MASTER CLASS

Prenatal Diagnosis

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In our contemporary society, where women and their physicians continue to seek as much information as possible early in their pregnancies, the field of prenatal diagnosis has rapidly become a well-established and central part of obstetrics. Prenatal diagnosis performed in the first trimester has become common practice—a far cry from the days in the not-so-distant past when the ultimate outcome of the fetus was not learned until the day of delivery.

As obstetricians and perinatologists, we benefit from being aware of and fully informed about the evolving technology that continues to move the field of prenatal diagnosis forward. The array of current prenatal diagnostic tools includes both invasive and noninvasive techniques that enable parents to assess the genetic, chromosomal, and biochemical aspects of their fetus considerably before the time of viability.

Parents and their physicians are using this information to guide them in pursuing potential therapeutic applications and interventions or, in some cases, interruption of the pregnancy.

Now there is a new technique called array-based comparative genomic hybridization, or array-CGH, which is entering the prenatal arena with promises of more comprehensive and faster detection capabilities than we now are afforded with the two current "gold standard" techniques: microscopic karyotype analysis and rapid fluorescent in situ hybridization.

Array-CGH is far from perfect in evaluating chromosomal material. It can only detect instances where there is a significant addition or deletion of genetic material. And, of course, it can only evaluate those genes encoded on the array.

As with every other prenatal diagnostic tool developed to date, the future use of this new technique involves many questions, including which variants are normal as opposed to abnormal, the technique's potential role as a screening tool, and other often vexing ambiguities and issues. However, its use in prenatal diagnosis will build upon a body of national experience in the postnatal setting.

To familiarize us with the new technology and discuss its role in prenatal diagnosis, I have invited Dr. Karin J. Blakemore to serve as the guest professor of this month's Master Class.

Dr. Blakemore is the director of maternal-fetal medicine and the Prenatal Genetics Service at Johns Hopkins University School of Medicine in Baltimore—an institution that is gearing up to use array-CGH as part of its armamentarium for prenatal diagnosis.

She is joined by her colleague Denise Batista, Ph.D., who is an assistant professor in the Johns Hopkins department of pathology and codirector of the university's prenatal cytogenetics laboratory. Dr. Batista also serves as the director of the cytogenetics laboratory at the Kennedy Krieger Institute in Baltimore. ■

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Array-CGH, Karyotype Analysis, and FISH

For years, microscopic karyotype analysis and rapid fluorescent in situ hybridization techniques have been the standard for prenatal diagnosis of chromosomal abnormalities. Today, with the availability of a new technique called array-based comparative genomic hybridization (array-CGH), the practice of prenatal diagnosis is poised to take another leap forward.

BY KARIN J. BLAKEMORE, M.D.

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The array-CGH test, which is already being used postnatally, will give obstetricians, geneticists, and their patients the opportunity in the prenatal setting to detect significantly more and smaller changes in the amount of chromosomal material present in individuals—and in significantly less time than a standard chromosome karyotype would take.

It may someday take the place of our standard techniques for cytogenetic analysis, but for now, it is a valuable addition to the available diagnostic tests.

Advances Over FISH

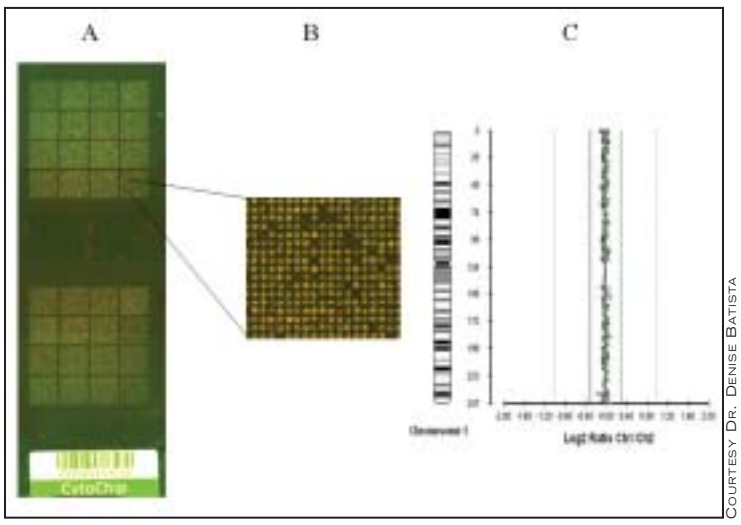
The technology, which has also been called chromosomal microarray, was first used to analyze gains and losses in chromosomal material in tumors and tumor cell lines. It is now a valuable tool in the postnatal testing of individuals with birth defects.

Between one-half and two-thirds of children with serious developmental abnormalities go undiagnosed and have a normal karyotype, so from a postnatal perspective, this new test has been welcomed at Johns Hopkins University and the Kennedy Krieger Institute, both in Baltimore, as well as at other institutions. Having a diagnosis facilitates the most appropriate therapy and allows parents to plan for future pregnancies and possible prenatal testing.

Yet it is the prenatal period for which array-CGH may have an even greater impact. Phenotypic features are not as apparent in the womb as at birth, making it more difficult to target testing with technology like rapid fluorescent in situ hybridization (FISH).

Along with standard karyotype analysis, the FISH technique has been the mainstay of cytogenetic analysis. It provides a targeted look at areas of the karyotype that are known to be associated with disease as a result of either the duplication or deletion of genetic material. In other words, it detects gains and losses in chromosomal material for just one or a few chromosome regions at a time.

Performing array-CGH is like doing FISH hundreds of times at once. Array-CGH testing may target the same chromosomal regions (and thus similar disorders) as a series of FISH tests, but array-CGH will target these regions at a much higher resolution, enabling the detection of much smaller deletions and duplications; it can also assess many regions associated with genetic disorders in a single test.



COURTESY DR. DENISE BATISTA

Figure A shows a hybridized array of >4,200 BAC clones; B, one area enlarged; C, plot for chromosome 1 based on fluorescence ratios (patient vs. control DNA) showing normal copy number.

If we see on a prenatal ultrasound that a fetus has cardiac problems, for example, we might suspect the DiGeorge syndrome. The obstetrician today would probably perform an amniocentesis and order both a karyotype and FISH with a specific probe for the DiGeorge syndrome, which we know is caused by a deletion on chromosome 22, just as he or she would do in the postnatal period for a child with the syndrome's more obvious phenotypic features.

In the near future, the obstetrician facing this prenatal situation will likely proceed differently than he or she would in the postnatal period. The obstetrician will use array-CGH instead of FISH in order to cast a wider net—one that can catch a deletion on chromosome 22, as well as other possible deletions which may cause the heart defect.

Right now, the available array-CGH platforms can detect more than 40 syndromic chromosomal disorders. Just as with FISH, a normal result rules out only those conditions that correspond to the deletions or duplications that are covered on the array.

How Array-CGH Works

The technique involves labeling the patient's DNA in one fluorescent dye, labeling DNA from a normal control with a different fluorescent dye, allowing the DNA from both to mix, and then applying the mixture to a slide that contains small segments of DNA from known chromosomal regions.

The slide serves as the platform or the array. The mixture of the patient's DNA and the normal control DNA is allowed to match up, or hybridize, with the complementary DNA segments on the slide.

A scanner then reads the intensities of the two different dyes, determining their relative strength at each of the DNA spots on the array. If a patient has less DNA in a specified region of the genome—a deletion of chromosomal material—then the color of the control sample will be stronger at that point on the array. If a patient has more DNA in this specific region—a duplication of chromosomal material—then the color of the patient's sample will be stronger at that location.

Analysis can be performed on direct chorionic villi or amniotic fluid, or alternatively on cultured cells. For direct analysis, it might be necessary to amplify the amount of DNA obtained before running it on an array. In this case, it is essential that the amplification is uniform and does not introduce any bias.

Although many laboratories are using cultured cells at this point, some studies are demonstrating the feasibility of relying on uncultured samples, and ultimately, this is the direction in which we're heading. Direct testing of fetal DNA will save time and give us rapid results.

The Limitations of Array-CGH

Unlike standard karyotyping, array-CGH cannot detect defects in which the total amount of chromosomal ma-

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terial is unchanged. The test cannot, for instance, detect balance rearrangements, such as balanced reciprocal translocations, balanced Robertsonian translocations, and inversions.

In a couple with multiple miscarriages, a karyotype is still the appropriate test to perform on the parents' blood because a balanced rearrangement is what you would be looking for. You would not request array-CGH because balanced rearrangements are not detectable with this technique. On the other hand, array-CGH could be very useful on the products of conception from a miscarriage because very small deletions and duplications could be found.

Array-CGH also cannot detect point mutations, or small changes in the genes, like those that cause hemophilia or sickle cell disease. It is designed to detect the syndromes caused by duplications or deletions of larger amounts of chromosomal material. And it will not detect abnormalities that are not covered by the array.

Chromosomal mosaicism, in which only some cells show a particular abnormality, may or may not be more readily detected by array-CGH than by standard techniques.

On one hand, mosaicism may be more readily detected with array-CGH than with standard karyotype analysis because abnormal cells often do not divide as well and may be lost during the culture process that is part of the standard karyotyping methodology. On the other hand, experts believe that array-CGH may not detect mosaicism below a certain level—below the level, some say, at which the abnormality affects fewer than 15%-30% of cells.

Array-CGH will also inevitably detect normal variants (benign duplications and deletions that are not associated with any abnormal phenotype). Some variants will be difficult to explain. This has been true for karyotyping as well, and just as we have in the past, we will want to minimize parents' anxiety over the unknowns.

When we find variants of uncertain significance, we will turn to the parents, checking their blood samples for the same losses or gains of chromosomal material.

Key Points for Array-CGH

▶ **Detects:** Unbalanced rearrangements, aneuploidy, gains and losses of regions represented in the array.

▶ **Won't detect:** Balanced rearrangements, point mutations, (possibly) low-level mosaicism.

▶ **Pick-up rate:** Estimated as 5%-10% from postnatal studies of developmentally delayed/dysmorphic children.

▶ **Confirmation:** By FISH probes.

▶ **Parental studies:** Might be necessary to sort out normal variants versus clinically significant changes.

▶ **Copy number variants:** Might find copy number variants of unknown significance.

▶ **Platforms:** Several commercial and home-brew arrays available with different genomic coverage.

The Near Future

The clinicians and cytogeneticists who are using and offering array-CGH are on a learning curve. Experts seem to have been successful in ensuring that the test works for the disorders that are covered; there is an enormous amount of information and data being shared by centers and labs on what variants are associated with the normal phenotype, and on other issues as well.

At Johns Hopkins University and the Kennedy Krieger Institute, we have postnatal experience to draw upon as we bring array-CGH into the prenatal arena. Of the children with developmental delay and dysmorphic features who have had array-CGH, we have been able to give a specific syndromic diagnosis to approximately 5%-8%, depending on the array platform we utilize. In about 12%, we have detected variants that we know—through parental testing and the use of databases—are normal. In a much smaller percentage (3.4%) of these children, we have found variants that we cannot yet explain.

Until we learn more, we plan to limit prenatal array-CGH to cases in which there is a known abnormality on ultrasound, rather than offer the test more broadly as a screening tool for chromosomal abnormalities in high-risk pregnancies. And although we are moving in the postnatal setting toward more of a whole-genome screening, we will use targeted arrays in the prenatal setting.

Within this context—that of ultrasound-detected anomalies and targeted arrays—we can expect that 5%-10% of tests will provide a clear diagnosis.

The question of whether array-CGH could replace a karyotype in prenatal testing is an interesting one. For now, there are too many questions and issues (mosaicism and normal variants, for instance) to do away with karyotyping. We believe the role of array-CGH is to enhance our current approaches to prenatal testing, and in this sense, it is an exciting development. ■

Reference: 1. Anderson FD, Gibbons W, Portman D. Safety and efficacy of an extended-regimen oral contraceptive utilizing continuous low-dose ethinyl estradiol. *Contraception*. 2006;73:229-234.