PRACTICE PARAMETER:
EVALUATION OF THE CHILD WITH GLOBAL DEVELOPMENTAL DELAY
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This appendix has been prepared to provide some background information regarding the tests described in the recently published practice parameter.

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Appendix A: Information Regarding Selected Tests

1. Metabolic Testing

While no standard has been set for what constitutes routine testing for an inborn error of metabolism, the studies most commonly performed on a screening basis include measures of serum glucose, bicarbonate, lactate, pyruvate, ammonia, creatine kinase, and amino acids and of urine pH, ketones, and organic acids. Measures of serum long chain fatty acids, carnitines, and acylcarnitines, of urine mucopolysaccharides, and of cerebrospinal fluid lactate and amino acids are more often considered to be part of a second tier of tests, performed when initial screening tests are positive or when there are specific clinical suspicions. The tests that are frequently available only on a send-out basis are further described.

Amino Acids

Amino acid profiles can be performed on serum, urine, cerebrospinal fluid, or amniotic fluid and are available at numerous laboratories throughout the world. Concentrations of amino acids are determined by high performance liquid chromatography (HPLC) separation and colorimetric reactions to ninhydryl or other reagents. Most laboratories require 2-3 milliliters of blood, drawn in a heparin containing tube (green top) and separated immediately, 5-10 ml of urine without preservatives, or 1 ml of cerebrospinal fluid without preservatives. Samples should be maintained at a temperature of -20°C and transported to the laboratory on dry ice by overnight carrier. Results, including individual amino acid concentrations and an interpretation of the profile, are usually available within 2-5 days.

Organic Acids

Screening tests for organic acids in the urine are widely available. Uncharged organic acids are separated by their solubility in organic solvents and then measured by combination gas chromatography and mass spectrometry. A random sample of 5-20 ml of urine, without preservatives, should be frozen at -20°C and shipped on dry ice. Results are usually available within 2-3 days.

Acylcarnitines

A serum profile of free carnitine and acylcarnitines, evaluating disorders of fatty acid oxidation, is widely available and most often performed using tandem mass spectrometry. Blood spot cards at room temperature are sufficient and results are usually available in 3-5 days.

Mucopolysaccharides

Urine mucopolysaccharide screening tests look for greatly elevated levels of glycosaminoglycans excreted by patients with mucopolysaccharidoses. The tests are widely available and most often use dyes which undergo metachromasia in the presence of these substances. A 5-10 ml random sample of urine is required, however a first void of the day sample is preferred due its greater concentration. Results are usually available within 2-5 days.

2. Genetic Testing

Routine karyotype analysis is available from most genetic laboratories. Other more specific genetic tests may be available on a clinical basis from only a limited number of laboratories. An excellent source of information regarding common genetic syndromes, genetic test availability, and individual laboratory sample requirements, testing procedures, and contact information is available via the internet at the publicly funded website www.genetests.org

Routine cytogenetic testing to detect aneuploidy, large deletions, and large rearrangements is widely available and can be performed on blood or tissue samples. Most laboratories offer high resolution analysis of blood specimens, which examines DNA banding patterns within cells undergoing the prometaphase stage of mitosis. High resolution chromosome analysis requires 1-5 ml of blood in a tube with heparin maintained at room temperature, and provides results within 10 days.

Fragile X

Fragile X syndrome is associated with large expansions of the number of CGG triplet repeats within the FMR-1 gene on the X chromosome. Testing is widely available and uses a combination of polymerase chain reaction (PCR) and Southern analysis. Quantification of the number of CGG repeats identifies large expansions, greater than 200, which are associated with developmental delay as well as smaller expansions, from 44 to 199, as are seen in carriers. Most laboratories require 2-6 ml of blood in a tube with EDTA (purple-top), which can be stored and sent at room temperature, and provide results within 2-3 weeks.

Rett Syndrome

Testing for mutations in the MECP2 gene on chromosome Xq28, found in approximately 80% of patients with clinically diagnosed Rett syndrome, is currently available on a clinical basis from 11 labs in the world, 6 of which are in the United States. Testing consists of initial screening using PCR amplification of three exons contained in the *MECP2* gene coding region, followed by denaturing high performance liquid chromatography (DHPLC) analysis. Positive and negative samples then undergo automated DNA sequencing. These methods have a sensitivity of 99%. A blood sample

of 2-6 ml in a tube with EDTA (purple-top) should be stored and sent at room temperature. Results are available within 4-6 weeks.

Subtelomeric Chromosomal Analysis

Subtelomeric chromosomal rearrangements and submicroscopic deletions can be detected by fluorescence *in situ* hybridization (FISH) with telomere region-specific probes, available on a clinical basis in many laboratoreis. Testing generally requires 1-5 ml of blood in a tube with heparin (green-top), stored and sent at room temperature, and provides results in about 10 days.

Prader-Willi and Angelman Syndromes

Prader-Willi syndrome (PWS) is associated with characteristic facial dysmorphisms, hypogonadism, developmental delay, feeding problems and hypotonia in infancy followed by hyperphagia and obesity in childhood. The genetic basis is the absence of the paternally derived portions of chromosome 15q11-q13. In 70-75% of cases paternal chromosome contains a deletion within this region. Another 20-25% are cases of maternal uniparental disomy (UPD) and have received two copies of chromosome 15 from their mother and none from their father. Approximately 2% of cases have an abnormality in the imprinting process which causes nonexpression of paternal genes in the PWS critical region..

Angelman syndrome (AS) has features of characteristic facial dysmorphisms, developmental delay, speech impairment, ataxia or tremor, and frequent laughter or smiling. Other frequently associated findings are acquired microcephaly and seizures. The genetic basis for AS is similar to that of PWS, involving the loss of the maternal contribution of chromosome 15q11-q13. Approximately 70% of cases are the result of a maternally derived deletion, 3-5% of cases have paternal UPD, with two copies of chromosome 15 from their father and none from their mother, and 7-9% of cases have an abnormality in the imprinting process which causes nonexpression of maternal genes in the AS critical region. These genetic causes would all exhibit abnormal DNA methylation patterns. Another 5% of AS patients have point mutations of the *UBE3A* gene, found within the 15q11-q13 region, while the genetic basis of 10-14% of AS patients remains undetermined.

Southern blot analysis to detect abnormal methylation patterns within the PWS/AS critical region will identify patients with either chromosomal deletions or with UPD. This will detect nearly all patients with PWS but has a sensitivity of only 80% for patients with AS. Methylation testing requires 2-6 ml of blood in a tube with EDTA (purple-top) and 2-3 weeks for results. FISH analysis for PWS or AS, which can demonstrate the presence of small deletions, insertions, and duplications, requires 1-5 ml of blood in a tube with heparin (green-top) and 10 days for results. These testing methods are widely available.

Analysis of the UBE3A gene, via PCR amplification and automated sequencing, is available from a more limited number of laboratories on a clinical basis for patients with documented negative methylation testing. This requires 6 ml of blood in a tube with EDTA (purple-top) and 4 weeks for results.

Selected Internet Resources		
Site Name	**Internet Address**	**Features**
General		
Child Neurology Foundation	www.childneurologyfoundation.org	Provides news and links to resources for families and clinicians
Child Neurology Society	www.childneurologysociety.org	A resource for professionals treating children with neurologic disorders
Family Village	www.familyvillage.wisc.edu	Disease specific listings of organizations, support groups, web pages, and chat rooms. General information regarding disability and adaptive equipment
Gene-Tests	www.genetests.org	Searchable catalog of genetic disorders with summary descriptions, reviews of the appropriate genetic tests, and directories of genetic labs and prenatal testing clinics
OMIM	http://www.ncbi.nlm.nih.gov/Omim/	Catalog of genetic disorders searchable by clinical features with links to academic publications
Fragile X Syndrome		
National Fragile X Foundation	www.nfxf.org	Provides information for families and physicians regarding the features, diagnosis, and treatment of Fragile X Syndrome
ACMG	www.acmg.net	Full text of the 1994 policy statement from the ACMG on diagnostic and carrier testing in Fragile X Syndrome
Rett Syndrome		
International Rett Syndrome Association	www.rettsyndrome.org	Current and detailed information on the clinical features, genetics, diagnostic testing, and treatment of Rett Syndrome
Prader-Willi and Angelman syndromes		
Prader-Willi Syndrome Association	www.pwsausa.org	Describes the clinical features, genetics, testing, and treatment of Prader-Willi Syndrome in English and Spanish
Angelman Syndrome Foundation	www.angelman.org	Provides families and clinicians with resources regarding the clinical and genetic diagnosis of Angelman Syndrome Includes the 1996 ACMG report on diagnostic testing for both Prader-Willi and Angelman syndromes

OMIM = Online Mendelian Inheritance in Man, ACMG = American College of Medical Genetics

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