Clinical implementation of chromosomal microarray technology in prenatal diagnosis (Review)

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Abstract. Chromosomal microarray technology represents the technical convergence of molecular genetics and cytogenetics, and is rapidly revolutionizing modern cytogenetics. Expected genomic aberrations are accurately identified and provide readily interpretable results that are suitable for clinical risk stratification and therapeutic strategies. The application of array technology in prenatal genetic diagnosis provides distinct advantages over conventional cytogenetic analysis in detecting both the majority of microscopic and submicroscopic chromosomal abnormalities. In the last few years, the validity of array technology has become obvious to medical and laboratory communities involved in prenatal diagnostic testing. However, whether or not microarray analysis is sufficient for the detection of cytogenetic abnormalities in prenatal diagnosis and if traditional cytogenetics continue to be important in this new era has yet to be confirmed. In the present study, we systematically reviewed the current status of microarray technology in the identification of pathogenic genomic imbalances and discussed practical considerations for its routine implementation in prenatal diagnosis.

Contents

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1. Introduction

The development of the bacterial artificial chromosome (BAC) system was partly developed through the Human Genome Project with a view to construct genomic DNA libraries and physical maps for genomic sequencing (1). The presence of BAC clones has become a valuable tool for identifying genomic imbalances in pregnancies to detect chromosomal abnormalities in at-risk fetuses.

The use of this technology has increased successful detection of risk-related abnormalities and provided an alternative for an enhanced level of screening for chromosomal abnormalities in high-risk pregnancies (2). Microarray-detected chromosomal abnormality rates are estimated to range between 5 and 17% in prenatal diagnosis, compared to normal results obtained from previous routine cytogenetic testing (3).

The implementation of array comparative genomic hybridization (CGH) in postnatal diagnosis has been thoroughly evaluated in the adolescent and adult population, and is now recommended as the first-line diagnostic test for clinically suspected genetic disorders (4,5). However, there are no available concise guidelines establishing the chromosomal microarray analysis (CMA) applications and platforms for a prenatal setting. The controversial question concerns whether or not CMA technology is likely to or should replace the standard karyotype in prenatal diagnostic practice and whether karyotyping and fluorescent *in situ* hybridization (FISH) remain essential.

In this article, we reviewed the current literature regarding array genomic hybridization in prenatal diagnosis and discussed the benefits and issues regarding the use of microarrays for the prenatal diagnosis of genetic diseases.

2. Conventional cytogenetic analysis

Cytogenetic analysis has provided fundamental insight into the molecular pathogenesis of prenatal diagnosis and has been a useful diagnostic tool for the identification of chromosomal abnormalities in at-risk pregnancies (3). Cytogenetic methods including karyotyping, FISH, CGH and multiplex-FISH or spectral karyotyping (SKY) have previously provided valuable diagnostic and prognostic information for the detection of genomic defects in prenatal diagnosis (6,7). Since the

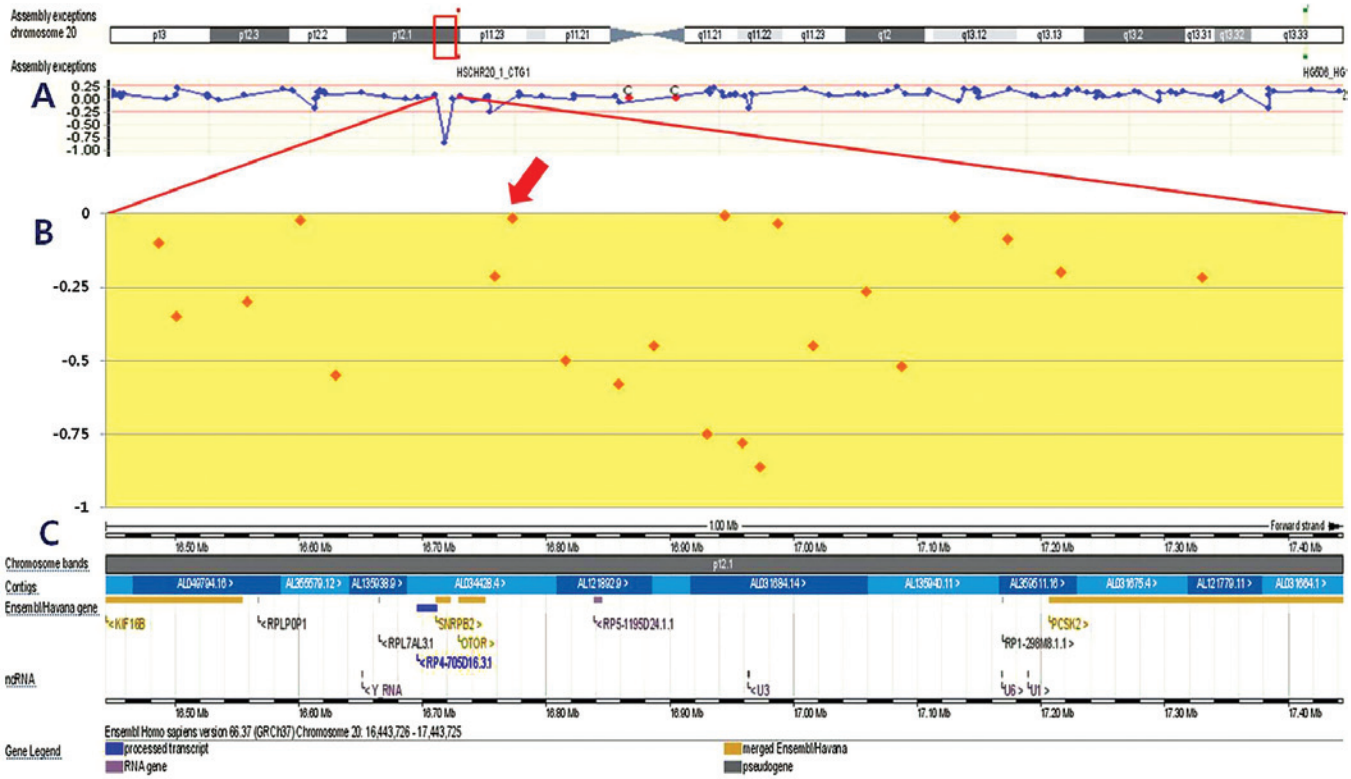

Figure 1. Chromosomal imbalance is detected by array CGH but not by G-banded karyotyping. (A) Increased resolution allowed for the sizing of the segment duplicated at the 20p12.1 region. (B) Arrow points to the close-up view of the duplication at 20p12.1. (C) Genes located within the deleted region (20p12.1) as shown by the UCSC genome browser.

development of chromosome banding techniques in the late 1960s, microscopic analysis has been used as the gold standard for prenatal diagnosis, while *in situ* hybridization methods have proven to be a useful and reliable technique for identifying and characterizing genomic imbalances. However, these conventional methods have technical limitations, thus resulting in the underestimation of the degree of chromosomal changes. In addition, these methods are also limited by their ability to detect individual DNA screening targets only rather than the entire genome. Furthermore, hidden mosaics, patients with uniparental disomy and complex patterns of meiotic crossing over led to chromosomal aberrations, none of which could be detected by standard cytogenetics or comparative CGH methods. In order to detect such abnormalities, a high-resolution technique is required.

3. Chromosomal microarray analysis

CMA circumvents the limitations of conventional cytogenetic techniques. It simultaneously evaluates regions across the entire genome with a higher resolution and an excellent throughput in patients with suspected genome imbalance. This method accurately identifies novel genomic aberrations of possibly uncertain clinical significance not described previously and provides readily interpretable results, suitable for clinical risk stratification and treatment planning (8) (Fig. 1).

The principle behind the array CGH technology is the detection of chromosomal deletions and duplications by comparing equal amounts of genomic DNA from a patient and a healthy control (9). In the array CGH, the two genomes (patient and control) are labeled and co-hybridized onto a glass microscope slide, on which cloned DNA segments have been immobilized (10). The analytic principle involves competition between a differentially-labeled fragmented test and a control diploid DNA, with imbalances due to copy number changes in the test DNA resulting in a shift in the fluorescence spectra (11).

The evaluation is performed by a scanner and the information is then computer-integrated to determine any quantitative deviations in the DNA of the test sample. The primary advantage of CMA is the enhanced detection of copy number anomalies: the deviations that are measurable by molecular means are orders of magnitude smaller than those detectable by light microscopy (12). Common protocols for the application and interpretation of genomic arrays in prenatal diagnosis are capable of decreasing the risk of unexpected findings.

4. Clinical utility of CMA in prenatal diagnosis

Array technology is rapidly taking over cytogenetic laboratories, resulting in ability for greatly improved visualization and validation. The increased diagnostic potential of the microarray has naturally led to the need for its use in the prenatal setting. In recent years, the application of microarray-based genomic copy number analysis has proven to be beneficial, allowing for proper counseling and providing the parents with all the tools for a conscious decision regarding their pregnancy.

There are several studies available aiming to assess the diagnostic ability of array CGH in the screening of hidden chromosomal aberrations in prenatal genetic diseases with an apparently normal karyotype (13-15). Depending on the

ascertainment criteria and the level of resolution achieved in the cytogenetic assessment, the microarray prevails in the detection of copy number anomalies, by identifying pathogenic abnormalities in up to 16% of fetuses with an abnormal ultrasound and normal karyotype (13).

In the study by Vialard *et al* (14), array CGH diagnosed two *de novo* unbalanced karyotypes and four additional abnormalities that could not be identified with conventional cytogenetic methods in classic microdeletion syndromes and subtelomeric rearrangements in 39 fetuses with multiple congenital abnormalities after the pregnancy was terminated. A previous analysis of eight prenatal studies using the array technology from various platforms also concluded that array CGH detected a 3.6% increase in genomic imbalances when conventional karyotyping was normal, regardless of the indication for referral. When the referral indication was abnormal in the ultrasound, this percentage increased to 5.2% (13).

More recently, a Canadian study using array CGH, has demonstrated the identification of an additional 8.2% of positive diagnosis in 49 fetuses with major malformations that were not visible with karyotyping (12). In the experiment of Le Caignec *et al*, (16) the array platform detected all cytogenetic abnormalities previously analyzed by G banding and revealed new rearrangements in 7-10% of the cases from *chorionic villus* culture in 41 products of conception. Emerging data from D'Amours *et al* (17) also reported a relatively high percentage of findings of unclear clinical significance in 12.2% of the tested fetuses. These observations demonstrated that the potential of the array CGH to reveal the cryptic and/or complex nature of chromosome arrangements otherwise undetectable by chromosome analysis markedly increases the elucidation of prenatal genetic diseases.

Additional cases have also emphasized the importance of further investigation on microarray technology since other imbalances underlying more serious consequences may be present. Maitz *et al* (18) reported a characteristic case concerning a 21-week gestation fetus with a complex congenital heart defect and no other ultrasound abnormalities. The karyotype carried out by conventional cytogenetic analysis was normal. FISH analysis by the Di George/VCFS probe, combined with a control probe mapping to the 22q13.3 region (ARSA) was performed, excluding the 22q11.2 deletion and showing only one signal from the ARSA locus. Microarray analysis demonstrated that a 6.5 Mb interstitial deletion was in fact present at 22q13.3, leading to hemizygosity in several genes (19). Findings of a similar study by Wat *et al* (20) also demonstrated that the high frequency of cardiac and diaphragmatic defects associated with 8p23.1 interstitial deletions that were detected by microarray analysis were not identified by conventional chromosome analysis. These findings prove that in isolated ultrasound heart abnormalities and a normal karyotype, FISH analysis is not adequate, and should therefore be substituted by microarray analysis.

Considering the advantages and the lack of additional risk of array CGH for the patients, it is reasonable to suggest that this test be offered to all women already undergoing invasive testing (21). In the study conducted by Van den Veyver *et al* (2), only 4 (22%) of 18 abnormal prenatal array CGH cases had abnormal ultrasound findings as the sole indication for testing, suggesting that testing should not be limited to pregnancies with known abnormal ultrasound findings. Wat *et al* (20) also suggested that array CGH be performed on all prenatal cases with congenital cardiac and/or diaphragm defects. When offered to choose between karyotyping and array CGH, 74% of the parents chose the latter method (21).

Another crucial instance requiring the application of microarray is the presence of a *de novo* reciprocal translocation or a *de novo* supernumerary marker chromosome in the fetal karyotype (22). Previous studies (23-25) demonstrated that cryptic deletions are present either at the translocation breakpoints or elsewhere in the genome in approximately 40% of the *de novo* reciprocal translocations detected in patients with phenotypic abnormalities, explaining the phenotype-genotype correlation. Since the breakpoints of the great majority of reciprocal translocations are non-recurrent, it is obvious that only the array platforms covering most of the genome have the potential to detect deletions associated with reciprocal translocations.

A high-resolution array platform covering the entire genome would therefore provide much more informative results than one containing only low coverage limited to prenatal disease-associated regions. Although microarray technology does not have the potential to detect polyploidy and balanced chromosomal rearrangements, these are relatively infrequent causes of abnormal phenotypes in a typical referral population. The frequency of pathogenic *de novo* reciprocal translocations due to the breakage of a dosage-sensitive gene or its long-range controlling region is extremely low (22), and polyploidy is almost always lethal during fetal life and is generally detected on ultrasound investigation (26). In case of such a suspicion, conventional karyotyping detects balanced chromosomal rearrangements. The majority of truly balanced translocations generate no phenotypic abnormality (27) and their identification leads to difficult clinical decisions during pregnancy. The American College of Obstetricians and Gynecologists (ACOG) (28) suggested that conventional karyotyping remains the principal tool for prenatal diagnosis and targeted arrays be offered as an adjunct in cases with abnormal prenatal anatomical findings and a normal conventional karyotype (21).

Given the potential described in this review, we anticipate the array CGH to be the initial prenatal diagnostic approach for the identification of chromosomal abnormalities in the near future. Although clinical application of array CGH as a universal routine test for genetic diagnosis is premature, further investigation may allow for an evaluation of the overall diagnostic yield of microarray technology over routine prenatal testing with conventional karyotype, as well as cost effectiveness (29).

5. Conclusions and final considerations

In this review, we presented the potential utility of array CGH for the detection of chromosomal abnormalities in prenatal diagnosis. This new platform, with its potential to decrease the risk of unexpected findings, is likely to be the first-line test for detecting chromosomal abnormalities in prenatal diagnosis.

In order to reach a consensus regarding the optimum configuration of an array, additional investigations carried out on large-scale populations that have undergone both karyotyping and a commercially reproducible array are required.

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