and fetuses with multiple congenital malformations. In both situations is expected a high prevalence of genetic abnormalities and it can explain the high detection rate of fetuses with copy number imbalances in that study.

There is still no consensus among research groups even for interpretation of the results. The definition of "results of uncertain significance" is not clear in the methodology adopted by some of the studies. It can happen due to very little is known about the natural history and range of clinical variability associated with recently described submicroscopic deletions and duplications detected by array CGH. It is worth noting that the presence of a deletion or duplication alone does not necessarily mean that the copy number alteration causes the observed phenotype and we can not also assure to consider a copy number imbalance as to be pathogenic on the basis only of the association with fetal malformation indentified by ultrasound examination. However, the fetus presenting a significant structural anomaly has a high *a priori* risk of having a pathogenic genetic abnormality and, as is true for any test, the found copy number imbalance is more likely to be a true positive pathogenic one.

In evaluating the scope and content of the regions affected by genomic alterations, Machado et al. (Machado et al., 2010a) identified in 13 of the 48 fetuses (27%) studied by CGH genomic amplifications or deletions that would lead to a modification of the initial cytogenetic results with clinical impact. For these cases, the implications of molecular diagnosis involve experts from the reference to the therapeutic intervention for specific syndromes, to tracking of other malformations. The implications also extend towards reducing the number of diagnostic procedures that the patient could be subjected. For the family, the diagnosis can decrease anxiety and allow for an adequate risk counseling and planning for future offspring. Cytogenetic changes considered clinically significant and ranged from 80Kb to 30Mb in size and were mostly genomic losses.

The clinical applicability of array CGH technique in prenatal diagnosis seems well established for refining diagnosis in cases with suspicious or inconclusive diagnosis of chromosomal structural changes. In recent literature, we find an increasing number of descriptions of cases or series that reinforce this clinical applicability prenatal array CGH (Machado et al., 2010b, Kitsiou-Tzeli et al., 2008). Other situations, for instance, include the study of supposedly balanced translocations by conventional karyotyping, but revealed his unbalanced pattern to the array CGH (Simovich et al., 2007) and the exact sizing and location of chromosome structural abnormalities.

Other clinical application can be checked using the fetuses' selection according to specific defect, as demonstrated for the molecular characterization of fetuses with holoprosencephaly (Machado et al., 2011a) and congenital diaphragmatic hernia. (Machado et al., 2011b) The array CGH could contribute to the knowledge of the submicroscopic genomic instability characterization of specific congenital abnormalities. The indicated significant chromosomal regions are supported when considered that a copy number imbalance in such region was recurrent in fetuses with the same phenotype and when the same genotype-phenotype correlation has been already described. This way, it could identify some clones with uncertain but putative significance that provided a list of chromosomal regions of clinical interest for further molecular evaluation. Additional and confirmatory researches are needed to further establish the role of genes from this chromosome region in the pathogenesis of each specific congenital defect.

The identification of CNVs can greatly complicate the interpretation of the results of array CGH techniques. Among fetuses with changes in the number of copies, 70 to 87% had at least one clone that contained altered, in whole or in part, a chromosomal region with copy number variation (CNV) described in the available databases (see Table 1). This issue is

particularly critical for prenatal diagnosis, where the "normal" or not the result defines the perinatal approach. An adequate discrimination between harmless and the real variations aberrations is essential for proper counseling.

Considering all the above and based on the American College of Obstetrics and Gynecology Statement published in 2009 (ACOG, 2009), the array CGH can not replace classic cytogenetics in prenatal diagnosis. According the ACOG opinion, the usefulness of array CGH as a first-line tool in detecting chromosomal abnormalities in all amniocentesis or chorionic villus samples is still unknown. The additional detection rate of chromosomal abnormalities using array CGH, as compared with conventional karyotype for routine fetal chromosomal analysis, awaits a larger population-based study (Table 2).

**ACOG RECOMMENDATIONS: (ACOG, 2009)**

- » Conventional karyotyping remains the principal cytogenetic tool in prenatal diagnosis.
- » Targeted array CGH, in concert with genetic counseling, can be offered as an adjunct tool in prenatal cases with abnormal anatomic findings and a normal conventional karyotype, as well as in cases of fetal demise with congenital anomalies and the inability to obtain a conventional karyotype.
- » Couples choosing targeted array CGH should receive both pretest and posttest genetic counseling. Followup genetic counseling is required for interpretation of array CGH results. Couples should understand that array CGH will not detect all genetic pathologies and that array CGH results may be difficult to interpret.
- » Targeted array CGH may be useful as a screening tool; however, further studies are necessary to fully determine its utility and its limitations.

Table 2. ACOG recommendations for array CGH in prenatal diagnosis.

The International Standard Cytogenomic Array (ISCA) Consortium, an international group of experts in the array CGH field, held two international workshops and conducted a literature review of 33 studies, including 21,698 patients tested by chromosomal microarray. They provided an evidence-based summary of clinical cytogenetic testing comparing CMA to G-banded karyotyping with respect to technical advantages and limitations, diagnostic yield for various types of chromosomal aberrations, and issues that affect test interpretation. They concluded that the available evidence strongly supports the use of CMA in place of G-banded karyotyping as the first-tier cytogenetic diagnostic test for patients with developmental delay/intellectual disability, autism spectrum disorders or multiple congenital anomalies MCA. However, the ISCA Consortium recognizes that current evidence is not sufficient to allow recommendations regarding prenatal multiple congenital anomalies, and traditional cytogenetic methods, such as G-banded karyotyping and fluorescence in situ hybridization (FISH), are still the standard for prenatal diagnosis (Miller et al., 2010).

**5. Challenges**

It is recognized that array CGH can detect submicroscopic changes that can be missed on routine chromosomal analysis, especially in prenatal samples where the band resolution may be compromised. However, the exact role of the array CGH in the flowchart of prenatal

diagnosis has not been established. Multicenter studies making a direct comparison of the performance of array CGH technique to conventional cytogenetic analysis in a prenatal setting are needed. So far, there's no evidence that it may replace conventional G-banded karyotype analysis, but it can complement and expand current methods for a precise prenatal diagnosis and syndromes' characterization.

The availability of the recent array CGH platforms for fetal chromosomal investigation, including the oligonucleotide arrays (Bi et al., 2008), has brought other challenge controversies over the ideal genetic testing for prenatal diagnosis (Pergament, 2007, Ogilvie et al., 2009, Friedman, 2009). Until now, the oligonucleotide array CGH has not been proven beneficial in relation to protocols with BAC clones for prenatal diagnosis (Bi et al., 2008). Many of the known genomic disorders that can be detected on target arrays do not show readily detectable fetal abnormalities on prenatal ultrasound examinations. Ordering array CGH only in the presence of ultrasound abnormalities may limit the diagnostic potential of this assay (Van den Veyver et al., 2009). Moreover, additional research is needed to further reach a consensus on the optimum platform of an array for clinical use in prenatal diagnosis. It's desirable that, in the future, customized chips with markers across loci discovered should be designed for prenatal diagnosis.

Also, further studies are needed to validate the clinical application of CGH arrays for different clinical situations of prenatal diagnosis, such as advanced maternal age, biochemical screening changed, first-trimester sonographic markers and change in situations of family anxiety in the presence of normal biochemical or ultrasound screening (Pergament, 2007).

Another challenge to overcome is to design a uniform and effective strategy for interpretation of results involving the copy-number variants. The time and effort required for distinguishing the pathogenic and benign findings increases as the resolution of the array CGH increases, but uninterpretable results occur with all array CGH platforms (Friedman, 2009).

Despite the challenges still to overcome, it is clear that the array-based CGH has been asserting itself as a valuable tool in the identification and molecular characterization of chromosomal abnormalities in fetuses with birth defects, opening a new chapter in the historical interface between the Cytogenetics and the Fetal Medicine.

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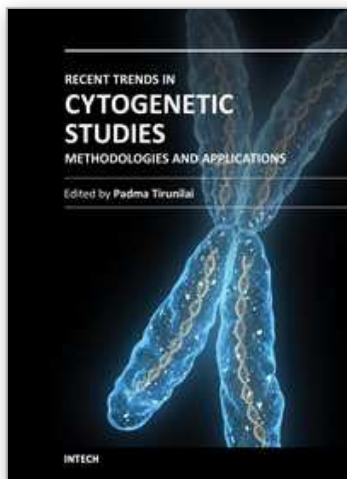
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## **Recent Trends in Cytogenetic Studies - Methodologies and Applications**

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Recent Trends in Cytogenetic Studies - Methodologies and Applications deals with recent trends in cytogenetics with minute details of methodologies that can be adopted in clinical laboratories. The chapters deal with basic methods of primary cultures, cell lines and their applications; microtechnologies and automations; array CGH for the diagnosis of fetal conditions; approaches to acute lymphoblastic and myeloblastic leukemias in patients and survivors of atomic bomb exposure; use of digital image technology and using chromosomes as tools to discover biodiversity. While concentrating on the advanced methodologies in cytogenetic studies and their applications, authors have pointed out the need to develop cytogenetic labs with modern tools to facilitate precise and effective diagnosis to benefit the patient population.

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