Microarray-Based Prenatal Diagnosis in Developing Countries

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Abstract: Detection of chromosomal alterations in prenatal diagnosis requires speed and precision, imposing the development of high-throughput screening methods. Thus the array CGH technology, used to diagnose genomic rearrangements in children with multiple congenital anomalies, idiopathic mental retardation and developmental delay, caught the attention of specialists worldwide who are now trying to implement it in routine prenatal practice in order to obtain rapidly useful information for a more accurate genetic counseling. Array comparative genomic hybridisation (Array CGH) has the capacity to detect genomic rearrangements at a higher resolution than other methods used in prenatal testing, like standard G banding, multiplex ligation dependent probe amplification (MLPA) or fluorescent in-situ hybridization (FISH), being able to detect chromosomal imbalances smaller than 3 Mb. Despite all these evidences that prove its utility in prenatal diagnosis, this method is not used worldwide either because of lack of experienced specialists in interpreting array results or because, especially in developing countries, the national health insurance systems does not settle the high cost of this analysis and patients cannot afford it.

This paper is a summary of recent progresses made using the array CGH technology in prenatal testing to detect if genomic imbalances are involved in the development of congenital malformations. Our aim is to reveal the importance of using advanced technologies like array CGH in routine prenatal practice, and thus to contribute to its implementation in developing countries like ours.

Keywords: Array comparative genomic hybridization, prenatal screening, prenatal diagnostic, conventional G-banding, fetal karyotype.

INTRODUCTION

Prenatal diagnosis of genetic disorders is a complex and challenging medical area, requiring speed and precision in obtaining accurate clinical information that will be used in genetic counseling. Since 1966, worldwide the gold standard in prenatal cytogenetic diagnosis was metaphase karyotype analysis obtained by culturing fetal cells isolated from chorionic villus samples (CVS) in the first trimester or amniocentesis in the second or third trimester [1, 2]. Standard G-banded karyotype analyze needs between 10 – 14 days to offer a result and is able to detect only aneuploidies (chromosome number alterations) and structural abnormalities that are microscopically visible, for example loss or gain of genetic material (deletions or duplications higher than 5-6 Mb in size) and balanced or unbalanced translocations and inversions [3]. Thus, the small chromosome changes that are at the limit of resolution of light microscopy cannot be excluded, requiring the use of adjunctive methods like FISH, quantitative fluorescent polymerase chain reaction (QF-PCR) or MLPA to make a complete genetic assessment in prenatal diagnosis, with the risk to become more time-consuming, expensive and labor-intensive and so the appropriate case management will not be performed at time. Thereby, recently, array comparative genomic hybridization (array CGH), an amazing method used in postnatal clinical cytogenetics to detect genetic changes (submicroscopic chromosomal imbalances) responsible for multiple congenital anomalies, idiopathic mental retardation and developmental delay, spurred interest of specialists in fetal pathology now trying to optimize it’s application in prenatal diagnosis. Also, compared with conventional karyotyping, use of array-CGH technology is more advantageous due to a higher resolution that increases detection rates of chromosomal variations and it doesn’t require that fetal cells obtained by invasive prenatal procedures to be cultured in order to obtain results. This last aspect leads to faster results and can be useful in cases of fetal demise. Origin identification of small chromosomal markers which indicate a copy-number gain for that region can be also performed through array-CGH. Several studies showed that compared to conventional karyotyping, prenatal diagnosis with arrays reveals 8 – 35% extra diagnoses in abnormal ultrasound pregnancies [4-8].
Despite all the advantages described above, microarray technology has limitations like the incapacity to detect mosaicism below 20%. Also, it only detects abnormalities of those regions that are represented and there is a lack of uniformity between various platforms with different degrees of resolution, and thus require a basic understanding of the coverage and resolution of the array used for diagnosis. A major limitation of this method, especially in developing countries like those from East Europe, is represented by the high costs.

Array CGH technology, an automated and high-throughput analysis, is not yet used alone in prenatal genetic diagnosis and is recommended as an adjunct tool in prenatal cases with abnormal ultrasound findings and normal conventional karyotype.

OBJECTIVE

Although there are numerous evidences showing that array CGH can be used in prenatal diagnosis, not many specialists in fetal medicine from developing countries recommend it either because the costs are too high and both patient and health insurance system cannot afford to pay them or because array results are difficult to interpret (it detects copy number variants with no major phenotypic effect or uncertain clinical significance). The aim of this review is to collect data which would sustain that array CGH method might help gain new insights into genetic assessment of prenatal cases and may be used to obtain accurate information for genetic counseling and pregnancy management.

METHODS FOR REVIEW

We have conducted this review based on a search of PubMed, OxLIP+/SOLO (Bodleian Libraries) database (from 1997 to 2013) of relevant articles based on prenatal diagnosis by array CGH method and in their references as well.

RESULTS AND COMMENT

Array-CGH (Array-comparative genomic hybridization) is a genome-wide screening for copy number variations (it appreciates gains and losses of chromosomal material), using slides arrayed, containing thousands of base pair fragments of the human genome adhered on a solid support, such as a glass slide, in an ordered fashion [9]. This technique does not necessitate obtaining metaphase chromosomes, it uses small segments of DNA as targets for analysis [10]. Each individual DNA fragment, which is located in a specific position on the microarray slide, corresponds to a known DNA sequence that has been mapped to a specific chromosomal region [11]. The DNA extracted from a sample and the reference DNA from a normal control are labeled with two different fluorescent dyes and mixed together. In the next step the mix containing denatured DNAs is applied to a microarray slide in order to hybridize with the arrayed single-strand probes [12]. The information on the relative copy number of sequences in the sample will be provided based on the fluorescence ratio of the test and the reference hybridization signals [9]. Thus, the information derived from a single array-CGH experiment is equal to that obtained from thousands of FISH tests [9].

The resolution power is obtained by considering both probe size and genomic distance between DNA probes, being detected even copy number changes lower than 1 kb. These technological advances have increased test sensitivity and specificity, but, also they raised financial challenges and difficulties in results interpretation and clinical management of the pregnancies due to detection of copy number variants with uncertain clinical significance. Thus, as a result of microarrays increased development and widely application, multiple whole genome copy number variation (CNV) studies in normal populations [12] are emerging worldwide.

CNVs are segments of DNA of 1 kb or larger present at a variable copy number in comparison with a reference genome. It was reported that approximately 12% of the human genome exhibits CNV [13]. With such widespread CNV density, interpretation in a clinical setting can be challenging in terms of classifying variants as pathogenic, benign or novel variants of unknown significance (VUS). CNVs were described both in disease and normal conditions. The majority (>99%) of benign CNVs are inherited and most of them are less than 500 kb [14]. Usually de novo chromosomal imbalances are more likely to be clinically significant, while familial CNVs are less likely to have a clinical relevance. Several studies have shown the clinical relevance of inherited CNVs and therefore de novo origin of a CNV is not by itself a good indicator of its clinical relevance [15-17]. Despite the development of very useful CNV databases containing an increasing number of arrays from both affected and healthy individuals, the clinical significance of a substantial number of CNVs remains unknown.

Thus, detection of copy number variants with no major phenotypic effect or uncertain clinical significance makes the interpretation of array CGH results in
prenatal diagnostic much more challenging than in postnatal, management of such prenatal cases becoming extremely difficult due to the fact that these cases need a large amount of databases research and literature reviewing, in order to give an adequate genetic counseling.

On the other hand, array CGH is able to detect only “unbalanced chromosomal changes”. It is assumed that de-novo (no inherited) balanced chromosomal rearrangements (such as reciprocal translocations or insertions) may disrupt gains or losses at breakpoints [12]. However, in practice, many apparently balanced rearrangements detected by G-banding were not truly balanced in DNA level and microarray testing was used to detect small regions with loss or gain of genetic material and so to clarify the exact nature of the rearrangement.

Detection of gains or losses at the breakpoints of apparently balanced rearrangements requires microarray slides with probes that cover these breakpoints. Array CGH did not detect 69, XXX triploidy [18], as triploidy is associated with an extra copy of all chromosomes, therefore being no copy-number imbalance between the different chromosomes within the sample. The most significant current disadvantage of array CGH, beside costs, was identification of novel, previously unreported, VOUS. This raised occasionally many difficulties in counseling and in clinical management of cases. Thus the development of CNV databases did facilitate us the framing of VOUS into benign or pathogenic variants.

Recently publications seem to conclude that although array CGH has only a small advantage over karyotyping when employed for prenatal population as a whole [12]. It seems to be more advantageous to perform microarray method for cases with abnormal ultrasound results due to the probability of finding additional pathological CNVs in 1–3% cases compared to conventional karyotyping [4].

At the time of writing in developing countries, none of the prenatal genetic tests (conventional karyotype, FISH, QF-PCR, MLPA or array CGH) is free of charge for patient, through the Health Insurance Institution, the process of subsidize is still ongoing. Also, in developing countries, aCGH is a much more expensive technique than conventional G-band karyotyping and inaccessible in many of the non-university settings. However, the published detection rates are high and the costs of array CGH seems to decrease. As in developed countries [12], a comprehensive cost/efficiency study is required to investigate the additional cost per case identified using array CGH compared with G band karyotyping and whether this is a cost that would be acceptable by national healthcare services. Within this analysis the cost of follow-up testing would need to be included when a pathological CNV, a VOUS or an uncommon benign CNVs is detected. It could be possible that conventional G-banding followed by targeted FISH test addition to be comparable, or even more cost-effective than performing array CGH with the risk of VOUS. The financial assessment would need to take into account firstly the diminishing cost of array CGH and secondly the decreasing number of VOUS due to increasing international databases and settlement of robust internal mapping of benign CNVs [12] by large centers.

Literature searches revealed that prenatal diagnose follows a standard attitude in developing countries: screening by combined test. We considered as gold standard, performing CVS in all cases screened positive at the combined test or if major or minor structural anomalies were diagnosed through ultrasound at this age. In all cases where anomalies were identified, is offered a rapid test (FISH or QF-PCR) and/or a full G-band karyotyping. In addition, array CGH could be performed in selected cases, if conventional G-banded karyotype did not show any change.

Based on data from several studies, can be said that array CGH is useful for genetic diagnose of fetal anomaly found on ultrasound, with normal karyotype [19-21], but cannot currently be, at least for developing countries, a substitute for G-band karyotyping due to increased costs. In our view, array CGH should be used as an adjuvant in specific cases (sonographic fetal anomalies, apparently balanced chromosomal rearrangements or supernumerary marker chromosomes).

Thus, array CGH remains for developing countries a screening method, not a diagnostic tool because of costs that remain high at the moment, and if parental blood sampling is mandatory in the study, they will raise even more.

Although array CGH has distinct advantages in certain applications over classic cyto genetics but is not currently a replacement for classic cytogenetics in prenatal diagnosis. In our view, this technology has a complementary role in prenatal diagnosis, next to G banding. At present, array CGH represents the gold standard in cases with ultrasonic markers for chromosomal anomalies or structural abnormality and normal karyotype at conventional genetic tests [19-21].
Recent data indicate that array CGH technology recorded a dramatic drop down regarding costs of devices and software, allowing an increasing number of universities from different developing countries to use this technology. This reality gives the hope that soon will be obtained arguments for generalize ability and for convincing political bodies from developing countries like ours to make the investigation free of charge through health insurance, in selected cases.

REFERENCES


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