Use of Array Genomic Hybridization Technology in Prenatal Diagnosis in Canada

Abstract

Objective: To summarize for obstetrical care providers the current literature on array genomic hybridization in prenatal diagnosis and to outline the recommendations of the Canadian College of Medical Geneticists regarding the use of this new technology with respect to prenatal diagnosis.

Evidence: PubMed and Medline were searched for articles published in English between 2004 and 2010, using the key words DNA QF-PCR, quantitative fluorescent polymerase chain reaction, fetal chromosomal abnormalities, prenatal diagnosis, array genomic hybridization, fetal structural anomalies, and copy number variants. Results were restricted to systematic reviews, randomized control trials/controlled clinical trials, and observational studies. Searches were updated on a regular basis, and articles were incorporated in the guideline to September 2011. Grey (unpublished) literature was identified through searching the websites of health technology assessment and health technology assessment-related agencies, clinical practice guideline collections, clinical trial registries, and national and international medical specialty societies.

Values: The quality of evidence in this document was rated using the criteria described in the Report of the Canadian Task Force on Preventive Health Care (Table 1).

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. (III-D)

2. Array genomic hybridization may be an appropriate diagnostic test in cases with fetal structural abnormalities detected on ultrasound or fetal magnetic resonance imaging; it could be done in lieu of a karyotype if rapid aneuploidy screening is negative and an appropriate turnaround time for results is assured. (II-2A)

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. (III-A)


Key Words: Array genomic hybridization, fetal structural anomalies, copy number variants
Table 1. Key to evidence statements and grading of recommendations, using the ranking of the Canadian Task Force on Preventive Health Care

<table>
<thead>
<tr>
<th>Quality of evidence assessment*</th>
<th>Classification of recommendations†</th>
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<tbody>
<tr>
<td>I: Evidence obtained from at least one properly randomized controlled trial</td>
<td>A. There is good evidence to recommend the clinical preventive action</td>
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<td>II-1: Evidence from well-designed controlled trials without randomization</td>
<td>B. There is fair evidence to recommend the clinical preventive action</td>
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<td>II-2: Evidence from well-designed cohort (prospective or retrospective) or case-control studies, preferably from more than one centre or research group</td>
<td>C. The existing evidence is conflicting and does not allow to make a recommendation for or against use of the clinical preventive action; however, other factors may influence decision-making</td>
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<tr>
<td>II-3: Evidence obtained from comparisons between times or places with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of treatment with penicillin in the 1940s) could also be included in this category</td>
<td>D. There is fair evidence to recommend against the clinical preventive action</td>
</tr>
<tr>
<td>III: Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees</td>
<td>E. There is good evidence to recommend against the clinical preventive action</td>
</tr>
<tr>
<td>L. There is insufficient evidence (in quantity or quality) to make a recommendation; however, other factors may influence decision-making</td>
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</table>

*The quality of evidence reported in these guidelines has been adapted from The Evaluation of Evidence criteria described in the Canadian Task Force on Preventive Health Care.

†Recommendations included in these guidelines have been adapted from the Classification of Recommendations criteria described in the Canadian Task Force on Preventive Health Care.

BACKGROUND

The investigation of fetal karyotypes has undergone significant changes since the 1970s when it first became possible to apply techniques of chromosome preparation to cultured amniocytes obtained by ultrasound-guided amniocentesis. At that time it was not possible to dissociate the search for deviation from the normal chromosome number 46 from the investigation of chromosomal structural rearrangements. Subsequent advances in nuclear FISH techniques and, more recently, QF-PCR have provided the opportunity to dissociate these 2 analyses and to tailor chromosomal investigations to the indications for testing. It is currently recommended that QF-PCR should replace conventional cytogenetic analysis in all cases of prenatal diagnosis performed solely for an increased risk of aneuploidy in chromosomes 13, 18, 21, X or Y.1 Conventional cytogenetic analysis (karyotyping and locus-specific FISH) is therefore reserved for more specific and higher-risk indications, such as multiple malformations seen on ultrasound, or for instances in which there is a high risk of unbalanced structural chromosome anomalies (e.g., one member of the couple is a carrier of a balanced translocation). However, conventional cytogenetic analysis is still limited by the level of resolution achieved and by the acuity and experience of the observer.

Array genomic hybridization, a new testing modality, can now be added to—and in some instances replace—conventional cytogenetic analysis. This technology reveals chromosomal duplications or deletions (extra or missing DNA sequences, respectively, also known as copy number anomalies) across the entire genome and has greater sensitivity than traditional microscopic chromosome analysis.2 The literature on the prenatal use of array genomic hybridization suggests it is a useful adjunct to conventional cytogenetic analysis.

TECHNICAL AND INTERPRETIVE CONSIDERATIONS OF ARRAY GENOMIC HYBRIDIZATION TECHNOLOGY

Array genomic hybridization is a DNA-based test that compares DNA extracted from cells such as amniocytes with control DNA that has been put on an “array.” The evaluation is performed by a scanner, and the information is then computer-integrated to determine if any quantitative deviations (extra or missing DNA sequences) exist in the DNA of the test case. The primary advantage of array genomic hybridization is increased detection of copy number anomalies: the deviations that can be measured by molecular means are orders of magnitude smaller than those detectable by light microscopy.

Array genomic hybridization is now widely applied in postnatal diagnosis, and the Canadian College of Medical Geneticists has endorsed it as “the first line laboratory investigation for the patient whose [developmental delay/ mental retardation], autism, multiple congenital anomalies or dysmorphic features is unexplained after a thorough history and physical examination.”3 In contrast, the application of...
Studies comparing the detection of unbalanced chromosomal rearrangements by conventional cytogenetics with detection by array genomic hybridization in prenatal populations are still relatively few, and sample sizes are small. \(^6\)–\(^{12}\) However, depending on the ascertainment criteria and the level of resolution achieved in the cytogenetic assessment, array genomic hybridization is superior in the detection of copy number anomalies, finding a pathogenic abnormality in up to 16% of fetuses with an abnormal ultrasound and normal karyotype (Table 2). \(^6\)–\(^{12}\) Of note, triploidy can be detected only by a particular type of genomic hybridization (SNP-based platform). Balanced chromosome rearrangements such as translocations or inversions (in which genetic material is only rearranged, not lost or gained) cannot be identified by array genomic hybridization.

One of the most significant impediments to the use of this technology in prenatal diagnosis is that array genomic hybridization analysis of normal individuals has uncovered variations in the number of copies of particular sequences known as copy number variants. These occur throughout the human genome, \(^{13}\)–\(^{16}\) making it imperative to distinguish between pathogenic and benign CNVs by incorporating family studies, information about the gene content of the region in question, and data from CNV databases and current literature. Given the high incidence of CNVs in a normal population, the detection of a CNV by microarray in pregnancies at low risk for a structural chromosomal abnormality (e.g., advanced maternal age, positive maternal serum screen, previous trisomy, or the presence of “soft markers” on fetal ultrasound) would likely be associated with a low positive predictive value since the vast majority of fetuses in these situations are clinically unaffected. Furthermore, the interpretation and follow-up of CNVs is labour-intensive and requires well-developed data management strategies and resources. The relatively new application of array genomic hybridization to prediction of phenotypes is hampered by the absence of very large population studies of fully validated CNV databases for each of the platforms used. In addition, there is still debate about the relative merits of different types of platforms and follow-up algorithms. It is generally agreed, however, that FISH or conventional cytogenetics are often required to confirm the abnormality detected on array genomic hybridization results and/or exclude a rearrangement predisposing to segmental aneuploidy. Therefore FISH or conventional cytogenetics can include parental testing for comparison of results, which will assist in the interpretation of findings. When no comparison can be made between parental and fetal results, the clinical significance of the genomic hybridization findings may not always be clear.

### 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. (III-D)**

2. **Array genomic hybridization may be an appropriate diagnostic test in cases with fetal structural abnormalities detected on ultrasound or fetal magnetic resonance imaging; it could be done in lieu of a karyotype if rapid aneuploidy screening is negative and an appropriate turn around time for results is assured. (II-2A)**

### Table 2. Summary of array findings in pregnancies with abnormal ultrasound findings and normal or balanced karyotypes

<table>
<thead>
<tr>
<th>Pregnancies with abnormal ultrasound and normal or balanced karyotype, n</th>
<th>Pregnancies with pathogenic findings detected by array, n (%)</th>
<th>Pregnancies with likely benign array finding, n (%)</th>
<th>Pregnancies with an array finding of unknown clinical significance, n (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>155</td>
<td>4 (2.6)</td>
<td>13 (8.4)</td>
<td>1 (0.6)</td>
<td>5</td>
</tr>
<tr>
<td>110</td>
<td>2* (1.8)</td>
<td>12† (7.9)</td>
<td>1† (0.6)</td>
<td>6</td>
</tr>
<tr>
<td>106</td>
<td>11 (10.4)</td>
<td>12 (11.3)</td>
<td>13‡ (12.2)</td>
<td>7</td>
</tr>
<tr>
<td>77</td>
<td>1 (1.3)</td>
<td>NA</td>
<td>1 (1.3)</td>
<td>8</td>
</tr>
<tr>
<td>50</td>
<td>5 (10)</td>
<td>1 (2)</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>49</td>
<td>4 (8.1)</td>
<td>4 (8.1)</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>31</td>
<td>5§ (16.1)</td>
<td>4 (12.9)</td>
<td>1 (3.2)</td>
<td>11</td>
</tr>
</tbody>
</table>

*Both cases with pathogenic findings were amongst the 110 cases with ultrasound anomalies.
†The indication for the prenatal array is not specified so the denominator is 151, pregnancies having prenatal arrays as opposed to 110 with ultrasound anomalies.
‡High incidence as parental studies were not done to determine if unbalanced karyotype was inherited.
§SNP array revealed uniparental disomy in 2 of 5 cases.
NA data not available.

DECEMBER JOGC DÉCEMBRE 2011
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. (III-A)

These recommendations are consistent with the American College of Obstetricians and Gynecologists 2009 committee opinion.17

Glossary

Aneuploidy: A chromosome number that is not an exact multiple of 23, usually resulting from a meiotic non-disjunction error in the production of gametes or from an early mitotic post-fertilization event.

Chromosome: A linear structure containing a single strand of DNA. A human normally has 46 chromosomes, in 23 pairs.

DNA: The molecule that encodes our genes.

Karyotype: The chromosome constitution of an individual or the photomicrograph of an individual’s chromosomes, systematically arranged in 23 pairs.

Monosomy: The absence of a single chromosome.

Mosaicism: The presence of 2 or more genetically different cell lines in an individual or tissue.

Numerical chromosome aberration: A chromosome number which is not 46.

Structural chromosome aberration: A chromosome number, usually, of 46 in which segment(s) of chromosome(s) are missing (deleted), extra (duplicate) or rearranged (translocated, inverted, or inserted). In a balanced karyotype with a structural rearrangement, no DNA is lost or gained, it is simply rearranged. In an unbalanced karyotype, the structural rearrangement results or in the presence of extra or missing DNA.

Triploidy: A chromosome number of 69 (3 copies of each chromosome).

Trisomy: The presence of an extra chromosome.

REFERENCES


