AMERICAN ACADEMY OF PEDIATRICS

Committee on Genetics

Molecular Genetic Testing in Pediatric Practice: A Subject Review

ABSTRACT. Although many types of diagnostic and carrier testing for genetic disorders have been available for decades, the use of molecular methods is a relatively recent phenomenon. Such testing has expanded the range of disorders that can be diagnosed and has enhanced the ability of clinicians to provide accurate prognostic information and institute appropriate health supervision measures. However, the proper application of these tests may be difficult because of their scientific complexity and the potential for negative, sometimes unexpected, consequences for many patients. The purposes of this subject review are to provide background information on molecular genetic tests, to describe specific testing modalities, and to discuss some of the benefits and risks specific to the pediatric population. It is likely that pediatricians will use these testing methods increasingly for their patients and will need to evaluate critically their diagnostic and prognostic implications.

ABBREVIATION. FISH, fluorescence in situ hybridization.

Recent developments in genetic research have accelerated the discovery of individual genes and enhanced our understanding of how genes work and how gene abnormalities lead to disease. There is a growing list of molecular diagnostic tests, with estimates that 10 to 12 new tests become available each year. Such tests in an individual can provide the following: 1) diagnostic confirmation in a symptomatic patient, including genotype-phenotype correlation in some disorders; 2) carrier testing; and 3) presymptomatic testing for late-onset disorders. They also can be used for population-based screening to predict future genetic disease or assess the risk for complex conditions such as cancer, cardiovascular diseases, and neurodegenerative disorders in otherwise healthy people. Although this subject review focuses on molecular genetic testing that can be used to diagnose many genetic disorders, it should be remembered that this type of technology may not be appropriate for diagnosis of all, or even most, genetic conditions. For example, molecular genetic testing is available for diseases such as cystic fibrosis, sickle cell anemia, and Tay-Sachs disease, but the initial diagnosis of these disorders usually is established by other methods.

The molecular genetic technology underlying genetic testing is complex, as are the issues of pretest counseling about the indications, benefits, and limits of testing. Expert interpretation and explanation of results to individuals and families is essential. Furthermore, predictive genetic testing in children and adolescents leads to complicated medical, psychological, ethical, and legal issues.[1,2] Media publicity combined with entrepreneurial marketing of molecular genetic tests to physicians accentuates the need for the practicing pediatrician to be informed and aware of the technologies and issues related to testing. This subject review will familiarize pediatricians with diagnostic molecular genetic testing and the clinical and ethical issues to be considered in the diagnosis of children and adolescents. A number of other reviews are available regarding the laboratory techniques of this testing and can serve as excellent background resources for understanding the methods by which molecular genetic diagnostic testing is accomplished.[3–5]

BACKGROUND INFORMATION

It is estimated that anywhere from 50 000 to 100 000 genes are contained in the 46 chromosomes present in each human cell. A genetic locus is the place on homologous chromosome pairs where genes are located. Each gene is composed of 2 alternative copies known as alleles, one originating from the maternally derived chromosome and the other originating from the paternally derived chromosome of each chromosome pair. Genes are composed of DNA, and the products of genes are most often proteins that may be used for a variety of purposes, including structural development, regulation of cellular function, enzyme activity, and control of metabolic pathways.

Although most changes in the DNA base-pair composition of genes do not result in disease and are known as *polymorphisms,* some gene changes alter gene function to such a degree that clinical disease is manifested, and these are known as *mutations.* Most genetic disease is caused by single base-pair deletions, additions, or substitutions. However, some disorders are caused by large-scale gene abnormalities, such as deletions of the entire gene, that can be detected by newer methods of diagnosis, such as molecular cytogenetic analysis.

As in all diagnostic testing, it is most important that the clinician have a reasonable index of suspicion based on clinical signs and symptoms that suggest a specific diagnosis. For proper interpretation of molecular genetic test results, it is also important that clinicians understand the probabilistic nature of

The recommendations in this statement do not indicate an exclusive course of treatment or serve as a standard of medical care. Variations, taking into account individual circumstances, may be appropriate.

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these tests and the full implication of positive and negative results.

TYPES OF MOLECULAR TESTING

Indirect Analysis

Indirect analysis, often referred to as *linkage analysis,* is used when the location of a gene is known, although the gene itself and its function are not, or when the gene is known but the mutations are too heterogeneous to make direct analysis practical. In addition to its critical importance as a research tool, linkage analysis sometimes may be used to identify heterozygotes (carriers) and in prenatal diagnosis. This diagnostic strategy often requires more than one affected individual in more than one generation. Under such circumstances, it is possible to use markers within or around the gene to track its inheritance within a family.

Markers for DNA sequences in or near genes include restriction fragment length polymorphisms, variable number of tandem repeats, or microsatellite repeats. Any marker known to be linked to a disease gene can be used to track the transmission of the mutant allele within the family studied.

Direct Mutation Analysis

Direct mutation analysis is the approach used when the gene responsible for the condition has been identified and specific mutations within the gene have been characterized. The techniques used to detect these mutations are diverse, including allele-specific oligonucleotide hybridization analysis, heteroduplex analysis, Southern blot analysis, multiplex polymerase chain reaction analysis, and direct sequencing. A detailed description of these techniques is beyond the scope of this subject review but is available elsewhere.[6,7]

Direct mutation analysis is preferred over indirect analysis, as it is mutation-specific and does not require testing of parents or family members. As with all molecular genetic testing, however, it is also subject to limitations that must be recognized when ordering such testing. One of the major limitations of direct mutation analysis is that some diseases are caused by many mutations, not all of which are detected by a particular molecular test. A characteristic example of this complexity is in the molecular diagnosis of cystic fibrosis. In Caucasians of northern European background, about 70% of all cystic fibrosis mutations are accounted for by deletion of 3 base pairs that results in loss of a phenylalanine at position 508 of the cystic fibrosis transmembrane regulator protein. The other 30% of mutations number in the hundreds, making it impractical to screen a single person for all disease-causing alleles.[8] In other populations, such as those of African and Asian descent, there is an even smaller percentage of affected persons who can be detected from a single test or even a panel of the most common mutations, further complicating the use of molecular technology for clinical diagnosis and determination of carrier status. This huge diversity of mutations at a single genetic locus is known as *allelic heterogeneity.* Another limitation of mutation analysis is the result of another type of genetic heterogeneity, known as *locus heterogeneity,* in which mutations at 2 or more genetic loci can produce the same phenotype, as has been demonstrated for congenital sensorineural deafness, for which more than a dozen loci have been identified.[9]

It is important to recognize that mutation analysis, like most other forms of molecular diagnosis, can confirm a diagnosis when the test result is positive. However, a negative test result may not determine conclusively that the patient is unaffected. Table 1 provides a list of some of the genetic disorders for which direct mutation analysis is available. There are many other disorders that may be diagnosed by molecular genetic techniques. Geneticists and other subspecialists usually will have specialized information about testing for disorders within their area of spe-

TABLE 1. Selected Disorders Diagnosable by Direct Mutation Analysis

Disorder	Associated Features	Gene Symbol (Locus)
Achondroplasia	Macrocephaly, short-limbed dwarfism	FGFR3 (4p16)
Apert's disease	Acrocephaly, craniosynostosis, extensive syndactyly of fingers and toes	FGFR2 (10q26)
Charcot-Marie-Tooth disease, type 1A	Progressive sensory and motor neuropathy	PMP22 (17p11)
Crouzon's disease	Bicoronal synostosis, proptosis, hypertelorism	FGFR2 (10q26)
Cystic fibrosis	Recurrent pulmonary infections, exocrine pancreatic insufficiency	CFTR (7q31)
Familial adenomatous polyposis	Adenomatous polyps of the colon, high risk for colorectal cancer in early adulthood	APC (5q21)
Fragile X syndrome	Mental retardation, long-appearing face, large ears, macro-orchidism	FMR1 (Xq27)
Friedreich ataxia	Progressive ataxia, insulin resistance, concentric cardiomyopathy	FRA1 (9q13)
Hemophilia A	Deficient thrombostasis, hemarthrosis	F8C (Xq28)
Huntington's disease	Progressive loss of motor and cognitive function, usually beginning in adulthood	HD (4p16)
Muscular dystrophy (Duchenne's and Becker's)	Progressive muscle weakness	DMD (Xp21)
Myotonic dystrophy	Frontal balding, cataracts, progressive myotonia, infertility, cardiac conduction defects	DMPK (19q13)
Neurofibromatosis, type 1	Café au lait spots, neurofibromas, Lisch nodules, optic gliomas	NF1 (17q11)
Neurofibromatosis, type 2	Vestibular schwannomas and other intracranial and spinal tumors	NF2 (22q12)
Saethre-Chotzen syndrome	Craniosynostosis, ptosis, variable digital anomalies	TWIST (7p22)

cialty practice and are appropriate resources for evaluation, test selection and interpretation, and counseling of patients with suspected genetic disease.

Molecular Cytogenetic Analysis

Standard cytogenetic analysis is used to detect abnormalities in chromosome number or microscopically visible duplications or deletions of chromosomal material. With the advent of molecular cytogenetic techniques, such as fluorescence in situ hybridization (FISH), it is now possible to detect chromosomal rearrangements that are beyond the resolution of light microscopy used for standard cytogenetic analysis.

The use of FISH analysis for genetic diagnosis is made possible when a unique sequence of a gene or group of genes is known and when the disease in question is the result of a deletion of this critical region. This unique sequence, known as a *critical region,* is synthesized in the laboratory and labeled with a fluorescent marker. A sample from a patient is cultured as in standard cytogenetic analysis, and the fluorescent-labeled probe is added to the sample. If the unique sequence is present, the fluorescent probe will hybridize with it and be visible when viewed under a fluorescent microscope. A characteristic example of this type of genetic diagnosis is in a patient suspected to have Williams syndrome, which is known to be associated with a deletion of the elastin gene. When a sample is taken from an affected person and combined with the elastin probe, only 1 fluorescent signal will be visible, whereas 2 signals will be visible in the unaffected person, indicating that both copies of the elastin gene critical region are present. Table 2 provides a partial list of disorders that can be diagnosed by FISH analysis. In addition to detecting large-scale deletions of chromosome material, FISH also can be used to detect abnormalities of chromosome number or large-scale rearrangements that may not be detectable by standard cytogenetic techniques.

BENEFITS AND RISKS OF MOLECULAR DIAGNOSIS

Persons who are at increased risk for a genetic disorder live with uncertainty about their health and that of their children and extended family members. The use of molecular testing may eliminate this uncertainty. When one's health status is known, an appropriate treatment plan can be developed, including timely health supervision, anticipatory guidance, and institution of preventive measures.

Molecular genetic testing seldom poses significant physical risks. However, presymptomatic testing or carrier screening, particularly for diseases with serious health implications, can have profound effects and should not be performed without pretest counseling. The decision to have a test and its results can reverberate throughout the family. As a part of pretest counseling, it should be recognized that genetic testing may reveal information about the extended family, as well as the person being tested, and that a genetic test inadvertently may disclose family secrets involving paternity or adoption. Emotions elicited by test results can shift family dynamics. Family members identified as carrying the gene may feel anger, while one who is identified as a noncarrier may feel guilt for avoiding a disease that affects a close relative. It is also important to recognize that positive test results may be used to discriminate against the patient and family member in the areas of insurability, job hiring and promotion, and adoption of children.[10] Many states have adopted, or are in the process of adopting, laws that protect the privacy of genetic information.

SPECIAL CONCERNS FOR CHILDREN AND ADOLESCENTS

Special considerations must be given to genetic testing of children and adolescents. Because minors may be incapable of giving informed consent, they generally should not be tested except under the following specific circumstances that have been outlined in publications from a number of national organizations, including the American Academy of Pediatrics[2,11–13]: 1) testing should be offered when there are immediate medical benefits, such as institution of measures that can prevent the disease, delay its onset, limit its severity, or prevent secondary disabilities; and 2) testing also may be offered when there is a benefit to another family member and no anticipated harm to the minor. When the results of

TABLE 2. Microdeletion Syndromes Diagnosable by FISH

Syndrome	Site	Comments
Prader-Willi	(del)15q11 (pat)	Hypotonia, obesity, hypogonadism, mental retardation, small fingers
Angelman's	(del)15q11 (mat)	Hypertonia, global developmental delay, ataxia, episodes of inappropriate laughter, seizures, microcephaly, movement disorder
22q deletion syndromes: DiGeorge Velocardiofacial	(del)22q11	Hypoparathyroidism, absent thymus—T-cell defect, velopharyngeal incompetence or cleft; typical facies, conotruncal heart defect
Williams	(del)7q11	Prominent lips, wide mouth, developmental delay, supravalvar aortic stenosis, growth delay, infantile hypercalcemia
Miller-Dieker	(del)17p13	Lissencephaly, microcephaly, decorticate and decerebrate postures, associated cardiac, kidney, and genital anomalies
Smith-Magenis	(del)17p11	Brachycephaly, broad face and nasal bridge, flat midface, mental retardation, hyperactivity, self-destructive behavior, insomnia
Langer-Giedion	(del)8q24	Characteristic facial features, exostosis, cone-shaped epiphysis, polydactyly, microcephaly, mental retardation
Alagille (arteriohepatic dysplasia)	(del)20p12	Neonatal and infantile cholestasis, peripheral artery stenosis and cardiovascular anomalies, typical facies

genetic testing will be used solely for future reproductive decisions or when parents request it and there are no benefits to the child, in most circumstances it should be deferred until the child can request such testing as an autonomous individual who is able to appreciate the emotional and social consequences, as well as the genetic facts, of the results.

Central to all types of genetic testing is the process of genetic counseling to ensure that the patient has adequate information to give truly informed consent, that he or she is psychologically prepared to cope with the results, and that patients and sometimes other family members receive assistance in understanding the medical, psychological, social, and legal implications of these findings.

SUMMARY

Molecular genetic testing is increasingly available in pediatric practice because of recent developments in genetic research and their rapid translation into clinical practice. The technology behind molecular genetic testing is complex, and such testing has its own limits. Furthermore, testing brings with it complex ethical, legal, and social issues, particularly for children and adolescents. Pediatricians must understand these issues and, through proper consultation with experts and specialists, aim to prepare families adequately before ordering molecular genetic testing.

REFERENCES

1. Wertz DC, Fanos JH, Reilly PR. Genetic testing for children and adolescents: who decides? *JAMA*. 1994;292:875–881
2. American Academy of Pediatrics, Committee on Bioethics. Ethical issues with genetic testing in pediatrics. *Pediatrics*. In press
3. Korf B. Molecular diagnosis, I. *N Engl J Med*. 1995;332:1218–1220
4. Korf B. Molecular diagnosis, II [published correction appears in *N Engl J Med*. 1995;333:331]. *N Engl J Med*. 1995;332:1499–1502
5. Cooper DN, Schmidtke J. Diagnosis of human genetic disease using recombinant DNA: fourth edition. *Hum Genet*. 1993;92:211–236
6. Malcolm S. Molecular methodology. In: Rimoin DL, Connor JM, Pyeritz RE, eds. *Emery and Rimoin's Principles and Practice of Medical Genetics*. 3rd ed. New York, NY: Churchill Livingstone; 1997:67–86
7. Beaudet AL, Scriver CR, Sly WS, Valle D. Genetics, biochemistry, and molecular basis of variant human phenotypes. In: Scriver CR, Beaudet AL, Sly WS, et al, eds. *The Metabolic and Molecular Basis of Inherited Disease*. 7th ed. New York, NY: McGraw-Hill, Inc; 1995:53–228
8. Genetic testing for cystic fibrosis: National Institutes of Health Consensus Development Conference Statement on genetic testing for cystic fibrosis. *Arch Intern Med*. 1999;159:1529–1539
9. Steel KP, Bussoli TJ. Deafness genes: expressions of surprise. *Trends Genet*. 1999;15:207–211
10. Weaver KD. Genetic screening and the right not to know. *Issues Law Med*. 1997;13:243–281
11. Andrews LB, Fullarton JE, Holtzman NA, Motulsky AG, eds. Institute of Medicine Committee on Assessing Genetic Risks, Division of Health Sciences Policy. *Assessing Genetic Risks: Implications for Health and Social Policy*. Washington, DC: National Academy Press; 1994
12. Holtzman NA, Watson MS, eds. *Promoting Safe and Effective Genetic Testing in the United States: Final Report of the Task Force on Genetic Testing*. Baltimore, MD: Johns Hopkins University Press; 1998
13. American Society of Human Genetics Board of Directors, American College of Medical Genetics Board of Directors. Points to consider: ethical, legal and psychosocial implications of genetic testing in children and adolescents. *Am J Hum Genet*. 1995;57:1233–1241

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