EDITORIAL

New technologies for the assessment of chromosomes in prenatal diagnosis

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Cytogeneticists have many tools that can be used to identify chromosome abnormalities. Microscopic analysis of banded chromosomes has been used for over 40 years as the main tool for uncovering cytogenetic aberrations in prenatal testing. In this special issue of *Prenatal Diagnosis*, we have worked with leading authors to compile state-of-the-art information on the new cytogenetic technologies available in the clinical and diagnostic laboratories to identify alterations of the genome leading to human disease. We have called this issue 'New cytogenetic technologies' because each of the methods described is aimed at uncovering chromosome imbalance, either whole chromosome aneuploidies, or locus-specific gains and losses of the genome.

The articles addressing aneuploidy detection showcase the use of fluorescence *in situ* hybridization-based microfluidics by Ho and co-workers[1] at the National University of Singapore, the application of quantitative fluorescent-polymerase chain reaction in prenatal diagnosis by Mann and Ogilvie[2] at Guy's & St. Thomas' National Health Foundation Trust, London, and multiplex ligation-dependent probe amplification in prenatal testing by Willis and Eng[3] at Baylor College of Medicine, Houston. Beyond aneuploidy testing is a new technology using bacterial artificial chromosomes (BACs), termed BACs-on-Beads, which can detect gains or losses of whole chromosomes or chromosomal segments in a limited, targeted approach. In a collaborative study, Vialard and co-authors[4] from several European cytogenetic laboratories describe their use of the BACs-on-Beads methodology in over 1600 prospective prenatal samples. The assay detects the common aneuploidies of chromosomes 13, 18, 21, X and Y, along with gains or losses of nine genomic regions from ten microdeletion disorders. The authors found nearly a 10% detection rate among the samples tested and among these, 7.3% represented gains or losses of the microdeletion regions tested. Finally, a review by Chiu and Lo[5] at the Chinese University of Hong Kong delivers important insights into the use of high-throughput sequencing for the noninvasive detection of trisomy 21.

The largest number of articles in this special issue is focused on the use of microarrays in prenatal testing. Brady and Vermeesch[6] from the University Hospital in Leuven, Belgium provide a comprehensive review of the technological aspects of genomic microarrays, discussing the various array platforms and protocols used in the cytogenetics laboratory. Some of the manuscripts focus on specific applications of arrays, such as Schmid *et al.*[7] on the use of array data in fetuses with congenital heart defects, and Gruchy *et al.*,[8] who compare the use of arrays after cultured prenatal specimens or extraction of cell-free fetal DNA. In another application, Reddy and colleagues[9] review the literature on the clinical application of arrays after fetal death. Because many cultures fail to grow after a spontaneous abortion or stillbirth and cytogenetic analysis is not possible, arrays may be particularly suited to the study of fetal demise because the extraction of DNA for use in microarrays does not rely on the ability to culture cells.

As one of the first laboratories offering prenatal testing using microarrays, Breman and colleagues[10] from Baylor College of Medicine present their experience with over 1000 prospective prenatal array tests performed. They compare their findings to that of the published medical literature. With a variety of indications for study by microarray, they found that 4.2% of cases had an abnormality detected by microarray after abnormal karyotypes were excluded. This is a significant finding because many of these abnormalities would be undetectable with routine banded chromosome analysis. To understand the ordering practices of physicians desiring microarray testing for their patients, Shaffer and co-workers[11] from Signature Genomic Laboratories in Spokane, Washington and colleagues from Canada and Israel report on over 1400 prenatal microarrays performed for a variety of indications on two different microarray platforms. Although the majority of pregnancies were referred for testing because of abnormal ultrasound findings, the

authors were able to draw some conclusions about the use of a lower-resolution as compared with a higher-resolution array in prenatal testing. They summarized the differences in the ordering practices between the three countries from which the majority of cases were referred. Finally, Faas and colleagues[12] from Radboud University in Nijmegen report their use of high-resolution single nucleotide polymorphism arrays to study 118 samples from pregnancies with abnormal ultrasound findings and normal quantitative fluorescent-polymerase chain reaction results; among these, 5% had an abnormal array finding that was relevant to the sonographic anomaly.

Because of the emphasis on the use of microarrays in prenatal testing and rapid adoption of this technology in fetal diagnosis, we invited two groups to write opinion papers on the genetic counseling issues that should be considered when using microarrays in prenatal diagnosis. We find it comforting that the paper by McGillivray *et al.*,[13] with authors mostly from Australia and New Zealand, and the article by Wapner *et al.*,[14] with American authors who participated in the National Institutes of Health clinical trial on the use of microarrays in prenatal testing, had many overlapping thoughts about the use of microarrays in prenatal diagnosis. Both papers acknowledge that the situation is more complex in the prenatal setting because neither the physician nor the laboratory has all of the information regarding the phenotype. Ultrasound can reveal structural anomalies of the fetus but cannot reveal the potential for future developmental or intellectual disabilities. In addition, laboratories are using varying array platforms with different resolutions. Thus, the detection rates of identifying variations of unknown significance (VOUS) will differ from laboratory to laboratory. In addition, the criteria established by each laboratory for classifying and reporting such findings will differ as well. Some VOUS, such as duplications that are not known to be associated with disease and are inherited from an asymptomatic parent, may be reported as likely benign by some laboratories, but not others. This may partially explain the different rates of VOUS reported by Breman *et al.*[10] compared with Shaffer *et al.*[11] who report a higher frequency of VOUS. Counseling in these cases is more difficult and requires integration of all available information, including data from the literature on penetrance for an abnormal phenotype and experience from the diagnostic laboratory. The finding of VOUS may be used to make decisions about pregnancy termination and thus the need for comprehensive pretest counseling to discuss the possibility of findings of uncertainty. Both the McGillivray and Wapner articles[13,14] acknowledge that uncertainty in prenatal testing is not a new concept but also point out that even the use of family history, parental testing, and inheritance pattern of the VOUS may sometimes not clarify the result to the satisfaction of the patient. Nevertheless, both papers support a woman's autonomy in decision-making regarding their pregnancies. McGillivray and colleagues[13] argue that this autonomy extends to receiving information from high-resolution prenatal array testing and that it would be unethical to withhold information from this test that is relevant to this decision-making process based on this ethical principle. Both papers argue that this brings with it the need for pretest counseling. Wapner *et al.*[14] support a formal consent process prior to testing that may include a choice about whether to receive results of unclear significance or adult-onset conditions.

The recent advances in the detection of cytogenetic anomalies through molecular methodologies is reflected in the large number of articles that are being submitted to *Prenatal Diagnosis* on this topic, and the large volume of papers found in the medical literature over the past couple of years. As this topic continues to evolve, we hope to bring you more articles on the increased detection of chromosome abnormalities using new technologies and insights from the United States National Institutes of Health clinical trial on microarrays in prenatal testing to improve the management and health of women and their fetuses (Eunice Kennedy Shriver National Institute of Child Health & Human Development, Project Number: 5R01HD055651-05, Clinical Trial ID: NCT01279733).

REFERENCES

1. Ho SSY, Chua C, Gole L, Biswas A, Koay E, Choolani M. Same-day prenatal diagnosis of common chromosomal aneuploidies using microfluidics-fluorescence in situ hybridization. Prenat Diagn 2012;32:321–8.
2. Mann K, Ogilvie CM. QF-PCR: application, overview and review of the literature. Prenat Diagn 2012;32:309–14.
3. Willis AS, Van den Veyver I, Eng CM. Multiplex ligation-dependent probe amplification (MLPA) and prenatal diagnosis. Prenat Diagn 2012;32:315–20.
4. Vialard F, Simoni G, Gomes DM, Abourra A, De Toffol S, Bru F, Romero MCM, Nitsch L, Bouhanna P, Marcato L, Popowski T, Grimi B, Martünez-Conejero JA, Benzacken B, Genesio R, Grati FR. Prenatal BACs-on-Beads™: the prospective experience of five prenatal diagnosis laboratories. Prenat Diagn 2012;32:329–35.
5. Chiu RWK, Lo YMD. Noninvasive prenatal diagnosis empowered by high-throughput sequencing. Prenat Diagn 2012;32:401–6.
6. Brady PD, Vermeesch JR. Genomic microarrays: a technology overview. Prenat Diagn 2012;32:336–43.
7. Schmid M, Stary S, Blaicher W, Gollinger M, Husslein P, Streubel B. Prenatal genetic diagnosis using microarray analysis in fetuses with congenital heart defects. Prenat Diagn 2012;32:376–82.
8. Gruchy N, Decamp M, Richard N, Jeanne-Pasquier C, Benoist G, Mittre H, Leporrier N. Array CGH analysis in high-risk pregnancies: comparing DNA from cultured cells and cell-free fetal DNA. Prenat Diagn 2012;32:383–8.
9. Reddy UM, Page GP, Saade GR. The role of DNA microarrays in the evaluation of fetal death. Prenat Diagn 2012;32:371–5.
10. Breman A, Pursley AN, Hixson P, Bi W, Ward P, Bacino CA, Shaw C, Lupski JR, Beaudet A, Patel A, Cheung SW, Van den Veyver I, Patel A. Prenatal chromosomal microarray analysis in a diagnostic laboratory; experience with >1000 cases and review of the literature. Prenat Diagn 2012;32:351–61.
11. Shaffer LG, Dabell MP, Rosenfeld JA, Neill NJ, Ballif BC, Coppinger J, Diwan NR, Chong K, Shohat M, Chitayat D, Cheung SW. Referral patterns for microarray testing in prenatal diagnosis. Prenat Diagn 2012;32:344–50.
12. Faas BHW, Feenstra I, Eggink AJ, Kooper AJA, Pfundt R, van Vugt JMG, de Leeuw N. Non-targeted whole genome 250 K SNP array analysis as replacement for karyotyping in fetuses with structural ultrasound anomalies: evaluation of a one-year experience. Prenat Diagn 2012;32:362–70.
13. McGillivray G, Rosenfeld JA, Gardner RJM, Gillam LH. Genetic counselling and ethical issues with chromosome microarray analysis in prenatal testing. Prenat Diagn 2012;32:389–95.
14. Wapner RJ, Driscoll DA, Simpson JL. Integration of microarray technology into prenatal diagnosis: counselling issues generated during the NICHD clinical trial. Prenat Diagn 2012;32:396–400.

