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Controversies and challenges of array comparative genomic hybridization in prenatal genetic diagnosis

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INTRODUCTION

The introduction of array comparative genome hybridization (CGH) into prenatal genetic diagnosis presents both challenges and controversy to the clinical cytogenetic community. The challenges are diverse and multiple–not only the obvious expenses of equipment, training, and the microarrays themselves, but most importantly, demonstrating efficacy–and controversial in that the application of array CGH to prenatal genetic diagnosis may cause more harm than good by compromising patient care. The most important issue that has to be addressed is whether there is there a demonstrable need for the introduction of array CGH in prenatal genetic diagnosis; specifically, what are the benefits and risks associated with the potential of significantly improving the prenatal detection rate of genetic aberrations leading to physical and mental disabilities by means of array CGH compared with conventional chromosome analysis?

For more than four decades, the prenatal diagnoses of chromosome aberrations have been primarily accomplished by tissue culture of cells representing the fetal genotype followed by what is termed "conventional chromosome analysis." When amniocentesis was introduced into clinical practice, staining techniques available at the time resulted in unbanded chromosomes, i.e., chromosomes appeared as solid entities, and their identification was based principally on relative length and centomere position. With the introduction of chromosome banding techniques in the late 1960s, a unique banding pattern not only characterized each chromosome, but it also became possible to detect structural chromosome aberrations such as deletions and duplications in the range of 5 to 10 megabases. However, there are significant limitations to conventional chromosome analysis as currently practiced. From a patient's perspective, the major disadvantage of prenatal diagnosis is that cells representing the fetal genotype have to be cultured for up to 2 weeks before the chromosome analysis can be initiated. Besides the fact that conventional chromosome analyses of prenatal specimens are not only labor intensive and costly, demanding a relatively high level of technical expertise and relatively expensive tissue culture and microscopy equipment, the time interval between sampling fetal tissues and diagnosis places a considerable emotional burden on prospective parents. This time element becomes particularly acute when an ultrasound examination in the first or second trimester identifies the presence of fetal anomalies associated with an unbalanced chromosome constitution and prompt confirmation of such a diagnosis is essential for purposes of clinical management. Fluorescent in situ hybridization (FISH) does provide rapid turn-around time but is limited either to aneuploidy for chromosomes 13, 18, 21, X, and Y or to an individualized FISH test developed to identify a known microdeletion or microduplication syndrome.

From the geneticists' perspective, the rational for the introduction of array CGH into the clinical practice of prenatal diagnosis is that there is an obvious need to improve the detection rate of genetic aberrations leading to physical and functional disabilities. In both prenatal and postnatal populations, conventional chromosome analysis accounts for a significant proportion of such cases, but not all. For example, in the first trimester of pregnancy, karyotypic abnormalities account for approximately 50% of fetuses with cystic hygromas,[1] 46%[2] to 83%[3] of spontaneous abortions, and 5%[4] of stillbirths. Whereas potentially lethal or handicapping major malformations are present in 2% to 3% of liveborn children, are the main cause of infant mortality during the first years of life, and are associated with long-term morbidity, chromosome aberrations only account for 0.5%. Among children with isolated idiopathic mental retardation, more than 75% remain undetermined after genetic testing. Therefore, after clinical evaluation and genetic testing, the underlying etiology still remains unexplained for most children with functional and physical disabilities. The potential efficacy of array CGH in markedly enhancing the detection of fetal chromosome aberrations has been recently demonstrated in a series of initial articles applied to prenatal specimens,[5–10] in addition to demonstrating complete concordance with both normal and abnormal results of conventional chromosome analyses. In studies of products of conceptions[9] and of fetuses with multiple malformations[8] after a normal chromosome constitution based on conventional chromosome analysis, array CGH identified new abnormalities in 8% to 9% of cases. In the case of prenatal diagnostic specimens, the overall detection rate of clinically relevant disorders was approximately 7%, more than three times that of conventional chromosome analysis.[10]

The promise of array CGH as applied to prenatal genetic diagnosis is manifold: array CGH in direct mapping of genetic aberrations to genome sequence is capable of higher resolu-

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tion, of being amenable to automation and to quality control, of high throughput, of shorter reporting times (overnight), and of requiring relatively small amounts of fetal genomic DNA. Array CGH has the capability of defining genetic syndromes that remain undefined using conventional cytogenetic techniques. For prospective patients, the main advantage is that a single overnight run can detect every imbalance–gain or loss of DNA–that is identified by G-banding, FISH, and related methods. Array CGH will be a particular advantage when conventional chromosome analysis is not possible because of tissue culture failure or when there is a limited number of cells in mitosis. Array CGH also holds the promise of providing information for more accurate genetic counseling and reproductive risk assessment. From the geneticist's perspective, a more comprehensive understanding of genomic aberrations will permit tracing their ultimate effects on gene regulation and transcription and that information may, at some later date, eventually lead to minimizing or eliminating their harmful clinical effects.

Although array CGH has the capability of defining genetic syndromes that remain undefined using conventional cytogenetic techniques, there are two principal challenges with its introduction into the field of prenatal diagnosis. The first is one of a technical nature: namely, what level of genomic resolution should be applied in the case of prenatal specimens, unbiased whole-genome arrays using oligonucleotides, or custom-designed arrays targeting genomic regions of known clinical significance and using BAC arrays.[11,12] The main advantage of high-resolution microarrays composed of oligonucleotides randomly distributed throughout the genome is the potential for identifying a higher rate of chromosome aberrations compared with arrays targeting regions of known clinical significance. To facilitate the clinical interpretation based on unbiased, high-resolution, whole-genome array CGH, clinical trials comprising significant numbers of normal and abnormal pregnancies will be essential, particularly for cases in which genomic alterations are novel and have not been previously recognized to be associated with a specific disease state. In particular, the human genome is characterized by many forms of polymorphisms of questionable clinical significance, including single nucleotide polymorphisms, small insertion-deletion polymorphisms, and variable numbers of repetitive sequences, either low copy repeats (also known as segmental duplications) ranging from 1 to 400 kb in length and large-scale copy number variations (CNVs) ranging from several kilobases to hundreds of kilobases of genomic DNA among presumably phenotypically normal individuals.[13–16] Polymorphisms of questionable clinical significance may account for up to 1% of all loci in whole-genome arrays; therefore, there is a critical need to acquire considerable clinical information to correlate any duplication or deletion detected by array CGH with fetal outcome. Furthermore, it has been estimated that at least one de novo segmental deletion occurs per approximately eight newborns and one segmental duplication per approximately 50 newborns.[17] It is the unknown clinical meaning of low copy repeats that is likely to generate the most interpretative difficulties and the most anxiety for prospective parents undergoing prenatal array CGH. Before undertaking large-scale clinical trials to establish the efficacy of array CGH in prenatal specimens, the question as to what information would be provided to prospective patients and when in the pregnancy, prenatally or postnatally, would have to be resolved.

The proposal of first introducing targeted array CGH in prenatal genetic diagnosis is premised on the use of a platform in which there is precise understanding of the clinical significance of specific changes in genomic architecture under investigation, compared with the explorative nature of whole-genome arrays.[12] Whereas the latter will identify more chromosome aberrations than targeted arrays, the improved detection rate may be more than offset by the anticipated exponential increase in uninterpretable results resulting from the presence of segmental duplications and CNVs of unclear clinical significance concurrent with the anticipated increase in parental anxiety and expense to evaluate whether an abnormality is benign or pathologic. Targeted array CGH is designed to avoid most known CNVs and interrogate specific targeted loci, unique pericentromeric regions, unique subtelomeric regions, and recognized microdeletion or microduplication syndromes. A targeted array approach to prenatal diagnosis would also materially reduce the need for parental follow-up that currently characterizes the application of most whole-genome array assays to postnatal specimens. In one such study of 100 individuals with mental retardation using a dense oligonucleotide array, a total of 3125 putative CNVs were identified, ranging from 19 to 43 and a median of 30 CNVs per study subject.[18] Most of the CNVs identified in this study were unique and had not been independently confirmed, emphasizing the enormous difficulties involved in interpreting a "staggering number of ambiguous and unhelpful results."[12] With clinical experience with the targeted array CGH of prenatal specimens and with an increase in knowledge of genomic architecture, transition to dense oligonucleotide arrays would be anticipated. Further increases in resolution through ultra high-density microarrays would likely increase the diagnostic yield in prenatal specimens, and, in time, the standard in microarray prenatal diagnostics may involve scanning of all exons in the human genome and the detection of clinically significant duplications and deletions at the single exon level.[11] If the cytogenetic community were to introduce the use of dense oligonucleotide array CGH into the clinical practice of prenatal diagnosis, this would require specific knowledge and understanding of the extent of copy number variations in regard to their location and their relevance in the context of disease.

Introducing array CGH into cytogenetic laboratories performing conventional prenatal diagnoses will require robust protocols, excellent understanding of technical factors, quality criteria defining a successful analysis, and reporting guidelines to enable correct interpretation of results obtained by different laboratories using different microarrays. Most critical, genetic counseling before and after the application of array CGH to prenatal specimens will be both demanding and difficult. For the present, what is needed is a national, multicenter clinical

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trial to address these issues and focused on (1) the accuracy of array CGH in regard to common chromosome abnormalities and common microdeletion syndromes; (2) whether array CGH should be applied to all prenatal specimens or limited to specific indications, e.g., fetuses with multiple structural malformations but normal karyotypes; and (3) the efficacy of array CGH in regard to processing time and laboratory costs, in regard not only to that of the array CGH analysis itself, but also to the charges for processing parental bloods and confirmation studies by FISH and conventional chromosome analysis; (4) the effectiveness of different genetic counseling models required to address parental concerns and needs after fetal array CGH analysis; and (5) the psychological aspects of the impact of fetal array CGH analyses after both positive and negative results. Array CGH must be interpreted within the context of traditional cytogenetics because the latter is the only means by which to determine the chromosomal sites of copy number changes identified by microarrays. Warburton[19] gives the example that a microarray may identify duplication on chromosome 15 but cannot distinguish among an unbalanced translocation, an insertion, or an additional extra chromosome. This has obvious implications for genetic counseling: different etiologies of chromosome aberrations are associated with different reproductive risks.

In today's health care environment, a cost-benefit analysis may not support the routine application of array CGH analyses to all prospective parents undergoing prenatal diagnosis: for one, current charges for a conventional prenatal chromosome study ($350 to $400) are approximately one-fourth that of an array CGH analysis ($1600-$1800). Either the laboratory costs of conducting array CGH would have to be considerably reduced, particularly the expense of the microarray itself, e.g., by partitioning microarray slides into subarrays for simultaneous processing of multiple samples, or a multicenter trial convincingly demonstrates that the benefits of array CGH applied to prospective parents undergoing prenatal diagnosis significantly outweigh laboratory expenditures. The question being asked is: At what level of detection should array CGH exceed conventional chromosome analysis if the former is to replace the latter; will a detection rate two times that of conventional chromosome analysis warrant the application of array CGH in applying to prenatal specimens? Will a detection rate three times of array CGH warrant using this technology in place of conventional chromosome analysis? In this case, whole-genome arrays may have an advantage over targeted arrays. Included in this consideration must be an unambiguous demonstration that, in the course of such a clinical trial, the effects of polymorphisms having no clinical significance, especially low copy repeats, were appropriately minimized without causing unnecessary emotional trauma to parents and inappropriate decisions as to the health of the pregnancy. What is also needed before array CGH is introduced into the clinical practice of prenatal diagnosis is a dataset that can differentiate ostensibly benign genomic variants from those either associated with a disease state or predictive of disease predisposition, while having identified those prospective patients who would most benefit from its application.

The second principal challenge to the introduction of array CGH into the practice of prenatal genetic diagnosis is based on two concerns: the consequences of the informational content generated by the microarrays and that microarrays represent a new dimension in eugenics.[20,21] The former challenge is based on the premise that the "more detailed the search for genetic variants the less likely it is to produce information that translates into useful knowledge about the health of a fetus;" rather, its application in prenatal diagnosis will likely produce a "flood of information that is overwhelming, anxiety-producing, inconclusive and misleading,"[21] all of which emphasizes the need to assess critically the role of genetic counseling and the potential positive and negative psychological impact on parents undergoing fetal array CGH analyses. The consequences of an "incidentalome," an incidental genomic finding, are that cytogeneticists will be inundated by the complexity of pursuing unexpected genomic changes; prospective parents will be subjected to unnecessary and expensive tests; and the incremental costs will provide minimal benefit to cytogeneticists or prospective parents.[22] These possible developments can be minimized by the following approaches: (1) the clinical consequences for each genetic variant identified by array CGH are documented, including benign and pathologic polymorphisms; (2) widely available information systems or datasets are created to estimate and explain the significance and risks of positive genomic results; and (3) the mode of delivery of array CGH results to referring obstetricians and prospective parents is carefully considered and conducted.[10] For these reasons, the more focused targeted array approach to prenatal genetic diagnosis seems more rational and attractive than the genome-wide approach, particularly when array CGH is first introduced into the clinical practice of prenatal genetic diagnosis.

With respect to the concern that microarrays represent a new dimension in eugenics, the arguments concerning the ethical implications of introducing array CGH into the practice of prenatal diagnosis seem to be similar to those previously articulated in the case of screening programs in the first trimester and second trimester or conventional prenatal genetic diagnostic programs.[23] The application of microarrays to prenatal diagnosis is likely to be another example in which science and technology continue to outpace ethical and legal considerations. It is reasonably arguable whether the new technology of array CGH in fact requires new approaches in ethical and legal considerations or rather represents a logical extension of current genetic counseling that addresses the needs of prospective patients without causing undue and inappropriate anxiety.

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