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Advances in Genetic Testing and Applications in Newborn Medicine

Kara Goodin, MD,* Margaret Chen, PhD,* Edward Lose, MD,* Fady M. Mikhail, MD, PhD,* Bruce R. Korf, MD, PhD*

Objectives  After completing this article, readers should be able to:

1. Recognize the application of new approaches to cytogenetic diagnosis.
2. Know when to consider ordering routine chromosomal analysis, fluorescence in situ hybridization, or array comparative genomic hybridization.
3. Be familiar with various approaches to molecular diagnosis and the factors to consider in ordering testing.
4. Describe the role of genomic imprinting in disease in newborns and the possibility of genetic testing.

Abstract

Because genetic conditions can alter the health of neonates, it is important for neonatologists to become familiar with the indications for testing and major issues in the interpretation of results. The two primary molecular cytogenetic techniques are fluorescence in situ hybridization and array comparative genomic hybridization, which allow detection of deletions, duplications, and rearrangements of small regions within the chromosome. Direct mutation analysis of DNA can be targeted to a specific variant known to be associated with disease. Epigenetic factors can affect gene expression without altering the genotype. For example, genomic imprinting (differential expression of a gene) causes several genetic disorders. Most molecular genetic testing performed in the newborn nursery is for purposes of diagnosis. Important differences in analytic validity, clinical validity, and clinical utility distinguish genetic testing from more traditional laboratory testing, and the implications of these differences must be considered when ordering such tests.

Introduction

Medical genetics began as a defined specialty in the 1950s, and the first genetic test was the analysis of chromosome number and structure beginning in 1959. Since that time, the number and complexity of genetic tests has increased steadily, especially since the sequencing of the human genome. Neonatologists are responsible for treating the sickest of newborns, so genetic conditions that alter the health of infants often are a consideration in the neonatal intensive care unit. Genetic tests are increasingly available to confirm a suspected clinical diagnosis, providing guidance in care of the newborn and counseling to the family. It is, therefore, important for the neonatologist to be familiar with the indications for testing and major issues in their interpretation. This review considers recent advances in cytogenetic and molecular genetic testing relevant to newborn medicine.

Cytogenetics

Chromosomal analysis enables the detection of changes in chromosome number and structure and has involved progressively increasing resolution over the past 4 decades. Routine cytogenetic study became possible with the advent of hypotonic treatment of dividing cells to spread the chromosomes and methods of cell culture in the 1950s. Banding techniques introduced in the late 1960s increased the precision of analysis. Banding allows for a more detailed view of the chromosomes, with finer appreciation of deletions, duplications, and gross rearrangements, but it still does not access the informa-
tion at the level of the individual gene. The current techniques of routine cytogenetic analysis have a resolution of about 5 to 10 megabases of DNA. Recent advances, particularly fluorescence in situ hybridization (FISH) and array comparative genomic hybridization (array CGH) are increasing that resolution rapidly. Indications for various cytogenetic approaches in newborn medicine are outlined in Table 1.

Fluorescence In Situ Hybridization

Cytogenetic testing progressed in the 1990s with the addition of FISH analysis (Fig. 1). This technique hybridizes purified single-stranded DNA sequences labeled with a fluorescent dye (probes) to target homologous single-stranded chromosomal DNA. This allows the detection of deletions, duplications, and rearrangements of small regions within the chromosome. One major benefit of FISH testing is a finer resolution than can be achieved with standard chromosomal analysis. In addition, results can be available in 2 to 3 days instead of 2 to 3 weeks. FISH requires knowledge of the region of interest so the appropriate probe can be used. Its resolution is limited by the need for relatively large probes necessary to generate sufficient fluorescence for adequate visibility.

FISH testing can be performed one probe at a time or with multiple probes to assess various regions at the same time. Locus-specific FISH probes can be used to identify submicroscopic deletions or duplications; whereas centromeric FISH probes can be used for chromosome enumeration in interphase cells. Also, chromosomes can be “painted” by “cocktails” of chromosome-specific probes to identify the source of rearranged chromosome segments. Subtelomere analysis is the evaluation of the ends of the chromosomes (subtelomeres) via FISH. Multiple probes specific for the subtelomere regions are used to assess for deletions, duplications, or rearrangements in these areas. Subtelomere analysis is not used frequently in the newborn period; it is used most often in the evaluation of patients who have developmental delay or failure to thrive.

FISH testing can be performed on interphase cells as well as on condensed metaphase chromosomes. Probe sets specific for chromosomes 13, 18, 21, X, and Y, all associated with liveborn aneuploidy syndromes, can be hybridized to interphase cells to provide rapid diagnosis. Although useful in the rapid diagnosis of aneuploidy, such analysis misses chromosomal rearrangements in-

Table 1. Indications for and Limitations of Major Cytogenetic Tests

| Technique                                | Major Indications                                                                 | Limitations                                                                 |
|------------------------------------------|----------------------------------------------------------------------------------|                                                                            |
| High-resolution banding                  | Suspected aneuploidy syndrome; parent who has known balanced rearrangement; multiple congenital anomalies | 5- to 10-Mbase resolution; requires several days to complete                |
| Interphase FISH aneuploidy screen        | Rapid screen for aneuploidy involving chromosomes 13, 18, 21, X, and Y           | Does not detect structural rearrangements of chromosomes                   |
| FISH analysis of microdeletions or microduplications | Suspected microdeletion syndrome, such as DiGeorge or Williams                   | Requires strong clinical suspicion to guide targeted analysis of specific chromosomal region |
| Subtelomere FISH                         | Detects subtelomere deletions in children who have unexplained multiple congenital anomalies | Misses rearrangements outside subtelomere region                            |
| Targeted array comparative genomic hybridization | High-resolution detection of a variety of clinically significant deletions or duplications; used to diagnose suspected syndrome or search for cause of multiple congenital anomalies | Does not detect all possible deletion or duplication syndromes               |
| Whole genome array comparative genomic hybridization | Unexplained multiple congenital anomalies                                      | Does not detect changes at level of single gene; may detect benign variants of no pathologic significance |

FISH = fluorescence in situ hybridization
volving these and other chromosomes, so results should not be taken as a definitive cytogenetic study.

**Array Comparative Genomic Hybridization**

The resolution of cytogenetic analysis has advanced further with the advent of array CGH. Array CGH involves competitive hybridization of two different genomic DNA sequences (one from the patient and one from a control) to the same reference sample of the probes fixed onto a glass slide (Fig. 2). Such genomic sequences are labeled with different colored fluorescent dyes (eg, red and green). Deletions and duplications are appreciated through the comparison of the red/green color ratio and, therefore, the relative copy number of sequences present. If equal amounts of test and control sequences are present, yellow is appreciated. If there is more of the test (red) sequence, red is seen; if more of the control sequence is present, green is seen. The slides are scanned by a specialized laser scanner and the data analyzed by special software.

The major advantages of array CGH include finer resolution than FISH and the ability to evaluate a large number of chromosome regions for deletions and duplications simultaneously. Current arrays are limited by the specific sequences attached to the slide, and the size of those sequences determines resolution. Targeted arrays focus on chromosomal regions associated with known microdeletion or microduplication syndromes as well as all subtelomeric regions. Whole genome arrays permit analysis of deletions or duplications anywhere in the genome without requiring predetermination of a region of interest. One limitation to this approach is the detection of copy number changes of unknown significance that could be pathologic or might represent benign variants. It is likely that array CGH gradually will replace standard cytogenetics and FISH for detection of copy number changes. It is important to recognize, though, that the technique will not detect balanced rearrangements, such as inversions, balanced translocations, and balanced insertions.

**Molecular Genetic Testing**

**Linkage Analysis**

The ultimate level of resolution is the direct analysis of genetic material by DNA testing. Initial approaches were based on genetic linkage, in which a genetic marker located near a disease gene is tracked through a family to follow inheritance of the disease. This approach is useful...
if the disease gene has not yet been identified but has been mapped or if it is difficult to identify mutations, perhaps due to a wide diversity of pathologic changes in different patients. The increasing number of disease loci that have been identified has reduced the number of linkage-based tests. Further, linkage requires study of multiple family members and takes considerable time. Therefore, it is not widely used in newborn medicine.

**Direct Mutation Analysis**

Widespread gene identification has allowed for the development of direct mutation analysis. In some cases, mutation analysis can be targeted to a specific variant known to be associated with disease. This is the case for detection of the beta globin mutation responsible for sickle cell anemia. A variety of techniques can be used for mutation detection. Direct DNA sequencing is becom-
Clinical validity is the degree to which the test correctly assesses risk of health or disease. It is related to familiar concepts of sensitivity, specificity, and positive and negative predictive value. Some limitations of genetic tests are different from other medical tests, however. “False-positive” test results include finding variants that do not correlate with disease. There are many benign genetic variants, some of which are rare. Rigorous criteria must be applied to determine that a specific variant is pathogenic. Criteria include finding the variant only in affected individuals, determining that the variant has a significant effect on the function of the gene product, and demonstrating that it segregates with disease in a family. The specific criteria may need to be customized to the individual gene, however. In some cases, there is substantial documentation of pathogenic significance, but in others, pathogenicity is uncertain. Clinicians need to be prepared to review laboratory results critically in light of clinical decision-making.

A common reason for a “false-negative” result is that mutation testing might not detect all possible variants in a gene. A wide diversity of mutations can affect the function of a gene or gene product, and it may not be practical to detect all possible variants. Also, some disorders are caused by mutations in any one of several distinct genes, some of which might not be included on a testing panel or might not yet have been discovered. The clinician must be aware that failure to identify a gene mutation may not mean that the condition is not present.

Clinical utility is the degree to which the test guides medical management. A test may be available to indicate risk of disease, but no approach to management may be available that improves the outcome based on the results of testing. In some cases, an individual might be subject to anxiety, stigmatization, or discrimination based on test results but have no benefit from medical surveillance or treatment. This applies particularly to predispositional tests, which are used best when results lead to implementation of a surveillance or management approach that can lead to an improved outcome or can provide reassurance that such an approach is unnecessary due to low risk. In addition, there may be implications for extended family members, some of whom may not wish to know their disease risk. Many laboratories employ a formal consent process, even though the testing is being offered for clinical purposes, not for research. Most, but not all, states in the United States have laws that deal with the privacy of genetic information, some explicitly preventing the use of genetic test results as a basis for employment or insurance decisions. These laws are highly vari-
able, however. Federal legislation recently was passed by the United States Congress.

**Control of Gene Expression Through Epigenetics**

Certain epigenetic factors affect gene expression without altering the genotype. Epigenetic changes consist of alterations in the properties of a gene that affect expression but do not represent a permanent change in genetic information. DNA methylation at CpG dinucleotides in gene promoter regions, as well as the addition of phosphate, acetyl, and ubiquitin groups to histone proteins, represent several epigenetic mechanisms by which cells regulate gene expression.

Epigenetic changes have been found to contribute to several medical disorders through the phenomenon of genomic imprinting. Genomic imprinting is differential expression of a gene, depending on whether it is inherited from the mother or the father. This is also known as a “parent-of-origin” effect. Imprinting occurs in a minority of genes soon after conception. Although imprinted genes usually are methylated differentially, an alteration of chromatin structure also may be involved.

Several genetic disorders result from consequences of genomic imprinting (Table 3). The phenotypic features associated with small deletions of some chromosomes are believed to be due, in part, to a lack of expression of essential imprinted genes on the intact homologue. In addition, the phenotypic features associated with uniparental disomy of some chromosomes also are believed to result from a lack of expression of essential imprinted genes. Uniparental disomy is the inheritance of both chromosomes in a pair from the same parent rather than from each parent. For example, Prader-Willi syndrome, a condition characterized by developmental delay, infantile hypotonia, and childhood obesity, can result from either a deletion of a region on the long arm of chromosome 15 (15q11–13) of the paternally inherited copy or maternal uniparental disomy for chromosome 15. In both cases, the affected child fails to express crucial gene products because the maternally inherited gene(s) in this chromosome region are imprinted and, therefore, are not expressed. Angelman syndrome is a clinically different condition characterized by severe mental retardation, seizures, ataxia, and apraxia. Like Prader-Willi syndrome, Angelman syndrome results from differential expression of specific genes at 15q11–q13, but Angelman syndrome is associated with a deletion of the maternal copy.

<p>| Table 2. Molecular Diagnostic Tests Commonly Used in Newborn Medicine |
|-----------------|-----------------|-----------------|
| <strong>Indication</strong>  | <strong>Condition</strong>    | <strong>Approach</strong>     |
| Hypotonia       | Myotonic dystrophy | Targeted mutation analysis: trinucleotide repeat in DMPK |
|                 | Spinal muscular atrophy 1 | Deletion analysis of exon 7 in SMN1 gene |
| Multiple anomalies | CHARGE syndrome | Gene sequencing of CDH7 |
|                 | Noonan syndrome | Sequencing of four genes in the Ras pathway: PTPN11, KRAS, RAF1, and SOS1 |
| Abnormal genitalia with or without other anomalies | Camptometic dysplasia | Gene sequencing of SOX9 |
|                 | WAGR or Denys-Drash syndrome | Gene sequencing of WT1 |
| Small infant or dwarfism | Achondroplasia, hypochondroplasia, thanatophoric dysplasia | Targeted sequencing of FGFR3 |
|                 | Ellis-van Creveld syndrome | Sequencing of EVC and EVC2 |
|                 | Shwachman-Diamond syndrome | Sequencing of SBDS |
| Craniofacial anomalies | Apert syndrome plus many other FGFR2-related conditions | Targeted mutation analysis of FGFR2 |
|                 | Saethre-Chotzen syndrome | Sequencing and deletion/duplication analysis of exon 1 in TWIST1 |
|                 | Waardenburg syndrome | Sequencing of PAX3, MITF, endothelin 3, endothelin receptor B, and SOX10 |
| Congenital heart disease | Holt-Oram syndrome | Sequencing of TBX5 |
|                 | Left ventricular outflow tract obstruction (aortic valve anomalies or hypoplastic left heart) | Research sequencing of GJA1, NOTCH1 |
|                 | Marfan syndrome neonatal form | Sequencing of fibrillin 1 |
|                 | Atrioventricular septal defect with heterotaxy | Sequencing of CRELD1 |</p>
<table>
<thead>
<tr>
<th>Genetic Disorder</th>
<th>Online Mendelian Inheritance in Man #</th>
<th>Chromosome</th>
<th>Gene(s)</th>
<th>Imprinted*</th>
<th>Protein</th>
<th>Characteristic Clinical Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angelman syndrome</td>
<td>105830</td>
<td>15q11–13</td>
<td><em>UBE3A</em></td>
<td>Pat</td>
<td>Ubiquitin-protein ligase E3A</td>
<td>Severe mental retardation, seizures, ataxia, apraxia</td>
</tr>
<tr>
<td>Prader-Willi syndrome</td>
<td>176270</td>
<td>15q11–13</td>
<td><em>SNRPN</em> Others (including <em>MKRN3</em> and <em>NDN</em>)</td>
<td>Mat</td>
<td>Small nuclear ribonuclear protein polypeptide N</td>
<td>Infantile hypotonia, developmental delay, childhood obesity</td>
</tr>
<tr>
<td>Beckwith-Wiedemann syndrome</td>
<td>130650</td>
<td>11p15.5</td>
<td>H19</td>
<td>Pat</td>
<td>H19 encodes an untranslated mRNA</td>
<td>Overgrowth, hemihypertrophy, macroGLOSSIA, omphalocele, neonatal hypoglycemia, predisposition to tumorigenesis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7p11.2-p13</td>
<td>IGFII</td>
<td>Mat</td>
<td>Insulin-like growth factor II</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CDKNIC</td>
<td>Pat</td>
<td>Cyclin-dependent kinase inhibitor 1C</td>
<td></td>
</tr>
<tr>
<td>Silver-Russell syndrome</td>
<td>180860</td>
<td>11p15.5</td>
<td>H19</td>
<td>Pat</td>
<td>H19 encodes an untranslated mRNA</td>
<td>Prenatal and postnatal growth retardation (with normal head circumference), triangular-shaped facies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7p11.2-p13</td>
<td>GRB10 (Candidate locus)</td>
<td>Mat</td>
<td>Growth factor receptor-bound protein-10</td>
<td></td>
</tr>
<tr>
<td>Albright hereditary osteodystrophy</td>
<td>103580</td>
<td>20q13.3</td>
<td>GNAS1</td>
<td>Pat</td>
<td>Alpha subunit of the stimulatory G-protein</td>
<td>Round face, obesity, short stature, and short fourth and fifth metacarpals are found in PHP Ia and pPHP</td>
</tr>
<tr>
<td>(including pseudohypoparathyroidism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hypocalcemia, hyperphosphatemia, resistance to parathyroid hormone, and mental retardation are found only in PHP Ia</td>
</tr>
<tr>
<td>[PHP] Type Ia and pseudo-pseudohypoparathyroidism [pPHP])</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The imprinted (inactivated) parental allele is indicated: Mat=maternal; Pat=paternal*
of chromosome 15 or paternal uniparental disomy. Recent studies employing genomic DNA sequencing have confirmed that approximately 10% of patients affected by Angelman syndrome have mutations in the maternally expressed copy of the \textit{UBE3A} gene.

Other genetic syndromes have been associated with genomic imprinting. For example, up to 10% of patients who have Silver-Russell syndrome (SRS) have maternal uniparental disomy for chromosome 7. SRS is characterized by intrauterine and postnatal growth retardation and triangular-shaped facies. Recent studies indicate that hypomethylation of the \textit{H19} gene promoter on chromosome 11p15 also is associated with SRS. Beckwith-Wiedemann syndrome is another disorder of genomic imprinting associated with chromosome 11p15. In contrast to patients who have SRS, individuals affected by Beckwith-Wiedemann syndrome have hypermethylation of the \textit{H19} gene promoter and display an overgrowth phenotype and predisposition to tumors. Hypermethylation of \textit{H19} may result from paternal uniparental disomy. Although the genetic mechanisms are complex, such hypermethylation ultimately leads to loss of imprinting of the oncogene \textit{IGFII} (insulin-like growth factor II). As a result, patients are at an elevated risk to develop Wilms tumor, a childhood neoplasm of the kidney, as well as several other malignancies.

\textbf{Summary}

Genetic testing has made great strides since its inception more than 40 years ago. Current techniques include chromosome analysis, FISH, array CGH, linkage analysis, direct mutation analysis, and evaluation of epigenetic modifications. Current genetic testing has aided in clinical, preclinical, and prenatal diagnosis. Although the neonatologist need not be familiar with the fine details of how genetic tests are performed, it will be increasingly important to have a basic appreciation of the range of possible tests, indications for their use, and pitfalls in their interpretation.

\textbf{Suggested Reading}


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1. Cytogenetic tests enable the detection of abnormalities in chromosome number and structure. Different types of cytogenetic tests are available for confirming a suspected clinical diagnosis, for providing guidance in the care of the newborn, and for counseling the family. In the evaluation of an infant who has developmental delay or failure to thrive, the cytogenetic test that, in combination with high-resolution banding, should give the highest detection rate of both visible and cryptic cytogenetic abnormalities is:
   A. Centromeric fluorescence in situ hybridization.
   B. Interphase fluorescence in situ hybridization aneuploidy screen.
   C. Subtelomeric fluorescence in situ hybridization.
   D. Targeted array comparative genomic hybridization.
   E. Whole genomic array comparative genomic hybridization.

2. Molecular genetic tests enable the detection of deletions, duplications, or rearrangements of the entire gene or segments within the gene as well as changes at the nucleotide level, including base substitutions and mutations in the promoter region that affect control of the gene expression. Among the indications for molecular genetic tests are making a diagnosis in a symptomatic individual, predictive testing in an individual at risk for inheriting a mutation, testing for late-onset disorders, and prenatal evaluation. Of the following, the molecular genetic test targeted for detection of trinucleotide repeats in the DMPK gene is most likely to identify:
   A. Camptomelic dysplasia.
   B. CHARGE syndrome.
   C. Holt–Oram syndrome.
   D. Myotonic dystrophy.
   E. Thanatophoric dwarfism.

3. Genomic imprinting indicates differential expression of a gene, depending on whether the gene is inherited from the mother or the father. Uniparental disomy represents the inheritance of both chromosomes in a pair from the same parent rather than from each parent. Of the following, paternal uniparental disomy for chromosome 15 is most characteristic of:
   A. Albright hereditary osteodystrophy.
   B. Angelman syndrome.
   C. Beckwith–Wiedemann syndrome.
   D. Prader–Willi syndrome.
   E. Silver–Russell syndrome.
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