

Genomic microarrays in prenatal diagnosis

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Abstract

The application of microarray-based techniques for the diagnosis of genomic rearrangements has been steadily growing in popularity since its introduction in 2004. Given the many advantages of these techniques over conventional cytogenetics, there is increasing pressure towards their application in prenatal diagnosis. However, there remain several important issues that must be addressed. For example, microarray-based techniques (comparative genomic hybridization-based arrays and single nucleotide polymorphism-based arrays) allow detection of even very small genomic imbalances that can determine pathological clinical conditions. In addition, there are other copy number variations which represent normal variation, with no detectable effects on phenotype. Given the still incomplete knowledge of the changes in our genome and the associated phenotypes, microarray-based diagnosis is likely to find variants of uncertain and unknown clinical significance. The interpretation of these variants is now a major challenge for the medical geneticist, who often find it difficult to establish precise correlations between genotype and phenotype. There is sufficient available evidence to justify the use of microarray-based diagnostics for a select number of specific conditions, but there is also an inevitable trend towards ever wider application. It is very important that this drift does not progress in an unchecked and uncontrolled manner under the thrust

of commercial interests. Therefore, we recommend that scientific societies be vigilant and take an advisory role in the adopting of these technologies as new scientific knowledge becomes available.

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Key words: Copy number variations; Genomic microarray analysis; Prenatal diagnosis

Core tip: Given its advantages over conventional karyotyping, there is an increasing interest in determining whether microarray technology will be similarly advantageous for the detection of fetal genomic imbalances in a prenatal setting. Several issues remain to be addressed, such as for which pregnancies comparative genomic hybridization-based arrays should be carried out (*i.e.*, whether for all pregnancies or only for those with ultrasound abnormalities). Another area of uncertainty is the choice of array platform. This article aims to contribute to the discussions on genomic microarrays in prenatal diagnosis by examining the literature and existing guidelines, and giving an opinion on possible future developments and on how best to handle them.

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INTRODUCTION

The objective of prenatal diagnosis (PD) is to provide prenatal diagnostic testing services for genetic conditions that enable families to make informed choices consistent with their individual needs and values, and to support them in deal with the outcome of such testing.

PD is offered with the intention of determining the presence or absence of a pathological condition in the fe-

tus. Prenatal tests may be performed using invasive (such as amniocentesis, chorionic villus sampling or fetal blood sampling) or non-invasive procedures (such as analysis of cell-free fetal DNA in maternal blood or fetal imaging). PD is mainly offered to pregnant women in one of the following four groups: (1) Advanced maternal age; (2) Women and/or partners from families known to genetic or other relevant specialist services before pregnancy because of significant family history of a condition; (3) Women who are identified during pregnancy as having a fetus at risk of a genetic condition (for example, through disclosure of family history, possibly including genetic test results, during an antenatal consultation or following positive prenatal screening); and (4) Women whose fetus is identified as at risk of a genetic condition due to abnormal ultrasound findings.

As chromosomal anomalies are a major cause of perinatal morbidity and mortality, as well as the most frequent cause of intellectual disability in our population, cytogenetic diagnosis using cultured cells obtained by prenatal invasive tests has been regarded as the standard method for PD since its first application. In 1966, Steele *et al*^[1] reported the feasibility of performing chromosomal analysis of amniotic fluid cells. One year later Jacobson *et al*^[2] performed the first PD of a chromosomal abnormality (a balanced translocation), shortly followed by the first PD of trisomy 21^[3].

Karyotyping has proven highly reliable for the genome-wide detection of numerical chromosome abnormalities (aneuploidies) and large structural rearrangements in fetal cells. However, chromosome analysis has some important limitations. It takes about 15 d to culture the cells, visualize the chromosomes and perform the analysis, thus leading to anxiety in the pregnant women. The resolution of a karyotype is limited and chromosomal anomalies in the grey zone (between 5 and 10 Mb in size) lead to interpretation difficulties. Karyotyping also requires skilled analysts, which increases costs and can lead to organizational difficulties in small laboratories^[4].

More recently, molecular cytogenetic methods including interphase fluorescence *in situ* hybridization (FISH), quantitative fluorescent polymerase chain reaction (QF-PCR) and multiplex ligation-dependent probe amplification (MLPA) have been introduced for the rapid detection of aneuploidies of chromosomes 13, 18, 21 and sex chromosomes. These techniques can provide a result in 1-3 d but are disadvantaged by the need to perform locus-specific analysis, leaving a residual risk for a clinically significant chromosomal abnormality. In addition to the common aneuploidies, many submicroscopic chromosomal rearrangements that lead to copy-number gains or losses have been shown to cause distinct and recognizable clinical phenotypes.

The sensitivity in detecting copy-number alterations has increased significantly with the advent of genomic microarray analysis (GMA). Together with improved assemblies and annotation of genome sequence data, these methods allow rapid identification of new syndromes

that are associated with submicroscopic genomic changes in children with idiopathic intellectual disabilities (ID), autism, developmental delay (DD) and/or multiple congenital anomalies (MCA)^[5].

Genomic microarrays detect gains and losses of genomic regions through the hybridization of fluorescently labeled patient DNA onto targets with known genomic coordinates, spotted onto a solid substrate (typically a glass slide). By measuring the signal intensity ratio of patient DNA to a reference sample, gains or losses of genomic material can be identified.

Comparative genomic hybridization-based arrays (CGH-arrays) involve hybridization of a patient's DNA onto predetermined targets representative of the whole genome or of target regions [bacterial artificial chromosomes (BAC) clones of 100-200 kb or synthetic oligonucleotide probes of 25-75 bp] spotted onto glass slides. The patient DNA is extracted from the relevant sample, labeled with a fluorochrome, mixed with a reference DNA pool (labeled with a different fluorochrome) and then hybridized on the microarray slide^[6].

Single nucleotide polymorphism-based arrays (SNP-arrays) were originally designed to detect common SNPs (> 1% in the population) and were mainly used in genotyping individuals for genome-wide association studies of many common multifactorial diseases. In addition to SNP typing, these platforms can also be used to perform copy number analysis. Gains and losses of genomic regions can therefore be detected as is the case for CGH-arrays. SNP arrays also detect copy neutral loss of heterozygosity (or absence of heterozygosity), uniparental disomy and regions identical by descent. However, although SNP arrays detect uniparental isodisomy, parental samples are required for the detection of uniparental heterodisomy. When using SNP-arrays, only a single hybridization is performed for the patient DNA (single channel or color) and the signal intensities are then compared with a reference dataset^[7].

Microarray technology has several advantages over conventional karyotyping, including improved resolution and potentially higher detection rates of chromosomal variation. Using arrays, an additional 15% of causally related chromosomal abnormalities are detected over routine microscopic and MLPA or FISH for subtelomeric screening in patients with DD and/or MCA^[8]. In another study performed in postnatal patients (children and adults) with a diagnosis of unexplained neurodevelopmental disability, the positive diagnostic yield of CGH-array has been reported to be about 10% higher than that of standard karyotyping^[9].

In addition to providing higher resolution, the genomic microarray offers other potential advantages over conventional karyotyping, such as automation (and thus faster turnaround times) and elimination of the need to culture amniocytes or chorionic villi. Because microarray analysis does not require dividing cells, it is also useful in cases of fetal death, when it is often not possible to culture cells^[10]. Given the advantages of microarray-based technologies over karyotyping, there is increasing

Table 1 Data from the literature

Ref.	Key results
[11]	Array CGH could detect causative CNVs in children with ID, and other disabilities and congenital malformations
[12]	Feasibility of performing CGH-array for prenatal diagnosis on DNA extracted from AF cells
[13]	Evaluation of the literature up to 2009. Pathogenic CNVs or VOUS were detected in 3.6% of cases with a normal karyotype. Microarrays detected an additional 5.2% pathological CNVs or VOUS in pregnancies with fetal anomaly on ultrasound
[14-21]	Diagnostic utility of CGH- and SNP-arrays in a prenatal setting
[22]	Cohort studies, published from 2009 onwards, have demonstrated an increased detection rate over standard karyotyping ranging from 0.9% to 26.5%
[23]	Evaluation of the utility of a 1-Mb BAC and 60-K oligonucleotide array in 3171 pregnancies. The detection rate was low (0.52%) in uneventful pregnancies, but increased to 8.2% when a fetus had an abnormality on ultrasound scan
[24]	Additional information in 7.7% of cases using a SNP-array with a resolution of 150/200 kb to analyze DNA from 207 cases with fetal anomalies
[25]	Comparison of microarray with standard karyotyping in 4406 women undergoing PD for common indications over a period of 3 yr (2008-2011) The analysis identified all of the common autosomal and sex-chromosome aneuploidies and the unbalanced rearrangements detected by standard karyotyping in the 4282 non mosaic samples. Microdeletions or duplications of clinical significance were found in 96 of 3822 fetal samples with normal karyotypes (2.5%), including 6.0% of cases in which fetal anomalies were detected on ultrasonography. There were 94 copy-number variants of uncertain clinical significance that required further evaluation. The pathogenicity of 1.5% of CNVs remained uncertain
[26]	Exploration of the utility of microarray analysis in groups of pregnancies with a priori low risk for detection of submicroscopic chromosome abnormalities. A total of 3000 prenatal samples were processed in parallel using both microarray and conventional karyotyping. Samples were processed using a BAC platform with a resolution of about 1 Mb across the genome and about 100 kb in 139 regions associated with constitutional disorders. The percentage of detection was 0.5% (6/1118) in advanced maternal age and 0.7% (11/1674) in parental anxiety. No genetic imbalances were detected in any of the cases sampled for an abnormal maternal serum screening, nor for a family history of a genetic condition or chromosomal abnormality. A total of 24 (0.8%) fetal conditions would have remained undiagnosed if only a standard karyotype had been performed. 17 (0.6%) of such findings would have otherwise been overlooked if CMA was offered only to high risk pregnancies
[27]	Study on 5003 prospective cases received for a variety of indications. The overall detection rate of clinically significant CNAs was 5.3%. Detection rates were 6.5% and 8.2% for cases referred with abnormal ultrasounds and fetal demise, respectively. The overall rate of findings with VOUS was 4.2% but would reduce to 0.39% if only de novo CNAs were considered. In cases with known chromosomal rearrangements in the fetus or parent, 41.1% showed CNAs related to the rearrangements, whereas 1.3% showed clinically significant CNAs unrelated to the karyotype. 71% of the clinically significant CNAs found by microarray were below the resolution of conventional karyotyping of fetal chromosomes
[28]	Evaluation of a multicentric collection of a 1-yr series of fetal samples with indication for prenatal invasive sampling simultaneously using three screening methodologies: (1) karyotype and QF-PCR; (2) two panels of MLPA; and (3) microarray-based analysis with a targeted BAC microarray. On a total of 900 samples, technical performance was excellent for karyotype, QF-PCR, and GMA (about 1% failure rate) but relatively poor for MLPA (10% failure). Mean turn-around time was 7 d for microarray or MLPA, 25 d for karyotype and 2 d for QF-PCR, with similar combined costs for each approach. A total of 57 clinically significant chromosomal aberrations were found (6.3%), with microarray yielding the highest detection rate (32% above other methods). The identification of VOUS (17, 1.9%) tripled that of karyotype and MLPA, but most alterations could be classified as likely benign after proving they were inherited
[29]	Evaluation of the results of prenatal microarray analysis on > 1000 fetal samples referred for testing and comparison of these data to published reports. Clinically significant CNVs were observed in 85/1115 cases (7.6%). Eighteen of the 1115 cases had VOUS (1.6%). Indications yielding the most clinically significant findings were abnormal karyotype/FISH (26/61, 42.6%), family history of chromosomal abnormality (13/137, 9.5%), abnormal ultrasound (38/410, 9.3%), abnormal serum screening (2/37, 5.4%) and advanced maternal age (5/394, 1.3%). Of 1075 cases having no previously known cytogenetic abnormality or family history, 18 (1.7%) had clinically significant genomic changes undetectable by conventional prenatal chromosome analysis
[30-33]	Papers reporting experience on a small number of samples and addressing the main issues in this field
[34]	Prospective cohort study of 243 women undergoing microarray testing alongside karyotyping when a structural abnormality was detected on prenatal ultrasound scan and review and meta-analysis of the literature. The collective number of samples analysed were 17113. The overall agreement between the two tests was 93.4% (95%CI: 90.4%-96.5%). The results obtained in attempting to calculate the rate of microarray detection over karyotyping were highly heterogeneous, ranging from 0.4% to 50%. When the indication was structural abnormality seen on ultrasound scan the detection rate over karyotyping was 10% (95%CI: 8%-13%). A sub analysis performed using cohorts published between 2011 and 2012 showed a lower detection rate (7%, 95%CI: 5%-10%). The authors suggest that GMA could have a higher detection rate not just in cases of abnormal scan findings but also with other indications for invasive testing, and conclude that it is likely that microarray testing will replace karyotyping in high risk pregnancies

BAC: Bacterial artificial chromosomes; CNAs: Copy number alterations; CGH-arrays: Comparative genomic hybridization-based arrays; CMA: Chromosomal microarray analysis; CNVs: Copy number variations; MLPA: Multiplex ligation-dependent probe amplification; PD: Prenatal diagnosis; QF-PCR: Quantitative fluorescent polymerase chain reaction; SNP-arrays: Single nucleotide polymorphism-based arrays; VOUS: Variants of uncertain (unknown) significance; ID: Intellectual disabilities.

interest in determining whether these technologies will offer similar advantages in the detection of fetal genomic imbalances in a prenatal setting.

DATA FROM THE LITERATURE

Starting in 2004, reports began to appear describing evi-

dence that array CGH could detect causative deletions and duplications in children with ID, and other disabilities and congenital malformations^[11] (Table 1).

Rickman *et al*^[12] have shown the feasibility of performing CGH-array for PD on DNA extracted from AF cells with the demonstration that in 29/30 samples, the CGH-array result was fully concordant with the karyotype.

Hillman *et al*^[13] published a critical appraisal of the literature evaluating the use of CGH-array in PD up to and including 2009. When CGH-array was used prenatally for any indication (*e.g.*, maternal age, parental anxiety or an identified ultrasound abnormality), pathogenic copy number variations (CNVs) or variants of uncertain (unknown) significance (VOUS) were detected in 3.6% (95%CI: 1.5%-8.5%) of cases in which conventional karyotyping was considered normal. When the indication for prenatal CGH-array was a fetal anomaly on ultrasound, microarrays detected an additional 5.2% (95%CI: 1.9%-13.9%) pathological CNVs or VOUS over conventional karyotyping. Heterogeneity (and hence large confidence intervals) was attributed to the varying resolution of the CGH-array methodology. In addition, there was considerable variation in the literature as to whether an attempt had been made to identify and investigate the presence of benign CNVs (by reviewing parental samples).

Other cohort studies, published from 2009 onwards, have demonstrated an increased detection rate over standard karyotyping ranging from 0.9% to 26.5%^[14-21]. Some of the studies with much larger detection rates may represent selection of patients rather than being a reflection of a true prospective series^[22].

More recently, Lee *et al*^[23] looked at the utility of both a 1-Mb BAC and 60-K oligonucleotide array in 3171 pregnancies. Although the added utility of CGH-arrays over karyotyping was small when there was an uneventful prenatal examination (0.52%), the proportion of cases in which additional information was provided by CGH-arrays increased to 8.2% when a fetus had an abnormality on ultrasound scan (USS).

Srebniak *et al*^[24] used a SNP-array with a resolution of 150/200 kb to analyze DNA from 207 cases with fetal anomalies, and detected additional information in 7.7% of cases, a similar percentage to that in the Lee and colleagues's cohort.

Over a period of 3 years (2008-2011), Wapner *et al*^[25] compared microarray with standard karyotyping in 4406 women undergoing PD for common indications, including advanced maternal age (46.6%), fetal abnormalities detected on ultrasonography (25.2%) and positive prenatal screening results (18.8%). Microarray analysis was performed using either a customized oligonucleotide-based microarray with spacing of approximately 1 probe per 75 kb, or a SNP-array with a comparable resolution. The analysis was successful in 4340 of 4391 cases with an adequate sample (98.8%), and identified all of the common autosomal and sex-chromosome aneuploidies and the unbalanced rearrangements detected by standard karyotyping in the 4282 non mosaic samples. As expected, microarray analysis did not identify balanced translocations (0.93% in this sample). The series also included seventeen triploid samples (0.4%), none of which were identified on microarray. Microdeletions or duplications of clinical significance were found in 96 of 3822 fetal samples with normal karyotypes (2.5%; 95%CI: 2.1%-3.1%), including 6.0% of cases in which fetal anomalies were detected on

ultrasonography. There were 94 copy-number variants of uncertain clinical significance that required adjudication by a Clinical Advisory Committee, and after discussion 61 (65%) were classified as pathogenic. A subsequent update of copy-number variants of uncertain significance resulted in reclassification of 30 copy-number variants as pathogenic and 8 as benign. With this additional information, the pathogenicity of 1.5% of copy-number variants detected on microarray analysis in karyotypically normal samples remained uncertain.

To assess whether chromosomal microarray analysis (CMA) improves the detection rate of prenatal chromosomal aberrations, Fiorentino *et al*^[26] explored the utility of microarray analysis in groups of pregnancies with *a priori* low risk for detection of submicroscopic chromosome abnormalities (usually not considered an indication for testing). A total of 3000 prenatal samples, including 2650 amniotic fluids (88.3%), 308 chorionic villus sampling (10.3%), 32 cultured amniocytes (1.1%), and 10 DNAs extracted by other laboratories from uncultured amniocytes (0.3%), were processed in parallel using both GMA and conventional karyotyping. The indications for prenatal testing included: advanced maternal age, maternal serum screening test abnormality, abnormal ultrasound findings, known abnormal fetal karyotype, parental anxiety, family history of a genetic condition and cell culture failure. Samples were processed using a whole-genome BAC platform with a resolution of about 1Mb across the genome and about 100 kb in 139 regions associated with constitutional disorders. In high risk groups (with abnormal ultrasound findings and fetal karyotype) the percentage of detection was 5.8% (7/120). In low risk groups the percentage was much lower: 0.5% (6/1118) in advanced maternal age and 0.7% (11/1674) in parental anxiety. No genetic imbalances were detected in any of the cases sampled for an abnormal maternal serum screening, nor for a family history of a genetic condition or chromosomal abnormality. A total of 24 (0.8%) fetal conditions would have remained undiagnosed if only a standard karyotype had been performed. About 17 (0.6%) of such findings would have otherwise been overlooked if CMA was offered only to high risk pregnancies.

Shaffer *et al*^[27] reported a study on 5003 prospective cases received from 2004 to 2011 for a variety of indications. The overall detection rate of clinically significant copy number alterations (CNAs) among unbiased, non-demise cases was 5.3%. Detection rates were 6.5% and 8.2% for cases referred with abnormal ultrasounds and fetal demise, respectively. The overall rate of findings with unclear clinical significance was 4.2% but would reduce to 0.39% if only *de novo* CNAs were considered. In cases with known chromosomal rearrangements in the fetus or parent, 41.1% showed CNAs related to the rearrangements, whereas 1.3% showed clinically significant CNAs unrelated to the karyotype. Finally, 71% of the clinically significant CNAs found by microarray were below the resolution of conventional karyotyping of fetal chromosomes.

In a comparative study of currently available methodologies for detection of chromosomal abnormalities after invasive prenatal sampling^[28], a multicentric collection of a 1-year series of fetal samples with indication for prenatal invasive sampling was simultaneously evaluated using three screening methodologies: (1) karyotype and QF-PCR; (2) two panels of MLPA; and (3) microarray-based analysis with a targeted BAC microarray. A total of 900 pregnant women provided informed consent to participate (94% acceptance rate). Technical performance was excellent for karyotype, QF-PCR, and GMA (about 1% failure rate) but relatively poor for MLPA (10% failure). Mean turn-around time was 7 d for microarray or MLPA, 25 d for karyotype and 2 d for QF-PCR, with similar combined costs for each approach. A total of 57 clinically significant chromosomal aberrations were found (6.3%), with microarray yielding the highest detection rate (32% above other methods). The identification of variants of uncertain clinical significance (17, 1.9%) tripled that of karyotype and MLPA, but most alterations could be classified as likely benign after proving they were inherited.

Breman *et al*^[29] evaluated the results of prenatal microarray analysis on > 1000 fetal samples referred for testing and compared these data to published reports. Clinically significant CNVs were observed in 85/1115 cases (7.6%) overall, and in 45/1075 cases (4.2%) if 40 abnormal cases with known chromosome abnormalities or familial genomic imbalances were excluded. Eighteen of the 1115 cases had variants of unclear clinical significance (1.6%). Indications yielding the most clinically significant findings were abnormal karyotype/FISH (26/61, 42.6%), family history of chromosomal abnormality (13/137, 9.5%), abnormal ultrasound (38/410, 9.3%), abnormal serum screening (2/37, 5.4%) and advanced maternal age (5/394, 1.3%). Of 1075 cases having no previously known cytogenetic abnormality or family history, 18 (1.7%) had clinically significant genomic changes undetectable by conventional prenatal chromosome analysis.

In 2013 several papers reported experience on a small number of samples and tried to address the main issues in this field^[30-33]. Finally, Hillman *et al*^[34] quite recently reported a prospective cohort study of 243 women undergoing microarray testing alongside karyotyping when a structural abnormality was detected on prenatal USS. This cohort is presented in the context of a systematic review and meta-analysis of the literature defining overall detection rates by microarray over karyotyping. When clinical indication for testing was abnormal fetal USS their cohort study noted a 4.1% increase in detection rate; lower than the rate of 10% (95%CI: 8%-13%) by meta-analysis. The VOUS rate was 2.1% (95%CI: 1.3%-3.3%) when the indication for GMA was abnormal scan. The VOUS rate was 1.4% (95%CI: 0.5%-3.7%) when any indication for prenatal GMA testing was meta-analysed. The authors, suggest that GMA could have a higher detection rate not just in cases of abnormal scan findings but also with other indications for invasive testing, and conclude that it

is likely that microarray testing will replace karyotyping in high risk pregnancies.

GUIDELINES

The accumulated evidence from many studies applying GMA together with chromosomal analysis in PD, demonstrate that there is improved detection of clinically significant genome imbalances when using GMA; proving the usefulness in using this technique in a PD setting. However, several issues remain to be addressed before implementing CGH-array in PD, such as: (1) in which pregnancies should CGH-array be carried out, whether for all pregnancies or only for pregnancies with ultrasound abnormalities; (2) which array platform to use; (3) an appropriate calling criteria must be established; (4) which confirmatory methods to use for the CGH-array findings; and (5) pretest counseling^[30].

Scientific societies have joined the discussions regarding microarray-based technologies in PD.

The American College of Obstetricians and Gynecologists stated that, although CGH-array has distinct advantages over classic cytogenetics in certain applications, the technology is not currently a replacement for classic cytogenetics in PD^[35].

The Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada and the Prenatal Diagnosis Committee of the of the Canadian College of Medical Geneticists make three principal recommendations: (1) Array genomic hybridization is not recommended in pregnancies at low risk for a structural chromosomal abnormality; for example, advanced maternal age, positive maternal serum screen, previous trisomy, or the presence of “soft markers” on fetal ultrasound; (2) Array genomic hybridization may be an appropriate diagnostic test in cases with fetal structural abnormalities detected on ultrasound or fetal magnetic resonance imaging and could be done in lieu of a karyotype if rapid aneuploidy screening is negative and an appropriate turnaround time for results is assured; and (3) Any pregnant woman who qualifies for microarray genomic hybridization testing should be seen in consultation by a medical geneticist before testing so that the benefits, limitations, and possible outcomes of the analysis can be discussed in detail. The difficulties of interpreting some copy number variants should also be discussed. This will allow couples to make an informed decision about whether or not they wish to pursue such prenatal testing^[36].

The European Society of Human Genetics stated that arrays were of proven value for investigation of fetal abnormalities and encouraged the establishment of local guidelines for the use of genome-wide array analysis in the prenatal setting. The most important recommendations helpful when establishing local or national guidelines are: (1) Establish the indications for the use of genome-wide array analysis in the prenatal setting; (2) An array platform with a minimal resolution of 200 kb is recommended; (3) Laboratory specialists should have suf-

ficient experience with the interpretation of array results; (4) Parental blood sampling is highly recommended; (5) Pretest counseling, including providing written information and parental consent are a prerequisite; (6) The laboratory and the clinicians should agree on what to report and what not to report before offering array diagnostics; and (7) There should always be optimal communication between the laboratory specialists and the clinicians^[37].

The cytogenetics working group of the Italian Society of Human Genetics (SIGU) recommended the use of CMA in prenatal testing: (1) never as a substitute for conventional karyotyping; (2) for specific diagnostic purposes in selected pregnancies and not for general screening in all pregnancies; and (3) only in prenatal cases with specific indications, such as: (a) single (apparently isolated) or multiple sonographic fetal abnormalities; (b) *de novo* chromosomal rearrangements (even if apparently balanced) detected by standard karyotyping to investigate the possible presence of cryptic imbalance(s) related to the structural chromosome abnormality; and (c) supernumerary marker chromosomes in order to characterize their origin and genetic content^[38].

CONCLUSION

The amount of information that can be obtained from the human fetus is growing at a remarkable rate. Although the times when the fetus was regarded as a mysterious object are long gone, the concept that the fetus is a genetically distinct entity from the pregnant woman and that can be studied on an individual basis is fairly recent.

For both clinical and technical reasons, PD has always focused on chromosomal disorders, which represent a very important cause of prenatal morbidity and mortality^[39]. The standard cytogenetic techniques have been used for many years for the diagnosis of chromosomal defects, accompanied in recent years by molecular cytogenetic techniques. In the postnatal field, other techniques such as those based on microarrays have been proposed as a first level test in children with ID and MCA^[9].

Microarray-based techniques such as CGH-arrays and SNP-arrays allow detection of very small genomic imbalances (at the level of genes and even exons) that can determine pathological clinical conditions^[40-42]. In addition to these pathogenetic CNVs, there are other CNVs which represent normal variations, without negative effects on the phenotype. Moreover, many CNVs are associated with variable expressivity and incomplete penetrance, leading to a difficult prevision of the phenotype. Given the still incomplete knowledge of the so-called “variomat” (the set of all the changes in our genome) and the associated phenotypes, microarray-based testing is likely to identify variants of uncertain and unknown clinical significance. The interpretation of these variants is a challenge for medical geneticists, who often find it difficult to establish precise correlations between genotype and phenotype.

These difficulties, already significant in a postnatal context, become critical in the prenatal setting, where the

fetal phenotype is difficult to explore and where there are huge dilemmas regarding the advice to be given. For these reasons, despite increasing interest in applying these techniques in PD, their actual use is as yet not widespread. The use of microarray-based techniques in PD is currently a topic of much debate, between supporters of the technology and its application and those that recommend a more cautious approach.

One of the most important issues concerns the pregnancies to be considered for this test. Is it appropriate and convenient to apply the routine examination by microarray in all pregnancies that are subjected to invasive PD or it is better to restrict their use to pregnancies that have particular characteristics, such as the presence of fetal ultrasound abnormalities? The currently available data does not support the implementation of these methods in low-risk pregnancies; however, under other conditions they are clearly advisable, as indicated in the Position Statement of the cytogenetic working group of the SIGU^[38].

Moreover, the number of chromosome abnormalities not detectable by microarray analysis suggests that microarray technology should remain a complementary analysis and not a replacement for current PD tests^[43].

Regarding the choice of platform, there have been no systematic studies to identify a specific platform most suitable for PD. The difficulty lies in finding the appropriate resolution, which must be high enough to detect small imbalances (already identified as a possible cause of disease patterns) but not so high as to generate large numbers of CNVs of uncertain significance. To address this problem, some groups have opted for the use of targeted platforms, which show only well-characterized imbalances linked to specific clinical situations. Although this approach avoids many of the problems presented to the examiner, it severely limits the diagnostic power of the technique (one of the main arguments for its introduction). In addition, the knowledge of CNVs is rapidly expanding and ever new microdeletion/microduplication syndromes are being discovered and described. This would involve a continuous update of the targeted platforms, which is practically unfeasible. A fair compromise could be represented by the platforms with an acceptably high (but not overly high) resolution of the entire genome (at least 200 kb) with a greater number of probes in regions of particular clinical interest^[37,44].

While oligonucleotide arrays with high-density exonic coverage remain the gold standard for the detection of CNVs, SNP-arrays allow for detection of uniparental disomy and consanguinity, while also providing a higher sensitivity in detection of low-level mosaic aneuploidies^[11]. Moreover, SNP-arrays allow identification of polyploidies and chimerisms.

There is increasing interest in the use of mixed platforms (oligo-SNP), which combine the advantages of the oligonucleotides in terms of diagnostic accuracy of CNVs, with those of the SNPs. These platforms deserve a thorough evaluation on a large number of cases and may become the best choice for PD.

Another important point to consider is the information process. PD is an extremely delicate issue, and any defect in communication between doctor and patient can produce very serious problems. In particular, genetic counseling relating to the examination by microarray in a prenatal environment is difficult and represents a challenge for even the most experienced geneticist. Therefore, there is a clear need for specific training and draft guidelines that will help to improve and standardize the professional standards in this sensitive area.

In conclusion, genomic rearrangements represent an important aspect of human pathology and the application of microarray-based techniques for diagnosis is likely to continue growing in significance. Given the undeniable advantages of these techniques over conventional cytogenetics, there is an increasing pressure towards their application in PD. However, introduction of these technologies into clinical practice should proceed with caution and be offered only by experienced laboratories and after proper validation, showing robust, reliable and reproducible results^[26]. While there is sufficient evidence in the literature to recommend the use of these technologies in specific conditions, it is important to avoid an unchecked drift towards widespread use driven by commercial interests. It is critical that application is tightly regulated and that scientific societies remain vigilant and participate in the decision making process.

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