ORIGINAL ARTICLE

Prenatal detection of unbalanced chromosomal rearrangements by array CGH

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Background: Karyotype analysis has been the standard method for prenatal cytogenetic diagnosis since the 1970s. Although highly reliable, the major limitation remains the requirement for cell culture, resulting in a delay of as much as 14 days to obtaining test results. Fluorescent in situ hybridisation (FISH) and quantitative fluorescent PCR (QF-PCR) rapidly detect common chromosomal abnormalities but do not provide a genome wide screen for unexpected imbalances. Array comparative genomic hybridisation (CGH) has the potential to combine the speed of DNA analysis with a large capacity to scan for genomic abnormalities. We have developed a genomic microarray of approximately 600 large insert clones designed to detect aneuploidy, known microdeletion syndromes, and large unbalanced chromosomal rearrangements.

Methods: This array was tested alongside an array with an approximate resolution of 1 Mb in a blind study of 30 cultured prenatal and postnatal samples with microscopically confirmed unbalanced rearrangements.

Results: At 1 Mb resolution, 22/30 rearrangements were identified, whereas 29/30 aberrations were detected using the custom designed array, owing to the inclusion of specifically chosen clones to give increased resolution at genomic loci clinically implicated in known microdeletion syndromes. Both arrays failed to identify a triploid karyotype. Thirty normal control samples produced no false positive results.

Conclusions: Analysis of 30 uncultured prenatal samples showed that array CGH is capable of detecting aneuploidy in DNA isolated from as little as 1 ml of uncultured amniotic fluid; 29/30 samples were correctly diagnosed, the exception being another case of triploidy. These studies demonstrate the potential for array CGH to replace conventional cytogenetics in the great majority of prenatal diagnosis cases.

Mainly as a result of screening programmes for the prenatal detection of chromosome abnormalities, approximately 40 000 amniocentesis and chorionic villus samples are processed annually in the UK.¹ The vast majority of these samples (around 90–95%) yield a normal karyotype by full microscopic analysis. A small proportion of cases reveal a chromosome abnormality, about 80% of which are autosomal trisomies for chromosomes 13, 18, and 21. The remaining abnormal karyotypes involve sex chromosome copy number changes and structural chromosomal rearrangements, such as deletions, duplications, inversions, and balanced and unbalanced translocations.

Microscopic analysis has been the gold standard for prenatal diagnosis since the development of chromosome banding techniques in the late 1960s.² Although highly reliable, this procedure has a number of limitations (reviewed by Shaffer and Bejjani³). Owing to the need for cell culture, the average reporting time for results in the UK can be up to 14 days. In addition, microscopic karyotyping is labour intensive and thus costly, requires skilled interpretation, and is not easily amenable to automation. The resolution is limited, with deletions and duplications <10 Mb not being reliably detected. Although high resolution methods have been shown to detect abnormalities of 3–5 Mb, these procedures are not suitable for routine screening applications (reviewed by Shaffer and Lupski⁴). When a structural chromosome abnormality is suspected, techniques such as fluorescent in situ hybridisation (FISH) or quantitative fluorescent (QF) PCR can be deployed, but a significant proportion of structural changes are not anticipated at the time of sample collection.

In attempts to overcome some of these limitations, alternative methods for aneuploidy detection based on FISH and QF-PCR have been applied to prenatal diagnosis. QF-PCR uses primer pairs designed to amplify sequences at several polymorphic loci in a single reaction, and is a rapid, efficient, and inexpensive method that is readily amenable to automation.^{5–7} FISH screening for common aneuploidies has also been applied to prenatal testing.⁸ The major limitation of QF-PCR and FISH compared with microscopic karyotype analysis is that they may not detect unbalanced chromosomal rearrangements such as microdeletions, which, although uncommon, account for approximately 1–2% of abnormalities detected by microscopic analysis of prenatal samples, and can have serious clinical consequences.⁹ Both of these techniques have been validated and applied to clinical samples, generally in addition to, rather than replacing, microscopic analysis.

Comparative genomic hybridisation (CGH) was developed as a genome wide screening strategy for detecting DNA copy number imbalances.¹⁰ The DNA content of a test and reference genome are compared by differentially labelling the genomic DNA with distinct fluorochromes, before competitively hybridising the labelled samples onto normal metaphase chromosomes and analysing the resulting ratio of the fluorochromes. While CGH has been used mainly to analyse the DNA content of tumours as a tool in cancer

Abbreviations: BAC, bacterial artificial chromosome; CGH, comparative genomic hybridisation; FISH, fluorescent in situ hybridisation; PAC, plasmid artificial chromosome; QF-PCR, quantitative fluorescent PCR

research,¹¹ the technique has recently been shown to be valuable for the detection of copy number imbalances in fetal tissue following loss of pregnancy.¹² However, CGH still requires metaphase chromosomes as targets for hybridisation, limiting high resolution CGH methods to around 3 Mb.¹³

Microarray CGH is similar in principle to conventional CGH,^{14,15} but uses arrayed DNA sequences instead of metaphase chromosomes as targets for hybridisation, thus providing a direct link between detected aberrations and the physical and genetic maps of the human genome. Array CGH has a number of significant potential advantages over conventional prenatal testing, providing a technique that is not only sensitive and comprehensive, but may be amenable to automation, thus decreasing cost, labour, and the reporting time of results.

Array CGH has already been shown to be a useful tool in clinical genetics for detecting deletions and duplications in patients with mental retardation or learning difficulties, beyond the limits detectable by microscopy,^{16,17} and for the analysis of individuals with known chromosome abnormalities using a custom designed microarray.¹⁸ Array CGH analysis of fetuses with multiple malformations identified genomic rearrangements in around 16% of cases, which had not been observed by karyotype analysis.¹⁹ In a study of products of conception from spontaneous miscarriages using a low density array containing targeted clones of clinical significance, array CGH was able to detect all abnormalities previously identified by microscopic karyotype analysis, and detected additional abnormalities in approximately 10% of cases.²⁰ The technique therefore holds some promise of combining the speed, sensitivity, and potential for partial automation of a DNA based test, with the genome screening characteristics of microscopic karyotyping.

Although it is becoming accepted that array CGH will have a place in clinical genetic testing, it is far from clear how this will be best applied. The coverage and resolution of array CGH are dependent on the design and density of the array used. Although superficially appealing, an array covering the entire genome at very high resolution would have potential disadvantages in clinical use; more array probes are likely to generate a higher number of false positives, and large arrays are more expensive to fabricate, quality control, and interrogate. Recent investigations showing significant levels of copy number polymorphism in normal populations^{21,22} reinforces the desire to test only a limited number of clones, the results of which do not give rise to needless complications in interpretation. We reason that, particularly for prenatal screening when time for further investigation is limited and ambiguous results cause severe anxiety, the ideal array would contain the minimum number of clones that will deliver the required diagnosis.

We therefore designed and carefully validated an array of approximately 600 large insert clones, concentrating on areas of known clinical significance with dense representation across the common microdeletion regions, and with a lower representation of about one clone per 10 Mb over the remainder of the genome to detect unexpected major chromosome imbalance. We compared the performance of this array with that of a previously described array of 1 Mb resolution,²³ by parallel, blind analysis of 30 prenatal or postnatal samples known to have unbalanced rearrangements. Because a major advantage of array technology would be lost if cell culture were required, we further tested the array on DNA extracted from 30 samples of 1–2 ml of uncultured amniotic fluid (many with abnormal chromosome constitution), surplus to requirements after diagnostic testing (publication in progress).

We believe these results provide proof of principle that array CGH, using a custom designed array, is rapid. Although

better than cytogenetics in some respects and worse in others, it will 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 we do not claim the current array is ideal, and further work is required to reach a consensus on the optimum configuration of an array for clinical use. Large trials will be needed to demonstrate sensitivity and specificity in clinical operating conditions.

METHODS

Array design and production

The prenatal array described in this study was developed using published protocols.²³ In brief, large insert bacterial and plasmid artificial chromosome (BAC and PAC) clones were chosen from the published Golden Path of the human sequence, to cover each chromosome at a resolution of one clone every 10 Mb. Additional clones were selected for the major common microdeletion syndrome regions, as far as possible covering identified critical regions and microdeletion breakpoints with overlapping clones. Approximate clone locations are shown in fig 1 (clone list in supplementary data). Isolated clone DNA was first amplified by degenerate oligonucleotide primed PCR, followed by secondary PCR with a 5'-amine modified primer. Array clones were spotted in duplicate onto CodeLink activated glass slides. The 1 Mb resolution arrays used in this study are as published previously.²³

Array CGH

DNA labelling and array hybridisation

DNA samples (0.225 µg (chromosome "add in" experiments, normal controls, and cultured samples) or 0.100 µg (uncultured samples) prenatal array (0.45 µg, 1 Mb resolution array) were labelled and the microarray hybridisation performed as described previously.²⁴

Image acquisition and data analysis

Arrays were scanned using an Agilent scanner (Agilent Technologies, West Lothian, UK) and images quantified using SPOT software.²⁵ Further analysis was performed using a custom designed Microsoft Excel spreadsheet, in which data were normalised by dividing the ratio of each spotted clone by the median ratio of all autosomal clones. Spots were then excluded from further analysis where duplicate spot ratios differed from the duplicate mean by >10% or where the fluorescent intensities were below those of *Drosophila* control spots.

Array validation

Chromosome add in experiments

To test the specificity and sensitivity of clones represented on the prenatal array, a series of experiments were performed in which flow sorted chromosome DNA was added to self versus self hybridisations to simulate gain of each chromosome in turn.²³ Chromosomes were flow sorted as described previously,²⁶ and DNA isolated from aliquots containing around 250 000 chromosomes using previously published methods.²⁷ Prior to labelling, DNA representing either approximately two or four additional copies of an individual chromosome was combined and labelled together with reference DNA and competitively hybridised against the same reference DNA. Following hybridisation and data analysis, the standard deviation for each array was calculated from the ratios comprising the 95th percentile. Thresholds were placed at $\pm 4SD$ from the median of the 95th percentile values. Using these settings, a clone would be expected to exceed the threshold once in every 24 hybridisations as a result of statistical variation. Clones exceeding the thresholds set for the individual experiment on chromosomes other than the

Figure 1 Ideogram showing the approximate locations for clones represented on the prenatal microarray.

add in chromosome were then identified and excluded from subsequent analyses. This method was used to identify potentially mismapped clones and clones hybridising to multiple genomic loci.

Normal controls

DNA isolated from 30 normal, healthy blood donors was hybridised against reference DNA. As described for the series of chromosome add in experiments, the standard deviation for each array was calculated from the ratios comprising the 95th percentile. Thresholds were placed at $\pm 4SD$ from the median of the 95th percentile values, and clones exceeding the thresholds set for the individual experiment identified.

Patient samples

Cultured samples

Samples were selected from amongst those available with consent, to represent a broad spectrum of cytogenetic abnormalities including autosomal trisomy, sex chromosome abnormality, marker chromosomes, and triploidy, with particular emphasis on those such as microdeletions, unbalanced structural rearrangements, and mosaicism, which pose difficulties in detection by either array CGH or cytogenetic examination. DNA was isolated from prenatal cultured amniocytes, cultured chorionic villi, or postnatal blood specimens for samples previously confirmed by either microscopic karyotype analysis or FISH as carrying a chromosomal rearrangement. The results of these investigations were blinded prior to further analysis by array CGH. Clones exceeding experimental thresholds were identified using methods as described above.

Uncultured samples

Prior to array CGH, all samples were analysed by QF-PCR and conventional cytogenetic analysis, the results of which were

concealed from the person performing array CGH. For array CGH, DNA was extracted from chorionic villi (a single small fragment) or 1–2 ml uncultured amniotic fluid using a

Figure 2 Chromosome 13 add in experiment, demonstrating the response of clones represented on the prenatal array to additional copies of chromosome 13. (A) Shows the response of all clones to approximately two additional copies of chromosome 13, whereas (B) shows the response of chromosome 13 clones only to approximately two or four additional copies of chromosome 13.

commercial kit (QIAamp Blood DNA Extraction Mini Kit; Qiagen, www.qiagen.com) with slight modification to the manufacturer's protocol. Briefly, cell pellets were resuspended in 200 µl PBS and the final elution volume was 100 µl. As previously described, clones exceeding experimental thresholds were identified. All samples retrieved from patients undergoing diagnostic procedures were surplus to diagnostic requirements, and were consented for use in research studies.

RESULTS

Array validation

Chromosome add in experiments

To comprehensively test hybridisation characteristics of the entire clone set represented on the prenatal array, chromosome add in experiments were performed by the addition of DNA isolated from flow sorted chromosomes to a series of self versus self hybridisations. Individual chromosomes were flow sorted, with the exception of chromosomes 9–12. Owing to their similar size and base pair composition, these chromosomes are difficult to resolve into distinct populations and were therefore co-sorted (reviewed in Ibrahim and van den Engh²⁸). A representative genomic profile for a chromosome 13 add in experiment is shown (fig 2). All clones mapping to chromosome 13 showed the expected increased ratio in response to the additional copies of the chromosome. However, three clones mapping to chromosomes 18 (RP11-16L7), 20 (RP13-329D4), and 22 (AC008103) also showed a significant ratio change identifying cross homology with chromosome 13 or incorrect mapping. Using this method for all chromosomes, we identified 69 incorrectly mapped or cross hybridising clones (~10% of total clones), which either did not respond to the expected chromosome add in, responded to DNA from a different chromosome, or hybridised to multiple chromosomes. Clones identified using this method were excluded from all subsequent analyses and would not be included in future arrays.

Normal controls

Validation experiments were performed in which DNA isolated from normal healthy blood donors was hybridised against reference DNA and clones whose log₂ ratios (test/reference) exceeded the thresholds set for each particular experiment were identified. In 30 normal versus normal reference hybridisations, two clones exceeded the set thresholds. The log₂ values for these clones were +0.37 and +0.38, which although significant, were substantially less than the theoretical ratios of +0.58 expected to indicate a single copy gain. Using the thresholds discussed earlier, we would expect around one false positive result in every 24 hybridisations, due to statistical variation. It would, however, be necessary to perform replicate or dye swap hybridisations and to carry out alternative investigations such as FISH to distinguish a false positive result from the possibility that these clones are reporting variation between normal individuals.

Patient samples

Array CGH was performed using both a 1 Mb resolution array and a custom designed prenatal array in a blind study of 30 cultured prenatal or postnatal samples with known unbalanced karyotypes. Of the 30 aberrations, 22 were detected using the 1 Mb resolution array, whereas 29/30 aberrations were identified using the prenatal array (table 1). Eight cases of autosomal trisomy (chromosomes 13, 18, and 21; fig 3A), five examples of sex chromosome copy number abnormality (monosomy X, XXY, and XYY), and microdeletions previously identified by FISH at 15q11 (Angelman and Prader-Willi syndromes; fig 3B), and 5p (cri-du-chat syndrome) were observed by array CGH using both arrays. However, microdeletions detected using the

prenatal array at 4p (Wolf-Hirschhorn syndrome), 7q11 (Williams syndrome; fig 3C), 22q11 (DiGeorge syndrome) and 17p11 (Smith-Magenis syndrome) were not observed using the 1 Mb array as these regions are not covered by clones representing the 1 Mb array clone set.

Details of specific cases

Case 9

Microscopic karyotype analysis revealed an isodicentric chromosome 13, leading to trisomy for 13ptel-13q32 and monosomy for 13q32-13qtel. Array CGH identified clones with ratios indicative of a single copy duplication or deletion.

Case 12

Case 12 had an unbalanced translocation between chromosomes 11 and 22, leading to trisomy for 11q23-11qtel and monosomy for the terminal region of the long arm of chromosome 22.

Case 22

Karyotype analysis showed 34/40 cells having an additional, small marker chromosome, positive for a chromosome 12 centromere repeat probe and a whole chromosome 12 FISH "paint". Array CGH using the prenatal array detected duplicated ratios for a single chromosome 12 clone, although the hybridisation ratios did not reach the values expected for a single copy gain, indicating either partial clone duplication or a mosaic karyotype. A similar result was observed with 12 chromosome 12 clones using the 1 Mb resolution array.

Case 27

Case 27 showed 25/44 cells having a marker chromosome, which was positive for a whole Y chromosome FISH paint. This was identified using array analysis by the deletion of 3 or 14 clones (prenatal and 1 Mb array respectively).

Case 28

Case 28 also exhibited a mosaic karyotype with 68/73 cells having an extra chromosome 9. Clones on chromosome 9 showed increased ratios by array CGH, but lower than those expected for a single copy gain.

Case 7

Neither array was able to identify an abnormality in case 7, which had a triploid karyotype.

Uncultured prenatal samples

Array CGH was performed using the prenatal array in a blind study of 30 uncultured prenatal samples. Samples had been previously analysed by QF-PCR and by microscopic karyotype analysis (publication in progress). They included 20 normal controls, 8 cases of trisomy 21, 1 case of triploidy with 4 copies of chromosomes 5 and 21, and a 45,X/46, X dic Yp mosaic. In 29/30 cases, there was concordance between array CGH and previous analyses (table 2, fig 4). Array CGH failed to detect triploidy for case 50, but this sample was identified as being abnormal, as ratios for clones representing chromosomes 5 and 21 were indicative of a single copy gain.

DISCUSSION

We have demonstrated the potential utility of array CGH for the detection of chromosomal abnormalities in prenatal diagnosis. By comparing established methods of prenatal testing (microscopic karyotype analysis, FISH, and QF-PCR) with array CGH, using both a small, custom designed array and a larger array containing clones covering the entire genome at an approximate resolution of 1 Mb, we were able to show that the great majority of abnormalities can be detected using array based methods.

Figure 3 Array CGH data for samples 5 (A), 1 (B) and 2 (C) (table 1), on both the prenatal (a) and 1 Mb (b) arrays. The x axis represents the chromosome (A) or the distance in Mb from the p telomere (B and C). The y axis represents the hybridisation ratio given as a log₂ scale. Significant gains are shown in green whereas significant losses are red. (A) Trisomy 21; (B) deletion at 7q11.23 observed on the prenatal array but absent at 1 Mb resolution; (C) deletion at 15q11.2–15q13.1 visible on both arrays.

The 1 Mb array used in this study was previously shown to be successful for the detection of submicroscopic deletions in patients with mental retardation or learning difficulties.¹⁶ In our study, this array detected only 22/30 known aberrations, missing small microdeletions at 4p (Wolf-Hirschhorn syndrome), 7q (Williams syndrome), 17p (Smith-Magenis

syndrome), and 22q (DiGeorge syndrome) because of low clone density in these regions.

In contrast, the smaller, purpose designed prenatal array identified 29 of the 30 aberrations. This lower density array is comprised of clones for genome wide analysis at an approximate resolution of only around 10 Mb (~450 clones)

Table 1 Summary of cytogenetic analyses and array CGH data using the prenatal and 1 Mb microarrays on cultured prenatal and postnatal blood samples

Array CGH			
Sample	Prenatal array	1 Mb array	Cytogenetic analysis
1	Deletion of 3 clones at 7q11.23	Normal	46,XY,ish del(7)(q11.23q11.23)(ELN-)*‡
2	Deletion of 10 clones at 15q11.2-15q13.1	Deletion of 7 clones at 15q11.2-15q13.1	46,XYdel(15)(q11.2q13).ish del(15)(q11.2q11.2)(E24-)*‡
3	Deletion of 10 clones at 15q11.2-15q13.1	Deletion of 7 clones at 15q11.2-15q13.1	46,XX,del(15)(q11q13).ish del(15)(q11q13)(D15S10-)*‡
4	Deletion of 10 clones at 15q11.2-15q13.1	Deletion of 7 clones at 15q11.2-15q13.1	46,XY,del(15)(q11.2q13).ish del(15)(D15S10-)*‡
5	Trisomy 21	Trisomy 21	47, XY, +21†§
6	Trisomy 21	Trisomy 21	47, XX, +21†§
7	Normal	Normal	69, XXX†§
8	Trisomy 18	Trisomy 18	47, XX, +18†§
9	Dup(13ptel-13q31.3), del(13q32.3-13qtel)	Dup(13ptel-13q32.1),del(13q32.1-13qtel)	46, XX, psu idic(13)(q32) †§
10	Monosomy X	Monosomy X	45, X†§
11	Trisomy 21	Trisomy 21	47, XX, +21†§
12	Dup(11q24.1-11qtel), del(22q13.2-22qtel)	Dup(11q23.3-11qtel),del(22q13.31-22qtel)	46, XY, add(22)(q13.3).ish der(22)t(11;22)(q23;q13.3)(pVYS207M-, wcp11+,pVYS239A+)de novo†§
13	Deletion at 22q11.21	Normal	46, XY, ish del(22)(q11.2q11.2)(D22S931-, D22S941-)*‡
14	Trisomy 13	Trisomy 13	47, XY, +13†§
15	Deletion of 3 clones at 7q11.23	Normal	46, XX,ish del (7)(q11.23q11.23)(ELN-,LIMK1-,D7S613-) *‡
16	Deletion of 7 clones at 17p11.2	Normal	46,XX,del(17)(p11.2p11.2).ish del(17)(p11.2p11.2)(D17S29-)*‡
17	Deletion of 10 clones at 4ptel-4p16.3	Normal	46,XX,ish del(4)(p16.3)(WHSCR-)*‡
18	Deletion at 22q11.21	Normal	46,XX,del(22)(q11.2q11.2).ish del(22)(q11.2q11.2)(TUPLE 1-)*‡
19	Deletion at 22q11.21	Normal	46,XX,ish del(22)(q11.2q11.2)(TUPLE1-)*‡
20	XXY	XXY	47, XXY†§
21	Trisomy 13	Trisomy 13	46, XY, der(13;13)(q10;q10),+13†§
22	Duplication of 1 clone at 12p11.21, ratios indicative of less than a single copy gain	Duplication of 8 clones at 12p11.22-12p11.1, ratios indicative of less than a single copy gain	47,XY,+mar.ish der(12)(wcp12+,D12Z3+)/46, XY†§
23	Monosomy X	Monosomy X	45, X†§
24	Trisomy 18	Trisomy 18	47, XX, +18†§
25	XXY	XXY	47, XYY†§
26	XXY	XXY	47, XXY†§
27	Deletion of 3 clones at Yq11.221-Yq12	Deletion of 14 clones at Yq11.221-Yq12	46, X, +mar.ish der (Y)(wcpY+) ²⁵ /45, X ¹⁹ †§
28	Ratios for chromosome 9 clones show values indicative of less than a single copy gain	Ratios for chromosome 9 clones show values indicative of less than a single copy gain	47, XX, +9[68]/46, XX ⁵ †§
29	Trisomy 13	Trisomy 13	47, XX, +13†§
30	Deletion of 13 clones at 5p15.33-5p15.2	Deletion of 17 clones at 5p15.33-5p15.1	46, XY, del(5)(p15.1) †§

*Postnatal sample; †prenatal sample; ‡performed by FISH; ± performed by microscopic karyotype analysis.

but with an additional set of around 200 clones, chosen to cover major trisomy and microdeletion regions at increased density. This array, designed with a lower overall complexity but containing clinically important clones, identified all but one of the known chromosomal abnormalities.

Particularly in light of emerging information about large copy number variation in normal human populations,^{21, 22} large arrays are likely to generate some difficulties in interpretation, at least until much more is learned about genomic copy number variation. Small, low resolution arrays are likely to be less prone to technical error, produce fewer false positive results, will be cheaper to standardise and fabricate, and can be designed to avoid genomic regions with known polymorphic copy number variation. This and earlier studies¹⁸ demonstrate that a targeted array can combine these advantages, with a high detection rate for the chosen diagnostic categories.

It is important to address some of the limitations of array CGH before this technique is considered for clinical diagnosis (reviewed in Vermeesh *et al* and Bajjani *et al*^{29, 30}). Array CGH is unable to detect polyploidy and balanced chromosomal rearrangements, as the process of data normalisation produces results only sensitive to relative dosage imbalance between different regions of the sample under test. Polyploidy (almost always triploidy) is virtually always lethal during fetal life and is generally suspected on ultrasound

investigation. Where such suspicion arises, other diagnostic procedures such as QF-PCR must be used. While conventional karyotyping is able to detect balanced chromosomal rearrangements, the majority of truly balanced translocations produce no phenotypic abnormality,³¹ and their discovery can lead to difficult clinical decisions during pregnancy. Detection of copy number variation by array CGH does not of itself allow non-disjunction to be distinguished from unbalanced structural abnormality, which is important for genetic counselling.

On the other hand, array CGH using a carefully selected clone set is likely to be more sensitive than microscopic karyotyping for detecting microdeletions in the tested regions. Microdeletion syndromes are contiguous gene syndromes characterised by the loss of specific chromosomal segments, and result in clinically distinct phenotypes, some of which are at least as severe as those observed with Down syndrome (reviewed in Malcolm³²). Many of the chromosomal rearrangements associated with microdeletion syndromes are too small to be reliably identified by routine microscopic karyotype analysis and are therefore often only detected after birth by FISH analysis using locus specific probes.³³⁻³⁵

Precisely which chromosomal regions to include in a designed prenatal array will require continued discussion including laboratory, clinical, and patient viewpoints. In the

Figure 4 Array CGH data for samples 35 (A), 46 (B), 52 (C), and 39 (D) (table 2), on the prenatal array. The x axis represents the chromosome (A, B, and C) or the distance in Mb from the p telomere (D). The y axis represents the hybridisation ratio given as a $\log_2$ scale. Significant gains are shown in green whereas significant losses are red. (A) 46, XY, (B) 46, XX (C) 47, XY +21 and (D) 46, XY +duplication of 5 clones at Yptel-Yq11.223.

array described in this study, we included major eponymous microdeletion regions. However, we deliberately excluded regions where copy number change is associated with mild or no phenotypic abnormality. Inclusion of more pericentromeric clones would improve the likelihood of detecting supernumerary marker chromosomes.

Array CGH using a small array is therefore likely to detect all major aneuploidies, and to be more sensitive than microscopy at detecting microdeletions within areas of suspicion. It will not detect polyploidy, balanced structural rearrangements, and some unexpected relatively small unbalanced rearrangements that happen not to fall over areas of clone coverage. Mosaicism, by its nature, is likely to present problems for either technique. In this series, we noted clone ratios suggestive of less than a single copy gain in two of the three mosaics tested, with the third case of mosaicism being identified as a deletion, but further data is needed to define the real sensitivity of array CGH for mosaicism. In practice, array CGH (in common with QF-PCR and FISH) will be used on uncultured material, and is therefore likely to produce some differences from

cytogenetics performed on cultured cells with the opportunity for differential cell lineage proliferation. We would estimate that, over all categories of abnormality, the two techniques will have rather similar sensitivity for detection of clinically important changes.

Array CGH analysis of cell free fetal DNA isolated from amniotic fluid samples has shown promising results, with fetal sex and whole chromosome changes being determined.³⁶ However, higher levels of clone to clone variability were noted when compared with cultured amniocytes, so it is possible that smaller changes involving only a few clones might not be reliably identified using this method. In this study, DNA isolated from both cultured and uncultured amniocytes was used for hybridisation. We found that array CGH can work well on DNA from uncultured amniocytes. As we had to work with material surplus to diagnostic requirements, we performed array CGH on DNA derived from as little as 1 ml of uncultured amniotic fluid; in practice, performance would doubtless be better with more material. The ability to perform array CGH on uncultured prenatal cells has not previously been established.

Table 2 Summary of cytogenetic analyses, QF-PCR, and array CGH data using the prenatal microarray on uncultured prenatal samples

No.	Prenatal array	Karyotype as ascertained by conventional karyotyping	Concordance between array CGH, QF-PCR and karyotype
31*	Normal female	46,XX	Yes
32*	Trisomy 21	47,XY +21	Yes
33*	Normal female	46,XX	Yes
34*	Normal female	46,XX	Yes
35*	Normal male	46,XY	Yes
36*	Normal male	46,XY	Yes
37*	Normal male	46,XY	Yes
38†	Normal male	46,XY	Yes
39*	Duplication of 5 clones at Yptel-Yq11.223	45,X/46,X dic Yp mosaic	Yes
40*	Normal female	46,XX	Yes
41*	Normal female	46,XX	Yes
42*	Normal male	46,XY	Yes
43*	Normal female	46,XX	Yes
44*	Trisomy 21	47,XY +21	Yes
45*	Trisomy 21	47,XY +21	Yes
46*	Normal female	46,XX	Yes
47*	Normal male	46,XY	Yes
48*	Trisomy 21	47,XY +21	Yes
49*	Trisomy 21	47,XY +21	Yes
50*	Ratios indicative of single copy gain for chromosomes 5 and 21	71,XXY +5 +21	No
51*	Trisomy 21	47,XY +21	Yes
52*	Trisomy 21	47,XY +21	Yes
53*	Normal female	46,XX	Yes
54*	Normal female	46,XX	Yes
55*	Normal male	46,XY	Yes
56*	Normal male	46,XY	Yes
57*	Trisomy 21	47,XY +21	Yes
58*	Normal male	46,XY	Yes
59*	Normal male	46,XY	Yes
60*	Normal male	46,XY	Yes

*Amniotic fluid sample; †CVS sample.

Array CGH has the potential to combine the speed and capacity for automation of a DNA based technique, with the genome wide scanning capability of conventional cytogenetics. 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 a 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.

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