The clinical utility of microarray technologies applied to prenatal cytogenetics in the presence of a normal conventional karyotype: a review of the literature

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ABSTRACT

The clinical utility of microarray technologies when used in the context of prenatal diagnosis lies in the technology’s ability to detect submicroscopic copy number changes that are associated with clinically significant outcomes. We have carried out a systematic review of the literature to calculate the utility of prenatal microarrays in the presence of a normal conventional karyotype. Amongst 12,362 cases in studies that recruited cases from all prenatal ascertainment groups, 295/12,362 (2.4%) overall were reported to have copy number changes with associated clinical significance (pCNCs), 201/3090 (6.5%) when ascertained with an abnormal ultrasound, 50/5108 (1.0%) when ascertained because of increased maternal age and 44/4164 (1.1%) for all other ascertainment groups (e.g. parental anxiety and abnormal serum screening result). When additional prenatal microarray studies are included in which ascertainment was restricted to fetuses with abnormal ultrasound scans, 262/3370 (7.8%) were reported to have pCNCs. © 2013 The Authors. Prenatal Diagnosis published by John Wiley & Sons Ltd.

INTRODUCTION

Over the past few years, microarray analysis, also known as molecular karyotyping or chromosomal microarray analysis, has gradually replaced conventional G-banded karyotyping as the frontline diagnostic test for children and adults presenting with a wide range of neurodevelopmental phenotypes with or without associated congenital abnormalities.1–4 In many countries, notably in the USA and Europe, the transfer from karyotyping to microarrays in the postnatal constitutional setting is now widespread, but the application of this technology to prenatal cytogenetics has lagged behind the postnatal implementation largely because of the perceived difficulties of interpreting variants of unknown significance (VOUS) in the context of an ongoing antenatal diagnosis. Prenatal microarrays have been more readily adopted in specific situations. For example, the use of microarrays in cases of known chromosomal abnormalities that were initially identified by karyotyping allows for further characterisation of the chromosomal breakpoints and the genes involved. Also, a number of smaller prenatal studies have restricted the comparison of microarrays and conventional karyotyping to fetuses ascertained with ultrasound abnormalities.5–13

Recently, several large-scale prenatal microarray studies have been published in which the diagnostic yields and the utility of microarrays and conventional karyotyping to fetuses ascertained with ultrasound abnormalities.

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In this paper, we have attempted to determine the underlying rate of copy number changes with associated clinical significance (pCNCS) that can be detected by microarrays in the prenatal diagnostic setting but have restricted our inclusion criteria to those cases where a pCNC has been detected in the presence of a normal conventional karyotype. This review therefore excludes the evaluation of cases where normal microarray results may mask a balanced chromosome rearrangement that could have clinical significance, for example apparently balanced translocations or inversions in conjunction with normal microarray profiles. We have presented our results to try and estimate the overall detection rate of prenatal pCNCS within the subclassifications of primary referral criteria.

**METHODS: INCLUSION AND EXCLUSION CRITERIA**

The search for suitable papers focused on prenatal studies using microarray technology. The advanced search function of the NCBI database, online resource PubMed (http://www.ncbi.nlm.nih.gov/pubmed/advanced), was used with the word ‘prenatal’ combined with the following terms to describe microarrays: ‘microarray’, ‘array’, ‘chromosomal microarray’, ‘array comparative genomic hybridisation’, ‘cma’, ‘CGH’ and ‘aCGH’. The titles and abstracts of the identified articles were then checked against predetermined criteria for eligibility. Bibliographies of relevant papers were manually interrogated for further papers not identified by electronic searches. Experts in the field were also contacted for completeness of the literature review (Lisa G. Shaffer and Jill A. Rosenfeld).

Our initial review of the literature identified 22 suitable papers5–26 published between 2005 and 2013, which were then subdivided into those where all categories of prenatal ascertainment were included in the study design14–17 and those where the analyses were restricted to fetuses presenting with an abnormal ultrasound scan.5–12 It should be noted that only papers where the microarray results, conventional karyotypes and the final clinical interpretations were all recorded have been included in this review. The strict inclusion criteria of papers also required the ability to identify the number of cases from each ascertainment category with a pCNC in the presence of a normal karyotype, calculate the total number of karyotypically normal cases tested by CMA belonging to each category and correlate specific raw copy number change data supplied by the authors to numbers provided in their summary tables. If it was not possible to elucidate this information from the paper, or obtain it through personal communication with the authors, then the paper was not included in the review. Based on this inclusion criteria, the relatively large data sets from Breman et al.,19 Park et al.18 and Armengol et al.20 were not included in the final tables.

There were a number of difficulties encountered when trying to make direct comparisons between studies including the following: (i) We have used and presented, without further interpretation or comment, the genome coordinates and clinical interpretation of pCNCS as published by the individual groups and have reproduced the original calls and associated interpretations in the Appendices. (ii) The studies included have used a variety of microarray platforms comprising differences in design and resolution (e.g. from ‘targeted’ 1 Mb BAC arrays to custom designed 44k oligo or 125k SNP arrays). It should be noted that we have only included abnormalities involving a change in copy number and have therefore excluded abnormalities such as uniparental disomy, which were detected by SNP arrays. (iii) We made the decision not to include copy number changes that were classified purely as VOUS. It should be noted here that some authors (e.g. Wapner et al.13) include a number of potentially significant VOUS within their category of copy number changes with clinical significance, and we have included these cases within the ‘pCNC’ group to try and determine the underlying rate of cases deemed to have clinical consequences. VOUS rates frequently depend on both the coverage of the array and the laboratory reporting policy employed, making comparisons across studies difficult to do meaningfully. The stratification of the relative risk associated with any particular VOUS can also determine whether it is considered to fall within a low, moderate or high risk of adverse clinical consequences, and this stratification is reflected in the evidence-based subclassification of VOUS as outlined by the International Standards for Cytogenomic Arrays (https://www.iscasonsortium.org/). In many of the papers reviewed, no such risk stratification was presented with the exception of Wapner et al.13 who employed an expert review panel to stratify risks associated with VOUS encountered throughout the course of their study. Although the clinical management of VOUS especially within the prenatal setting is important, we felt that the focus of this review should be restricted to those cases that were interpreted and therefore reported to have clinically significant prenatal pCNCS in the presence of a normal karyotype.

**RESULTS**

A list of the published results can be found in the online appendix. The inclusion criteria used in this review meant that a number of relatively large-scale studies were not included principally because it was not possible to correlate individual karyotypic and microarray results18 and/or to break down results by different ascertainment groups.19,20 Other studies were not cited individually because their results were included in other large-scale studies (e.g. Kleeman et al.,13 Maya et al.21 and Coppinger et al.22 are included in the study by Shaffer et al.15).

Overall prenatal detection rate for pCNC in the presence of a normal conventional karyotype

In Table 1, it can be seen that we have limited our review to four large-scale studies in which the data are broken down into three main ascertainment categories. It should also be noted that because of differing study designs, there is significant variability with respect to the numbers reflected in the ascertainment groups; for example, ~51% and ~38% of cases recruited by Wapner et al.14 and Fiorentino et al.17 respectively, were for advanced maternal age compared with only 6% by Shaffer et al.15. By comparison, the proportion of cases recruited because of an abnormal ultrasound ranged from ~2.5% by Fiorentino et al.17 to ~80% by Shaffer et al.15. Despite this variation in study design we decided to pool these data, which results in 25% of cases recruited following an abnormal ultrasound scan, 41% with advanced maternal age and 34% for the other ascertainment categories.

These pooled data show that in the presence of a normal conventional karyotype, 295/12362 (2.4%) of cases reported...
overall were found to have a pCNC compared with 201/3090 (6.5%) following an abnormal ultrasound, 50/5108 (1.0%) when ascertained because of increased maternal age and 44/4164 (1.1%) for all other ascertainment groups.

Fetuses presenting with ultrasound abnormalities

The results for the eight targeted studies included in these analyses5–12 are summarised in Table 2. Amongst these targeted studies, the detection rate for pCNCs ranged from 6.1% to 13.3%, but by pooling these data, 61/640 (9.5%) cases were interpreted to have a pCNC. In Table 2, we have also included the abnormal ultrasound abnormality ascertainment category from Wapner et al.14 Shaffer et al.15 Lee et al.16 and Fiorentino et al.17 from which it can be seen that 262 of the 3730 (7.0%) abnormal ultrasound cases overall were classified as having pCNCs.

DISCUSSION

The primary purpose of this review was to estimate the ‘added’ diagnostic value of microarray technology when applied to cytogenetic prenatal diagnosis especially in those cases where conventional G-banded analysis provides a normal chromosome result. The headline figures are compelling in that from all ascertainment groups comprising 12362 cases, microarrays revealed 295 (2.4%) of cases with cryptic abnormalities interpreted to have clinical significance to the ongoing pregnancy, and this increases to 7.0% of cases following an abnormal ultrasound scan, and in ~1% of combined cases with advanced maternal age or other referrals such as parental anxiety, history of chromosome abnormality or an abnormal serum screening result. The detection of these abnormalities is in addition to those seen by conventional karyotyping, as all of the cases considered here were karyotypically normal by routine chromosome analysis.

Current conventional prenatal diagnosis requires an invasive procedure (amniocentesis or chorionic villus sampling) with an associated risk of miscarriage of approximately 0.5% to 1%.27 In many countries, the offer of an invasive procedure is usually preceded by maternal serum and/or ultrasound screening designed to stratify the risk primarily of Down syndrome in the

**Table 1** Summary of pCNC microarray findings in routine prenatal diagnosis in the presence of a normal karyotype

| Study         | Sample size | Platform                      | Total pCNC (%) | Abnormal ultrasound (%) | Maternal age (%) | Other (%) |
|---------------|-------------|-------------------------------|----------------|-------------------------|------------------|-----------|
| Wapner et al. | 3822        | Targeted with 1 Mb backbone   | 96 (2.5)       | 45/755 (6.0)           | 34/1966 (1.7)    | 17/1101   |
| Shaffer et al.| 2587        | Various                       | 142 (5.5)      | 131/2081 (6.3)         | 0/161 (0.0)      | 11/345    |
| Lee et al.    | 3080        | BAC targeted/60k oligo        | 35 (1.1)       | 20/180 (11.1)          | 10/1891 (0.5)    | 5/1009    |
| Fiorentino et al. | 2873    | BAC targeted                  | 22 (0.8)       | 5/74 (6.8)             | 6/1090 (0.6)     | 11/1709   |
| Total         | 12362       | Various                       | 295 (2.4)      | 201/3090 (6.5)         | 50/5108 (1.0)    | 44/4164   |

*Normal conventional karyotypes only.

*bIncluding parental anxiety, history of chromosome abnormality, and abnormal serum screening result.

**Table 2** Prenatal microarray studies focused on abnormal ultrasound (AUS) only

| Sample size | Platform                     | No. pCNC | pCNC % |
|-------------|------------------------------|----------|--------|
| Tyreman et al. | 106 Affymetrix Gene Chip 6.0 | 11       | 10.4   |
| D’Amours et al. | 49 BAC/105 and 135k oligo | 6        | 12.2   |
| Valdivia et al. | 15 1 Mb BAC | 2        | 13.3   |
| Faas et al. | 50 44k oligo | 5        | 10.0   |
| Srebniak et al. | 30 250k SNP | 2        | 6.7    |
| Le Caignec et al. | 199 105k SNP | 16       | 8.0    |
| Rooyink et al. | 49 BAC targeted | 3        | 6.1    |
| Subtotal     | 640 60k oligo | 16       | 11.3   |
| Other studies |   |   |   |
| Wapner et al. | 755 Targeted with 1 Mb backbone | 45       | 6.0    |
| Shaffer et al. | 2081 Various | 131      | 6.3    |
| Lee et al. | 180 BAC targeted/60k oligo | 20       | 11.1   |
| Fiorentino et al. | 74 BAC targeted | 5        | 6.8    |
| Subtotal     | 3090 | 201 | 6.5    |
| Combined studies | 3730 | 262 | 7.0    |

*Normal conventional karyotypes only.
index pregnancy. However, the increasing use of high resolution fetal ultrasound, particularly in the late second and early third trimesters, may reveal detailed abnormal phenotypes that may be associated with more subtle chromosome imbalances. Conventional prenatal cytogenetics has long been seen as the gold standard for chromosome diagnosis, but the resolution afforded by conventional G-banded chromosomes means that the vast majority of imbalances <6–10 Mb will go undetected.

From the results presented here, it is clear that the majority of studies to date have focused their ascertainment on cases with abnormal fetal ultrasound scans. This approach provides the highest rate of clinically relevant copy number changes in the presence of a normal conventional karyotype. However, although the overall pick-up rates in the non-ultrasound ascertainment groups is ~1%, on a population level, this represents a large number of cases where clinically relevant copy number changes will go undetected if microarray technology is applied only to fetuses with an abnormal ultrasound. Furthermore, current prenatal diagnosis requires an invasive procedure with an ~0.5% to 1% associated risk of miscarriage.

Rapid technological advances, especially the use of massively parallel sequencing to analyse cell-free DNA circulating in maternal plasma, raise the possibility that prenatal microarrays may have a limited time frame for clinical implementation. There are already a number of proof-of-principle studies that have demonstrated the feasibility of using non-invasive next generation sequencing approaches to detect not only the common aneuploidies but also unbalanced chromosome abnormalities including micro-deletions and micro-duplications.29–31 The pace at which these technologies will be applied to non-invasive prenatal testing will increase exponentially over the coming months providing significant challenges to all healthcare providers for assessing the most effective way of implementing these novel approaches. In any event, it is clear that the current model of screening followed by an invasive test and conventional karyotyping will soon be replaced by molecular approaches.

CONCLUSION

Despite the perceived difficulties of implementing prenatal microarrays diagnostically, especially those associated with the discovery of VOUS and rarer incidental findings, the headline figures presented here indicate that microarray technology could and indeed should be the frontline prenatal test in the presence of a fetal structural abnormality. Furthermore, we argue that with a pick-up rate of at least 1% greater than that being achieved by karyotyping in the other ascertainment groups, and with the ever increasing availability of data to help with copy number change interpretation, the data presented here also provide support to the notion that microarrays should be the frontline test for all prenatal diagnoses regardless of ascertainment category.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Microarray testing gives an increase in detection rate of unbalanced structural abnormalities in both the postnatal and prenatal contexts.

WHAT DOES THIS STUDY ADD?

- This work attempts to calculate the overall detection rate, and the detection rates for different ascertainment categories, of clinically significant prenatal copy number changes detected by microarrays in the presence of a normal conventional karyotype by combining data obtained from several published studies.
REFERENCES

1. Shaw-Smith C, Redon R, Rickman L, et al. Microarray based comparative genomic hybridisation (array-CGH) detects submicroscopic chromosomal deletions and duplications in patients with learning disability/mental retardation and dysmorphic features. J Med Genet 2004;41(4):241–8.

2. de Vries BB, Pfundt R, Leisink M, et al. Diagnostic genome profiling in mental retardation. Am J Hum Genet 2005;77(4):506–18.

3. Miller DT, Adam MP, Aradhya S, et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am J Hum Genet 2010;86(5):749–64.

4. Shaffer LG, Bejjani BA, Torchia B, et al. The identification of microdeletion syndromes and other chromosome abnormalities: cytogenetic methods of the past, new technologies for the future. Am J Med Genet C Semin Med Genet 2007;145C(4):335–45.

5. Tyrenier M, Abbott KM, Willatt LR, et al. High resolution array analysis: diagnosing pregnancies with abnormal ultrasound findings. J Med Genet 2010;47:349–57.

6. D'Amours G, Kibar Z, Mathonnet G, et al. Whole-genome array CGH identifies pathogenic copy number variations in fetuses with major malformations and a normal karyotype. Clin Genet 2012;81:128–35.

7. Evangelidou P, Sismani C, Ioannides M, et al. Clinical application of whole-genome array CGH during prenatal diagnosis: study of 25 selected pregnancies with abnormal ultrasound findings or apparently balanced structural aberrations. Mol Cytogenet 2010;3:24.

8. Valduga M, Philippe C, Bach Segura P, et al. A retrospective study by oligonucleotide array-CGH analysis in 50 fetuses with multiple malformations. Prenat Diagn 2010;30:333–341.

9. Faas BH, van der Burgt I, Kooper AJ, et al. Detection of genomic imbalances by array based comparative genomic hybridisation in fetuses with ultrasound anomalies. Eur J Med Genet 2010;53(5):250–58.

10. Srebniak MI, Boter M, Oudesluijs GO, et al. Genomic SNP array as a gold standard for prenatal diagnosis of foetal ultrasound abnormalities. Mol Cytogenet 2012;5:14.

11. Le Caignec C, Bocero M, Saugier-Veber P, et al. Chromosomal microarray analysis as a first-line test in pregnancies with a priori low risk for the detection of submicroscopic chromosomal abnormalities. Eur J Hum Genet 2012; doi:10.1038/ejhg.2012.253 [Epub ahead of print].

12. Armgell I, Nevado J, Serra-Juie C, et al. Clinical utility of chromosomal microarray analysis in invasive prenatal diagnosis. Hum Genet 2012;131:513–23.

13. Brennan A, Pursley AN, Hixson P, et al. Prenatal chromosomal microarray analysis in a diagnostic laboratory; experience with >1000 cases and review of the literature. Prenat Diagn 2012;32:351–61.

14. Park SJ, Jung EH, Ryu RS, et al. Clinical implementation of whole-genome array CGH as a first-tier test in 5080 pre and postnatal cases. Mol Cytogenet 2011;4:121.

15. Maya I, Davidov B, Gershovitz L, et al. Diagnostic utility of array-based comparative genomic hybridization (aCGH) in a prenatal setting. Prenat Diagn 2010;30:1131–7.

16. Copppinger J, Alliman S, Lamb AN, et al. Whole-genome microarray analysis in prenatal specimens identifies clinically significant chromosomal alterations without increase in results of unclear significance compared to targeted microarray. Prenat Diagn 2009;29:1156–66.

17. Reddy UM, Page GP, Saade GR, et al. Karyotype versus microarray testing for genetic abnormalities after stillbirth. N Engl J Med 2012;367:2185–93.

18. Filges I, Kang A, Klug V, et al. Array comparative genomic hybridization in prenatal diagnosis of first trimester pregnancies at high risk for chromosomal anomalies. Mol Cytogenet 2012;5:38.

19. Leung TY, Vogel I, Lau TK, et al. Identification of submicroscopic chromosomal aberrations in fetuses with increased nuchal translucency and apparently normal karyotype. Ultrasound Obstet Gynecol 2011;38:314–319.

20. Vaisenka SA, Davis S, Hendrix NW, et al. Application of chromosomal microarray in the evaluation of abnormal prenatal findings. Clin Genet 2012; doi:10.1111/cge.12027 [Epub ahead of print].

21. Tabor A, Alfirevic Z. Update on procedure-related risks for prenatal diagnosis techniques. Fetal Diagn Ther 2010;27(1):1–7.

22. Rosendal JA, Coe BP, Eichler EE, et al. Estimates of penetrance for pathogenic copy-number variations. Genet Med 2012; doi:10.1038/gim.2012.164 [Epub ahead of print].

23. Srinivasan A, Bianchi D, Huang H, et al. Noninvasive detection of fetal subchromosome abnormalities via deep sequencing of maternal plasma. Am J Hum Genet 2013;92:1–10.

24. Dan S, Chen F, Choy KW, et al. Prenatal detection of aneuploidy and imbalanced chromosomal arrangements by massively parallel sequencing. PLoS ONE 2012; doi:10.1371/journal.pone.0027835 [Epub ahead of print].

25. Lau TK, Jiang FM, Stevenson RJ, et al. Secondary findings from non-invasive prenatal testing for common fetal aneuploidies by whole genome sequencing as a clinical service. Prenat Diagn 2013;33(6):602–8.

26. Chen S, Lau TK, Zhang C, et al. A method for noninvasive detection of fetal large deletions/duplications by low coverage massively parallel sequencing. Prenat Diagn 2013;33(6):584–90.

27. Lee C-N, Lin S-Y, Lin C-H, et al. Clinical utility of array comparative genomic hybridisation for prenatal diagnosis: a cohort study of 3171 pregnancies. BJOG 2012;119:614–25.

28. Fiorentino F, Napoletoni S, Caiazzo F, et al. Chromosomal microarray analysis as a first-line test in pregnancies with a priori low risk for the detection of submicroscopic chromosomal abnormalities. Eur J Hum Genet 2012; doi:10.1038/ejhg.2012.253 [Epub ahead of print].