EXPERT REVIEWS

The future of prenatal diagnosis: karyotype, microarray or both? Technical and ethical considerations

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Prenatal diagnosis is now offered to high-risk pregnancies, including advanced maternal age, ultrasound anomalies and positive Down's syndrome screening, and karyotype on cultured fetal material is the test of choice to screen these pregnancies. However, microscope analysis can only detect gross chromosome abnormalities, highlighting the need for more sensitive techniques. It has recently been established that the higher resolution of microarray-based platforms can increase the diagnostic yield, offering more information to couples, and it is being discussed as a replacement to the standard karyotype. Conversely, the very high sensitivity of microarray-based analysis allows us to detect small microdeletions/microduplications (copy number variations) with unknown functional role and difficult genotype/phenotype correlation. In addition, the new copy number variation syndromes are often associated with variable outcomes, ranging from normal to severely affected individuals. This means that the microarray-based analysis introduced routinely in prenatal diagnosis needs to answer the question: are laboratory staff, clinical geneticists and counselors really experienced enough to manage these new scenarios?

Keywords: copy number variations • genetic test • karyotype • microarray-based analysis • prenatal diagnosis • uncertain clinical significance

Genomic DNA microarray platforms have been developed in the last decade to detect microdeletions/microduplications (copy number variations [CNVs]) throughout the whole genome with a higher resolution power with respect to the standard techniques [1]. The use of microarray analysis has proven to be an important tool to map relevant genomic regions associated to new, recognizable microdeletion/microduplication syndromes [2]. Therefore, this analysis is recommended as the first-tier genetic test in patients with congenital abnormalities, developmental delay, intellectual disabilities, autism and other conditions [3].

Given the higher analytical sensitivity, the application of this technique in prenatal diagnosis is still debated. The few available guidelines suggest a careful use of microarray analysis in prenatal diagnosis [4,5]. Microarray-based analysis is currently advised in pregnancies with anomalies detected by ultrasound or *de novo* structural chromosome rearrangements, although only after conventional karyotyping.

Conversely, its use in pregnancies with no known risk factors is more controversial, as the higher sensitivity associated with microarray analysis can detect even CNVs of uncertain clinical significance, leading to a dilemma in counseling those families [6,7]. Hereafter, the authors discuss one of the more extensive studies, which was performed on this topic by Wapner *et al.* [8].

Methods & results

The study by Wapner *et al.* was conducted in a blinded fashion, aiming to compare microarray analysis to conventional karyotyping in the detection of common aneuploidies, and to further evaluate the significance of additional clinical information produced by microarray analysis [8].

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The final goal of the study was to assess whether microarray-based analysis might replace conventional karyotyping even in normal pregnancies and to calculate the incremental diagnostic yield of microarray analysis as compared with conventional karyotyping.

Over a period of 3 years (2008–2011), Wapner *et al*. enrolled 4450 pregnant women undergoing prenatal diagnosis for common indications, including advanced maternal age, fetal abnormalities detected on ultrasonography and positive screening results. These categories are representative of the normal population attending prenatal clinics [8].

Microarray analysis was performed using either a customized oligonucleotide-based microarray (44K; Agilent Technologies, CA, USA) or a SNP array (6.0 GeneChip®; Affymetrix, CA, USA) with an effective resolution comparable to the oligonucleotide-based array.

Microarray analysis was mainly performed on uncultured samples and results were obtained in 93.2% of chorionic villus (CVS) and in 83.4% of amniotic fluid samples. Unsuccessful DNA extraction or failed microarray analysis was reported in 1.2% of samples. Samples with mosaicism found at karyotype were excluded. For these reasons, the comparison between conventional karyotyping and microarray results could only be performed in 4282 samples [8].

Microarray analysis identified all autosomal and sex-chromosome aneuploidies and all unbalanced rearrangements detected by standard karyotyping. Interestingly, microarray analysis performed on uncultured CVS revealed eight mosaic aneuploidies that proved to be homogeneous on long-term culture standard karyotyping.

As expected, microarray analysis did not detect any balanced rearrangements (0.93% of the present survey) or triploid cases (0.4%).

In 3822 fetuses with a normal karyotype, microarray analysis identified CNVs in 1399 samples. All CNVs not encompassing known pathogenic regions and/or disease genes were considered only when they had a size of >1 Mb. Among them, 88.2% were classified as common benign and 27.7% were considered as likely benign. Thirty five pathogenic CNVs were found in 35 fetuses (0.9%). CNVs of uncertain significance were found in 94 cases (2.5%) and were referred to the Clinical Advisory Committee which participated in the study. After discussion, 61 were classified as pathogenic. Overall, a microdeletion/duplication with clinical significance was found in 96 of the 3822 fetuses with normal karyotype (2.5%).

A stratified analysis found that 6.0% of fetuses with growth and/or structural anomalies and normal karyotype had clinically relevant results on microarray analysis.

In pregnancies with positive screening results and/or advanced maternal age and normal karyotype, clinically relevant CNVs were found in 1.6–1.7% of cases.

CNVs associated with autism and neurocognitive alterations were relatively prevalent in this cohort of samples. They were detected in 1.3% (51 of 3822) of karyotypically normal pregnancies: 3.6% with and 0.8% without structural anomalies.

Significance of the results

Data discussed by Wapner *et al*. indicate that microarray analysis is a reliable and time-saving technique that can be used to detect common chromosome imbalances and that it is comparable to conventional cytogenetics in detecting chromosome aneuploidies.

Of note, a discrepancy was found in eight out of 374 (2%) cases with aneuploidies, where the mosaic state detected by microarray analysis was not confirmed by conventional karyotyping. All these cases were derived from uncultured CVS. It is well known that CVS analysis should be based both on uncultured and cultured samples. Therefore, replacing karyotype with microarray analysis on uncultured CVS may increase the number of cases to be repeated, due to the possibility of genomic imbalances confined to extraembryonic tissues [9].

A major point to discuss is the authors' decision not to include the mosaic samples detected by karyotyping (58 out of 4391: 1.3%). In fact, the non-negligible percentage of these cases would have deserved a more in-depth discussion about the sensitivity of the microarray-based technique in identifying mosaicism.

The relevant number of chromosome abnormalities not detectable by microarray analysis, such as balanced rearrangements (0.9%) and triploidy (0.4%), clearly suggest that microarray should be intended as a complementary analysis and not a replacement test in prenatal diagnosis.

Wapner *et al*. confirmed that microarray-based analysis is a useful technique to be used in pregnancies with ultrasound anomalies, with an incremental yield of approximately 6%, consistent with previous reports [10,11]. A major concern is represented by the CNVs detected in 1.6–1.7% of low-risk pregnancies. Many of those CNVs, considered a risk factor for neurodevelopmental conditions, are associated with variable expressivity and incomplete penetrance, leading to an unpredictable phenotype.

Finally, microarray analysis detected CNVs with uncertain clinical significance in 1.5% of pregnancies. This represents the final rate after stringent selection of found CNVs and a detailed revision of all available clinical and molecular data performed by the Clinical Advisory Committee. That figure is comparable to previous reports [12], suggesting that it will not be further reduced, depending on the current knowledge on the human genome structure and function.

Expert commentary

The usefulness of a diagnostic test revolves around its ability to reduce the diagnostic uncertainty. Although microarray analysis is undoubtedly characterized by higher analytical sensitivity, other factors should be taken into consideration while evaluating the performance of a genetic test in a clinical setting and clinical utility should be prioritized over maximum sensitivity. Moreover, other than analytical validity (analytical sensitivity, analytical specificity, quality control, robustness) and clinical validity (clinical sensitivity, clinical specificity, negative and positive predictive values, prevalence, penetrance), ethical, legal and social implications should carefully evaluated [101].

Wapner *et al*. introduced the novel definition of 'potential of clinical significance' of a test result. The clinical significance of a

test hinges upon its practical consequences on the decision-making process, and should be discussed in terms of meaningfulness, validity and usefulness.

The authors believe that Wapner *et al*. did not give a satisfactory answer to the following question: 'Is the (genetic) test useful in the management of a disease, a patient or a family?'

Therefore, the authors believe that the statement of 'potential of clinical significance' is ambiguous and unsound.

One could speculate that adding an element of uncertainty to the diagnostic process would not be advantageous, if not simply detrimental to our patients' health and well being.

Five-year view

The contained cost of microarray platforms, their growing diffusion even in small laboratories and the undeniable technical advantages of microarray-based analysis as compared with conventional karyotyping will make this analytical approach increasingly accessible for routine prenatal diagnosis. Therefore, it is urgent to delineate guidelines that are widely accepted by the international scientific community. We believe that the issue should be considered in a patient-oriented fashion, rather than addressed with a merely research-oriented approach, or in presence of potential conflicts of interest. More extensive collection of data, both in prenatal and postnatal diagnosis, will help to establish the correct resolution to be used in prenatal diagnosis and will clarify the functional role of many CNVs that currently remain of uncertain significance.

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Key issues

- Microarray-based analysis has recently been developed to detect submicroscopic genomic microdeletions/microduplications, the so-called copy number variations (CNVs).
- The opportunity to offer a microarray-based analysis to all pregnancies is largely debated.
- Nowadays microarray analysis is advised in cases with fetal ultrasound anomalies and/or chromosome rearrangements detected by karyotyping.
- In cases with ultrasound malformations, the incremental yield offered by microarray analysis is approximately 6%.
- Microarray-based analysis is not able to detect balanced chromosome rearrangements and triploidies.
- CNVs are generally classified as pathogenic, likely pathogenic, likely benign and benign.
- In approximately 1.5% of the cases, the clinical significance of a CNV remains uncertain.
- A robust diagnostic test should be able to reduce the diagnostic uncertainty.
- In the near future, it will be urgent to establish guidelines and clarify the functional role of CNVs.

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Website

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