ARRAY COMPARATIVE GENOMIC HYBRIDISATION (aCGH): AN ANALYSIS OF THE CURRENT TECHNOLOGY AND ITS FUTURE IN PRENATAL DIAGNOSIS

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1 AN INTRODUCTION TO ARRAY COMPARATIVE GENOMIC HYBRIDISATION (aCGH)

Array comparative genomic hybridisation (aCGH) represents a major advance in the field of cytogenetics and offers tremendous promise in prenatal diagnosis for detecting genetic alterations leading to serious genetic conditions. The technology, also sometimes known as molecular karyotyping, can detect differences in DNA copy number at hundreds or thousands of points in the genome simultaneously. This technology promises to replace standard karyotyping, which uses standard microscopy to view chromosomes directly in order to detect structural variations that lead to conditions such as Down syndrome (which can be caused by trisomy 21, that is three copies of chromosome 21 instead of two, or a joining of chromosomes 21 and 14) or Turner syndrome (loss of one copy or part of one copy of the X chromosome in girls), or genetic duplications, deletions, insertions or translocations (some of which can be associated with hereditary diseases or cancers). Compared to standard karyotyping, aCGH can detect genetic variations at a much higher resolution.

aCGH was initially studied and utilised in cancer genetics to determine how chromosome structure and function contributed to tumour development.[1] Although it is still used for this purpose, it is hoped that aCGH will be valuable in other clinical contexts, including prenatal screening and diagnosis. In research, aCGH has demonstrated an unparalleled ability to perform comprehensive, high-resolution scans of both the whole genome and specific chromosomal regions. Physicians are looking to aCGH to increase their ability to detect clinically significant genetic alterations. Although it is already a powerful research tool, the technology is still in its early stages and has not fully transitioned to clinical use. However, in the United States, aCGH is already being offered as a clinical test in postnatal and prenatal settings. More research is necessary to determine how and in what capacity aCGH technology can and should be used for clinical prenatal screening or diagnosis. A necessary aspect of this development is a thorough consideration of the ethical implications of the new technology; this will come to the forefront as clinical use of aCGH increases.

2 PRENATAL DIAGNOSTIC TECHNIQUES AND aCGH

In the last four decades, techniques designed to detect chromosomal abnormalities in prenatal diagnosis have developed significantly from the early use of chromosome banding techniques to the more advanced technologies available today. Because chromosome analysis plays an integral role in the detection and diagnosis of many genetic disorders, technology that allows clinicians to identify cytogenetic imbalances with increased precision and speed is a high research priority. Banding techniques,

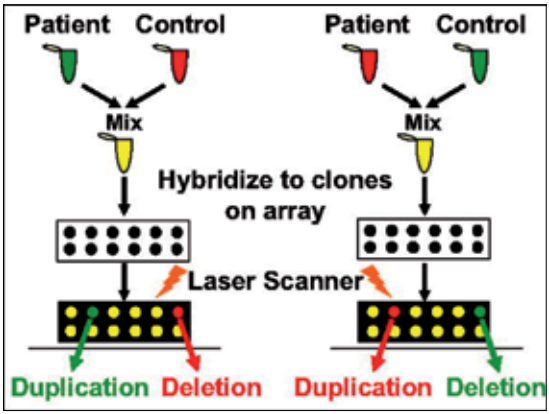
which have been used in prenatal diagnosis since the 1970s, can detect aneuploidy (an abnormal number of chromosomes), and large structural rearrangements of chromosomes such as deletions, duplications and translocations, by evaluating metaphase cells.[2,3,4] This type of chromosome analysis reliably detects deletions and duplications >10Mb, but smaller structural rearrangements will not be detected without higher resolution capability.[5] Banding techniques are able to detect alterations as small as 3–5Mb, but this higher-resolution technology is not routinely applied because it is labour-intensive and therefore costly.[2,3,4] Other limitations regarding the use of these methods include the need for cultured cells (metaphase cells), requiring additional time, and the difficultly of interpreting data.[3,4] To address these limitations, as well as the need for higher resolution methods that would permit reliable detection of chromosomal abnormalities smaller than 3Mb, researchers have developed and used alternative techniques, such as fluorescence *in situ* hybridisation (FISH) and quantitative fluorescent polymerase chain reaction (QF-PCR).[2,3]

When used for prenatal diagnosis, both FISH and QF-PCR methods are useful tools in detecting chromosome alterations. The process of FISH involves hybridising fluorescently labelled DNA probes to targeted regions on human chromosomes enabling researchers to screen for aneuploidy and to detect submicroscopic abnormalities in those specific locations.[4] Of particular interest for prenatal diagnosis are the common aneuploidies of chromosomes 13 (Patau syndrome), 18 (Edward syndrome), 21 (Down syndrome), X and Y, which account for the vast majority of chromosome abnormalities affecting the human population.[2, 4] The advantages of this technique include increased resolution (size of individual probes (~35–200kb)) and rapid detection of alterations by locus-specific probes.[4,6] QF-PCR is another technique employed in prenatal diagnosis. It works by using primer pairs that quantitatively amplify polymorphic, chromosome-specific sequences in order to detect genomic alterations.[4] Similar to FISH, QF-PCR is used for the more common aneuploidies mentioned above, and for sex-chromosome aneuploidy.[4] It is a relatively quick and efficient alternative to more labour-intensive processes because it does not require cultured cells and is amenable to automation.[3,4] There are, however, a number of limitations to both FISH and QF-PCR procedures. The primary challenge is that neither of these two methods provides a comprehensive, whole-genome screen, resulting in a failure to detect approximately 1–2 per cent of abnormalities in prenatal diagnosis.[4] Because both techniques require targeted probes, some alterations detected by conventional karyotype analysis will not be detected.[3,4,7] Furthermore, the successful use of FISH and QF-PCR probes requires suspicion regarding a particular region so that specific probes can be utilised. The limitations of these prenatal diagnostic techniques prompted researchers to continue developing methods that would enable them to perform a high-resolution, comprehensive screen of the human genome in order to detect more chromosome abnormalities.

The introduction of aCGH, a technique similar to conventional comparative genomic hybridisation (CGH), has demonstrated considerable potential in its ability to detect copy number variation in DNA at significantly increased resolution.[3,6] The first step of this relatively new process involves taking two genomic DNA samples – a patient (test) sample, and a reference (control) sample – then differentially labelling them with fluorescent dyes (cyanine-3 and cyanine-5, for example).[3] The two genome samples are then mixed together and hybridised to a genomic microarray, consisting of DNA fragments fixed on the surface of a slide. These DNA fragments or targets can exist in a variety of forms, including (the most commonly used) bacterial artificial chromosomes (BACs); PCR-generated sequences; cDNA clones; and oligonucleotides.[2,7] When hybridised, the two genomic samples compete for binding to the targets on the slide, thus creating a profile of relative dye intensities that can be read by a laser scanner.[8] Deletions and duplications are detected by differing signal intensities of the respective dyes. To increase reliability, the process can be repeated a second time by reversing the dyes and analysing the scanned product, thereby confirming the initial result.[8] This procedure is illustrated in Figure 1 with the initial and duplicate processes represented.

Figure 1: *The aCGH process*
DNA from a patient sample and a control sample are labelled with a different fluorophore, Cy3 (green) versus Cy5 (red), mixed and hybridised to an array of cloned genomic DNA fragments (shown as black spots). Two different hybridisations with dye reversal are performed (compare left to right panels). The hybridised slides are scanned with a laser scanner and the signal intensities of all fragments for each of the fluorophores (shown as coloured spots) are analysed using specialised software. A gain (duplication) in the patient of DNA fragment(s) present on the slide will give a more intense signal compared to the control; a loss (deletion) in the patient will produce a less intense signal compared to the control.

Source: *Van den Veyver I.B., Beaudet A.L. Comparative genomic hybridization and prenatal diagnosis. Curr Opin Obstet Gynecol 2006 18: 185–91*



Different arrays have been constructed for different purposes, and researchers have developed arrays to analyse specific chromosomes and segments of chromosomes, referred to as targeted arrays, as well as arrays that cover the entire human genome, referred to as whole-genome arrays.[2,7] Targeted arrays are site specific, enabling researchers to examine a precise chromosomal region of particular interest. Genome-wide screens, in contrast, provide a comprehensive analysis of the whole genome. This ability to perform an extremely high-resolution screen of the entire genome sets aCGH technology apart from other prenatal diagnostic techniques, and is of tremendous value to researchers. In the research context, it can be used for the discovery of genes or the detection of copy gains and losses.[7] It is not yet clear what role an aCGH whole-genome screen will play in the use of aCGH for prenatal diagnosis due to limitations that will be discussed later.

As aCGH technology continues to develop, even higher-resolution microarrays are now constructed using a 'tiling' approach in which researchers can arrange target clones in an overlapping manner. The use of these tiling-arrays can increase resolution from ~1Mb to the size of a single clone (~100–200kb), dramatically increasing the ability to detect chromosomal deletions and duplications in specific chromosome locations.[8] It is important to note with this new method, however, that detection is most reliable when the abnormality spans more than one clone.[8] In theory, therefore, resolution is restricted only by the size of an individual target and the density of contiguous targets. This leads to the following relationship: the smaller the target clone and the higher the density of coverage on the array, the higher the resolution.[7]

3 DATA ANALYSIS IN aCGH

In addition to the aCGH process described, of hybridising two samples of DNA, there is also a large data synthesis component in the process that involves sophisticated software technology. The applications of this software, as recently reviewed by Chari et al. in *Computational Methods for the Analysis of Array Comparative Genomic Hybridization*,[9] can be broken down into three primary application stages: data pre-processing, visualisation, and analysis. Different software packages execute some or all of these general functions.

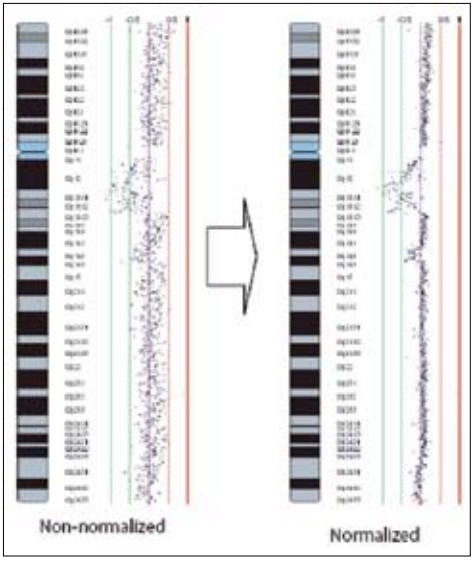
Data Pre-processing

This stage of the aCGH process occurs after the two genomic samples have been mixed and hybridised to the microarray. Preparation for an accurate interpretation of the relative dye intensities requires image normalisation and the removal of biases potentially interfering with the real biological data. The goal is to remove all non-biological sources of variation before interpretation so that only the actual chromosomal abnormalities are revealed.

Figure 2 demonstrates how normalised data (right) can provide a cleaner, more accurate profile of actual copy number variation in the sample than non-normalised data (left).[9]

Figure 2: *aCGH and data pre-processing*

Source: *Chari R., Lockwood W.W. and Lam W.L. Computational methods for the analysis of array comparative genomic hybridization. Cancer Informatics 2006 2: 48–58*

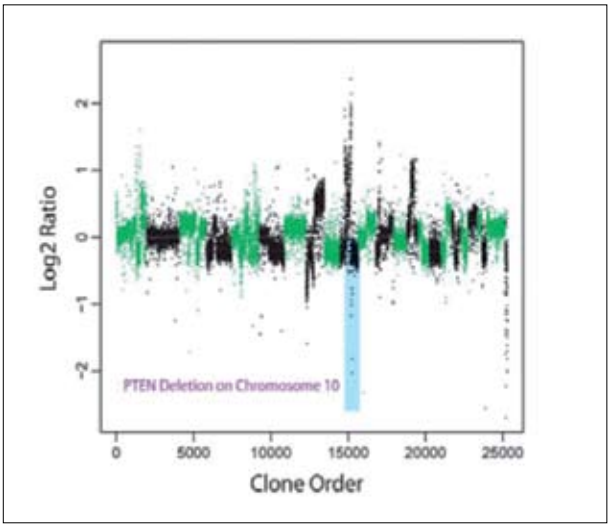


Visualisation

In order to create an accurate visualisation of the processed data, two different methods are currently available for visually representing the copy number variation detected by the aCGH experiment. These two visualisation techniques are 'graphical representation' and 'interactive display'. The first of these, graphical representation, is used for lower-density arrays and illustrates the data in an XY graph, by plotting the chromosome position (clone order) on the X-axis and the $\log_2$ signal ratio on the Y-axis. Figure 3 is an example of such a graph, showing a PTEN deletion on chromosome 10 (indicated by the blue line).[9]

Figure 3: *Graphical representation of aCGH*

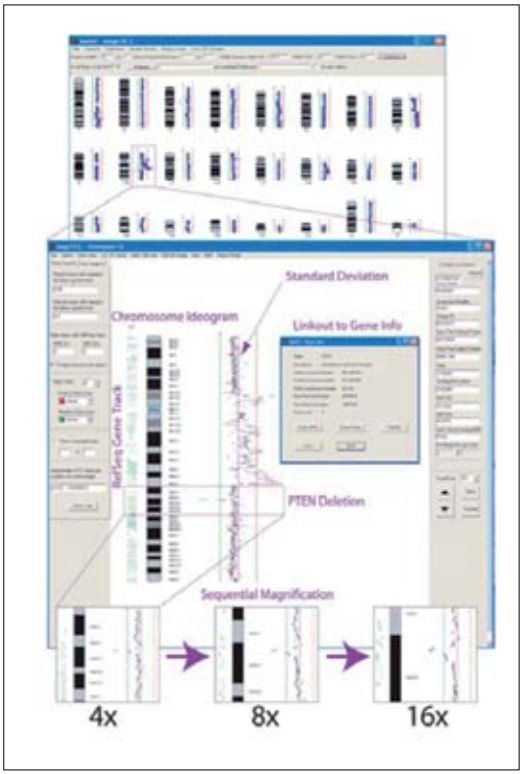
Source: *Chari R., Lockwood W.W. and Lam W.L. Computational methods for the analysis of array comparative genomic hybridization. Cancer Informatics 2006 2: 48–58*



The second approach to visualisation, interactive display, is capable of visually representing higher-density arrays containing tens of thousands of DNA targets. Each chromosome or chromosome segment in the array can be represented individually and then magnified for closer analysis. A chromosome ideogram is placed to the left of each signal ratio plot. Different software can include additional functions such as links to online public databases (OMIM, NCBI Entrez and UCSC Genome Browser) and gene track. Figure 4 shows what this visualisation software looks like.[9]

Figure 4: *Visualisation using interactive display*

Source: *Chari R., Lockwood W.W. and Lam W.L. Computational methods for the analysis of array comparative genomic hybridization. Cancer Informatics 2006 2: 48–58*



Analysis

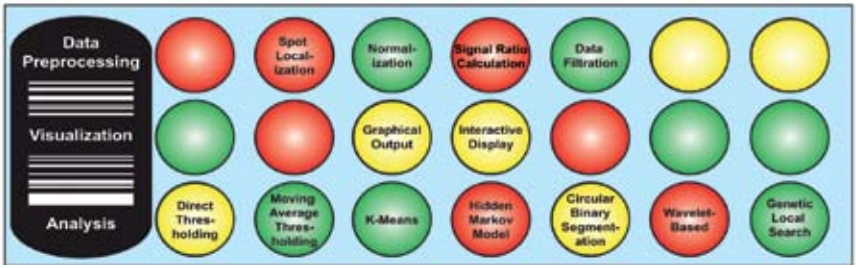
The most important phase of the aCGH process is the analysis and interpretation of data and the detection of chromosome abnormalities in the form of deletions and duplications. There are a number of analytical strategies that are frequently used for data analysis of genetic alterations (direct thresholding, moving average-based thresholding, k-means clustering, hidden Markov model, circular binary segmentation and wavelet based and genetic local search). By way of illustration, the following is a brief explanation of two of these methods.

1. *Direct thresholding:* This is the most straightforward analytical method. It involves setting a specific threshold pre-defined by the differing signal ratios represented by single-copy gains and losses. A single-copy gain theoretically appears as a 3:2 ratio, with a $\log_2$ value of 0.585; while a single-copy loss appears as a 1:2 ratio, with a $\log_2$ value of -1.
2. *Moving average-based thresholding:* In contrast to direct thresholding, this analytic approach implements an average ratio calculated for a specific distance with a sliding window. The larger the window (i.e. the more data points calculated into the average ratio) the smoother the curve, but the less sensitive the detection of alterations. In order to achieve higher sensitivity, a smaller window can be used, but the probability of false positives increases.

Inherent in the process of data analysis is the risk of detecting false positives as well as false negatives.[9] In order to correct for this potential error, other methods such as FISH and QF-PCR can be applied to validate the evidence of chromosome alteration, as shown in Figure 5.

Figure 5: *Data pre-processing visualisation analysis*

Source: *Chari R., Lockwood W.W. and Lam W.L. Computational methods for the analysis of array comparative genomic hybridization. Cancer Informatics 2006 2: 48–58*



As the use of aCGH transitions from the research setting to the clinical setting, software will continue to be developed to meet these needs. Important considerations for the future development of this software include increased accessibility for researchers with less computational experience, increased automation, security of information and integration of previous knowledge of genetic disorders to help interpret analysed data.[9]

4 ADVANTAGES AND LIMITATIONS OF aCGH

A promising prenatal diagnostic technique is offered in the form of aCGH, as it addresses many of the limitations of previous diagnostic methods such as microscopic karyotype analysis, FISH and QF-PCR. The advantages of aCGH can be broken down into four general categories: higher-resolution capability; comprehensive screening; faster turn-around time; and potential for automation.

Higher-resolution capability

The ability to detect chromosome abnormalities at a higher resolution using aCGH substantially increases the ability to diagnose potential genetic disorders. Microscopic karyotyping allows reliable resolution at >10Mb, and has been used to detect alterations as small as 3–5Mb, although this is not done routinely.[3] FISH probes can theoretically achieve resolution as small as the individual probes used in the procedure, ~35–200kb, but the process is limited by number and specificity of probes.[6] The process of aCGH has demonstrated high-resolution detection of copy number variation (both deletions and duplications) as small as 100–200kb.[8] This technique has been successful not only in detecting all aneuploidies, but also in detecting over forty chromosome alterations and forty-one subtelomeric rearrangements.[8] The technology is also continually developing and the potential for an even higher resolution with oligonucleotide targets may be of clinical value in the future.[8]

Comprehensive screening

One major limitation in the use of FISH in prenatal diagnosis is that screens are substantially limited by the number of probes that researchers can utilise simultaneously.[7] In addition, researchers must suspect a specific region in order to determine which FISH probes to utilise in a given experiment.[7] Researchers do not always have this knowledge prior to testing. Although this technique can detect the common chromosome abnormalities associated with many genetic disorders, it is limited in its comprehensive nature, for it can only reliably detect microdeletions and could potentially miss chromosome duplications.[7] Similarly, QF-PCR cannot perform genome-wide screens and, therefore, can potentially fail to spot ~1–2 per cent of copy number imbalances.[4] The aCGH technology, however, now provides a technique through which the entire human genome can be screened in one trial.[7] Different research has utilised different array techniques in order to accomplish this. For example, some research has utilised arrays with uniform coverage, such as one clone/1Mb, for the entire genome.[3,7] Other research has demonstrated a full-genome screen with contiguous coverage.[7] This genome-wide screening capability at very high resolution has been of tremendous value to researchers, and has played an integral role in the discovery of genes and the detection of chromosome deletions and duplications throughout the human genome.

Faster turn-around time

In contrast to microscopic karyotyping and conventional CGH, aCGH, in addition to FISH and QF-PCR, can be performed on uncultured samples.[3] This is a significant advantage for the use of this technology in the clinical setting because reporting time can be significantly decreased.[3] In addition, recent research demonstrates that successful aCGH analysis of prenatal material can be performed on as little as 1ml of uncultured material.[3]

Potential for automation

Moreover, aCGH is amenable to automation, making it a highly valuable research and clinical diagnostic tool. This characteristic feature of aCGH technology will decrease the labour and expense involved as well as decreasing the reporting time for results.[3]

As well as these advantages, however, there are also limitations to aCGH technology. One important consideration is that the genomic alterations aCGH can detect are limited in number. Because of the very nature of the aCGH methodology, the process will not detect balanced chromosomal rearrangements such as reciprocal and Robertsonian translocations and inversions.[3,4,7,8] In addition, aCGH does not reliably detect polyploidy and low-level mosaicism below 30 per cent.[3,4,8] Because detection is dictated in part by the size of BAC clones, chromosome alterations that are smaller than individual targets may also not be revealed in the analysis.[7] There are also limitations that are unique to different types of arrays: targeted, and whole-genome, arrays. The primary limitation of targeted arrays is that chromosome alterations that exist outside the particular scope of the microarray will not be detected. For whole-genome arrays, a number of limitations with the current technology make it unlikely such arrays will soon be widely used for clinical diagnosis. In the light of recent research, demonstrating the high copy-number variation in the normal human genome,[10,11] it is increasingly difficult to interpret data from genome-wide arrays that detect copy-number alterations. Whether such variation should be considered benign or the cause of disease introduces an additional complexity to the analysis of aCGH data.[2,7] Also, whole-genome arrays utilise more probes which can lead to an increased number of false positives, additional expense and complicated issues with regard to quality control.[3]

	ADVANTAGES	LIMITATIONS
aCGH	– can detect copy-number variation at multiple loci simultaneously – faster turn-around time – can detect deletions, duplications and amplifications at locus – potential for automation – comprehensive – lower cost and labour and report times – can detect more complex chromosomal defects (double trisomies)[8]	*cannot detect:* – low-level mosaicism 30 per cent – balanced chromosomal rearrangements (reciprocal and translocations and inversions) – point mutations[7] *may not be detected:* – triploidy or polyploidy – abnormalities smaller than the size of the specific BAC target
Targeted array	– reliably detects more than forty chromosomal defects, forty-one subtelomeric rearrangements and all aneuploidy in one diagnostic assay – proved clinical use in cases of mental retardation and learning disability and known chromosome alterations with targeted array	– regions not represented by chromosome target will not be analysed
Whole-genome array	– ability to perform whole-genome screens at unprecedented resolution	– difficult to interpret data given new research on the normal human copy-number variation in the genome – requires skilled interpretation – lower resolution compared to targeted-array CGH – not as good for clinical use: more array probes means a higher number of false positives; and more expensive to fabricate, control quality and interrogate

Table 1: *Summary of advantages and limitations of aCGH*

5 TRANSITION FROM RESEARCH TO CLINICAL USE

Of critical importance is the discussion about how this relatively new technology can have an impact on clinical diagnosis and what role it should play in prenatal diagnostics. Although aCGH technology has been available since the 1990s, it is only just transitioning from research to clinical use. While this represents exciting progress in the field of clinical cytogenetics, there are a number of important considerations that must be addressed before the technology can be implemented safely and effectively, while minimising the potential harms. In the research context, the introduction of aCGH technology has led researchers to the discovery of genes and provided a much broader picture of the human genome with regard to chromosome structure and function.[7]

The application of aCGH in clinical diagnosis, however, calls for further research and developments in available technology. As discussed earlier, the use of whole-genome arrays for clinical use is less appropriate at this time given its current limitations. These limitations are illustrated by de Vries et al. in their study looking at unexplained mental retardation in a sample of one hundred affected individuals.[12] Despite the fact that all one hundred people demonstrated normal microscopic karyotype results, array CGH detected *de novo* alterations in 10 per cent of the sample (seven deletions and three duplications) ranging in size from 540kb to 12Mb. In 97 per cent of the patients, however, large-scale copy-number variation was detected; and, upon DNA analysis of phenotypically normal parents, it became clear that the majority of these alterations were benign rather than disease causing. *De novo* abnormalities are those chromosome alterations that are not inherited from either parent, therefore making it more likely that such chromosome imbalances will lead to phenotypic expression. This study demonstrates some of the important challenges and limitations of using whole-genome arrays in clinical prenatal diagnosis.

Due to the prevalence of large-scale copy-number variation in the normal human genome,[10,11,23] interpretation of data can be extremely difficult. Not only is the interpretation challenging, it also requires significant resources (labour, funding and time) to test parents in order to determine whether a detected chromosome variation is clinically significant. This places a financial and technical burden on laboratories and can also cause parents and families significant stress and anxiety.[7] In contrast to the use of whole-genome arrays, researchers have constructed targeted arrays to examine specific chromosomal regions of known significance, which seem more suitable for use in clinical prenatal diagnosis. The use of targeted arrays in the clinical setting is still extremely limited, although there is continued progress. A review follows of four significant studies specifically designed to evaluate the use of targeted aCGH technology for clinical use.

Bejjani et al. Use of targeted array-based CGH for the clinical diagnosis of chromosomal imbalance: Is less more? (2005)[6]

In this study, the researchers designed a targeted microarray constructed specifically to evaluate chromosomal regions of known medical significance. After FISH verification of BAC clones for the array, 589 (65 per cent) of the original 906 BAC clones were used to test fifty control subjects with normal phenotypes and thirty-six subjects with known chromosome alterations. In the control cohort, aCGH detected deletions and duplications previously undetected by FISH (2p pericentromere, 5q telomere, 6p telomere, 7q pericentromere, 14q pericentromere and 14q telomere). In addition, the array detected all chromosome single-copy abnormalities in the test group. All detections by aCGH were later confirmed by FISH. From the results of this work, the authors concluded that:

> *Our data demonstrate that the rigorous assessment of BACs and their use in array CGH is especially important when the microarray is used for clinical diagnosis. In addition, this study illustrates that, when constructed carefully with proper attention to the quality of the BACs that are arrayed, array CGH is an effective and efficient tool for delineating chromosomal aberrations and an important adjunct to FISH and conventional cytogenetics.*[6]

Rickman et al. Prenatal detection of unbalanced chromosomal rearrangements by array CGH (2006)[3]

In this study, researchers looked at thirty samples of cultured foetal material with two arrays: a specific targeted array, and an array with overall 1Mb resolution. The targeted array was designed with ~600 BAC clones evaluating chromosome regions of particular interest for aneuploidy, previously known microdeletion syndromes and large structural rearrangements. All thirty samples had been tested microscopically and were known to have chromosome abnormalities. With the custom design array, 29/30 unbalanced chromosome rearrangements were detected, whereas the 1Mb array detected only 22/30. The one failed detection with the use of the custom array was a case of triploidy. For the 1MB array, the test failed to detect chromosome abnormalities in regions that were not represented by clones on the array (microdeletions at 4p, 7q11 and 22q11). In addition to the study of cultured samples, this study also established the value of the custom design array on thirty uncultured samples, delivering the same 29/30 detections on as little as 1ml of amniotic fluid. The failed detection in this part of the experiment was again triploidy. The authors of this work concluded that:

> *We believe these results provide proof of principle that array CGH using a custom designed array is rapid, and may have about the same overall capacity to detect clinically relevant chromosome abnormalities as conventional cytogenetics. It has the potential to replace karyotyping for prenatal cytogenetics, but considerable further work is required to reach consensus on the optimum configuration of an array for clinical use, and large trials are needed to demonstrate sensitivity and specificity in clinical operating conditions, before the clinical implementation of array CGH can be considered.*[3]

Schaeffer et al. Comparative genomic hybridization-array analysis enhances the detection of aneuploidies and submicroscopic imbalances in spontaneous miscarriages (2004)[13]

The goal of this study was to determine whether a targeted microarray could detect all of the known chromosome abnormalities previously determined by G-banding in forty-one 'products of conception'. It was also of interest whether the aCGH array would detect any additional abnormalities that the microscopic karyotyping did not detect. In fact, not only did the array detect all established chromosome abnormalities, it also discovered previously undetected aberrations in four of the forty-one cases (9.8 per cent). A lower-density targeted array was utilised in this study, as opposed to a higher-density and higher-resolution array, so as to limit the chromosome analysis to clinically relevant regions and clones. Because chromosome abnormalities cause the majority of miscarriages, and miscarriages occur in as many as 15 per cent of all clinically recognised pregnancies, aCGH has the potential to provide a more comprehensive understanding of the causes of such tragedies and offer more complete diagnoses in the clinical setting. The tremendous value of aCGH technology, therefore, reinforces its potential to make significant impact on clinical prenatal diagnosis. This article looks to future considerations and progress for aCGH:

> *Future studies can test the benefits of the array technique in regard to higher detection parameters, more accurate and less failure-prone diagnoses, and a greater ease of use, as compared with conventional cytogenetic analysis.*[13]

Shaffer et al. Targeted genomic microarray analysis for identification of chromosome abnormalities in 1500 consecutive clinical cases (2006)[14]

> *In this unique and important study, researchers did not explicitly collect subjects with particular known chromosome abnormalities; but, rather, studied 1500 consecutive cases referred to the laboratory for array analysis.[7] The idea behind this methodology was that these 1500 cases would provide a relatively representative cohort of real clinical cytogenetic cases. Of the 1500 patients, array analysis revealed that ~9 per cent of cases presented chromosome abnormalities: thirty-six patients (2.4 per cent) demonstrated polymorphisms or familial variants; fourteen patients (0.9 per cent) demonstrated abnormalities for which there was unknown clinical significance; and eighty-four patients (5.6 per cent) showed clinically relevant abnormalities. The detected abnormalities, specifically, included unbalanced rearrangements, subtelomeric deletions, microdeletions and reciprocal duplications as well as rare abnormalities and low-level trisomy mosaicism.[7,14] The custom design array included 832 BACs, which were representative of only 140 loci. The authors of this study concluded that:*
>
> > *Our results showed that microarray analysis likely doubles the yield of chromosome abnormalities that is currently detected by conventional cytogenetic analysis. … Therefore, in a clinical setting, a significant percentage of clinically relevant chromosomal abnormalities can be detected by judicious coverage of the genome.[7]*

These studies, as well as others, have quantitatively demonstrated that targeted aCGH has significant potential in the field of clinical cytogenetics. Clinicians using this screening method in prenatal diagnosis must recognise the limitations of the current aCGH technology and take special precaution to limit potential harms. This is essential in aCGH's clinical diagnostic utility. Many of these studies make a number of recommendations of important and relevant considerations for the application of this technology in a clinical setting.[7] They can be summarised as follows:

1. **Independent FISH verification:** Because BAC clones used in the construction of BAC arrays are collected from a variety of sources, such as academic or commercial databases,[7] there is no absolute guarantee that these BAC clones will map to the right genomic location, and not map to more than one location. In order to ensure the identity and reliability of individual BACs, individual clones should be verified by FISH before use in a clinical array.[7,8]
2. **Loci represented by more than one BAC clone:** By using multiple clones for specific loci, the statistical confidence in the results of the analysis increases. If only one clone is used, variation could be the result of systematic error rather than actual copy-number variation and chromosome abnormality.[7]

3. **Identification of polymorphic clones:** As demonstrated in the research,[10,11] there is large-scale variation in the normal human genome, which means that some BAC clones will also express this polymorphic quality, potentially complicating clinical diagnosis. It is essential that these polymorphic clones are identified, at which point they can be removed from the targeted array or remain on the array with particular recognition of their status.[7]

4. **aCGH plus karyotype:** Because current targeted arrays do not provide complete coverage of each chromosome, it is recommended that karyotype analysis be performed in addition to aCGH as a cautionary measure. In addition, if a chromosome abnormality is detected by aCGH, the finding should be subjected to external confirmation by karyotype analysis or FISH.[8]

In addition to these technical considerations, there are also questions with regard to accessibility and availability of this technology. Two primary questions arise here:[8]

1. Should aCGH technology be offered to women in addition to invasive amniocentesis (AC) and chorionic villus sampling (CVS)? Relevant concerns here include expense and increased stress and anxiety for parents and families, as well as the possibility of revealing non-paternity in evaluating relatives to determine benign or disease-causing chromosome abnormalities.[8]

2. Should aCGH technology, which currently requires invasive testing of AC or CVS, be offered to all pregnant women regardless of risk? Answering this question requires an assessment of the relative risks of both AC and CVS in relation to the risk of detecting a chromosome abnormality that would not be detected with normal Down syndrome screening protocols.[8]

Although there is significant potential for the clinical use of aCGH, there are many considerations to be addressed when discussing its successful transition from use as a research tool to use in clinical prenatal diagnosis.

6 GUIDELINES AND STANDARDS

Because aCGH is still in its very early stages, there are no mandatory guidelines or protocols with reference to its specific use in the United States; and there are no Federal or State laws that directly apply to the technology. Despite this, a growing body of aCGH research is indirectly developing standards for the acceptable uses of the current technology, both in research and in the future prospects for its clinical application. This research has outlined many of the benefits as well as the limitations and potential risks of the aCGH method, a number of which were discussed earlier. As this technology continues to develop, and transitions into an applied clinical tool, there will be an increasing need for policy decisions and guidelines to

direct researchers, clinicians and physicians toward the appropriate use of aCGH. Professional organisations, such as the American College of Medical Genetics (ACMG), the American College of Obstetricians and Gynecologists (ACOG), the American Academy of Pediatrics (AAP) and others, will play a significant role in influencing the direction of aCGH use in the research and clinical contexts. Although the recommendations of these professional organisations are not legally binding, and membership is voluntary, guidelines and recommendations published by these bodies carry significant weight in the medical community.

American College of Medical Genetics (ACMG)

Although the ACMG has not taken an explicit position on aCGH, the ACMG Laboratory Quality Assurance (QA) Committee publishes Standards and Guidelines for Clinical Genetics Laboratories (2006).[15] *The ACMG reports the purpose of these standards as follows:*

> *These* ***voluntary standards*** *have been established as an educational resource to assist medical geneticists in providing* ***accurate and reliable diagnostic genetic laboratory testing*** *consistent with currently available technology and procedures in the areas of clinical cytogenetics, biochemical genetics and molecular diagnostics.*[15]

These standards fall into the categories of personnel policies, general policies, shared methodologies, clinical cytogenetics, clinical biochemical genetics and clinical molecular genetics, as well as standards and guidelines for the genetic testing of specific genetic disorders such as Down syndrome, fragile X, Huntington disease, open neural tube defects and ultra rare disorders.[15] *Although aCGH is not mentioned in these sections, there are regulations and standards outlined for similar diagnostic tools such as FISH and PCR. Specifically, ACMG is now developing technical and clinical practice standards for the use of aCGH. In addition, recommendations for acceptable use could also be integrated into guidelines for particular disorders for which aCGH technology could be applicable, e.g. Technical Standards and Guidelines: Prenatal Screening for Down Syndrome (2006).*[16] *These standards cover wide-ranging technical considerations and also integrate issues such as information distribution for patients and health-care providers and informed consent.*

American College of Obstetricians and Gynecologists (ACOG)

Like ACMG, ACOG is a professional organisation that releases guidelines, committee opinions and publications on various medical topics relating to women's health care. In relation to standards and guidelines for aCGH technology, ACOG has a committee on genetics, which publishes committee opinions on a number of genomic related subjects. Although it too has not presented an opinion

on aCGH technology and its potential clinical utility, there could be a place for relevant guidelines, recommendations or opinions on this subject in the future. In the last two years, the committee on genetics has published opinions such as First-Trimester Screening for Fetal Aneuploidy (2004)[17] and Prenatal and Preconceptional Carrier Screening for Genetic Diseases in Individuals of Eastern European Jewish Descent (2004).[18] ACOG recommends that all pregnant women, regardless of age, be offered screening for Down syndrome in the first trimester.[24] ACOG recommends that non-invasive screening techniques such as nuchal translucency in conjunction with standard blood tests be offered to women in the general population, with AC or CVS as a follow-up test. ACOG also recommends that AC or CVS be offered to women known by other means to be of high risk for foetuses with Down syndrome (for example, those who have had a child with Down syndrome). This recommendation will increase the number of women potentially using aCGH.

American Academy of Pediatrics (AAP)

The AAP Policy is a collection of policy statements, clinical reports, clinical practice guidelines and technical reports by the AAP. Although this organisation also has not published explicit opinions on the use of aCGH in the clinical setting, or even in a research context, it did publish a clinical report in 2004 entitled Prenatal Screening and Diagnosis for Pediatricians (2004).[19] This document offers physicians and clinicians guidance in the appropriate handling of situations with regard to potential genetic abnormalities. The purpose of this report is:

> *To update the pediatrician about indications for prenatal diagnosis, current techniques used for prenatal diagnosis, and the status of maternal screenings for detection of fetal abnormalities.[19]*

Although aCGH technology is not yet widely used for prenatal diagnosis in the clinical setting, it is this type of clinical report that could help to construct the necessary guidelines and professional recommendations.

Many researchers have integrated standards and opinions about the use of aCGH into their publications, in place of actual guidelines. Publications cover such topics as the circumstances in which it is appropriate to utilise aCGH technology; whether genome-wide arrays or targeted arrays should be used; and what precautions should be taken in the clinical application of aCGH technology. Researchers are evaluating not only the technical side of the new diagnostic method, but also, more broadly, the clinical impact this technique could have on medicine in general and on patients and families. As the clinical use of this technique becomes a reality in prenatal diagnostics, more comprehensive guidance for researchers, clinicians and physicians will be necessary.

7 ETHICAL, LEGAL AND SOCIAL IMPLICATIONS

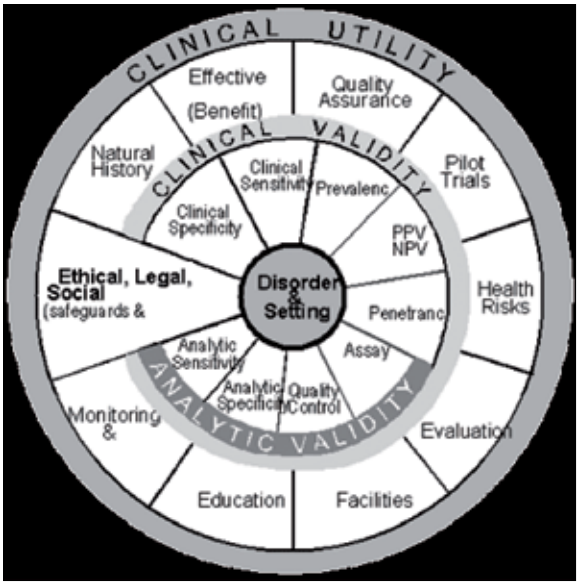
Before aCGH can be introduced for clinical prenatal diagnosis, a comprehensive analysis is necessary of the new technology's ethical, legal and social implications. Although aCGH holds significant medical promise, there are a number of complex and controversial issues that must be addressed. These questions are not necessarily unique to aCGH but rather apply to the rapidly developing field of genetics in general. Projects such as ACCE, a Centers for Disease Control and Prevention (CDC)-sponsored project, and the Human Genome Project's Ethical, Legal and Social Issues (ELSI) research, are examples of different frameworks by which to evaluate new genetic technology in terms of the broader implications of clinical use.

National Office of Public Health Genomics: ACCE project

This CDC-funded project has created a comprehensive model, as shown in Figure 6, to evaluate genetic tests and data according to four specific principles (from which it takes its name): analytic validity; clinical validity; clinical utility; and ethical, legal and social implications.[20] The model offers an abstract guideline for this assessment and can be of tremendous value in determining whether a particular genetic technology, in this case aCGH, is ready for clinical application.

Figure 6: *The ACCE evaluation process for genetic testing*

Source: *National Office of Public Health Genomics website: http://www.cdc.gov/genomics/gtesting/ACCE.htm*



Human Genome Project: ELSI research

This programme represents a small component of the larger Human Genome Project, providing a comprehensive outline of ethical, legal and social issues with regard to genetic testing and new genetic technology.[21] The goal of this research is to examine relevant issues and offer preliminary recommendations and considerations in order to promote the potential benefits of this technology while also minimising potential risks. These broad societal implications and concerns are broken down into a number of categories,[21] including but not limited to:

1. ***Privacy and confidentiality of genetic information***
   – *Who owns and controls genetic information?*
2. ***Psychological impact and stigmatisation***
   – *How does personal genetic information affect an individual and society's perceptions of that individual?*
   – *How does genomic information affect members of minority communities?*
3. ***Reproductive issues***
   – *Adequate informed consent for complex and controversial procedures; use of genetic information in reproductive decision-making; and reproductive rights*
4. ***Clinical issues***
   – *Education of physicians, patients and the general public in genetic capabilities, scientific limitations and social risks; and implementation of standards and quality-control measures in testing procedures*
5. ***Uncertainty***
   – *Should testing be performed when no treatment is available?*
   – *Are genetic tests reliable and able to be interpreted by the medical community?[21]*

These general models offer a starting point from which to examine the benefits and possible risks in genetics. However there are also a number of specific ethical and social issues that must be discussed in the light of the specific application and use of aCGH. The following is an analysis of various issues to be considered when examining the value and potential harms of clinical use of aCGH.

Justification of prenatal diagnosis

The first step in analysing the implications of aCGH technology is to understand why such a tool is useful and representative of advances in medicine. The prenatal screening capability of this method will provide physicians and parents with additional information about a pregnancy that could potentially affect a number of pregnancy-related issues:

> ***Pregnancy termination:*** *Because aCGH can detect chromosome abnormalities of known clinical significance leading to many severe disorders, parents may choose to terminate a pregnancy once they know that the foetus is affected.*
>
> ***Preparation for child:*** *In learning that a foetus is affected by a chromosome alteration that causes severe genetic disorders, parents will have the ability to properly prepare for the birth of an affected child. This could mean becoming educated on the disorder itself, researching options related to childcare and undertaking financial planning.*
>
> ***Pregnancy management:*** *Often knowledge of a chromosome abnormality can contribute to a physician's efficacy and success with regard to management of the pregnancy as well as the delivery.*
>
> ***PGD:*** *If aCGH technology progresses to the point where it can be used to clinically screen embryos for disease-causing genetic alterations, it could play an integral role in PGD. This potentially offers families who would otherwise choose not to become pregnant due to familial genetic disorders the option of having a healthy child.*

Alongside these justifications for prenatal diagnosis, there are additional ethical considerations with regard to pregnancy termination and PGD.

Acceptable or appropriate use of aCGH

As aCGH technology becomes available for clinical use, there will be a number of questions raised as to the acceptability of its use in prenatal diagnosis. In the current research and development phase, the technology is being used to identify genetic alterations that lead to serious conditions such as Down syndrome, and potentially fatal disorders such as Patau syndrome. However, there is potential for this technology to detect less severe genetic disorders; this raises serious concerns when parents may consider termination of pregnancy due to positive test results.[22] How severe is severe enough to justify ending a pregnancy in view of prenatal diagnostic results? As it becomes possible to screen for more minor genetic alterations, leading to less severe conditions, it may be necessary to evaluate for which disorders and genetic alterations it is appropriate to screen. The large

number of variants identified by aCGH, the clinical significance of which is unclear, will make decision-making more difficult for parents. Furthermore, the faster turn-around time of aCGH makes it more attractive for use in PGD, but will put increased pressure on prospective parents to make reproductive decisions in a short timeframe based on large amounts of imprecise information.

Test uncertainty

With aCGH, as with any diagnostic test, there is a certain risk of false positive results. At this stage of development of aCGH, there will be many results of uncertain clinical significance.[3] If the test were used to confirm a clinical diagnosis based on other clinical observations, the results of aCGH would be more interpretable than if the test were used as a prenatal diagnostic test. These tests introduce complicated issues for parents with regard to decision-making in the absence of correct or complete test results. A false positive, for example, may lead parents to terminate the pregnancy. In addition, it is not always the case that a particular abnormal genotype will lead to a particular phenotype. The inability to confirm that such a genomic alteration will lead to a specific phenotype could also lead to difficult decisions for the parents. In either case, these tests can also unnecessarily increase stress and anxiety for patients and families. Extreme caution and sensitivity is necessary both in the technical application of this technology (leading to fewer false positives, etc.), but also in the test result reporting phase, which requires the involvement of families.[14]

Privacy

When used as an alternative to traditional karyotyping, aCGH includes analysis across the entire genome to detect abnormalities in chromosomal integrity. Thus, in contrast to a more targeted examination, such as looking for specific point mutations in genes known to be associated with diseases such as cystic fibrosis or breast cancer, aCGH has the potential to yield unanticipated or unwanted information. Furthermore, it may yield information about family members. This is not unique to aCGH analysis, but the breadth and specificity of the analysis may give rise to much more, and more specific, unexpected information than traditional karyotyping. If used in the prenatal or PGD setting, parental samples are sometimes also analysed to aid in interpretation of the proband. In these cases, clinicians may face the situation of having to decide whether to disclose to the adults clinically significant information that arises in the context of testing their embryo or foetus.

Access to aCGH

As discussed, there are a number of questions that must be answered regarding to whom this technology will be offered and whether it should be available to all pregnant women, regardless of risk factors such as maternal age and previous familial genetic disorders.[8, 22] These questions will become more important as aCGH is increasingly used for clinical prenatal diagnosis outside of the research context. Because current aCGH technology uses samples from invasive testing (AC and CVS), a risk assessment will be necessary to determine whether aCGH is a potential option. Another component of this will be a financial assessment of the clinical use of aCGH, including laboratory costs and insurance coverage issues. The question of access to this technology is an important social and ethical consideration in the transition of aCGH from research to clinical use.

Education and counselling

For appropriate use of aCGH, genetic counselling will be needed to help families understand the process of genetic testing[19] and make informed decisions, especially in the context of prenatal testing. Understanding and interpreting the results of aCGH, in the context of genetic disorders and genetic tests, will be challenging for clinicians, patients and families. The many uncertainties about the clinical validity and utility of variation in DNA copy number, and the large amount of information generated by whole genome analysis, will make aCGH especially challenging for educators and counsellors. Parents and families facing decisions with regard to these issues must be fully informed on appropriate tests and the implications of positive results. However, the sheer volume of information about hundreds or thousands of loci in the genome will be difficult to process by counsellors and families.

8 RECOMMENDATIONS

At this stage of the development of aCGH technology, a number of recommendations can be made for clinical use. These recommendations should be revisited as information about its clinical validity and utility is obtained, and as the accuracy, resolution and cost of aCGH-based tests evolve.

First, aCGH should be used for prenatal testing only under research protocols where other data necessary for learning how to interpret aCGH data are also collected. This information might include results of other prenatal screening tests (such as nuchal translucency and multiple marker blood tests) and clinical data on parents. The aCGH data should be validated by and compared to some other method such as standard karyotyping, FISH and QF-PCR.

Secondly, before clinical use as a prenatal test, aCGH should be used and evaluated under conditions that allow assessment of the clinical significance of the results (i.e., where phenotype and clinical information about the patient is available, from newborns, children or adults). Given the large amount of copy-number variation in the general population, even among apparently healthy people, the finding of copy-number variants may in itself be of unclear clinical significance. In the prenatal setting, very little clinical information can be obtained to aid in the interpretation of aCGH results, and it is thus not the optimal setting in which first to bring the technology to clinical use.

Thirdly, laboratories beginning to use aCGH should adopt uniform and transparent technical standards, including standards for what constitute the 'normal' control samples with which patient samples are compared.

Fourthly, research and clinical laboratories using aCGH should anticipate the possibility of uncovering 'incidental' findings and make plans for handling them. For example, if parental samples are tested, in order to interpret the findings from a foetal sample tested to detect trisomy 21, the laboratory should make advance plans for whether and how to report unexpected findings of major clinical significance that arise in the parental samples. Laboratories should also decide whether any results will be withheld.

Fifthly, informed consent to aCGH in research or clinical settings should include provisions for how research subjects or patients want to handle 'incidental' findings, the possibility of unwanted results and results of unknown clinical significance.

9 CONCLUSION

The diagnostic power of aCGH technology offers an exciting and revolutionary approach to prenatal diagnosis, providing a much more fine-grained tool for genetic analysis than other currently available technologies. The detection capability, as a result of increased resolution, the comprehensive nature of the tests and the potential for faster reporting times, all make aCGH a promising new technique. However, despite the documented successes of this technology so far, more research is needed to understand its full scope so that aCGH can be implemented as a clinical tool in prenatal diagnosis. Because the technique has not yet transitioned to clinical use, there are as yet no established standards for its application. To be sure, ethical difficulties and ambiguities will attend its clinical use. Research will no doubt continue to improve the technology, but it is also important that the ethical, legal and social implications enter the discussion as aCGH transitions to clinical use.

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