review

Pre- and postnatal genetic testing by array-comparative genomic hybridization: genetic counseling perspectives

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Recently, a new genetic test has been developed that allows a more detailed examination of the genome when compared with a standard chromosome analysis. Array comparative genomic hybridization (CGH microarray; also known as chromosome microarray analysis) in effect, combines chromosome and fluorescence in situ hybridization analyses allowing detection not only of aneuploidies, but also of all known microdeletion and microduplication disorders, including telomere rearrangements. Since 2004, this testing has been available in the Medical Genetics Laboratory at Baylor College of Medicine for postnatal evaluation and diagnosis of individuals with suspected genomic disorders. Subsequently, to assess the feasibility of offering CGH microarray for prenatal diagnosis, a prospective study was conducted on 98 pregnancies in a clinical setting comparing the results obtained from array CGH with those obtained from a standard karyotype. This was followed by the availability of prenatal testing on a clinical basis in 2005. To date, we have analyzed over 8000 cases referred to our clinical laboratory, including approximately 300 prenatal cases. With the clinical introduction of any new testing strategy, and particularly one focused on genetic disorders, issues of patient education, result interpretation, and genetic counseling must be anticipated and strategies adopted to allow the implementation of the testing with maximum benefit and minimum risk. In this article, we describe our experience with over 8000 clinical prenatal and postnatal cases of CGH microarray ordered by our clinical service or referred to the Baylor Medical Genetics Laboratory and describe the strategies used to optimize patient and provider education, facilitate clinical interpretation of results, and provide counseling for unique clinical circumstances. ***Genet Med* 2008:10(1):13–18.**

Microarray-based comparative genomic hybridization (CGH microarray) is an evolving technology for the rapid multiplex detection of genomic imbalances.[1] This diagnostic strategy has contributed to our growing understanding of the role of genomic gains and losses in the etiology of genetic disorders.[1–3] Microarrays containing large-insert genomic clones can be used to reliably detect deletions or duplications that are tens to hundreds of kilobases in size, well below the level of detection of G-banded karyotype analysis.[4–7] Recently, arrays consisting of thousands of oligonucleotides distributed throughout the genome have been introduced and have the potential to refine the resolution of gains or losses to an even more detailed level.[8] The genomic clones or oligonucleotides contained in a clinical array generally span most regions that are subject to recurrent deletions and duplications resulting in a recognized syndrome. In addition, they have the potential to detect novel gains or losses that can then be correlated with a clinical phenotype.

The addition of CGH microarray to the available diagnostic tools for evaluation of a child or adult suspected of having a genetic condition offers several potential advantages to patients and physicians. The multiplex format of the test permits simultaneous evaluation of multiple disease specific loci and subtelomeric regions, resulting in a more efficient consideration of possible diagnoses and cost savings over ordering testing of each locus individually. As discussed earlier, these tools present the possibility of detecting novel gains or losses that may help characterize a new genomic syndrome. Moreover, with the addition of clones or oligos providing backbone genomic coverage, CGH microarrays have an advantage in terms of sensitivity, cost effectiveness, and higher resolution when compared with a standard karyotype. However, the complexities of the testing, including the availability of various technical platforms and different designs of clinically available arrays, the large number of loci, the broad range of syndromic

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phenotypes interrogated by arrays, the variation in detection rates of different syndromes, and the clinical interpretation of novel copy number variants present challenges for physicians and genetic counselors who must educate patients, perform clinical correlations, and communicate results to patients. Based on our experience with over 8000 pre- and postnatal samples referred to our laboratory for CGH microarray testing, we discuss the issues that are encountered with CGH microarray testing in the pediatric, adult, and prenatal clinical settings and explore strategies used to optimize patient and provider education and communication of results to patients and their families.

PRETEST EDUCATION—PEDIATRIC AND ADULT PATIENTS

The pretest education and consent process generally used for diagnostic studies on pediatric or adult patients with clinical indications for testing consists of a description of the type of testing and estimate of the diagnostic utility of the test based on the differential diagnosis. When applied to CGH microarray, the most common clinical indications for testing are developmental delay and dysmorphic features in a patient who does not demonstrate clinical findings that are immediately suggestive of a particular disorder. If cardinal features of a syndrome are present, it may be more appropriate for a physician to order disease-specific fluorescence in situ hybridization or DNA studies. At a minimum, the pretest discussion of CGH microarray testing should include a description of the objective of the test, the test methodology, the type of sample required, and the possible outcomes of testing. In our experience, the test is typically described as a more detailed analysis of the chromosomes than is possible with a karyotype. It is also discussed that genetic syndromes may be caused by imbalances of genetic material and that CGH microarray is designed to interrogate the genome for gains or losses that might help explain the cause of the patient's medical problems. The discussion of test methodology should include whether the array is "targeted" to regions of the genome that are known to be associated with genetic syndromes or if a whole genome screen is to be used and to what resolution.

A discussion of the possible outcomes of testing should include an explanation of the categories of results (see below). In approximately 5–10% of cases, it may be necessary to test parental samples to determine if a gain or loss is inherited from either parent and most likely benign, or a de novo event, more likely to be associated with the clinical phenotype. Laboratories may have different protocols regarding whether parental samples are required to accompany the child's sample or if they will be requested after detection of a variant. If parental samples are not requested at the time of submission of the child's samples, it is still advisable to inform the family of the possibility that their samples will be needed at a later time to aid in interpretation of results.

LABORATORY INTERPRETATION OF RESULTS

Result categories

When the CGH microarray results are complete and ready to be reported, the laboratory will classify the findings into various categories. These include (1) no clinically significant abnormality detected; (2) clinically significant abnormality detected, known to be associated with a genetic condition; (3) variation of uncertain significance detected in the patient, and also in a parent (based on our experience thus far, this has been seen in about 5–10% of cases and is generally of low concern, but should be discussed with a genetic counselor); and (4) variation of uncertain significance detected in the patient, but not present in either parent. This is relatively rare and requires detailed discussion with a physician or genetic counselor as it may represent a clinically significant event that is causative of the clinical phenotype.

Communication of results of known clinical significance

Many families of individuals with suspected genetic syndromes become frustrated after many years of testing that fails to lead to a diagnosis. In a survey of 2513 clinical cases submitted to the Baylor Medical Genetics Laboratories for chromosome microarray analysis (CMA) testing using versions 4 or 5 of the array, 8.5% of individuals were found to have a genomic imbalance of clinical significance.[9] Importantly, abnormal CMA findings were observed in 5.2% of patients with a previously reported normal karyotype. Although this new diagnosis did not necessarily influence treatment or outcome, the discovery of a diagnosis is of vital significance to many families. For practical concerns, a specific diagnosis, rather than a descriptive diagnosis such as developmental delay or dysmorphic features, often facilitates getting services at school or through insurance carriers. For issues related to genetic counseling, a specific diagnosis allows physicians and counselors to provide accurate recurrence risks for the couples, and provide risks for the extended pedigree. Finally, a specific diagnosis allows the medical emphasis to progress from a diagnostic phase to an evaluation and long-term management phase.

Identification of novel results of probable clinical significance

In contrast to the identification of gains or losses known to be associated with well-characterized disorders, CGH microarray can also detect novel findings that have not been previously described and may be of uncertain clinical significance. These cases are the most challenging for the laboratory to interpret and generally require additional studies to assign the appropriate interpretation based on available evidence. Generally, the lab will confirm the finding by one of several possible methods, including repeat analysis using the original method or an independent method such as fluorescence in situ hybridization or quantitative polymerase chain reaction. In addition to laboratory confirmation of the original result, the laboratory will search available databases to determine if the genomic area corresponding to the gain or loss has been reported in association with a clinical phenotype. The laboratory may also have

an internal resource to provide information on the frequency of gains or losses associated with a particular clone or oligo. For example, the clinical laboratory at Baylor College of Medicine maintains an interactive database now containing data from over 8000 clinical cases that tracks results by clone and can give information as to the frequency of detected gains or losses. This yields immediate information regarding the frequency that the clones in question have deviated from normal and gives an indication of whether the change is likely to be a rare polymorphism or a potentially unique finding. Clones with a high frequency of variation are less likely to be clinically significant and are generally removed from an array after sufficient experience. A gain or loss detected by a clone with a low frequency of variation is more suspect and further analysis of parental samples is generally performed to determine whether the finding is inherited from one of the parents or is a de novo event. A confirmed positive result in a child that is also found in a parent is generally interpreted as a familial variant that is likely to be benign. However, an important caveat to this general interpretation of inherited variation is the situation in which a parent is assumed to be phenotypically normal, but in fact has subtle findings either at present or historically that can be related to the child's more pronounced medical concerns. In our experience, this seems to be particularly relevant for inherited duplications in which parents who also carry the duplication have clinical findings. Thus, it is recommended that when parental samples are obtained, clinical screening of the parents for possible phenotypic features should be performed, which may reveal a subtle phenotype that could aid in interpretation of the finding.

Alternatively, if the finding is not seen in either parent, the index of concern regarding its significance as a causative factor in the child's phenotype is increased. In this testing scenario, the laboratory will issue a report that describes the findings and indicates the gain or loss is of probable clinical significance. The referring physician and/or genetic counselor must then put the CGH microarray result into clinical context before presenting the results to the family. The clinical correlation may include a review of the genes that map to the involved region and whether these may be related to the phenotype (also see Posttest Counseling section). Another important issue that the referring physician and/or genetic counselor should consider when interpreting these findings is whether misidentification of paternity may be a possible confounding factor. Because the laboratory interpretation of a de novo event is dependent on correct identification of parentage, this issue may be explored with the family or independently with the mother in a manner appropriate to each case.

POSTTEST COUNSELING

Positive results

The posttest counseling process in the case of a positive result of clear clinical significance is consistent with the process followed for other types of genetic testing. Depending on the diagnosis and review of the family pedigree, it may be appropriate to offer testing to at-risk relatives, particularly those with possible phenotypic findings. Specific counseling issues that may arise as a result of positive findings from CGH microarray include the finding of concomitant or presymptomatic diagnoses. Because of the large number of disorders or genomic regions interrogated on the array platforms, it is possible that a diagnosis unrelated to the original indication for testing may be ascertained. For example, a patient sample submitted to the Baylor laboratory for a child <1 year of age with dysmorphic features was found to have a deletion in the NF1 region. There was no family history of NF1, nor did the child meet clinical diagnostic criteria at the time. Moreover, the current arrays may also detect disorders, such as Charcot Marie Tooth type 1, that typically present in late childhood or adulthood, raising the possibility that a younger individual may receive a presymptomatic diagnosis. Although the risk of detecting secondary or concomitant diagnoses when performing a diagnostic test for a primary indication is not unique to CGH microarray, families should be made aware of this possibility before undertaking testing.

In our experience thus far with offering CGH microarray on a clinical basis, there have been several examples of novel findings that have initiated studies on a research basis to further characterize the extent of the genomic imbalance and family studies to ascertain genotype/phenotype relationships in the extended pedigree. Examples of these include MECP2 duplications in males,[10] duplications of the Williams syndrome locus, atypical deletions and duplications of 22q11, and others. These extended investigations require communication and a concerted effort between the clinical lab and the referring physician and genetic counselor to communicate the results to families, describe the objectives of the further studies, and obtain consent to proceed and help coordinate clinical visits and testing of appropriate family members. These types of interactions are of great value to the scientific and patient communities as more syndromes are defined and clearly characterized.

Negative results

Previous experience has shown that new conclusive diagnoses can be made in about 8–12% of cases. However, for the remaining majority of cases, a negative result was obtained. In cases where a chromosomal or genomic abnormality is strongly suspected, the question of additional testing is raised. The clinical arrays have undergone rapid evolution, with new versions containing greater coverage and additional, newly identified disease loci appearing at least annually. In addition to the option of repeating an array study when a significant enhancement of coverage is clinically available, referring physicians and genetic counselors may consider a high-density array that may be available on a research basis as an additional path to possible delineation of a genomic etiology.

One must also consider the detection rate for the syndromes interrogated by the array. Because of the molecular and cytogenetic heterogeneity of many different genetic conditions, it is possible that a syndrome may have a different etiology than the one being screened via the microarray. For some conditions,

such as velo-cardio-facial syndrome and Williams syndrome, detection will be very high because of the fact that most cases are due to a single etiology. For other conditions such as Prader-Willi or Angelman syndrome, the detection will be moderately high. However, for many conditions, genomic gains or losses have been described, but only in a minority of cases. These syndromes may be included on the current commercially available arrays, however, referring physicians and families must be aware that for many conditions, failure to detect an alteration by the array does not rule out a condition. If strong clinical indication exists, further studies to analyze other possible molecular etiologies of specific syndromes should be considered.

PRENATAL ISSUES

Since 2005, the Baylor laboratory has offered prenatal CGH microarray testing on a clinical basis. This was preceded by a year-long study conducted at Baylor in which we investigated the reliability and accuracy of this technology for testing on prenatal samples and compared it with standard prenatal karyotyping.[11,12] In addition, procedures were developed for the informed consent process, communication of the limitations and benefits of the new testing, and the communication of results. This study provided the basis for our informed consent process for prenatal CGH microarray clinical testing. The major difference between counseling for pre- and postnatal CGH microarray testing is related to the pretest counseling component. Although limited pretest counseling is conducted for pediatric and adult, the pretest education and counseling for prenatal testing is paramount. The pretest counseling and education, conducted by the patient's physician and/or genetic counselor, is supplemented by an informed consent document that provides a written summary of the testing process, potential benefits and limitations of testing, and possible testing outcomes. A signed copy of the consent form is required by the laboratory before initiating testing and a copy is given to patients for their records. For both patients and health care providers, many additional sources of educational materials are also available including printed materials and a dedicated laboratory website. The website contains detailed information on the genomic regions and corresponding disorders covered by the array, including links to other resources such as OMIM. In addition, the laboratory staff of directors and genetic counselors is available to respond by telephone or e-mail to inquiries before testing or to help clarify result interpretations. Peer-reviewed publications in the medical literature and presentations at national subspecialty meetings are also a resource for dissemination of new findings to health care providers.

Pretest counseling and informed consent for prenatal CGH microarray testing

For couples interested in learning about the option of prenatal CGH microarray testing, the prenatal genetic counseling session should include a review of chromosomes and genomic structure, a description of the type of array (bacterial artificial chromosome or oligo array) that will be used, and the extent of genomic coverage. Additional important points that should be fully discussed with families before testing include the spectrum of disorders that the array detects, including disorders with severe neurologic phenotypes and others with more mild or adult onset phenotypes. The amount of information that can be effectively conveyed about each disorder during a counseling session is limited by time constraints and the difficulties inherent in a patient's ability to comprehend a large amount of complex information presented in one session. It may be helpful to present broad categories of disorders, such as severe disorders presenting in infancy with neurologic phenotypes or phenotypes with physical disabilities and mild to moderate developmental delay, to orient patients to the types of conditions evaluated by the array. In addition to information about the spectrum of phenotypes, it is important to emphasize that the detection rates for disorders vary widely and in relation to the possible genetic mechanisms affecting a particular locus (see above). As noted earlier, counselors and physicians should be prepared to provide resources where families may obtain additional information should they desire. Many of the laboratory websites (e.g., www.bcmgeneticlabs.org) contain links for each disorder interrogated by their array, which may then provide additional links to more patient-oriented materials. Other issues that families should be aware of include the possibility that a genetic diagnosis could be made that proved to involve genetic information that the family feels, in retrospect, was unwanted information. Examples of this may be genetic diagnoses that do not cause significant birth defects, but may present with signs and symptoms of varying severity at a later stage of life. This may be a significant concern for some families, and may result in their choosing not to pursue testing.

Preliminary findings with regard to patient decision-making

Data regarding patient attitudes toward a prenatal CGH microarray testing are limited. In the study conducted by Sahoo et al.[11], data were collected regarding patients who accepted testing and those who declined. Fifty-three couples who received genetic counseling by a single genetic counselor were examined in more detail. These 53 couples were considered to be at increased risk for aneuploidy because of advanced maternal age, abnormal fetal ultrasound findings, abnormal serum screening, or a family history of a previous child with anomalies. After genetic counseling, 45 of 53 couples (85%) chose to have amniocentesis or CVS. Of these 45 couples, 33 (73%) elected to have CGH microarray testing in addition to the standard karyotype, whereas 12 declined testing even though the testing was at no additional charge. The most common reasons couples gave for accepting CGH microarray testing included the desire to obtain the most information possible on their pregnancy without introducing any additional procedures and findings of ultrasound abnormalities in the current pregnancy or a history of anomalies in a prior pregnancy. Frequent reasons for declining testing included the fear of increased anxiety while waiting for the results of the additional testing and the patient's perspective that the disorders tested on the array were

rare. The process of deciding whether to undergo CGH microarray testing may differ significantly for couples in whom an ultrasound abnormality has been found in the fetus when compared with couples who seek additional genetic information about the fetus. Below are three examples of decision-making for couples seen by Baylor genetic counselors.

Case 1

The patient was a 44-year-old woman who presented for counseling at 17 weeks gestation. The current pregnancy was conceived through in vitro fertilization using a donor egg. The patient and her 52-year-old husband were weighing the pros and cons of having amniocentesis due to parental concern. The patient was uncertain of whether she wanted to proceed with amniocentesis due to the associated risk for complications, but was also concerned about the lack of detailed family history information she had due to using a donor egg to conceive her pregnancy. The couple had been told about prenatal CMA testing as a way of obtaining further information about a pregnancy prenatally. In counseling this couple, we discussed not only the benefits, limitations, and risks of amniocentesis but also the benefits and limitations of prenatal CMA testing. Ultimately, the patient decided to proceed with amniocentesis and prenatal CMA testing, indicating that she felt more comfortable with the risk of amniocentesis knowing that she would be able to obtain more detailed information about a larger number of genetic conditions through the CMA testing. Both the routine chromosome analysis and CMA testing were normal. Subsequently, the patient contacted the counselor to state that having done the CMA testing and knowing it was normal allowed her to feel less anxious about the pregnancy.

Case 2

A 40-year-old patient was referred for genetic counseling to discuss the option of prenatal CMA testing. The patient had expressed an interest in learning about additional prenatal testing that was currently available. The benefits and limitations of prenatal CMA testing were discussed in detail with this patient and her 41-year-old husband. After detailed counseling, the patient and her husband ultimately declined prenatal CMA testing indicating that for them the possibility of a result of uncertain significance and the resulting anxiety they knew they would feel in that case was an important factor in their decision. They also indicated that knowing that the conditions included in this testing were not considered to be extremely common and that most were sporadic conditions made them feel confident that this was the right decision for them.

Case 3

The patient was a 39-year-old woman who presented for counseling at 17 weeks and 6 days gestation. Amniocentesis had been performed at 15 weeks gestation due to advanced maternal age. The results of the amniocentesis showed a normal male chromosome pattern (46,XY) with an elevated amniotic fluid alpha fetoprotein (multiples of the median = 5.16) and positive acetylcholinesterase. Fetal hemoglobin studies were negative. On ultrasound at 16 weeks gestation, the fetus was noted to have a lemon-shaped head. Fetal magnetic resonance imaging (MRI) was performed at 17 weeks gestation and showed a lemon-shaped skull but without evidence of a Chiari 2 malformation or any definite evidence of an open neural tube defect. At that time it had been recommended that they return for a follow-up fetal MRI at 21–22 weeks gestation. The patient and her husband came for counseling to discuss the results of the prenatal testing and the implications for the pregnancy. They were trying to decide whether to continue or terminate the pregnancy based on the information from the testing that had been completed, which was inconclusive, or whether to wait the additional 3–4 weeks until a repeat MRI would be performed. In reviewing the information that was currently known about the pregnancy, the option of prenatal CMA was discussed as a means of trying to find an explanation for the findings in the pregnancy. After discussing the details of prenatal CMA testing, the couple decided to proceed with prenatal CMA testing. They indicated that having the prenatal CMA as a testing option that may provide further information about the pregnancy allowed them to justify waiting another 3 weeks for a repeat fetal MRI before deciding whether to terminate the pregnancy. The prenatal CMA results revealed a gain in copy number of one chromosome 5 clone that was also found in the father and thus most likely represented a familial copy number variant. However, repeat fetal MRI revealed a frontal encephalocele and at least partial agenesis of the corpus callosum. The couple subsequently chose to terminate the pregnancy.

Future directions

The past 5 years has seen a very rapid evolution of CGH microarray technology and an equally rapid application of the technology to the clinical diagnostic setting. CGH microarray technology has already proven useful in the pediatric arena and is proving to be increasingly useful in the prenatal arena as well.[13] Future studies and experience will further elucidate the role that this highly sensitive tool to detect genomic disorders will come to play in our armamentarium of diagnostic tests, including whether it may replace the use of standard karyotyping. Genetic counseling issues that are especially pertinent to the use of this testing platform include how to incorporate adequate pretest counseling and consent and how to interpret and convey results to patients, especially those results of uncertain significance. With both pediatric and prenatal applications of this technology, thorough genetic counseling has proven to be beneficial in helping patients to understand the aim of the testing and also to better understand the possible range and implications of results. Overall, the goal is to help prepare patients for information they may receive and decide whether it is the type of information they want to have. Further studies are needed to delineate the pattern of patient acceptance and identify factors associated with patient decision-making, so that the most effective genetic counseling models can be developed. As this technology continues to evolve, it is important that we continue to explore and anticipate the genetic counseling issues posed by this new technology, particu-

larly in the prenatal realm of testing, with the goal of maximizing the benefits and reducing the potential risks of this testing for both the clinician and the patient.

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