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Evolving Applications of Microarray Analysis in Prenatal Diagnosis

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Abstract

Purpose of review—Evaluation of copy number variation by microarray analysis has significant advantages over standard metaphase karyotyping and is quickly becoming the primary means of postnatal genetic evaluation for neonates and infants with dysmorphic features or cognitive difficulties. Before this technology is routinely used for prenatal diagnosis, further evaluation of its value and the clinical dilemmas it may introduce requires further study. This article reviews the recent literature on array technology use in prenatal diagnosis.

Recent findings—The use of microarray analysis for routine prenatal diagnosis is still being investigated. Use in certain prenatal situations such as the fetus with structural anomalies or those who are stillborn appears to add important, clinically relevant information. There are a broad range of array designs available and recent research has focused on the appropriate design for prenatal testing. Patient counseling may occasionally be difficult because of the uncertain phenotype associated with some array findings.

Summary—We present a brief overview of microarray technology including benefits and limitations. Previous research regarding use of microarray in prenatal diagnosis including specific scenarios of anomalous fetuses and abnormal karyotype is reviewed. Current guidelines and the authors' recommendations are presented.

Keywords

microarray; aCGH; prenatal diagnosis

Introduction

Evaluation of copy number variation by microarray analysis has significant advantages over standard metaphase karyotyping and is quickly becoming the primary means of postnatal genetic evaluation for neonates and infants with dysmorphic features or cognitive difficulties. Use in certain prenatal situations such as the fetus with structural anomalies or those who are stillborn appears to add important, clinically relevant information. There are a broad range of array designs available and recent research has focused on the appropriate design for prenatal testing. Patient counseling may occasionally be difficult because of the

uncertain phenotype associated with some array findings. Before this technology is routinely used for prenatal diagnosis, further evaluation of its value and the clinical dilemmas it may introduce requires further study.

Basic Introduction to Arrays

Metaphase karyotyping is the current gold standard for prenatal cytogenetic analysis and is able to detect whole chromosome aneuploidy as well as deletions, duplications and other chromosomal rearrangements that are 5–10 megabases in size or larger. Microarray analysis (MA) has recently become available and is able to detect deletions or duplications that are 100 times smaller (in the kilobase range) than those identified on karyotype [1*]. It has now become well known that deletions and duplications too small to be detected by karyotype (submicroscopic) can be easily identified by MA and can cause significant anomalies. Many of these smaller changes occur frequently enough to have well characterized microdeletion or duplication syndromes (e.g. DiGeorge syndrome) while others may occur only sporadically but have equally significant phenotypic consequences.

As a group these cytogenetic alterations, whether visible or submicroscopic, are referred to as copy number variations (CNV's). CNV's are regions of DNA usually larger than a kilobase (1000 basepairs) in size that are present at an altered copy number in comparison with a normal reference genome [2]. For the autosomes, each segment of DNA should have 2 copies, one on each of the homologous chromosomes. Deletions result in the presence of only one copy and duplications, including whole chromosome trisomies, result in 3 copies. Many small CNV's are benign, some are disease causing, and yet others are of unknown significance. In general, alterations that are greater than 1MB, involve gene-rich areas and are de novo have the greatest likelihood of being disease causing [1*].

Comparative genomic hybridization (CGH) is a method for detecting deletions or duplications in the genome by directly comparing an unknown DNA sample to a normal sample and identifying those areas of the genome that are either under or over represented in the study sample. Therefore, only changes that cause deletions or duplications can be detected; point mutations, balanced translocations or inversions cannot be. For MA, DNA from a test sample and a normal reference sample are labeled with different fluorophores, mixed together, and hybridized to a glass slide printed with several thousand probes derived from most of the known genes and select non-coding regions of the genome. For any one probe, there should be equal amounts of DNA from both the patient and reference samples. Accordingly, the ratio of the fluorescence intensity of the test to that of the reference DNA is measured to determine the copy number changes for a particular location in the genome [2].

Array Coverage: Density/Backbone/List of Covered Regions

The probes on an array can be created using short 30 to 50 base pair (bp) segments known as oligonucleotides (oligos) which are constructed directly on the array slide. Alternatively, the probes can come from slightly larger DNA constructs (150–750bps) which are called BAC probes because they originate from bacterial artificial chromosomes. Rather than a single normal DNA sequence as a target, some arrays are now constructed using single nucleotide polymorphisms (SNP's).

Arrays can be differentiated by their probe composition. Targeted arrays are designed to maximize coverage of the parts of the genome that are known to be associated with phenotypic abnormalities and are intended to minimize findings of uncertain clinical significance. A targeted array can include as many or as few microdeletion and duplication syndromes as needed. Whole genome arrays screen the entire genome with the potential for over 6 million probes including both those known to be associated with specific disorders as well as the rest of the genome.

The density of the probes in both targeted and whole genome arrays can vary. For instance, arrays can be designed with varying numbers of oligos dispersed across the genome. These oligonucleotides will be spaced so that critical regions have a limited number of kilobases between oligos (thus detecting small deletions) and backbone regions have a greater distance between oligos. In this way, it is possible to customize arrays for a specific list of critical regions associated with disease and with specific backbone coverage so that deletions outside of the critical regions are not detected if they are smaller than a given size.

Construction of a Prenatal Microarray

The ideal construction of a prenatal array remains uncertain and is presently being evaluated in an NICHD funded study to be concluded this summer. As opposed to postnatal arrays used to evaluate infants with abnormal phenotypes, array designs for prenatal testing must minimize findings of uncertain clinical significance since the phenotype is incomplete or unpredictable in these cases and uncertain results can make counseling and parental decisions about pregnancy termination difficult. Presently, while not completely comparable, some guidance on the composition of a prenatal array is available from postnatal studies and a limited number of small series of prenatal cases.

Recent studies have suggested that 5–18% of children with multiple anomalies and/or developmental delay and a normal standard karyotype will have a disease causing CNV [3*, 4–10]. Because of this diagnostic advantage, it has been suggested that microarray analysis should become the first line approach to evaluating infants and children with suspected cytogenetic disorders [3*]. However, over 95% of these cases will identify benign or uncertain CNVs when a high density whole genome array is used potentially reducing their value in the prenatal setting [11].

Theoretically, low density targeted arrays may identify fewer CNV's of unknown significance and hence be preferable for prenatal diagnosis [12**]. However, the recent literature argues that whole genome arrays provide superior identification of clinically significant syndromes without increasing findings of unknown significance [13]. A further potential benefit of whole genome arrays is that the targeted regions may change as new syndromes are identified, so that constant editing of the array design will be required, whereas use of a whole genome array would reduce this need [14]. Further research and experience is needed before any design for routine prenatal use can be recommended.

Probe type may also vary between arrays. While oligo arrays may detect a significantly higher number of genomic alterations, BAC arrays have a quicker turnaround time, better

detection of mosaicism and have clear probes which allow for easy FISH confirmation [15]. Presently, it appears that oligo arrays are used most frequently for prenatal testing.

Current indications for Microarray Analysis in Prenatal Diagnosis

Microarray technology for prenatal testing is just transitioning into clinical care so that no well substantiated guidelines for its use exist. However, there is emerging information about its value in certain specific clinical scenarios.

Use of Arrays in Evaluation of Ultrasound Structural Anomalies

As with postnatal use of MA analysis, early prenatal experience has been in the evaluation of phenotypically abnormal cases. Overall, in fetal samples referred for standard indications including advanced maternal age, family history, ultrasound anomalies, etc., clinically significant CNV's, including those also seen on standard karyotype analysis, are identified in approximately 5–6% of cases with results of unknown significance occurring in approximately 1%–1.5% of these [16*, 17–18**]. When a fetal structural anomaly is present in these cases, MA will have a detection rate of approximately 1–3% beyond that of karyotype [16*,19,20]. The frequency of microarray specific findings in sampled pregnancies not having an ultrasound identifiable anomaly is not certain. Presently, based on expert opinion, in cases of anomalies identified on ultrasound, MA is recommended to identify microdeletion or duplication syndromes [1*,14].

Whether certain anomalies are more likely to be secondary to microdeletion or duplication is also being investigated. To date, the most common anomalies investigated have been cardiac, central nervous system, skeletal, urogenital, and renal. Increased nuchal translucency measurements and intrauterine growth restriction (IUGR) have also been evaluated [14,19,20]. These evaluations confirm the incremental value of MA compared to conventional cytogenetics or FISH [16*].

The potential of MA in evaluating fetal structural anomalies is illustrated in neonatal series [19]. Lu *et al.* demonstrated that 17.1% of 638 neonates with structural anomalies had CNVs. Of these, only 2.5% were whole chromosome aneuploidy. Of the remaining 93 anomalous neonates with genomic imbalances, 37 were well known common findings, 44 were rare findings and 12 had mosaic results.

Delineating Marker Chromosomes

Arrays are also useful in interpreting karyotype findings, including marker chromosomes. The phenotype associated with a marker chromosome varies depending on the origin of the marker chromosome and its gene content. Empirically, non-satellited markers have approximately a 15% risk of an abnormal phenotype whereas a satellited marker risk is approximately 11%. Similarly, whether or not the marker is from an acrocentric chromosome or contains heterochromatin versus euchromatin will modify the empiric risk. The characteristics, origin and content of marker chromosomes are not always fully determined by conventional cytogenetics. FISH can help identify their origin, however, prior knowledge of the region involved is required [21*]. MA has the ability to specifically

identify the region involved and the gene content. While further experience is required to confirm the value of arrays in evaluating these cases, initial experience has demonstrated the potential value [16*].

Reciprocal Balanced Translocations

At present, the likelihood of phenotypic sequelae being associated with an apparently balanced de-novo reciprocal translocation on a prenatal karyotype is based on empiric data and is approximately 6%. This residual risk exists because the breakpoints are located within a gene disrupting its function or alternatively there is a submicroscopic imbalance unable to be identified by conventional karyotype. Recent reports have suggested that in up to 40% of individuals with apparently balanced rearrangements and phenotypic abnormalities, an imbalance involving one of the breakpoints will be identified by MA [22]. This high rate of imbalance has not been confirmed in prenatal cases. De Gregori *et al.* reported 14 balanced rearrangements (2 with ultrasound abnormalities) identified from prenatal samples with no imbalances detected by array. At present, it is uncertain whether array evaluation of these rearrangements will be clinically valuable. Although it seems likely that confirming that a translocation is balanced by MA should reduce the residual risk and identifying an imbalance should increase it, further studies are necessary to confirm this.

Use in the Evaluation of Stillborn Infants

About 5% of structurally normal stillborn fetuses will have an abnormal karyotype as will 35–40% of stillbirths that are structurally abnormal or macerated. These numbers are likely to be underestimates since the quality of the karyotype in these cases is frequently poor and tissue culture is successful in only about 50% of cases; less in those with anomalies or maceration. Recently, Raca *et al.* reported on the successful use of MA in the analysis of 15 phenotypically abnormal stillborns in which standard karyotyping was unavailable. In 13% MA revealed an abnormality [23].

Benefits of Microarray in Prenatal Diagnosis

The primary advantage of MA over conventional karyotyping is the ability of an array to interrogate the genome at a higher resolution and therefore, array should have a higher sensitivity for identifying clinically significant abnormalities. This has already been illustrated in the prenatal setting in fetuses with structural anomalies including IUGR and increased nuchal translucency. It has also been demonstrated that MA can improve the genetic evaluation of balanced translocations and marker chromosomes which most likely will improve the ability to predict the phenotype.

Another important issue to consider is the turnaround time from prenatal sample collection to result availability. Currently, it takes 1–2 weeks for a karyotype result to become available, as cells must be cultured and scanned. For MA, cell cultures are not necessary since there is sufficient DNA in most villus and amniotic fluid samples from direct isolation. This is particularly true for analysis of stillborns in which cell culture may not be possible. In addition, microarray scanning is automated and does not require the same time and effort as scanning slides for karyotypes. Thus, turnaround times will be much shorter.

Limitations of Microarray

As is the case with karyotyping and other technologies, microarray has limitations for prenatal testing. One of the most frequently mentioned limitations is that MA will not detect truly balanced translocations. While this may not be clinically relevant for most prenatal cases, the knowledge of a translocation may provide important information for future generations and other family members. Also, some de-novo translocations may be balanced yet result in phenotypic consequence due to gene disruption. These cases would be missed. Standard MA will also not identify polyploidies since the relative gene content is balanced. Preliminary work suggests that use of SNP data, if present on an array, will identify these. Also, maternal cell contamination studies should reveal the majority of these. In both of the above instances, a karyotype performed in addition to the MA would solve these problems but whether this is cost effective is uncertain.

Although microarray is more sensitive than karyotype for detecting small gains or losses, it does not always determine the exact size and location of the added or lost segments. Microarray has also been criticized for not being able to detect low-level mosaicism. However, a recent study by Hoang *et al.* found that oligonucleotide array was able to detect mosaicism of 10% or greater and in some cases was able to detect mosaicism previously undetected by karyotype [24]. It should also be remembered that standard karyotyping in which 15 to 20 cells are counted will only detect about 30–40% mosaicism with 95% certainty. While it is clear that neither MA nor karyotype will identify all cases, the importance of such low-level mosaicism when found prenatally in an unselected population remains uncertain.

Another limitation of MA is the difficult counseling required when results of unknown clinical significance are discovered. As additional experience with MA is obtained, these should become much less frequent as many of these findings will be able to be reclassified as either benign or pathogenic. However, as with all prenatal testing, some findings will always be difficult to interpret because of their variable expressivity or incomplete penetrance. At present, a worldwide consortium (International Standards for Cytogenomic Arrays; ISCA) is collecting array findings and associated phenotypes and organizing them into a database within NIH.

Current Guidelines

The American College of Medical Genetics has recently published guidelines that specifically endorse using arrays in cases where individuals show "multiple anomalies not specific to a well-delineated genetic syndrome [such as] non-syndromic developmental delay and intellectual disability and autism spectrum disorders." The guidelines recommend the use of arrays in other cases as well [25]. These include evaluation of the children with growth retardation, speech delay, and other less-well studied indications. These recommendations confirm the value of arrays in postnatal evaluation but do not give guidance for prenatal testing

The American College of Obstetrics and Gynecology (ACOG), suggests that the usefulness of microarray as the first line test for prenatal evaluation of chromosomal abnormalities

remains unknown and conventional karyotyping remains the primary cytogenetic tool. However, targeted arrays in combination with genetic counseling can be offered in the setting of an abnormal ultrasound finding and a normal karyotype result. In addition, it can also be offered in cases of fetal demise with congenital anomalies when conventional karyotype is unobtainable. ACOG stresses that couples who choose to undergo targeted MA receive both pre- and post-test genetic counseling. Couples should follow up with their genetic counselors and discuss the results. They should understand that microarrays cannot detect all genetic pathologies and that a result of unknown clinical significance may cause anxiety. ACOG acknowledges that further studies are necessary to fully determine the clinical use of microarrays in prenatal diagnostics [26*].

Genetic Counseling for Prenatal Microarray Analysis

Perhaps the most difficult aspect of prenatal microarray use is the counseling dilemmas that can result. Generally, the microdeletion and duplication syndromes associated with severely abnormal phenotypes require counseling identical to abnormal karyotype results. Fetuses discovered to have less severe or later onset diseases, (e.g. Charcot Marie Tooth Type 1A), may present a slightly more complicated counseling session. Where counseling is most difficult is when results are of unknown significance such as those involving array findings not previously reported. Counseling is equally difficult for array findings that may have a large spectrum of phenotypic consequences, show variable expressivity, or have reduced penetrance (e.g. the relatively frequent 22q11.2 deletion). In these cases, it may be helpful to determine if the array finding is inherited or de novo, although inheritance from a mildly or unaffected parent does not assure a similar fetal phenotype. One example of a particularly difficult result would be 16p13.11 deletions where both normal individuals and those with mental retardation, autism, seizures and schizophrenia have been found to have this deletion. There may also be affected children from an unaffected parent even when both carry the same deletion. Because of these dilemmas, comprehensive pre and post-test genetic counseling is imperative in the use of prenatal microarray analysis.

Resources

Genome browsers such as the UCSC browser, DECIPHER, ISCA, dbVar, dbGaP, the European Cytogenetics Association Register of Unbalanced Chromosome Aberrations and Ensembl can help navigate which genes are included in the deleted/duplication region of an abnormal result. As described above, dbGap will be hosting the database of CNV's through ISCA. Groups such as Unique (rarechromo.org) can help as resources for patients and families of affected pregnancies.

Conclusion: The Authors' Views

The role of arrays in prenatal testing is evolving. It is already clearly demonstrated that MA has value in the evaluation of fetal anomalies identified by ultrasound and in the investigation of abnormal karyotype results. There use in the evaluation of stillbirths also seems to be appropriate for clinical care. In this authors opinion it seems highly likely that MA will become the first tier test for all prenatal testing including structurally normal

pregnancies. However, caution is needed in transitioning the use of MA into clinical care. Thought and study must be given to identify appropriate patient education and counseling. Equally important is the selection of the appropriate array design for prenatal testing. Such a design must balance the identification of pathologic imbalances against the number of uncertain and difficult to interpret results. Perhaps, more than one array choice will be required based on clinical findings and patient preferences. For instance, for patients who only want information on severe disorders, a low density targeted array would be appropriate. For those patients with anomalies and those who want all available information, a whole genome array would be preferred. Regardless of which array type is chosen, it has become clear that pre and post test counseling regarding both normal and abnormal results is warranted.

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Key Points

- Evaluation of copy number variation by microarray analysis has significant advantages over standard metaphase karyotyping and is quickly becoming the primary means of postnatal genetic evaluation for neonates and infants with dysmorphic features or cognitive difficulties.
- While it seems likely that microarray will become standard of care for prenatal testing, further evaluation of its value and the clinical dilemmas it may introduce requires further study.
- Pre and post test counseling are imperative whenever prenatal microarray testing is performed.