

# Array comparative genomic hybridization (a-CGH): state of the art and perspective

*Array comparative genomic hybridization (a-CGH):  
estado-da-arte e perspectiva*

## Editorial

There is convincing evidence suggesting a potential benefit of chromosomal microarray analysis for fetal abnormalities beyond conventional fetal karyotyping<sup>1-5</sup>. Microarray chromosomal genomic hybridization (a-CGH) may provide submicroscopic rearrangements especially duplicated or deleted portion of the DNA also known as copy number variants (CNVs). A limitation of chromosomal microarray analysis is the potential to identify variants of unknown clinical significance (VOUS). This occurred in 3.4% of cases in the NICHD trial. Such results were classified as “likely benign” in 1.8% of cases and “likely pathogenic” in 1.6%<sup>6</sup>. The result may be uncertain because the CNVs may be rare, novel or characterized by variable penetrance. Furthermore, in such cases, a parental search is mandatory to detect a carrier state or a *de novo* mutation and to calculate recurrent risk in a genetic counseling.

*Is genetic counseling advice before testing with a-CGH? Should an informed consent be obtained?*

Pre as well as post-test genetic counseling is mandatory when karyotyping is performed with a-CGH. Patients must be counseled concerning the incidence of VOUS, identification of diseases with variable clinical presentation, identification of consanguinity and/or non-paternity as well as adult-onset diseases. It is mandatory that healthcare givers do not performed the testing before genetic counseling and signed informed consent. In addition, as the amount of information depends by the type of array technique used and laboratory policy<sup>7</sup>, it is essential that doctors as well as patients be informed about this. Notwithstanding, the NICHD trial have demonstrated that women who received abnormal results reported a need for extensive support and counseling while referring a lack of good understanding of the potential for uncertain results<sup>8</sup>.

*Is there evidence to indicate that a-CGH should be performed in all cases as integrated prenatal karyotyping testing when fetal malformations are detected on ultrasound?*

Array chromosomal genomic hybridization (a-CGH) studies performed in fetuses with sonographic anomalies and normal karyotype have demonstrated to detect clinically significant CNVs in 2% of cases<sup>9</sup>. Moreover, when rapid fetal karyotyping is clinically indicated, oligonucleotide a-CGH for direct analysis of uncultured amniocytes has shown to be feasible. The use of oligonucleotide arrays increases the sensitivity and accuracy of detection over previous bacterial artificial chromosome (BAC)-based arrays and shorter reporting time<sup>10</sup>. In a series of 162 fetuses with sonographic anomalies of whom 6.8% had abnormal karyotype and 23.7% had abnormal

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microarray results, a-CGH have enabled the detection of 11.3% of fetuses with pathogenic CNVs, 9% with CNVs of uncertain clinical significance, 2.2% with CNVs establishing carrier status for recessive, X-linked, or susceptibility to late onset dominant disease, and 1.1% CNVs with pseudomosaicism due to *in vitro* cultural artifacts. In 13% of cases, a-CGH contributed to important new information and improved the detection of genomic imbalances of pregnancies with structural congenital anomalies<sup>11</sup>.

In a large series of 3,171 fetuses undergoing BAC-a-CGH and oligonucleotide- a-CGH in cases of abnormal ultrasound findings and/or abnormal fetal karyotype, numerical chromosome anomalies were detected in 1.2% of fetuses, microdeletion/duplication in 1.1%, large deletion/duplication and benign CNVs in 0.4%, while variation of unknown clinical significance were identified in 0.2% of fetuses. The a-CGH has proven to be effective in identifying submicroscopic genomic imbalance in fetuses with *de novo* balance translocations (1.8%), supernumerary marker chromosomes (50%), and abnormal prenatal ultrasound findings (17%). In addition, a-CGH detected microdeletions/duplications in 12 fetuses with normal karyotype. Prenatal a-CGH is effective in screening for submicroscopic genomic imbalance and may add 8.2% to the diagnostic field, compared with conventional karyotyping in fetuses with abnormal ultrasound results<sup>12</sup>. Healthcare authority may also consider and evaluate the economic impact of prenatal testing. In Italy, the laboratory costs are approximately 283 € for a genetic amniocentesis, 553 € for chorionic villus sampling (CVS) and 1,013 € for a-CGH.

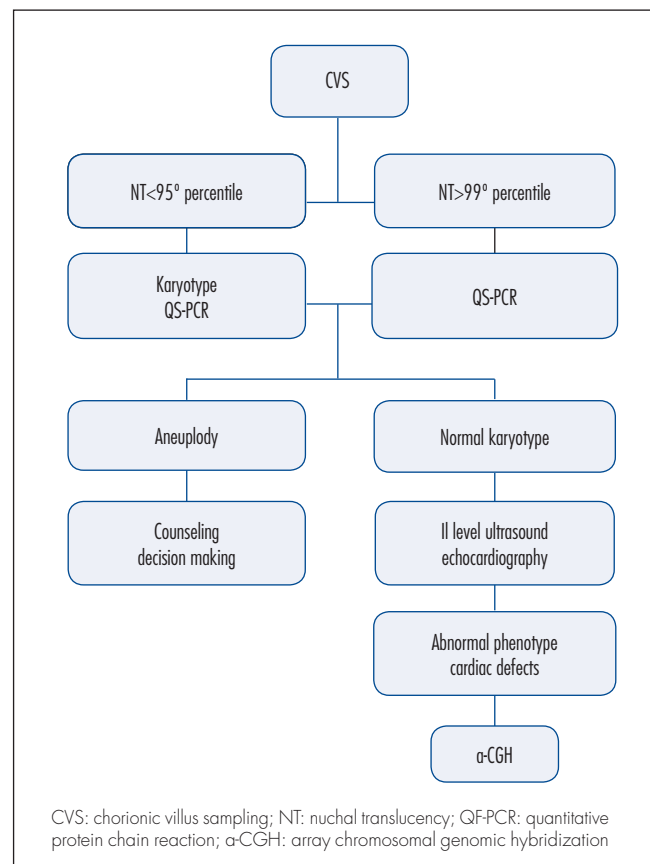
*Which type of a-CGH platform should be developed? Wide genomic or targeted characterization?*

Whole-genome a-CGH have been evaluated in fetuses presenting at least one major malformation detected on ultrasound, but for whom standard genetic analyses (including karyotype) failed to provide a diagnosis. Whole-genome a-CGH have demonstrated to identify a clinically significant chromosomal aberration in 8.2% of tested fetuses and a result of unclear clinical significance in 12.2% of tested fetuses supporting evidence of the value of whole-genome a-CGH as a prenatal diagnostic tool. A potential ideal approach would be to apply a-CGH in high risk pregnancies in conjunction with chromosomal analysis<sup>13</sup>.

Array-CGH has demonstrated to increase the detection rate for likely pathogenic CNVs up to 5%. To avoid interpretation problems, these arrays should cover all known pathogenic CNVs and have a low-resolution backbone for the detection of relatively large CNVs thus keeping the detection of CNVs of unclear significance to the minimum<sup>14</sup>. Although the American College of Obstetricians and Gynecologists (ACOG) Bulletin Committee advises to perform a-CGH even in cases with no congenital anomalies seen at ultrasound, critical issues have arisen from

this guideline. Firstly, a-CGH cannot detect balanced rearrangements such as Robertsonian translocations, balanced insertions, and inversions. Carriers of Robertsonian translocations are at high risk for uniparental disomy (UPD)<sup>15</sup>. Secondly, a-CGH cannot detect low level mosaicism that may occur in 1–2% of chorionic villus sampling and in 0.2% of amniotic fluid samples<sup>16</sup>. Furthermore, a-CGH may not be able to detect marker chromosome (0.1% of all prenatal diagnosis cases) even in the non-mosaic state and may not be able to visualize the type of rearrangement in the event where deletion or duplication detected by a-CGH is proven to be *de novo* after parental testing<sup>17</sup>.

Finally, prenatal a-CGH can detect VOUS in 1.5% of cases, in which the clinical utility of that technique could be disputed. In those cases that finding could be associated with parental anxiety and problematic genetic counseling<sup>18</sup>. As prenatal diagnosis should be seen as a whole, we believe that the higher analytical and clinical sensitivity of a-CGH technique must be associated with a comprehensive approach that includes better co-operation among genetic labs, genetic counselors, and obstetricians. Figure 1 presents the sequence used to the indication of a-CGH during the clinical obstetric practice.



**Figure 1.** Clinical-diagnostic flowchart has been developed for both clinicians and geneticists. The flowchart is intended to be used on a clinical basis or when counselling the parents-to-be. Note that it can be applied from first trimester onwards

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