

Newborn Screening Fact Sheets

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Abbreviations: OMIM, Online Mendelian Inheritance in Man; MS/MS, tandem mass spectrometry; CoA, coenzyme A; *BTD*, biotinidase gene; CAH, congenital adrenal hyperplasia; 21-OH, 21-hydroxylase; SW, salt wasting; SV, simple virilizing; AG, ambiguous genitalia; ACTH, adrenocorticotropic hormone; 17-OHP, 17-OH-progesterone; AABR, automated auditory brainstem response; OAE, otoacoustic emission; CH, congenital hypothyroidism; T_4 , thyroxine; HPT, hypothalamic-pituitary-thyroid; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; IRT, immunoreactive trypsinogen; GALT, galactose 1-phosphate uridylyltransferase; GALK, galactokinase; GALE, galactose-4'-epimerase; CBS, cystathionine β -synthase; BIA, bacterial inhibition assay; MSUD, maple syrup urine disease; BCKD, branched-chain α -keto acid dehydrogenase; BCAA, branched-chain amino acid; BCKA, branched-chain α -keto acid; E3, dihydrolipoyl dehydrogenase; E1, thiamine pyrophosphate-dependent decarboxylase; E2, transacylase; MCAD, medium-chain acyl-coenzyme A dehydrogenase; FAO, fatty acid oxidation; SIDS, sudden infant death syndrome; ADHD, attention-deficit/hyperactivity disorder; PKU, phenylketonuria; PAH, phenylalanine hydroxylase; BH4, tetrahydrobiopterin; SCD, sickle cell disease; HPLC, high-performance liquid chromatography; Hb, hemoglobin; HbF, fetal hemoglobin; HbA, normal adult hemoglobin; FA, fetal and adult hemoglobin; MCV, mean corpuscular volume; FAH, fumarylacetoacetate hydrolase; TAT, tyrosine aminotransferase; NTBC, 2-(2-nitro-4-trifluoromethylbenzyl)-1,3-cyclohexanedione

ABSTRACT

Newborn screening fact sheets were last revised in 1996 by the American Academy of Pediatrics Committee on Genetics. This revision was prompted by advances in the field since 1996, including technologic innovations, as well as greater appreciation of ethical issues such as those surrounding informed consent. The following disorders are discussed in this revision of the newborn screening fact sheets: biotinidase deficiency, congenital adrenal hyperplasia, congenital hearing loss, congenital hypothyroidism, cystic fibrosis, galactosemia, homocystinuria, maple syrup urine disease, medium-chain acyl-coenzyme A dehydrogenase deficiency, phenylketonuria, sickle cell disease and other hemoglobinopathies, and tyrosinemia. A series of topics related to newborn screening is discussed in a companion publication to this electronic publication of the fact sheets (available at: www.pediatrics.org/cgi/content/full/118/3/1304). These topics are newborn screening as a public health system; factors contributing to the need for review of the newborn screening system; informed consent; tandem mass spectrometry; DNA analysis in newborn screening; status of newborn screening in the United States; and the effect of sample timing, preterm birth, diet, transfusion, and total parental nutrition on newborn screening results.

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www.pediatrics.org/cgi/doi/10.1542/peds.2006-1783

doi:10.1542/peds.2006-1783

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

Key Words

newborn screening, screening, genetic disorder, biotinidase deficiency, congenital adrenal hyperplasia, congenital hearing loss, congenital hypothyroidism, cystic fibrosis, galactosemia, hemoglobinopathies, homocystinuria, maple syrup urine disease, medium-chain acyl-CoA dehydrogenase deficiency, phenylketonuria, sickle cell disease, tyrosinemia, tandem mass spectrometry
PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275). Copyright © 2006 by the American Academy of Pediatrics

zyme A dehydrogenase (MCAD) deficiency, phenylketonuria (PKU), sickle cell disease (SCD) and other hemoglobinopathies, and tyrosinemia. A series of topics related to newborn screening is discussed in a companion publication to this electronic publication of the fact sheets (available at: www.pediatrics.org/cgi/content/full/118/3/1304). These topics are newborn screening as a public health system; factors contributing to the need for review of the newborn screening system; informed consent; tandem mass spectrometry (MS/MS); DNA analysis in newborn screening; status of newborn screening in the United States; effect of sample timing, preterm birth, diet, transfusion, and total parenteral nutrition on newborn screening results.

BIOTINIDASE DEFICIENCY

Biotinidase deficiency (Online Mendelian Inheritance in Man [OMIM] database No. 253260)¹ is a disorder of biotin recycling. Biotin is a water-soluble vitamin of the B complex that acts as a coenzyme in each of 4 carboxylases in humans (pyruvate carboxylase, propionyl-coenzyme A [CoA] carboxylase, β -methylcrotonyl CoA carboxylase, and acetyl-CoA carboxylase).² Missing a diagnosis of biotinidase deficiency, a condition that is easily treated with vitamin supplementation, can have severe consequences, including seizures, developmental delay, and sensorineural deafness.

Incidence

Neonatal screening for biotinidase deficiency has been instituted in many states (25 at the time of this publication) as well as many countries (approximately 25) since the biochemical basis was elucidated by Wolf et al³ in 1983. Of slightly more than 8.5 million newborn infants screened worldwide up to 1990, 142 affected infants have been identified, with 76 having profound (<10% activity) deficiency (approximate incidence 1 in 112 000) and 66 having partial (10%–30% activity) deficiency (approximate incidence 1 in 129 000).⁴ Most affected individuals who have been identified are of European descent; however, individuals of Turkish, Saudi Arabian, and Japanese descent have been described.⁵

Clinical Manifestations

Biotinidase deficiency can present with clinical symptoms as early as the first week of life up to 10 years of age. Most infants first exhibit clinical symptoms between 3 and 6 months of age.² The most commonly affected systems are the central nervous system and skin. Affected children usually have myoclonic seizures, hypotonia, seborrheic or atopic dermatitis, partial or complete alopecia, and conjunctivitis.² Other features may include developmental delay, sensorineural hearing loss, lethargy, ataxia, breathing problems, hepatosplenomegaly, and coma.^{6,7} Laboratory findings vary and can include

ketolactic acidosis, organic aciduria, and mild hyperammonemia.²

Individuals with partial biotinidase deficiency can present with skin manifestations and no neurologic symptoms.⁸ Several children with profound deficiency have presented later in childhood or during adolescence with hemiparesis and eye findings (scotoma).^{9,10} With therapy, the eye problems resolved quickly, but the neurologic findings remained for a longer period of time.¹¹ There are even reports of adults with profound biotinidase deficiency who have never had symptoms but were diagnosed because their children had positive results of newborn screening.²

Pathophysiology

Each of the 4 carboxylases in humans requires biotin as a cofactor. The carboxylases are first synthesized as inactive apoenzymes. After synthesis, biotin is added to the inactive proteins through 2 partial reactions, each of which is catalyzed by the enzyme holocarboxylase synthetase. Ultimately, each of these active, biotin-containing enzymes is degraded. The biotin-containing products of degradation are acted on by biotinidase to liberate biotin, which is recycled and enters the free-biotin pool. Biotinidase deficiency results in inability to recycle endogenous biotin and to release dietary protein-bound biotin. Thus, the brain may be unable to recycle biotin adequately. This may lead to dependence on the biotin that crosses the blood-brain barrier, resulting in decreased pyruvate carboxylase activity in the brain and accumulation of lactate. The neurologic symptoms may be secondary to accumulation of lactic acid in the brain.²

Inheritance

Biotinidase deficiency is inherited as an autosomal recessive trait. The biotinidase (*BTD*) gene has been mapped (chromosome 3p25), cloned, and characterized.^{12–14} Sixty-two mutations of the *BTD* gene have been described to date.¹⁴ Interestingly, when testing a US population, mutations occur at different frequencies in children with symptoms than in children who were only identified through newborn screening. Two mutations accounted for 52% of the mutations found in symptomatic patients, and 3 other mutations accounted for 52% of mutations in children identified through newborn screening. Partial *BTD* deficiency is predominantly caused by the 1330G→C mutation on one allele in combination with one of the mutations causing profound deficiency on the other allele.¹⁴

Benefits of Newborn Screening

Biotinidase deficiency has been identified as an appropriate disorder for newborn screening by numerous countries and states because of its prevalence, the potentially tragic outcome if not diagnosed, and availability of effective, low-cost treatment. Unfortunately, once

symptoms have occurred, some of the findings are not reversible with therapy. This is particularly true in the case of the neurologic findings. For example, sensorineural hearing loss is common (detected in approximately 75% of symptomatic children with profound deficiency) and is usually irreversible.⁶

Screening

The best method of screening is a semiquantitative colorimetric assessment of biotinidase activity that can be performed on whole blood spotted on filter paper.^{2,15,16} Although the majority (>80%) of patients with biotinidase deficiency demonstrate organic aciduria when symptomatic, a significant percentage (20% in one study) may not; therefore, tandem mass spectrometry (MS/MS) testing should not be used for newborn screening of biotinidase deficiency.²

Follow-up and Diagnostic Testing

A positive screening result for biotinidase deficiency should be followed up with definitive testing for diagnosis. Quantitative measurement of enzyme activity should be performed on a fresh serum sample. Residual enzyme activity determines whether the patient has profound (<10% activity) or partial (10%–30% activity) biotinidase deficiency. Most patients with profound deficiency present early in life, whereas those with partial deficiency can present later or with a cutaneous phenotype and no neurologic findings.

Brief Overview of Disease Management

Children with profound biotinidase deficiency have been treated successfully with biotin. Pharmacologic doses of biotin (5–20 mg/day) were determined empirically.^{8,17} One patient required a dose of 30 mg/day to resolve dermatitis.¹⁸ For most patients, the currently prescribed dose is probably much more than is needed to overcome the deficiency. It should be stressed that the biotin must be in the free, not bound, form to be effective. There are no known adverse effects of the currently recommended dosage of 5 to 20 mg/day.¹⁹

Once therapy is instituted, cutaneous symptoms resolve quickly, as do seizures and ataxia. Some of the symptoms (as mentioned previously) are less reversible, including hearing loss and optic atrophy. Children who have developmental delay have been noted in some cases to achieve new milestones and regain lost milestones after beginning therapy.¹⁹ There are individuals reported who have profound biotinidase deficiency, have never been treated, and have never had any associated symptoms.¹¹

Partial biotinidase deficiency can probably be treated with lower doses of biotin (1–5 mg/day) and/or only during times of metabolic stress.¹⁹ There are children with partial deficiency who have never had any related illness. In others with partial deficiency, it has been

noted that mild intercurrent illnesses such as gastroenteritis can lead to development of typical clinical symptoms that resolve with biotin therapy.¹⁹

Current Controversies

As noted above, it is difficult to determine if individuals with partial biotinidase deficiency need daily therapy. When such individuals are identified in newborn screening programs, follow-up happens routinely and care is instituted. The negative psychological aspects of learning through newborn screening that an infant potentially has a genetic disorder and the parental anxiety generated should be weighed against the positive aspects, including that the treatment is simple and inexpensive and some individuals with partial deficiency would (at some point) have symptoms. Although this is mildly controversial, it is truly not of enough significance to negate the value of newborn screening for the disorder.

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CONGENITAL ADRENAL HYPERPLASIA

Congenital adrenal hyperplasia (CAH) is a family of inherited disorders of the adrenal cortex that impair steroidogenic enzyme activity essential for cortisol biosynthesis.^{20,21} Newborn screening focuses exclusively on the most common 21-hydroxylase (21-OH) deficiency CAH (>90% of all CAH cases [OMIM database No. 201910]),²² which impairs production of cortisol and often aldosterone.^{20,21} Prompt diagnosis and treatment of CAH is essential to prevent potential mortality as well as physical and emotional morbidity.^{20–23}

Incidence

Health organizations in 13 countries (including 36 US states) screen or will screen for CAH in their newborn screening programs. On the basis of newborn screening data, the incidence of CAH ranges from a low of 1 in 21 270 (New Zealand) to a high of 1 in 5000 (Saudi Arabia) live births.²⁴ The incidence is 1 in 15 981 live births (Hispanic > American Indian > white > black > Asian) in North America, 1 in 14 970 live births in Europe, and 1 in 19 111 live births in Japan.²⁵ An exceedingly high CAH incidence (1 in 282 live births) exists among Yupik Eskimos in western Alaska.²⁶

Clinical Manifestation and Variability

The spectrum of disease in CAH ranges from the “classic, severe” salt-wasting (SW) form, to “classic, less severe” simple-virilizing (SV), to “mild, nonclassic” forms.^{20,21}

Symptomatic Presentation and Morbidity

Neonates with the SW form exhibit adrenal crisis during the first through fourth weeks of life, peaking at approximately 3 weeks of age. This manifests as poor feeding, vomiting, loose stools or diarrhea, weak cry, failure to thrive, dehydration, and lethargy. These symptoms may not be evident until serum sodium concentrations are below 125 mEq/L. If untreated, circulatory collapse, shock, and death are inevitable. Permanent brain injury attributable to shock, lower cognitive scores, and learning disabilities are observed in some with the SW form.²⁰ Affected females have ambiguous genitalia (AG) (but

normal internal reproductive anatomy), prompting a clinical diagnosis in many. Affected males have no obvious physical signs of CAH. Therefore, without newborn screening and in the absence of a positive family history, all male and a minority of female neonates are undiagnosed until adrenal crisis. The SW form affects approximately 70% of patients with CAH that is diagnosed through newborn screening programs.^{25,26} If inadequately treated, postnatal virilization (girls), pseudo- or true-precocious puberty (boys), and premature growth acceleration (boys and girls) occur, leading to early growth cessation.^{20–23} Patients with the SV form do not manifest adrenal-insufficiency symptoms unless subjected to severe stress but exhibit virilization as in patients with SW.^{20,21} Males and some females with the SV form are not diagnosed until much later when symptoms of virilization, precocious pseudopuberty, or growth acceleration occur.^{20–23} The markedly advanced skeletal age of patients with the SV form diagnosed late contributes to their short adult stature. Late discovery of incorrect male sex assignment in females with the SW and SV forms causes extreme distress to the family and matured patients. Mild 21-OH deficiency produces no symptoms at birth and manifests as premature sexual hair, acne, and mild growth acceleration in childhood and hirsutism, excessive acne, menstrual disorder, and infertility later in life.^{20,21} This milder disorder may be missed by newborn screening programs.

Mortality

The mortality rate for infants with the SW form not detected through newborn screening was 11.9%, which was fivefold higher than that of the general population (2.29%).²³

Pathophysiology

21-OH deficiency results in cortisol deficiency with or without aldosterone deficiency. Cortisol deficiency from early fetal life leads to increased adrenocorticotropic hormone (ACTH) secretion,^{20,21} which then stimulates excess secretion of the precursor steroids including 17-OH-progesterone (17-OHP) and causes hyperplastic changes of the adrenal cortex.^{20,21} The precursor steroids can only be metabolized by way of the androgen biosynthetic pathway, resulting in excess androgen production that virilizes the genitalia.^{20,21} Aldosterone deficiency contributes to SW. The increased circulating 17-OHP concentration is diagnostic for 21-OH deficiency.

Inheritance and Genotype

21-OH deficiency is an autosomal recessive disorder caused by a mutation of the *CYP21* gene.^{20,21} There is an active *CYP21* gene and an inactive pseudo-*CYP21P* gene in normal individuals. Both genes are in the HLA complex on chromosome 6p21.3.^{20,21} Most mutations in the *CYP21* gene are the pseudogene sequences, suggesting

that the mutations in *CYP21* were caused by a gene conversion or recombination between *CYP21* and *CYP21P*. The genotypes from 5 different populations of individuals with CAH correlated well with the phenotype in approximately 90% of affected subjects but did not correlate well in the remaining patients.²¹

Rationale for and Benefits of Newborn Screening

The goals of newborn screening are to (1) prevent life-threatening adrenal crisis, thereby averting shock, brain damage, and death, (2) prevent male sex assignment for life in virilized female newborns, and (3) prevent progressive effects of excess adrenal androgens, which cause short stature and psychosexual disturbances in boys and girls. Kovacs et al²³ found the average serum sodium concentration at diagnosis of the SW form of CAH to be 135 mEq/L in individuals detected through newborn screening programs and 125 mEq/L in those detected after development of clinical symptoms. Thus, prevention of severe SW CAH by newborn screening was demonstrated. Worldwide newborn screening data showed that screening prompted early diagnosis of CAH before clinical suspicion in 67% of newborn infants with CAH, including many females with AG.²⁶ The mortality rate of individuals with CAH identified through newborn screening has not been established yet. Other newborn screening benefits include (1) improved case detection evidenced by twofold higher incidence versus that of case-survey reports (North America and Japan), (2) improved detection of patients with SW CAH (70% with newborn screening vs 43%–60% in patients with clinical symptoms), and (3) improved detection of males, as evidenced by a 1:1 sex ratio in subjects identified through newborn screening versus a male/female ratio of 0.6:1 in patients with clinical symptoms leading to diagnosis.

Screening

Screening for 21-OH deficiency is accomplished by measurement of 17-OHP concentration in the dried blood spot. Newborn screening for CAH requires a rapid process to prompt the diagnosis before the onset of SW symptoms. Sampling at less than 1 day is associated with a high rate of false-positive results, and sampling beyond 5 to 7 days of age reduces the benefit of screening. Normal preterm infants have higher concentrations of 17-OHP than do term infants; therefore, it is important to have 17-OHP reference concentrations in blood spots of preterm and term unaffected infants according to birth weight or gestational age.^{27,28} 17-OHP is not influenced if drawn several hours after transfusion.

Dissociation-enhanced lanthanide fluorescence immunoassay, radioimmunoassay, and enzyme-linked immunosorbent assay with a commercial kit are used to measure 17-OHP concentrations in blood spots.^{25,26} The screening 17-OHP assays are nonspecific, and the result

on a screening study is not equivalent to the diagnostic serum concentrations.^{21,26,29} Affected neonates had screening 17-OHP concentrations of 35 to 900 ng/mL of blood, with preterm infants having higher concentrations.^{27,29}

MS/MS may have the advantage of rapid 17-OHP detection and may eliminate the variable 17-OHP cutoff concentrations influenced by different reagents/assays. However, comparative studies of immunoassays versus MS/MS are necessary, and because of the complexity of the MS/MS assay for 17-OHP detection, MS/MS may be used as a complementary test. *CYP21* genotyping is not currently used in newborn screening, but it may be helpful in uncertain cases and for genetic counseling.

Almost all neonates with SW CAH have been identified with the first sample test.²⁶ Newborn screening for CAH is not intended to detect mild cases, although some are detected. In a study performed in Texas, testing again at 1 to 2 weeks increased detection of SV CAH and the mild form.²⁹ Despite the birth weight- or age-adjusted 17-OHP cutoff concentrations, preterm birth or low birth weight and samples taken at less than 1 day of age are major factors for false-positive results.^{24–30} In an international study, 7% of neonates later determined to have CAH (mostly the SV form) were not detected in newborn screening for a variety of reasons (human error, prenatal dexamethasone therapy, or high 17-OHP cutoff concentrations).²⁵

Follow-up and Diagnostic Testing

In most newborn screening programs, 2-tiered 17-OHP cutoff concentrations are established to guide evaluation in term and preterm newborn infants. Exceptionally high (urgent) and moderately high (suspected) 17-OHP concentrations are reported. Pediatricians need to be familiar with these concentrations as reported by their local newborn screening program. Most newborn screening programs that screen for CAH report the presumed positive results with instructions. Immediate evaluation (serum electrolytes, 17-OHP) is necessary in newborn infants with AG, in sick or asymptomatic male newborn infants with urgent or suspected 17-OHP concentrations, and in sick female infants with urgent 17-OHP concentrations. The evaluation is necessary in asymptomatic normal female infants with urgent 17-OHP concentrations and in sick female infants with normal genitalia and suspected 17-OHP concentrations, but these newborns are at low risk of having SW CAH. Normal females with suspected 17-OHP concentrations are not at risk of SW CAH but need at least a second screening to be sure that a mild deficiency is not missed.

Diagnosis

Quantitative serum 17-OHP concentration is used for the diagnosis of CAH. Concentrations are generally higher in individuals with the SW form.²⁹ Care must be

taken to use the appropriate term or preterm normal values for comparison.²⁶ With age, serum 17-OHP concentrations decrease in unaffected neonates but increase in those with CAH.³⁰ Concentrations in neonates with SW and SV CAH are higher than the concentrations in infants with the mild form.^{21,29} In neonates with mildly elevated 17-OHP concentrations (4–10 ng/mL), the ACTH-stimulation test helps to rule out nonclassic CAH.^{20,21} In asymptomatic infants, serial evaluation of electrolytes throughout the neonatal period is necessary if serum electrolyte concentrations remain normal.

Brief Overview of Disease Management

Treatment for CAH involves replacement of cortisol, which suppresses increased ACTH, 17-OHP, and androgen secretion. Replacement of aldosterone with an analog of mineralocorticoid (Florinef) is required for patients with SW CAH. Adequate medical therapy restores normal energy, glucose and electrolyte concentrations, and fluid balance and prevents excess adrenal androgen effects. Special medical care is needed in case of stress. The rate of mortality is 4.3% for treated patients.²³ In virilized female infants, surgical correction is generally performed before 1 year of age and, if necessary, again before menarche. With standard glucocorticoid therapy, adults with classic CAH do not always reach their genetic potential for height, and obesity is common. Inadequate medical therapy causes infertility. Experimental antiandrogenic/antiestrogenic drug therapy to improve height outcome is ongoing in children with CAH. Adrenalectomy is recommended when medical therapy is ineffective.

Carrier testing for CAH is performed most accurately using *CYP21* genotyping.

Pregnant women known to be at risk of having a fetus with CAH can receive prenatal dexamethasone therapy. First-trimester prenatal diagnosis is indicated for these women. An elevated 17-OHP concentration in amniotic fluid by a specific assay (>6–18 ng/mL) is also diagnostic, but normal concentrations do not exclude SV or nonclassic forms of CAH, and concentrations may be normal in mothers who are on dexamethasone therapy. Prenatal treatment is only indicated for female fetuses with classic virilizing CAH. Maternal dexamethasone therapy at 20 µg/kg per day beginning at 5 to 8 weeks' fetal age prevents or reduces AG in most affected females.³¹ Controversy regarding prenatal therapy is related to the fact that (1) this treatment must begin before fetal sex can be determined or CAH diagnosis can be made, and 7 of 8 fetuses are thus unnecessarily subjected to this therapy, and (2) long-term safety of early exposure to dexamethasone in utero is unproven to date.³¹ Maternal adverse effects include cushingoid features of excessive weight gain, intense striae, edema, discomfort, and emotional instability. In a consensus meeting concerning prenatal CAH therapy, representatives from the

US Lawson Wilkins Pediatric Endocrine Society and European Pediatric Endocrine Society recommended that designated teams undertake this specialized therapy using a national protocol approved by institutional review boards. Treatment is preceded by informed consent about the risks and benefits of the therapy, and prospective follow-up and evaluation are needed.³¹

Current Controversy

The major controversy regarding newborn screening for CAH is the cost and impact of evaluating those whose test results are false-positive.³² A second issue is the use of prenatal dexamethasone therapy for CAH. A large national multicenter study on long-term cognitive and psychological development and other health-related outcomes is required to resolve this issue.

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CONGENITAL HEARING LOSS

Congenital hearing loss, for the purposes of this fact sheet, is defined as permanent and is bilateral or unilateral, is sensory or conductive, and averages 30 dB or more in the frequency region important for speech recognition. Congenital hearing loss has many etiologies, with at least half associated with genetic risk factors. Congenital nonsyndromic hearing loss is usually categorized by mode of inheritance—autosomal recessive, autosomal dominant, X-linked, or mitochondrial.^{33–35}

Newborn hearing screening programs became possible after the development of hearing screening technologies. Although most states have begun screening for congenital hearing loss, the integration of these programs with ongoing screening and early intervention programs remains a challenge.³⁶

Prevalence

Estimates of the prevalence of moderate-to-profound bilateral hearing loss vary, depending on the criteria used to define the different degrees of hearing loss and the characteristics of the studied population.³⁷ The prevalence of congenital hearing loss also depends on race, birth weight, and other risk factors.³⁸ Profound and permanent congenital hearing loss is estimated to occur in approximately 1 in 1000 births.^{39,40}

Clinical Manifestations

The spectrum of congenital hearing loss ranges from mild to profound hearing loss. In syndromic hearing loss, the auditory pathology may be conductive and/or sensorineural, unilateral or bilateral, symmetrical or asymmetrical, and progressive or stable. The auditory pathology of nonsyndromic hearing impairment is usually sensorineural.^{41,42}

Pathophysiology

Approximately half of the cases of congenital hearing loss are thought to be attributable to environmental factors (acoustic trauma, ototoxic drug exposure [aminoglycosides], bacterial or viral infections such as rubella or cytomegalovirus).^{39,41,42} The remaining cases are attributable to genetic mutations. Although these cases may seem to be part of a recognizable syndrome, approximately 70% are nonsyndromic (the deafness is not associated with other clinical findings that define a recognized syndrome) and, therefore, clinically undetectable at birth. In the remaining 30%, 1 of more than 400 forms of syndromic deafness can be diagnosed because of associated clinical findings.^{39,42}

Inheritance

Approximately 77% of congenital nonsyndromic hearing impairment is autosomal recessive, 22% is auto-

mal dominant, and 1% is X-linked. As a general rule, individuals with autosomal recessive congenital nonsyndromic hearing impairment have profound prelingual deafness, and dominant mutations lead to a more variable phenotype. More than 90% of children with congenital profound autosomal recessive congenital nonsyndromic hearing impairment are born to parents with normal hearing, and the remaining 10% or less are born to deaf parents.⁴¹

There has been significant progress in identifying and sequencing autosomal dominant, autosomal recessive, and sex-linked genes for deafness.^{41,43} However, it is clear that more genes and mutations await discovery. This knowledge may lead to mutation-specific therapies that can delay or prevent certain forms of genetic deafness, such as the avoidance of aminoglycoside therapy in those with specific mitochondrial mutations.

Benefits of Newborn Screening

The goals of newborn screening are to identify those infants with hearing loss early for prompt intervention to diminish the morbidity associated with congenital hearing loss. Left undetected and untreated, hearing impairment can affect speech and many other cognitive abilities. For children without risk factors, hearing loss frequently escapes detection until the age when hearing children normally begin to talk (9 months or older).^{44–48} Current theory views auditory stimulation during the first 6 months of life as critical to development of speech and language skills. Children who are identified early as having hearing loss and receive intensive early intervention perform better on school-related measures (reading, arithmetic, vocabulary, articulation, percent of the child's communication understood by non-family members, social adjustment, and behavior) than children who do not receive such intervention.⁴⁹ Early intervention resulted in improvements in receptive language⁵⁰ and prevented developmental delays.⁵¹ However, the efficacy of universal newborn hearing screening to improve long-term language outcomes remains uncertain.^{52–54}

Screening

Newborn hearing screening is accomplished through the use of a variety of computerized equipment that uses automated auditory brainstem response (AABR), distortion product otoacoustic emissions (OAEs), or transient evoked OAEs. Screening is performed before discharge from the nursery.⁵⁵ Screening for congenital hearing loss is a simple process and in some cases may be performed by specially trained volunteers under the supervision of nurses or audiologists. Screening with AABR is accomplished by placement of soft earphones through which a series of soft clicks are introduced, usually at the 30- to 40-dB level. An auditory brainstem response detected through electrodes attached to the infant's forehead and

neck indicates that there is no significant sensorineural hearing loss. If OAE technology is selected as the screening test, a tiny microphone that detects sounds generated by the outer hair cells of the cochlea is introduced into the infant's auditory canal. Presence of those sounds indicates a functioning inner, middle, and outer ear. Each of these tests has advantages and disadvantages that should be considered carefully when selecting equipment. AABR tends to be somewhat more expensive and must be used in a quiet setting. OAE screening may result in higher false-positive rates if the infant's ear canal is blocked by fluid or debris.^{56,57} Some hospitals use a combination of screening tests or repeat the OAE screening to reduce the false-positive rate and thereby minimize the need for follow-up after hospital discharge, which may reduce costs overall.⁵⁸

Follow-up and Diagnostic Testing

Infants who do not "pass" the screening are either rescreened before discharge or given an appointment for rescreening as outpatients. Results of the screening are generally transmitted to the primary care physician of record, to the parents, and to the state health department. Failure to pass the screening results in a recommendation for referral to a qualified audiologist for confirmatory testing for congenital hearing loss.

In areas where universal newborn hearing screening is occurring, appropriate and timely diagnosis and intervention continue to be a major challenge. Attrition rates as high as 50% between initial referral and diagnostic confirmation still are not unusual.³⁶ Linkages between hospital-based screening programs and early intervention programs may not be well established, and data management and tracking of infants through the screening and diagnostic process also may be in the developmental stage.⁴⁹ As state programs assume more responsibility for the tracking and follow-up, these linkages will be more firmly established.³⁶

Brief Overview of Disease Management

Appropriate management of all persons identified with congenital hearing loss requires a comprehensive pediatric and genetic evaluation.³³ Core personnel include individuals with expertise in the genetics of hearing loss, dysmorphology, audiology, otolaryngology, and genetic counseling. Qualified interpreting services may be needed when the parents are deaf. On the basis of the outcome of the evaluation, other types of professional expertise also may be needed, including professionals with experience with syndromal hearing loss (eg, ophthalmology, cardiology, nephrology, neurology).

After a family history, patient history, and physical examination, it may be possible to ascribe an etiology to the hearing loss. However, in approximately 30% of patients, there will be no obvious etiology.³³ An important goal of the genetic evaluation is to attempt to dis-

tinguish isolated or simplex cases, in which the risk of deafness in subsequent offspring may be 25%, from sporadic cases, which have a low risk of recurrence.³³

After diagnosis of hearing loss, continuity of care for the affected infant is important to reduce morbidity. The pediatrician should ensure referral to the state early intervention program and/or the state program for children with special health care needs as appropriate. Referral to these programs at hospital discharge helps to minimize loss to follow-up.

Current Controversy

The US Preventive Services Task Force did not find evidence for the benefit of (nor evidence against the benefit of) universal newborn hearing screening.⁵³ They argued that, among low-risk infants, the prevalence of hearing impairment was very low, and substantial numbers of infants would be misclassified. They found that evidence for the efficacy of early intervention for patients diagnosed by screening was incomplete.

Additional controversy centers on the generally inadequate integration of these programs with ongoing newborn screening and early intervention programs.³⁶ The Newborn Screening Task Force suggested that child health-related programs such as newborn genetic and hearing screening programs would avoid unnecessary duplication of effort if they were more closely aligned with each other.⁵⁹

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CONGENITAL HYPOTHYROIDISM

Thyroid hormone deficiency at birth is one of the most common treatable causes of mental retardation. There are multiple etiologies of this disorder, both heritable and sporadic, varying in severity. There is an inverse relationship between age at diagnosis and neurodevelopmental outcome; the later treatment is started, the lower the IQ will be. Most infants seem to be protected for the first few weeks of life by the fraction of maternal thyroid hormone that crosses to the fetus. Because of the urgency in detection and initiating treatment to prevent mental retardation, screening newborns for this disorder was added to existing programs in the mid-1970s.

Incidence

Congenital hypothyroidism (CH) occurs in 1 in 4000 to 1 in 3000 newborns. Programs reporting a higher incidence may include some transient cases. CH seems to occur more commonly in Hispanic and American Indian/Alaska Native people (1 in 2000 to 1 in 700 newborns) and less commonly in black people (1 in 3200 to 1 in 17 000 newborns). Programs report a consistent 2:1 female/male ratio, which is unexplained but speculated to be related to an autoimmune risk factor. Newborn infants with Down syndrome are at increased risk of having CH (approximately 1 in 140 newborns).

Clinical Manifestations

Most affected infants appear normal at birth, without obvious manifestations of CH. This is likely the result of transplacental passage of some maternal thyroid hormone; cord thyroxine (T_4) concentrations are approximately one third of maternal concentrations. In addition, many infants have some functioning thyroid tissue. Gestational age is 42 weeks or greater in approximately one third of these infants. Their birth weight and length fall into the normal range, and their head circumference may be at a slightly higher percentile because of brain myxedema. Approximately 5% of these infants, generally those who are more severely affected, have recognizable features at birth, including large fontanels and wide suturae, macroglossia, distended abdomen with umbilical hernia, and skin mottling. As maternal thyroid hormone is excreted and disappears in the first few weeks, clinical features gradually become apparent. These infants are slow to feed, constipated, lethargic, and sleep more (“sleep through the night” early), often needing to be awakened to feed. They may have a hoarse cry, may feel cool to touch, may be hypotonic with slow reflexes, and may have prolonged jaundice because of immaturity of hepatic glucuronyl transferase. A goiter is seen in 5% to 10% of these infants, most commonly in those with an inborn error of T_4 synthesis. If hypothyroidism goes undiagnosed beyond 2 to 3 months of age, infants will begin to manifest slow linear growth. If this disorder is untreated, studies show a loss of IQ proportionate to the age at which treatment is started: if treatment is started at 0 to 3 months of age, mean IQ is 89 (range: 64–107); if treatment is started at 3 to 6 months of age, mean IQ is 71 (range: 35–96); if treatment is started at older than 6 months, mean IQ is 54 (range: 25–80). Other long-term neurologic sequelae include ataxia, gross and fine motor incoordination, hypotonia and spasticity, speech disorders, problems with attention span, and strabismus. Approximately 10% of these infants will have an associated sensorineural deafness, and approximately 10% will have other congenital anomalies, most commonly cardiac defects.⁶⁰ Some newborn screening programs also detect secondary or hypopituitary hypothyroidism in infants. These infants may have

associated midline defects, such as the syndrome of septo-optic dysplasia or midline cleft lip and palate. Other pituitary hormones, such as growth hormone, may also be missing.

Pathophysiology

The most common cause is some form of thyroid dysgenesis: aplasia, hypoplasia, or an ectopic gland; thyroid ectopy accounts for two thirds of thyroid dysgenesis. The cause of thyroid dysgenesis is unknown; rare cases result from mutations in the genes that control thyroid gland development, including thyroid transcription factor (TTF-2) and paired box-8 protein (PAX-8). Inborn errors of T_4 synthesis, secretion, or utilization account for two thirds of heritable cases. Errors in iodide trapping, organification of iodide to iodine by thyroid peroxidase (most common inborn error), coupling of monoiodothyronine and diiodothyronine, deiodinase, and an abnormal thyroglobulin molecule all have been described. In mothers with autoimmune thyroiditis, transplacental passage of a thyrotropin-receptor–blocking antibody is associated with transient hypothyroidism. Infants born to mothers with Graves' disease treated with antithyroid drugs also may have transient hypothyroidism. Worldwide, iodine deficiency resulting in endemic cretinism is the most common cause of hypothyroidism at birth. Exposure of the neonate to excess iodine, as with topical antiseptics, can also cause hypothyroidism.

Inheritance

Approximately 85% of cases are sporadic, and 15% are hereditary. Each of the inborn errors of T_4 synthesis is autosomal recessive except thyroid hormone receptor defects, which are autosomal dominant. In the cases associated with transplacental passage of a maternal blocking antibody, future siblings are at risk of having the same problem.

Rationale for and Benefits of Newborn Screening

Most newborn screening programs report no difference in global IQ score compared with sibling or classmate controls, whereas some report a reduction in IQ ranging from 6 to 15 points. Even if there are no differences in global IQ, some show differences in subtest components, such as language or visual-spatial skills. These results are more likely in severely affected infants,⁶¹ those started on too low an initial dose of levothyroxine sodium, or those who are not optimally managed or poorly compliant in the first 2 years of life. However, these differences in IQ nearly disappeared if higher starting doses of levothyroxine, averaging 11.6 $\mu\text{g}/\text{kg}$ per day, were used.⁶² Recent data suggest that a starting dose of 10 to 15 $\mu\text{g}/\text{kg}$ per day normalized serum thyrotropin by 1 month and resulted in a higher IQ as compared with infants started on a lower treatment dose.⁶³

Screening

Most screening programs in the United States measure T_4 initially, with a thyrotropin determination on infants whose T_4 level is less than the 10th percentile for that specific assay. Some US newborn screening programs and more in Canada now are screening with an initial thyrotropin measurement. Because there is a thyrotropin surge after birth that decreases over the next 5 days, infants with screening specimens obtained at less than 48 hours of age may have false-positive thyrotropin increases. Each screening program must establish its own T_4 and thyrotropin cutoff levels. Primary T_4 screening programs may identify infants with delayed thyrotropin increase (usually preterm infants) and secondary hypothyroidism. Primary thyrotropin screening programs identify infants with subclinical hypothyroidism (high thyrotropin, normal T_4). The false-positive rate is generally higher for primary T_4 programs compared with primary thyrotropin programs (0.30% vs 0.05%, respectively). Preterm infants have reduced T_4 concentrations and, thus, make up a disproportionate percentage of infants with false-positive results. Neither screening is affected by diet or transfusion, except total exchange transfusion.

Follow-up and Diagnostic Testing

Infants with abnormal screening results must have confirmatory serum T_4 testing and some measure of thyroid-binding proteins (eg, triiodothyronine [T_3] resin uptake), or a free T_4 level, and thyrotropin determination. Once a diagnosis of hypothyroidism is confirmed, studies may be undertaken to determine the underlying etiology. Most useful are imaging studies, either thyroid ultrasound or thyroid uptake and scan, using either technetium 99m pertechnetate or iodine 123. In general, information gained from these studies does not alter management, so they are considered optional; they should never delay onset of treatment. If there is evidence of maternal autoimmune thyroid disease, measurement of thyrotropin-binding inhibitor immunoglobulin in the mother and infant can identify those with likely transient hypothyroidism. If iodine exposure or deficiency is suspected, measurement of urinary iodine can confirm this etiology.

Brief Overview of Disease Management

Levothyroxine is the treatment of choice; only tablets should be used, because liquid preparations are not stable. The recommended starting dose is 10 to 15 $\mu\text{g}/\text{kg}$ per day^{62,63}; it is important that the initial dose correct hypothyroxinemia as rapidly as possible.^{64–66} Treatment can be started after confirmatory studies are obtained, pending results. Treatment goals are to keep the serum T_4 or free T_4 in the upper half of the reference range (10–16 $\mu\text{g}/\text{dL}$ [130–204 nmol/L] or 1.2–2.3 ng/dL [18–30 pmol/L], respectively) and the thyrotropin in the

reference range (<6 mU/L). Laboratory evaluation should be conducted (1) at 2 and 4 weeks after initiation of T₄ treatment, (2) every 1 to 2 months during the first year of life, (3) every 3 to 4 months between 1 and 3 years of age, and (4) 2 to 4 weeks after any change in dosage.⁶⁷ Prolonged overtreatment can lead to disorders of temperament and craniosynostosis and should be avoided. Close monitoring is essential in the first 2 to 3 years of life, a time at which the brain still has a critical dependence on thyroid hormone. If permanent hypothyroidism has not been established by 3 years of age, levothyroxine treatment can be discontinued for 1 month and endogenous thyroid function can be reevaluated.

Current Controversies

Preterm infants with hypothyroidism can have a delayed thyrotropin increase,⁶⁸ most likely because of immaturity of the hypothalamic-pituitary-thyroid (HPT) axis. Such infants may be missed by either the primary T₄ or thyrotropin screening approach. Some programs, therefore, have undertaken or are considering a routine second screening between 2 and 6 weeks of age in preterm infants. Programs that undertake a routine second screening report an additional 10% of cases. In addition, some studies suggest that infants less than 28 weeks' gestational age who lose the maternal contribution of thyroid hormone may benefit from treatment until the HPT axis matures.⁶⁹ Additional studies are needed before this can be considered standard of care. Last, some infants seem to have altered feedback of the HPT axis, manifested as persistently high serum thyrotropin concentrations despite apparent adequate treatment.

Special Issues/Concerns

Managing CH presents challenges with stakes that are far greater than management of acquired hypothyroidism. Laboratory evaluation occurs much more frequently, and target T₄ or free T₄ ranges are different for infants. Infants with an altered HPT axis and persistently high thyrotropin concentrations are difficult treatment challenges. With a goal of ensuring optimal treatment and, therefore, optimal neurodevelopmental outcome, these cases should be managed by pediatricians in consultation with pediatric endocrinologists.

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CYSTIC FIBROSIS

Cystic fibrosis (CF) (OMIM database No. 219700)⁷⁰ is a hereditary disease that has primary effects on the lungs, pancreas, intestine, liver, sweat glands, and male reproductive tract as well as important secondary effects on growth and nutrition.⁷¹ The clinical course is variable, but most patients succumb to lung disease in early adulthood.

Incidence

The incidence of CF is approximately 1 in 3500 in white newborn infants. The incidence in black and Hispanic newborn infants (approximately 1 in 15 000 and approximately 1 in 7000, respectively) is higher than previously suspected. There is a low incidence in Asian infants.

Clinical Manifestations

CF usually presents in infancy. Meconium ileus, a neonatal intestinal obstruction, occurs in approximately 17% of infants with CF. Beyond the perinatal period, CF presents as failure to thrive secondary to exocrine pancreatic insufficiency, chronic respiratory symptoms, or both. Nutritional deficits can be severe at presentation and may lead to edema and hypoproteinemia from protein-calorie malnutrition. Infants may present with hyponatremia from sweat salt loss. The most common chronic respiratory symptoms are cough and wheeze. If infants are not diagnosed in the newborn period, they often undergo months of illness with concomitant stress

on the parents. Patients are prone to chronic endobronchial infections with *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and other characteristic bacteria throughout childhood. Many of these patients suffer from recurrent intestinal blockages, and a small percentage of patients have severe liver disease. Diabetes is increasingly common during adolescence and young adulthood. Fifteen percent of these patients have mutations that do not lead to exocrine pancreatic insufficiency. They are at risk of recurrent pancreatitis, however. The median predicted age of survival is 33 years.⁷²

Pathophysiology

CF results from abnormalities in the CF transmembrane conductance regulator (*CFTR*) protein, a membrane glycoprotein that regulates ion flux at epithelial surfaces. Abnormalities in *CFTR* cause thick secretions that obstruct pancreatic ductules, leading to exocrine pancreatic destruction. In the airway, dehydration of airway surface liquid leads to chronic infection and neutrophil-dominated inflammation. Bronchiectasis and progressive obstructive lung disease then follow.

Inheritance

CF is autosomal recessive. More than 1000 mutations in the *CFTR* gene have been described, but one mutation, $\Delta F508$, accounts for more than 70% of affected chromosomes in individuals of European ancestry. Several dozen mutations have been characterized as pancreatic sufficient or insufficient on clinical grounds. The American College of Medical Genetics has developed standards and guidelines for population-based CF-carrier screening that include a panel of 25 mutations.⁷³

Rationale for and Benefits of Newborn Screening

The principal benefit of newborn screening and early diagnosis is improved height and weight at least through adolescence, demonstrated in a well-controlled clinical trial.⁷⁴ Improvement in height and weight likely occurs from early institution of pancreatic enzyme, fat-soluble vitamin and salt supplementation, as well as the general nutritional follow-up that is part of care at a CF center. In addition, it is likely that early diagnosis and attention to nutrition can help patients avoid severe nutritional complications of infancy, although this has not been shown in a controlled trial. Severe nutritional complications of CF in infancy include anemia from vitamin E deficiency, zinc deficiency, linoleic acid deficiency, hypoelectrolytemia, and protein-calorie malnutrition. In addition, vitamin E deficiency at symptomatic diagnosis of CF is associated with cognitive deficits. Thus, early diagnosis through newborn screening is likely to improve developmental outcome. Observational studies support improved pulmonary outcome after newborn screening. In addition, height in CF is correlated with improved pulmonary outcome. Thus, the increase in

height in patients identified through screening also may be beneficial. Another benefit of screening is that parents of children identified through screening have been shown to have greater trust in the medical establishment than parents whose children are identified only after symptoms appear.⁷⁵

Screening

Methodology

Determination of immunoreactive trypsinogen (IRT) concentrations from dried blood spots serves as the basis for the first tier in all newborn-screening programs for CF. IRT concentration is high in the blood of infants with CF, presumably from leakage of the protein into the circulation after exocrine pancreatic injury. Two approaches can be taken if the IRT concentration is high. The more common approach is to perform mutation analysis from the dried blood spot for a set of CF mutations. Another approach is based on persistent elevation of IRT concentration, which requires a second dried blood spot taken 2 to 3 weeks after birth.

The value at which the initial IRT concentration is considered abnormal varies from program to program. If mutation analysis is performed from the first dried blood spot, a second specimen is not required. Thus, the IRT cutoff can be set to include a substantial fraction of the newborn population. In some programs, the top 5% of all IRT concentrations are considered abnormal and mutation analysis is performed. In other programs, the cutoff is set at the top 1%.

Programs that are based on persistent elevation of IRT concentration require a second dried blood spot taken at 2 to 3 weeks of age in infants with a high concentration on the first specimen. These programs set the cutoff value for IRT at a higher concentration (0.5% of newborn infants) than programs that perform mutation analysis. Diagnosis through persistent elevation of IRT concentration can identify infants with CF who do not carry mutations included in most mutation-analysis panels.

Timing

Because IRT concentration is frequently high immediately after birth, specificity is improved if the test is performed after the first day of life.

Sensitivity and Specificity

The sensitivity of most CF screening programs, whether based on genotyping or persistent elevation of IRT concentration, is approximately 95%. The specificity of programs that rely on persistent elevation of IRT concentration without genotyping is approximately 99.5% after the first measurement of IRT concentration. The specificity of programs that perform genotyping after the initial elevation of IRT may be as high as 99.9%.

Follow-up and Diagnostic Testing (Short-term)

Timeline

For programs that perform mutation analysis, the diagnosis of CF can be made if 2 mutations are identified from the dried blood spot. If only one mutation is identified from the dried blood spot, then sweat testing, the definitive diagnostic test, should be performed as soon as possible. In programs that do not perform mutation analysis, sweat testing should be performed within a few days of the repeat IRT test. There is some urgency to making the diagnosis. Many patients are pancreatic-insufficient in the first weeks of life and are at risk of severe nutritional complications. Pancreatic enzyme-replacement therapy, fat-soluble vitamin supplementation, and salt supplementation should be initiated very soon after diagnosis in pancreatic-insufficient infants.

Test and Procedures

Sweat testing should be performed at more than 1 week of age. Almost all term infants will have adequate sweat amounts by that time.⁷⁶ Sweat collection amounts may be inadequate in preterm infants; in such a case, mutation analysis can be performed.⁷⁷ Currently, a sweat chloride value of more than 40 mmol/L is required for the diagnosis of CF in the newborn period; infants with values more than 30 mmol/L, however, require follow-up.⁷⁸ In programs that perform mutation analysis, confirmatory sweat testing should be obtained even in infants who test positive for 2 mutations.

Brief Overview of Disease Management

Nutrition is an important focus of management beginning in infancy. A recently developed test for fecal elastase may allow convenient determination of need for pancreatic enzyme supplementation. Pancreatic enzyme, fat-soluble vitamin, and salt supplementation will be started in most infants at diagnosis. Outpatient regimens become increasingly complex with age and often include several inhaled medications, nutritional supplements, attention to secretion clearance, and a number of ongoing oral medications to be taken daily. Patients with pulmonary exacerbation require hospitalization to receive intravenous antibiotic therapy and intensive secretion clearance. Every effort should be made to have the infant and family cared for at centers accredited by the Cystic Fibrosis Foundation.

Current Controversies

Three controversies have surrounded newborn screening for CF. One issue has been whether the growth and nutritional benefits of early diagnosis are sufficient to justify screening. Very recently, however, the Centers for Disease Control and Prevention has determined that newborn screening for CF is of benefit.⁷⁹ Follow-up studies of pulmonary and cognitive outcomes may further

address this issue. A second issue is carrier detection, which occurs in all programs that use mutation analysis as part of the screening. It is not known for sure whether identification of otherwise well infants as carriers of CF may do harm, but studies suggest that this is not the case. A third issue is that approximately 5% of newborn infants identified will have borderline sweat tests (sweat chloride levels of 30–40 mmol/L) and “mild” mutations. It is not clear yet how many of these infants will have important medical problems. Follow-up studies are underway.

Counseling

Parents will require education on all aspects of CF. The care team consists of the primary pediatrician and the CF center staff. Genetic counseling should be arranged for all families.⁸⁰

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GALACTOSEMIA

Lactose, or milk sugar, is broken down into its constituent simple sugars, glucose and galactose, before absorption in the intestine. Galactosemia, which is an increased concentration of galactose in the blood, has many causes. The genetic disorders that cause galactosemia vary in severity from a benign condition to a life-threatening disorder of early infancy. Early diagnosis and treatment of the latter condition can be life saving; hence, newborn screening for this disease has been instituted in many states.

Incidence

Three distinct enzyme deficiencies may lead to galactosemia. The most common of these, galactose 1-phosphate uridylyltransferase (GALT) deficiency (OMIM database No. 606999),⁸¹ occurs in approximately 1 in 47 000 newborn infants.⁸² This disorder is often referred to as “classic galactosemia.” Galactokinase (GALK) deficiency (OMIM database No. 230200)⁸¹ seems to be very rare, although there have been no large population studies to assess its incidence. One study found that 1% of North American people were carriers, suggesting a disease frequency of 1 in 40 000.⁸³ However, a newborn screening study conducted in Massachusetts detected no cases among 177 000 newborn infants.⁸⁴ The third disorder, galactose-4'-epimerase (GALE) deficiency (OMIM database No. 230350), occurs in 2 forms; one form is confined to red blood cells and has no symptoms, and the second form, which is exceedingly rare, is generalized, with only a few patients reported nationally.^{85,86}

Clinical Manifestations

Infants with classic galactosemia, or GALT deficiency, generally present within the first weeks after birth with a life-threatening illness. Feeding intolerance, vomiting and diarrhea, jaundice, hepatomegaly, lethargy, hypotonia, and excessive bleeding after venipuncture are characteristic findings. Laboratory studies indicate liver and renal tubular disease. Septicemia, particularly with *Escherichia coli*, is not uncommon. Cataracts are generally seen at presentation, but they may be mild in the first few weeks of life and only detectable with slit-lamp examination. Less frequently, patients with classic galactosemia may have a more chronic presentation, with failure to thrive, poor feeding, and developmental delay. Black individuals with classic galactosemia, in particular, frequently have a mild presentation.

Infants with GALK deficiency generally present with bilateral cataracts, which have been observed as early as 4 weeks of age. The cataracts are identical to those seen in classic galactosemia.⁸⁷ The great majority of infants with GALE deficiency have an enzyme deficiency that is confined to the red blood cells and causes no symptoms. Five individuals with generalized GALE deficiency had

developmental delay, hypotonia, and poor growth; 3 had sensorineural hearing loss.⁸⁸

Pathophysiology

The main metabolic pathway for the conversion of galactose to glucose uses 3 enzymes: GALK, GALT, and GALE. Individuals who lack GALK cannot convert galactose to galactose 1-phosphate. As a result, galactose is converted to galactitol by an alternative pathway. The accumulation of galactitol in the lens results in the development of cataracts. Individuals with classic galactosemia, or GALT deficiency, cannot convert galactose 1-phosphate to uridine diphosphate galactose. Galactose, galactitol, galactose 1-phosphate, and other metabolites accumulate. Although it seems clear that increased galactitol is responsible for the development of cataracts in all forms of galactosemia, it is not known which metabolites are responsible for the other clinical findings in classic galactosemia.⁸⁹

Inheritance

All forms of galactosemia are autosomal recessive in inheritance. More than 150 different mutations have been identified in GALT, the enzyme that is deficient in classic galactosemia. The most common GALT mutation in Europe and North America is Q188R, which is associated with the severe presentation of classic galactosemia. A mutation found in black and some Hispanic individuals is S135L.⁹⁰ This mutation is associated with a milder presentation of the disorder.

Benefits of Newborn Screening

Exclusion of galactose from the diet results in marked improvement of the life-threatening complications of classic galactosemia. However, this treatment has only limited efficacy in the prevention of long-term complications. These include impaired cognitive development, with mean IQ in the range of 70 to 90; verbal dyspraxia, a speech disorder attributable to a sensorimotor disturbance of articulation; growth delay, with ultimate height in the normal range; neurologic findings, including tremor and ataxia beginning in midchildhood to middle age; and ovarian failure, manifesting as delayed puberty, primary amenorrhea, secondary amenorrhea, or oligomenorrhea.⁹¹ Prepubertal children with GALT deficiency are also at increased risk of having decreased bone mineral density despite normal calcium intake.⁹²

Screening

Newborn screening for galactosemia may test for galactose, galactose 1-phosphate plus galactose, or GALT enzyme deficiency. Some laboratories test for all of these substances. Because GALT is deficient only in classic galactosemia, this newborn screening test alone will not detect the other 2 forms of galactosemia. The GALT enzyme test has the advantage of being independent of

the infant's diet. Therefore, the timing of the newborn screening sample collection will have no effect on the reliability of this test. However, because GALT analysis is performed using red blood cells, there may be a false-negative result for up to 3 months if the infant has received a blood transfusion. Tests for galactose and galactose 1-phosphate depend on the infant's diet; therefore, it is important to be sure that the infant is receiving galactose-containing formula or breast milk before testing. MS/MS can be used as a technology in screening for galactosemia.⁹³

Follow-up and Diagnostic Testing

All newborn infants with positive screening results should be evaluated rapidly by an experienced physician for feeding difficulty, signs of sepsis, jaundice, and hepatomegaly. Untreated classic galactosemia may progress very rapidly to hepatic toxicity, with death resulting from sepsis or bleeding. Immediate restriction of dietary galactose is critical and should not await diagnostic testing. Galactose restriction should be instituted immediately even in the asymptomatic child and should be continued until the extent of enzyme deficiency, if any, is known.

Diagnostic studies for classic galactosemia include quantitative analysis of GALT and red blood cell galactose 1-phosphate. In states where the screening test measures GALT activity, these studies will establish or rule out classic galactosemia. When the screening results, including estimates of galactose and galactose 1-phosphate and quantitative GALT activity, are normal, quantitative analysis of GALK and GALE are required to identify these forms of galactosemia. It is likely that another pathway exists that can be responsible for galactose disposal, but this pathway has not been characterized.⁹⁴

Brief Overview of Disease Management

Infants suspected of having galactosemia should be fed with a galactose-free formula until diagnostic testing confirms a specific diagnosis. Children who are seriously ill at the time of diagnosis of classic galactosemia require supportive care, which may include vitamin K supplementation and fresh-frozen plasma transfusions, antibiotics for presumed Gram-negative sepsis, and phototherapy for hyperbilirubinemia. After dietary galactose has been eliminated, most infants improve rapidly. Milk and milk products are excluded from the diet indefinitely, because significant ingestion of galactose at any age can be toxic.⁹² Because medications may contain galactose, the pediatrician should instruct parents to ask the pharmacist if a medication is galactose free before administering it to the child. Regular nutritional evaluation is necessary to ensure adequate calcium intake. Regular developmental evaluation and early speech assessment are also required. Girls should be monitored frequently

in late childhood and adolescence for pubertal development. Regular measurement of galactose 1-phosphate in red cells is the most common method used to assess dietary compliance.⁸⁵

Lifelong galactose restriction is also indicated for individuals with GALK and generalized GALE deficiencies. No treatment seems to be necessary for red blood cell GALE deficiency.

Current Controversies

In addition to milk products, certain fruits contain significant quantities of galactose.⁹³ There is no consensus about whether these fruits should be eliminated from the diet, because endogenous synthesis of galactose also occurs.⁹⁴ Some authors have suggested that an elemental formula (galactose free) may be preferable to soy formula in the treatment of galactosemia.⁹⁵

Special Issues

Galactose is a reducing substance, and the presence of reducing substances in the urine is sometimes suggested as a test for galactosemia. However, this test is neither sensitive nor specific, and it should not be used as a screening or diagnostic test for galactosemia.

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HOMOCYSTINURIA

The term “homocystinuria” designates a biochemical abnormality, not a specific disease entity. There are many causes of homocystinuria. All affect one of the transsulfation pathways that convert the sulfur atom of methionine into the sulfur atom of cysteine. This pathway is the chief route of disposal of methionine. The most common defect, cystathionine β -synthase (CBS) deficiency (OMIM database No. 236200),⁹⁶ results in high concentrations of serum methionine. One form of CBS deficiency is responsive to vitamin B₆.⁹⁷ Other metabolic variants of homocystinuria include defects of vitamin B₁₂ uptake or activation and tetrahydrofolate reductase deficiency (OMIM database No. 236250).

Incidence

Although homocystinuria is a rare disorder, carriers of the condition represent a much larger population. If one assumes a worldwide incidence of 1 in 300 000 individuals, the expected carrier frequency is 1 in 135. Because carriers are more prone to thromboembolic events, ascertainment of these individuals via identification of an affected person needs to be emphasized to primary health care professionals.⁹⁸

Clinical Manifestations

Clinical problems include multiple, recurrent thromboemboli.⁹⁹ Arterial or venous thromboses may involve the cerebral, pulmonary, renal, and myocardial circulation.¹⁰⁰ Patients may also exhibit ectopia lentis, glaucoma, cataracts, developmental delays/mental retardation, seizures, psychiatric disturbances, osteoporosis with bone deformities, scoliosis, high palatal arch, muscle weakness with a shuffling gait, and a marfanoid habitus. Death has been reported within the first year of life. Approximately 50% of untreated individuals die by 25 years of age; death is frequently a result of thromboembolic events. Developmental delay is reported in 65% to 80% of untreated individuals.¹⁰¹

Pathophysiology

Two mechanisms probably explain most of the clinical symptoms seen: (1) abnormal (hyper) coagulation because of “sticky” platelets; and (2) direct toxicity of ho-

mocystine and its metabolites, causing endothelial cell damage.

Inheritance

The specific enzymatic defect should be ascertained. However, all heritable forms of homocystinuria exhibit autosomal recessive inheritance. Prenatal diagnosis is available for CBS deficiency using cultured chorionic villus cells or amniotic fluid cells to measure the activity of this enzyme. The chromosome map location is 21q22. The incidence in Ireland, Australia, Great Britain, and New England is 1 in 50 000, the incidence in Japan is 1 in 1 million, and the worldwide incidence is 1 in 250 000.

More than 90 different disease-associated mutations of the CBS gene have been identified. The vast majority of these mutations are “private” mutations that occur in only a single or a very small number of families. The most prevalent mutations are the G307S and I278T mutations.¹⁰² Affected patients vary widely in the extent to which they manifest clinical abnormalities, suggesting considerable genetic heterogeneity. Some of the variability is accounted for by the relative reduction of enzymatic activity. Absent to relatively low residual activity (up to 10%) of CBS has been noted among different families. However, there are reports of individuals with the identical genotype resulting in a different phenotype within the same family.

Rationale for and Benefits of Newborn Screening

The potential for early clinical diagnosis is limited. Ocular abnormalities, because of their distinctive lens displacement, may lead to the diagnosis. The diagnosis should be considered in any child or young adult with thromboembolism affecting both the large and small arteries as well as the veins, particularly in association with developmental disabilities, mental retardation, or skeletal findings. Most patients, however, have nonspecific features so that definitive testing involving the measurement of serum or urine amino acids is not accomplished before the expression of more severe clinical symptoms. Treatment seems to reduce the risk of thromboembolic episodes. Because this is the major cause of mortality and morbidity in these patients, the survival rate may improve with early, effective treatment. The incidence of mental retardation may be prevented or reduced.¹⁰³ For patients with classic (homozygous) homocystinuria, early treatment with good biochemical control (lifetime plasma-free homocystine < 11 μ mol/L) seems to prevent mental retardation,¹⁰⁴ ectopia lentis seems to be delayed, and the incidence of seizures is reduced.

Screening

The bacterial inhibition assay (BIA) test may be used to detect increased concentrations of blood methionine.¹⁰⁵

Normal values for serum methionine concentration are noted to be less than 2 mg/dL. Newer methods include direct methionine assay by MS/MS.¹⁰⁶ The false-negative rate seems to correlate with the time that the specimen was obtained and the level of residual CBS activity present (ie, the B₆-responsive form). The false-negative rate increases with earlier newborn discharges. Approximately 1 in 5000 infants is found to have blood methionine concentrations more than 2 mg/dL. The use of a reduced cutoff level (1 mg/dL) increases the false-positive rate from 0.006% to 0.03%.¹⁰⁷ However, use of this cutoff should identify affected infants who have only slightly increased concentrations of methionine and reduce the frequency of false-negative results. It has been suggested that the increased false-positive rate does not represent an undue burden in terms of requests for repeat analysis.

Follow-up and Diagnostic Testing

Quantitative serum or plasma amino acid determination is used for diagnosis of homocystinuria. Plasma amino acids show increased methionine and homocystine concentrations with reduced concentrations of cystine and absent cystathionine. A urine organic acid profile with gas chromatography and MS/MS may be used to determine the presence or absence of methylmalonic acid.

Brief Overview of Disease Management

Treatment depends on the underlying cause of homocystinuria. As a first step, pyridoxine (vitamin B₆) responsiveness should be ascertained, because approximately 50% of patients respond to large doses of this vitamin. Nonresponsive patients with CBS deficiency should be treated with a methionine-restricted, cystine-supplemented diet. Folic acid and betaine therapy may also be helpful with all patients. In the disorders of cobalamin metabolism and transport in which methylmalonic acid and homocystine appear in the urine, hydroxycobalamin treatment (vitamin B₁₂, not cyanocobalamin) may be beneficial. Aspirin and dipyridamole have also been used to decrease the occurrence of thromboembolic phenomena. Clinical variability remains despite therapy. Not all affected individuals have increased methionine concentrations. The relationship between variability and the underlying metabolic processes or compliance has not yet been completely ascertained. One described mutation, G307S, is typically a pyridoxine-nonresponsive mutation, and individuals homozygous for the I278T mutation are usually responsive to pyridoxine therapy. The presence of some activity of the enzyme seems necessary for a clinical response to pyridoxine (vitamin B₆) administration. Individuals who are clinically responsive to pyridoxine generally have milder or more slowly progressive disease.

Current Controversies

Increased concentrations of methionine may be minimal during the first 3 days of life until there is adequate protein intake (milk feedings). This is especially true in patients who are responsive to vitamin B₆, who usually have some residual enzyme activity. This minimal increase probably accounts for the difference in screening frequencies between the United States and United Kingdom, where screening specimens are obtained at 5 to 7 days. It may be preferable to screen for this disorder at 2 to 4 weeks of age. Early discharge at 24 or even 18 hours results in many missed cases and decreases screening effectiveness.

Programs continue to evaluate the efficacy of screening and early treatment. Improvement in screening to decrease the numbers of missed cases is important. Recent evidence has shown that carriers (heterozygotes) for homocystinuria have an increased risk of thromboembolic events. Therefore, genetic counseling and screening should be offered to relatives of persons with homocystinuria.

Special Issues/Concerns

Specialized care is required that includes the ability to monitor amino acids and provide nutritional assessment and planning. Doses of pyridoxine higher than 900 mg have been associated with neuropathy; however, these higher doses are usually not required for adequate treatment. Thromboembolic phenomena are more prone to occur during anesthesia, surgical procedures, and prolonged immobilization.

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MAPLE SYRUP URINE DISEASE (BRANCHED-CHAIN KETOACIDURIA)

Maple syrup urine disease (MSUD) (OMIM database No. 248600),¹⁰⁸ also known as branched-chain ketoaciduria, is caused by a deficiency in activity of the branched-chain α -keto acid dehydrogenase (BCKD) complex. Deficiency of the BCKD complex results in accumulation of the branched-chain amino acids (BCAAs) leucine, isoleucine, and valine and the corresponding branched-chain α -keto acids (BCKAs).¹⁰⁹ A pathognomonic finding in individuals with MSUD is the presence of alloisoleucine, a compound that is not present in other individuals. There are 5 phenotypes observed in patients with MSUD: classic, intermediate, intermittent, thiamine-responsive, and dihydrolipoyl dehydrogenase (E3)-deficient. Although enzyme activities overlap to some degree in these phenotypes, in general, lower enzyme activity is associated with a more severe disorder.

Incidence

The worldwide frequency of MSUD (including classic and some variant forms), which is based on routine screening data from 26.8 million newborn infants, is approximately 1 in 185 000.^{109,110} Newborn screening of 756 163 newborn infants over an 8-year period in Georgia revealed a much higher frequency of 1 in 84 000.¹¹¹ In the population of Mennonites living in Lancaster and Lebanon counties in Pennsylvania, the incidence is 1 in 176 newborn infants, most likely attributable to a founder effect.^{112,113}

Clinical Manifestations

Classic MSUD (residual enzyme activity $\leq 2\%$) is the most severe and most common form. Affected infants are normal at birth, with symptoms usually developing between 4 and 7 days of age; however, lower intake of protein, as in breastfeeding, can delay the onset of symptoms until the second week of life. Initial symptoms are lethargy and poor sucking with little interest in feeding. Weight loss follows with abnormal neurologic signs (al-

ternating hypertonia and hypotonia; dystonic posturing of the arms) becoming more and more apparent. The characteristic odor of the urine, described as smelling like maple syrup, burnt sugar, or curry, is then noted. Finally, seizures and coma, leading to death (in untreated cases), occurs.¹⁰⁹ Laboratory findings include increased concentrations of BCAAs, ketosis, acidosis, and occasionally hypoglycemia.¹¹⁴ Patients with intermittent MSUD (enzyme activity 5%–20%) exhibit normal growth and intelligence. These children do not go into metabolic crisis unless the body is in a stressful situation, such as an infection (ie, otitis media) or after surgery.¹¹⁵ Although patients with intermittent MSUD generally present between 5 months and 2 years of age secondary to a minor infection, some individuals have not shown symptoms until the fifth decade. Concentrations of BCAAs are normal between episodes.

In contrast, patients with intermediate MSUD (enzyme activity 3%–30%) do not present with catastrophic illness during the neonatal period but have gradual neurologic problems, eventually resulting in mental retardation. In one study, most were diagnosed between 5 months and 7 years of age while undergoing evaluation for developmental delay or seizures.^{116,117} Several patients have had episodes of ketoacidosis, but acute encephalopathy is rare.¹¹⁸ Increased concentrations of BCAAs and BCKAs in serum and urine are present. Patients with thiamine-responsive MSUD (enzyme activity 2%–40%) have a clinical course similar to those with intermediate MSUD. These patients have decreased concentrations of BCKAs and/or BCAAs with thiamine therapy in varying dosages ranging from 10 to 1000 mg/day.^{119,120} In some instances, the patient does not show the full response to thiamine until therapy has commenced for 3 weeks.¹²¹ In all documented cases, patients required dietary intervention in conjunction with thiamine to achieve metabolic control.¹⁰⁹

E3-deficient MSUD (E3 deficiency) is rare, with fewer than 20 patients having been described.^{109,122,123} Clinically, newborn infants with E3 deficiency are similar to patients with intermediate MSUD, but severe lactic acidosis is also present. The infants develop a persistent lactic acidosis between 8 weeks and 6 months of age followed by progressive neurologic deterioration with hypotonia, developmental delay, and movement disorder. Laboratory findings include mild to moderately increased concentrations of BCAAs and increased lactate, pyruvate, α -ketoglutarate, α -hydroxyisovalerate, and α -hydroxyglutarate concentrations. The patients have a combined deficiency of BCKD, pyruvate, and α -ketoglutarate dehydrogenase complexes, leading to the more complex phenotype. Various combinations of dietary therapy, vitamin therapy (thiamine and biotin), and lipoid acid have been tried without success.¹²²

Pathophysiology

The BCKD complex is a macromolecule composed of 3 catalytic components: a thiamine pyrophosphate–dependent decarboxylase (E1) with α and β subunits, a transacylase (E2), and a dehydrogenase (E3). In addition, the BCKD complex contains 2 regulatory enzymes, a kinase and a phosphatase, that control activity of the complex.¹⁰⁹ The genes encoding E1 α , E1 β , E2, E3, and the specific kinase are cloned. Mutations with genotype/phenotype correlations have been described (see “Inheritance” below).

Inheritance

MSUD is an autosomal recessively inherited condition.¹⁰⁹ Mutations in the E1 α subunit result in the molecular phenotype referred to as MSUD type IA (OMIM database No. 248600). The type IA mutations almost always result in the severe classic form of MSUD. The most prevalent mutation is Y393N, the mutation in the Mennonite community in Pennsylvania. DNA testing has been developed for the Y393N mutation because of its prevalence.¹²⁴ Only a few mutations have been described in the E1 β subunit (type IB mutations; OMIM database No. 248611), all resulting in the classic neonatal MSUD phenotype. Mutations affecting the E2 core of the BCKD complex (type II MSUD mutations; OMIM database No. 248610) characteristically lead to a milder phenotype than types IA or IB. Most patients have the intermediate or intermittent phenotype, and several have been reported to respond to thiamine therapy. All type III mutations (defects in the E3 subunit; OMIM database No. 238331) lead to a distinct severe combined phenotype (MSUD plus primary lactic acidosis).

Benefits of Newborn Screening

Prognosis is poor for the patient with classic MSUD that goes undiagnosed and untreated, with death versus survival with severe neurologic damage as potential outcomes. Patients with classic MSUD who are not treated by 14 days of age generally have a less desirable outcome. In one study, the outcome with treatment was reported in more than 150 patients with classic MSUD and more than 25 patients with the variant forms.^{109,125} Most of these cases were detected by newborn screening or because of clinical presentation. In the patients with classic MSUD, one third had IQ scores higher than 90, and one third had scores between 70 and 90. Rapid recognition and treatment (as with newborn screening) is important. When both performance and verbal scores are available, verbal scores are consistently higher than performance scores.¹²⁶ The discrepancy between the 2 scores is not surprising, because cerebellar dysfunction is often an early sign of acute metabolic decompensation. Even with newborn screening leading to timely treatment, outcome is not perfect. Short attention span and minor learning disabilities were observed even in pa-

tients with normal intellect who were treated soon after birth.¹²⁶

Screening

State-of-the-art screening for MSUD is by MS/MS. The sum of the 3 isomers (leucine, isoleucine, and alloisoleucine) leads to a distinct diagnostic peak.¹⁰⁹ Classic MSUD, the intermediate form, and E3 deficiency can usually be detected by screening in the newborn period. Intermittent MSUD would not be detected, because the patients' concentrations are normal when they are not in crisis. Thiamine-responsive MSUD has been missed by newborn screening.¹¹⁰

Follow-up and Diagnostic Testing

A blood leucine concentration greater than 4 mg/dL, or a concentration of 3 to 4 mg/dL (305 mmol) in the first 24 hours of life, requires immediate medical follow-up.¹⁰⁹ Plasma amino acid analysis reveals findings diagnostic for MSUD: increased concentrations of BCAAs, low alanine concentrations, and the presence of alloisoleucine.

Brief Overview of Disease Management

Treatment consists of a carefully regulated diet that provides sufficient BCAAs for normal growth and development without exceeding the patient's degradative enzyme capacity.¹⁰⁹ Because natural protein must be limited, a medical food product (BCAA-free) supplement is necessary. A metabolic team, including not only a physician metabolic specialist but also a metabolic nutritionist, is crucial. A trial of thiamine supplementation (50–300 mg/day for at least 3 weeks) is recommended, because it is therapeutic for some patients and has no adverse effects. There are 2 aspects of treatment: long-term management and treatment during acute metabolic crisis. The goal of long-term dietary management is normalization of blood BCAA concentrations while providing nutrition adequate to sustain growth and development in children. Dietary therapy should be continued for life.¹²⁷ Patients with intermediate MSUD may only require protein restriction without supplementation of synthetic formula. Individuals with intermittent MSUD do not require a special diet except during episodes that may lead to metabolic crisis. Treatment during acute illnesses should be aggressive, because the metabolic decompensation can be life-threatening.¹⁰⁹ Toxic metabolites must be removed at the same time that catabolism is minimized and anabolism is promoted. Dialysis (first peritoneal and, more recently, continuous venovenous hemofiltration) has proven useful in BCAA/BCKA clearance.¹²⁸ Dietary treatment to break the cycle of catabolism and promote anabolism sometimes requires parenteral nutrition¹²⁹ or insulin combined with a large glucose infusion.¹³⁰

Current Controversies

MSUD has been treated since the early 1960s,¹³¹ and consequently, some neurologically intact MSUD-affected women have reached childbearing age and reproduced. As has been reported for other enzyme deficiencies, postpartum metabolic decompensation can be a problem.¹³²

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MEDIUM-CHAIN ACYL-CoA DEHYDROGENASE

Medium-chain acyl-CoA dehydrogenase (MCAD; OMIM database No. 201450)¹³³ deficiency is a disorder of fatty acid oxidation (FAO) first described in 1982–1983.¹³⁴ All together, 10 disorders affecting mitochondrial FAO and ketogenesis have been identified. Among these, MCAD deficiency seems to be the most important because it is the most common and it has been implicated in some cases of sudden infant death syndrome (SIDS) and Reye syndrome.¹³⁵

Incidence

MCAD deficiency has been diagnosed almost exclusively among individuals of northwestern European origin, with frequencies ranging from 1 in 46 000 to 1 in 6400.^{136,137} The heterozygote frequency is 1% to 2%. A few cases have been identified in other populations, including one Pakistani patient, one black patient, and isolated cases in individuals of Southern European and Northern African origin.^{138,139} Newborn screening in Japan did not identify any carriers.¹³⁸

Clinical Manifestations

The classic presentation is an episode of vomiting and lethargy after a period of fasting in a child between 3 and 15 months of age. The child may have had a previous viral infection (gastrointestinal or upper respiratory) resulting in decreased oral intake that would have little consequence in an unaffected child.¹³⁹ The episode may

result in coma, and the child may remain obtunded even after hours of treatment with intravenous glucose. Undiagnosed disease has a mortality rate of 20% to 25%, many times with death occurring during the initial episode.¹⁴⁰ In a clinical review of 94 families with MCAD deficiency, 19 families (20%) had one or more unexplained child deaths. The diagnosis of MCAD deficiency was made postmortem in all cases.¹⁴¹

There are few reports of first symptoms after 4 years of age and fewer recurrent episodes after 4 years of age. Symptoms that require hospitalization during the second decade are unusual. The earliest onset of symptoms and sudden death is in the neonatal period, although this is rare, and the latest documented onset of the first episode was at 14 years of age. Most deaths would be preventable if dietary therapy and measures to prevent fasting were begun before the onset of symptoms. Cases in which children have died have, in some instances, resembled cases of SIDS or Reye syndrome. There is marked clinical variability even within the same family. There are families reported with several affected children with one child in the family dying on the first episode before 2 years of age and other children as old as 10 years never having had an episode.¹³⁹

Although death is certainly the most important potential outcome of not screening for MCAD deficiency, there are findings in survivors that are very concerning regarding morbidity. A follow-up survey of 78 MCAD-deficiency survivors (all older than 2 years) revealed a number of unexpected problems, including developmental disabilities, speech and language delay, behavioral problems, attention-deficit/hyperactivity disorder (ADHD), proximal muscle weakness, chronic seizure disorder, cerebral palsy, and failure to thrive. The finding of ADHD was seen in 9 patients (12%), 8 of whom were female, in contrast to the usual male preponderance of ADHD in the general population. The development of muscle weakness was strongly correlated with length of time between symptomatic presentation and the institution of appropriate measures to prevent additional episodes of illness.^{141,142}

Pathophysiology

MCAD deficiency is one defect in the pathway of mitochondrial β -oxidation. It is primarily a disease of hepatic FAO, with the most frequent presentation being episodic hypoketotic hypoglycemia provoked by fasting. FAO disorders do not present under nonfasting conditions and, therefore, have escaped attention for many years. The plasma and urinary metabolites of MCAD deficiency are of 2 types: general indicators of impaired function of the β -oxidation pathway (eg, dicarboxylic acids) and specific metabolites (eg, octanoylcarnitine). The inability to break down fats to ketone bodies for an energy source while fasting eventually leads to hypoglycemia. In addition, medium-chain (C8–C12) acyl-CoA intermediates

accumulate in mitochondria, with the end result being inhibition of mitochondrial β -oxidation. Fatty acid is incorporated into triglycerides, resulting in accumulation of fat in the liver during acute episodes. The clinical presentation and many of the routine laboratory observations in MCAD deficiency are indistinguishable from those in Reye syndrome.¹⁴³ Encephalopathy and cerebral edema are secondary to accumulation of fatty acids within the central nervous system. Coma results from a combination of hypoglycemia and toxic effects of fatty acids or their metabolites.¹³⁴

Inheritance

MCAD deficiency is inherited as an autosomal recessive trait. The causative gene is known, and multiple mutations have been identified. In studies of clinically affected patients with MCAD deficiency, 90% of mutant alleles identified have a single missense mutation (A985G); other mutations identified seem to individually account for less than 1% of the mutant alleles.¹⁴⁴ Virtually all of the A985G alleles arose on a background with the same haplotype, which suggests a founder effect, with the mutation beginning in northwestern Europe and then spreading throughout the rest of the world.¹⁴⁵ Recent molecular studies performed as follow-up to newborn screening by MS/MS technology have found a lower percentage of individuals with the common A985G mutation.^{136,146} A second common mutation (T199C) has been observed in US populations identified initially by MS/MS screening. The T199C mutation is a mild mutation that produces a biochemical phenotype but has never been observed in clinically affected patients.¹⁴⁶

Benefits of Newborn Screening

The benefits of and rationale for using newborn screening for diagnosis of MCAD deficiency are obvious. As noted above, many individuals affected with MCAD deficiency will die during the presenting episode, sometimes having been misdiagnosed with SIDS or Reye syndrome. Not only is this a tragic outcome for the loss of the child, but the family also has a 25% recurrence risk for the condition or may already have affected children who have not yet had clinical symptoms. The condition is relatively common, with a frequency of 1 in 15 001 in prospective newborn screening of 930 078 blood spots from different areas of the United States.¹⁴⁶

Screening

The most efficient and sensitive method of screening for MCAD deficiency is MS/MS, measuring octanoylcarnitine (a compound normally not present) on the filter-paper blood spot. The optimal time for testing is the newborn period, because levels of octanoylcarnitine are significantly higher in the first 3 days of life than later (8 days to 7 years).¹⁴⁷ Individuals who are homozygous for

the common mutation (A985G) who are most likely to present clinically will have octanoylcarnitine concentrations higher than 2.3 $\mu\text{mol/L}$, and individuals with one copy of 985 and one copy of a milder mutation (eg, T199C) will have octanoylcarnitine present but most likely at a lower concentration ($\geq 1.0 \mu\text{mol/L}$). The latter group is more challenging to determine the best course of follow-up.

Follow-up and Diagnostic Testing

Any child with an octanoylcarnitine concentration of 1.0 $\mu\text{mol/L}$ or greater will require definitive diagnostic testing. Follow-up testing will consist of plasma acylcarnitine analysis, urinary organic acid analysis, and molecular testing. The plasma acylcarnitine analysis and urinary organic acid analysis will confirm the diagnosis. The molecular analysis should provide guidance regarding prognosis.

Brief Overview of Disease Management

Treatment for MCAD deficiency consists of avoidance of fasting and mildly decreased intake of dietary fat coupled with L-carnitine supplementation. MCAD deficiency results in a secondary deficiency of carnitine, because carnitine couples with toxic intermediates, resulting in their excretion while depleting carnitine stores. Although it remains questionable how helpful supplemental carnitine is during periods when the patient with MCAD deficiency is healthy, there is no doubt that exogenous carnitine is recommended during times of illness.¹³⁹ Another important point is that patients should be treated aggressively even during minor illnesses (eg, otitis media) to avoid a severe episode. There should be no hesitation to institute therapy with intravenous glucose and carnitine.

Current Controversies

Genotype/phenotype correlation is not straightforward, and the treatment of individuals with milder mutations remains controversial.^{148,149} There are questions yet to be answered, such as whether some (or all) individuals with the less deleterious mutations (either in combination with the common 985 mutation or in combinations with one another) who have a biochemical phenotype would ever have medical problems. In addition, would some such individuals have serious episodes and others would not because of unknown modifying factors? Until we know the answer to these and other questions, we would be remiss in not treating everyone identified, perhaps overtreating some individuals. Newborn screening for MCAD deficiency will be key in answering some of these questions.

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PHENYLKETONURIA

Hyperphenylalaninemia (OMIM database No. 261600),¹⁵⁰ an abnormal increase in the concentration of the amino acid phenylalanine (Phe) in the blood, may be a benign condition with little clinical significance. When the concentration of Phe is very high (>20 mg/dL or 1210 μ mol/L) and there is accumulation of phenylketones, the condition is called classic phenylketonuria (PKU).

Incidence

Despite the fact that newborn screening has been underway for more than 40 years in the United States, data only allow estimates of the incidence and prevalence of this disorder. This is partly because of the fact that states vary in their definitions of hyperphenylalaninemia and PKU. For PKU, the reported incidence ranges from 1 in 19 000 to 1 in 13 500 newborn infants. For non-PKU hyperphenylalaninemia, the estimated incidence is 1 in 48 000 newborn infants. There are large variations in the incidence of PKU by ethnic and cultural groups, with individuals of Northern European ancestry and American Indian/Alaska Native individuals having a higher incidence than black, Hispanic, and Asian individuals.¹⁵¹

Clinical Manifestations

PKU is rarely diagnosed before 6 months of age without newborn screening, because the most common manifestation without treatment is developmental delay followed by mental retardation. Untreated individuals may also develop microcephaly, delayed or absent speech, seizures, eczema, and behavioral abnormalities.

Pathophysiology

PKU results from a deficiency of activity of a liver enzyme, phenylalanine hydroxylase (PAH), leading to increased concentrations of Phe in the blood and other tissues. Certain mutations of the *PAH* gene usually result in non-PKU hyperphenylalaninemia, and others result in classic PKU. Because siblings with the same mutation at the *PAH* locus may have different clinical findings, it is likely that other genetic and environmental factors influence the severity of the disorder.¹⁵² In fact, a few individuals with PKU have no evidence of mental retardation, even without dietary treatment.¹⁵¹ However, there is evidence that certain genotypes are associated with higher increases of Phe concentration.¹⁵³ It is likely that Phe itself leads to the mental retardation and other findings of PKU. In excess, Phe disturbs transport of other amino acids across the blood-brain barrier and impairs synthesis of neurotransmitters.¹⁵¹ For the enzyme PAH to be active, the cofactor tetrahydrobiopterin

(BH4) is required. Impaired synthesis or recycling of BH4 results in increased concentrations of Phe and certain other amino acids. This condition does not respond to routine dietary management of PKU, and hence, states have instituted additional screening programs to identify infants with these rare disorders so that appropriate treatment can be initiated.

Inheritance

PKU is an autosomal recessive disorder, with the *PAH* locus on chromosome 12q24.1. More than 400 different mutations have been described, including deletions, insertions, missense mutations, splicing defects, and nonsense mutations. Most individuals with PKU are compound heterozygotes, meaning that a single individual will have different mutations of each copy of the *PAH* gene.¹⁵¹ The numerous possible combinations of gene mutations undoubtedly contributes to the variable clinical findings in PKU.

Benefits of Newborn Screening

Children with PKU who are treated appropriately after positive newborn screening results have average intelligence as measured by IQ tests, although their scores are somewhat lower than expected when compared with parent and sibling IQs. There is an inverse relationship between the age at which treatment is begun and the IQ level, even in PKU that is treated early.¹⁵⁴ Tremor of unknown origin has been reported in 10% to 30% of early-treated individuals with PKU.¹⁵⁵ Adolescents and young adults who are treated early and continuously seem to have no increased incidence of psychiatric, emotional, or functional disorders, and there is no increase in problems of self-concept.^{156,157} Although children with PKU are not at increased risk of developing dental caries, children with PKU may exhibit increased signs of tooth wear because of the erosive potential of the amino acid supplements in the diet.¹⁵⁸ Therefore, it is important for children and adolescents with PKU to have regular dental care.

Screening

There are 3 main methods used for screening newborns for PKU in the United States: the Guthrie BIA, fluorometric analysis, and MS/MS. The Guthrie BIA is inexpensive and reliable. Fluorometric analysis and MS/MS are quantitative and can be automated; both of these methods also produce fewer false-positive results than BIA.¹⁵¹ Preliminary data indicate that MS/MS produces fewer false-positive results than the fluorometric method in samples obtained in the first 24 hours of life.¹⁵⁹ Newborn screening laboratories in the United States use cutoff values from 2 mg/dL (125 μ mol/L) to 6 mg/dL (375 μ mol/L). A positive screening result should lead to rapid evaluation of the newborn for clinical status, age, and diet at the time of sample collection. Severe

deficiency of PAH will usually result in an increased concentration of blood Phe within the first 24 hours of life; however, infants with a less severe deficiency may take longer to develop an abnormal Phe concentration. It is for this reason that a repeat test for all infants initially screened in the first 24 hours of life has been recommended by some authorities.¹⁶⁰ Few states, however, currently require a second screen.

Follow-up and Diagnostic Testing

Early treatment of PKU is associated with improved intellectual outcome. Therefore, an infant with a positive newborn screening result should receive the benefit of rapid diagnostic testing. Diagnostic testing includes quantitative determination of plasma Phe and tyrosine concentrations. If the Phe concentration is increased, additional studies are indicated to determine if the infant has an abnormality in synthesis or recycling of BH4.

Brief Overview of Disease Management

Once the diagnosis of hyperphenylalaninemia is confirmed, metabolic control should be achieved as rapidly as possible. This is achieved through the use of medical foods, including medical protein sources that are low in Phe; small amounts of Phe must also be provided, which is achieved through the use of small amounts of natural protein. The infant with PKU can be given breast milk along with Phe-free formula under the direction of a metabolic dietitian. The response to dietary treatment is monitored through periodic measurement of blood Phe concentrations, assessment of growth parameters, and review of nutritional intake. There is no consensus concerning the optimal blood Phe concentration or the duration of strict dietary management. The most commonly reported blood Phe concentration recommendations for US centers are 2 to 6 mg/dL for individuals 12 years or younger and 2 to 10 mg/dL for persons older than 12 years.¹⁵¹ Most US centers recommend lifelong dietary treatment. This is particularly important for women, because fetuses exposed to increased concentrations of Phe are at significant risk of microcephaly, congenital heart disease, and reduced IQ.¹⁵¹ It is recommended that a woman with PKU achieve Phe concentrations of less than 6 mg/dL at least 3 months before conception and that concentrations be maintained between 2 and 6 mg/dL throughout pregnancy.¹⁵¹ The importance of management throughout the reproductive years illustrates the critical role of long-term follow-up in this disorder.

Current Controversies

As noted previously, there is no national or international consensus regarding the optimal concentration of Phe across the life span. Similarly, there is no consensus regarding discontinuation of dietary therapy. Although appropriately treated young adults with PKU lead nor-

mal and productive lives, there are no meaningful data regarding the incidence of long-term sequelae in individuals who remain on dietary therapy into middle and old age. Recent evidence suggests that some individuals with hyperphenylalaninemia and classic PKU may benefit from BH4 treatment in addition to dietary Phe restriction.¹⁶¹

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SICKLE CELL DISEASE AND OTHER HEMOGLOBINOPATHIES

The term sickle cell disease (SCD) (OMIM database No. 603903)¹⁶² encompasses a group of genetic disorders characterized by chronic hemolysis and intermittent episodes of vascular occlusion that cause recurrent episodes of severe pain and a wide variety of other disease manifestations. Specialized comprehensive medical care markedly reduces mortality in infancy and early child-

hood by preventing some disease-related complications and limiting the severity and sequelae of others.

Newborn screening for SCD also identifies infants with nonsickle hemoglobinopathies, hemoglobinopathy carriers, and, in some states, infants with α -thalassemia.^{163,164} Newborn screening results and clinical manifestations for some of these conditions are outlined in Table 1. Guidance for follow-up and diagnostic evaluation of infants with these screening results has been published^{163,164} and is often provided by state newborn screening programs or their designated hemoglobinopathy consultants.

Incidence

Overall, SCD occurs in 1 of 2500 to 1 of 2000 US newborns.^{165,166} Its incidence is highest in persons of African, Mediterranean, Middle Eastern, Indian, Caribbean, and Central and South American ancestry. The disease occurs less commonly in other ethnic groups, including individuals of Northern European descent. Accurate incidence data for many groups are unavailable. SCD is estimated to occur in 1 of 346 black infants and in 1 of 1114 Hispanic infants in the eastern United States.¹⁶⁷

Clinical Manifestations

Most infants with SCD are healthy at birth and become symptomatic later during infancy or childhood. The most common clinical manifestation is musculoskeletal or abdominal pain, which occurs unpredictably and is

often excruciating. Acute manifestations that may rapidly become life-threatening include bacterial sepsis or meningitis, splenic sequestration, acute chest syndrome, and stroke. Other acute complications include aplastic crises, priapism, and renal papillary necrosis. Chronic manifestations include anemia, jaundice, splenomegaly, hyposthenuria, hematuria, proteinuria, cholelithiasis, and delayed growth and sexual maturation. Avascular necrosis of the hip and shoulder, restrictive lung disease, and leg ulcers may cause chronic disability. Pulmonary hypertension is a risk factor for early death. It is important to note that the severity of SCD varies widely, even among individuals with the same genotype.

Pathophysiology

Sickle hemoglobin is caused by a point mutation in the β -globin gene, which leads to an amino acid change that causes hemoglobin to polymerize when deoxygenated. Sickle red blood cells are dehydrated and show oxidative damage and increased adhesion to endothelial cells. The cumulative effects of these cellular abnormalities are shortened red cell survival and intermittent episodes of vascular occlusion, which cause tissue ischemia and organ damage.

Inheritance

SCD is an autosomal recessive disorder. Heterozygous individuals have a generally benign, asymptomatic genetic carrier state, sometimes referred to as a sickle cell

TABLE 1 Newborn Screening for Conditions Other Than SCD

Screening Results	Possible Conditions	Clinical Manifestations
Nonsickle hemoglobinopathies		
F only	Preterm infant Homozygous β^0 -thalassemia	Repeat screening necessary Severe thalassemia
FE	EE E β^0 -thalassemia	Microcytosis with mild or no anemia Mild to severe anemia
FC	CC C β^0 -thalassemia	Mild microcytic hemolytic anemia Mild microcytic hemolytic anemia
FCA	C β^+ -thalassemia	Mild microcytic hemolytic anemia
α -Thalassemia syndromes		
FA + Bart's ^a	α -Thalassemia silent carrier α -Thalassemia minor HbH disease HbH Constant Spring	Normal complete blood cell count Microcytosis with mild or no anemia Mild to moderately severe microcytic hemolytic anemia Moderately severe hemolytic anemia
FAS + Bart's	α -Thalassemia with structural Hb variant	Clinical manifestations, if any, depend on the structural variant (eg, HbE) and severity of α -thalassemia
FAC + Bart's		
FAE + Bart's		
FE + Bart's		
Hemoglobinopathy carriers		
FAS	Sickle cell trait	Normal complete blood cell count; generally asymptomatic
FAC	HbC carrier	No anemia; asymptomatic
FAE	HbE carrier	Normal or slightly decreased MCV without anemia; asymptomatic
FA Other	Other Hb variant carrier	Depends on variant; most without clinical or hematologic manifestations

^a Hemoglobin Bart's, a tetramer of γ -globulin, is present in infants with α -thalassemia.

Modified from: National Heart, Lung, and Blood Institute. Neonatal screening. In: *The Management of Sickle Cell Disease*. Washington, DC: National Institutes of Health; 2002:7–14. NIH publication No. 02-2117.

trait. Homozygous and compound heterozygous individuals have symptomatic disease. Four SCD genotypes (sickle cell anemia, sickle-hemoglobin C disease, and 2 types of sickle β -thalassemia [sickle β^+ -thalassemia and sickle β^0 -thalassemia]) account for most SCD cases in the United States.^{163,164,168} Less-common forms of SCD are caused by coinheritance of hemoglobin S with other hemoglobin variants such as hemoglobin D-Punjab and hemoglobin O-Arab.

Benefits of Newborn Screening

The primary rationale for newborn screening and pre-symptomatic diagnosis is prevention of mortality from pneumococcal sepsis and splenic sequestration during infancy and childhood.^{167,169–172} Prophylactic penicillin has been shown to reduce the incidence of pneumococcal sepsis by 84%¹⁷⁰ and is used in conjunction with pneumococcal conjugate and polysaccharide vaccines and urgent evaluation and treatment of febrile illness with parenteral antibiotics. Family education about signs and symptoms of splenic sequestration results in earlier detection and reduced mortality from that complication.¹⁶⁹ Data from a number of statewide newborn screening programs confirm that mortality from SCD during the first 3 to 4 years of life, historically as high as 20%, is virtually eliminated by universal screening and appropriate follow-up and treatment.¹⁷³

Screening

Most newborn screening programs use isoelectric focusing to separate hemoglobins eluted from dried blood spots. A few programs use high-performance liquid chromatography (HPLC) or cellulose acetate electrophoresis as the initial screening method. Most programs retest screening specimens with abnormal results using a second complimentary electrophoretic technique, HPLC, immunologic tests, or DNA-based assays. The sensitivity and specificity of isoelectric focusing and HPLC are excellent,¹⁶⁷ but results and interpretation can be confounded by extreme preterm birth or previous blood transfusion.^{163,174}

Hemoglobins identified by these screening methods are reported in order of quantity. Because more fetal hemoglobin (HbF) than normal adult hemoglobin (HbA) is present at birth, most normal infants show FA results. Infants with SCD also show a predominance of F at birth; FS, FSC, or FSA are the most common results in children with SCD.

Follow-up and Diagnostic Testing

Infants with screening results indicative of possible SCD (FS, FSC, FSA) should have confirmatory testing of a second blood sample accomplished before 2 months of age. Confirmatory testing is performed by isoelectric focusing, HPLC, hemoglobin electrophoresis (cellulose acetate and citrate agar), and/or DNA-based methods.¹⁶⁴

Most infants with screening results that show HbFS have sickle cell anemia, but other possibilities include sickle β^0 -thalassemia, sickle $\delta\beta$ -thalassemia, and hereditary persistence of fetal hemoglobin, a benign condition. For this reason, testing of parents or DNA analysis may help clarify the diagnosis in selected cases. For infants with probable sickle cell disease, the selection of diagnostic tests and the interpretation of results ideally should be supervised by an expert in the diagnosis of hemoglobin disorders in childhood.

Family testing to identify carriers, for the purpose of defining an infant's diagnosis and/or providing genetic education and counseling, requires a complete blood cell count and hemoglobin separation by electrophoresis, isoelectric focusing, and/or HPLC.^{164,168} Individuals with hemoglobin variants such as S, C, and E are identified readily. Most individuals with heterozygous β -thalassemia show a decreased mean corpuscular volume (MCV) and increased levels of hemoglobin A₂ and/or hemoglobin F. Thus, accurate quantitation of hemoglobin F and hemoglobin A₂ is needed if the MCV is decreased or borderline decreased. Solubility testing is inadequate and should never be used for carrier testing, in part because it will not identify individuals with the hemoglobin C trait and β -thalassemia.

Brief Overview of Disease Management

SCD is a complex disorder with multisystem manifestations that require specialized comprehensive care to achieve an optimal outcome.¹⁶⁸ Family and patient education about the genetics, clinical manifestations, and treatment of SCD and its complications are important, particularly because prompt recognition of potentially life-threatening complications reduces morbidity and mortality. Important health maintenance issues include prophylactic medications, particularly prophylactic penicillin (should be started no later than 2 months of age), and timely immunizations, especially with the pneumococcal conjugate and polysaccharide vaccines. Periodic comprehensive medical evaluations facilitate documentation of important baseline physical findings and laboratory values, detection of signs of chronic organ damage, and development of individualized patient care plans. Timely and appropriate treatment of acute illness is critical, because life-threatening complications can develop rapidly. Some patients, including those who have suffered a stroke or who are identified as being at high risk of stroke by transcranial Doppler ultrasonography screening, receive chronic blood transfusions to prevent stroke and other complications. Selected patients with frequent or severe disease manifestations may benefit from hydroxyurea therapy and/or may be considered for stem cell transplantation, particularly if there is an HLA-matched sibling donor. Guidelines for the management of SCD were published recently.^{168,175}

Current Controversies

Because SCD is more prevalent in some racial and ethnic groups than others, some programs initially implemented selected or targeted screening rather than testing all newborn infants. However, experience with targeted screening showed a rate of missed cases as high as 30%, in part because of difficulties identifying infants' race or ethnicity. In addition, targeted compared with universal screening incurs additional costs and exposes screening programs, nurseries, and physicians to increased litigation risk for the preventable morbidity and mortality that results from delayed diagnosis. For these and other reasons, universal screening is strongly recommended and has been implemented in all 50 states, the District of Columbia, and the US Virgin Islands.

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TYROSINEMIA

There are 2 clinically recognized types of tyrosinemia. Type I (hepatorenal) tyrosinemia (OMIM database No. 276700)¹⁷⁶ is characterized by liver toxicity from increased concentrations of tyrosine and other metabolites with hepatocellular damage. Acutely, this produces jaundice and increased transaminase concentrations. Chronically, there is a high risk of hepatic cancer. Other features include the renal Fanconi syndrome and peripheral neuropathy.¹⁷⁷ Type I tyrosinemia is caused by deficiency of the enzyme fumarylacetoacetate hydrolase (FAH). Type II (oculocutaneous tyrosinemia, also known as Richner-Hanhart syndrome; OMIM database No. 276600) exhibits corneal lesions and hyperkeratosis of the palms and soles. It is caused by deficiency of the enzyme tyrosine aminotransferase (TAT). A third entity, neonatal tyrosinemia, should be mentioned. It is more common in preterm infants and, in fact, is the most common cause of abnormal initial newborn screening results for tyrosinemia and PKU.¹⁷⁸ All show increased concentrations of serum tyrosine that can be detected on newborn screening.

Incidence

Type I tyrosinemia has an incidence of 1 in 12 000 to 1 in 100 000 in those of northern European descent. The incidence of type II and neonatal tyrosinemia has not been established.

Clinical Manifestations

Type I

Type I tyrosinemia in the acute form is characterized by failure to thrive, vomiting, diarrhea, a cabbage-like odor, hepatomegaly, fever, jaundice, edema, melena, and progressive liver disease. If untreated, death from liver failure may occur in the first year of life. The chronic form is similar but with milder features characterized by hypophosphatemic rickets. Other features have included hypertrophic obstructive cardiomyopathy, abdominal crises, polyneuropathy, hypertension, and hepatoma (a late complication in one third of patients). Death occurs during the first decade of life. There are increased concentrations of tyrosine in blood and urine. Urinary tests for succinylacetone and tissue analysis (liver or fibroblasts) for FAH activity establish the diagnosis.

Type II

Type II tyrosinemia is a distinctive oculocutaneous syndrome. Eye findings may be limited to lacrimation, pho-

tophobia, and redness. Signs may include mild corneal herpetiform erosions, dendritic ulcers, and, rarely, corneal and conjunctival plaques. Neovascularization may be prominent. Long-term effects include corneal scarring, nystagmus, and glaucoma. The skin lesions usually begin with or after the eye lesions. Skin findings may begin as painful, nonpruritic blisters or erosions that crust and become hyperkeratotic. They are usually limited to the palms and soles, especially the tips of the digits, and to the thenar and hypothenar eminences. They may be linear or subungual. A skin biopsy is not diagnostic and may show nonspecific hyperkeratosis, acanthosis, and parakeratosis. Skin lesions may be difficult to distinguish from any of the more common forms of keratosis. Mental retardation is an inconstant feature; mild-to-moderate retardation, self-mutilating behavior, disturbances of fine motor coordination, and language deficits have been reported. Tyrosinemia is the diagnostic feature of this disorder. Tyrosine is the only amino acid that is found in increased concentrations in the urine in this disorder.

Neonatal

Clinical findings in neonatal tyrosinemia are nonspecific. Infants with persistent neonatal tyrosinemia may be somewhat lethargic and have difficulty swallowing, impaired motor activity, prolonged jaundice, and increased levels of galactose, phenylalanine, histidine, and cholesterol. Mild acidosis may be present in approximately half of the infants. Mild retardation and decreased psycholinguistic abilities have been noted in some studies.¹⁷⁹

Pathophysiology

Type I

This disorder, although not a primary disorder of tyrosine metabolism, is accompanied by increased concentrations of tyrosine and its metabolites, which inhibit many transport functions and enzymatic activities. It has been proposed that the degree of residual FAH activity determines whether the disease will be acute or chronic in the affected patient.

Type II

This disorder is associated with a deficiency of hepatic TAT, the rate-limiting enzyme of tyrosine catabolism. Tyrosinemia, tyrosinuria, and increases in urinary phenolic acids, *N*-acetyltyrosine, and tyramine persist for life. The metabolism of other amino acids and renal and hepatic function are otherwise normal.

Neonatal

It is generally assumed that this disorder is caused by a relative deficiency of *p*-hydroxyphenylpyruvate oxidase stressed by high-protein diets, with resulting high ty-

rosine and phenylalanine concentrations. Others have suggested a mild decrease in TAT activity.

Inheritance

Type I and II tyrosinemias are autosomal recessive, with a 25% risk of recurrence in siblings. The heterozygotes for type I have approximately half-normal levels of FAH activity in fibroblasts and lymphocytes. Prenatal diagnosis is complex, requiring at least 3 different procedures using amniotic fluid and cultured amniocytes or chorionic villus cells. These procedures involve the direct measurement of succinylacetone by combined gas chromatography and mass spectrometry in amniotic fluid, FAH enzymatic activity, and the measurement of the ability of succinylacetone to inhibit aminolevulinic dehydrase activity in cultured amniotic fluid or chorionic villus cells.¹⁸⁰

The carrier state for type II tyrosinemia has not been detected biochemically, and prenatal diagnosis is not currently available. The inheritance of neonatal tyrosinemia is unclear.

The chromosome map location for type I (FAH) is 15q23-25, the location for type II (TAT) is 16q22.1-22.3, and the location for neonatal (*p*-hydroxyphenylpyruvate) oxidase is 12q24-qter. Type I tyrosinemia is most prevalent in French Canadians, with an overall incidence of as high as 1 in 700 in certain regions of Quebec.¹⁸¹ Type II tyrosinemia cases have been described in several countries including the United States, Canada, Japan, Europe, and the Middle East. Neonatal tyrosinemia is most prevalent in Canadian Inuits.

Rationale for and Benefits of Newborn Screening

Death from complicating liver failure occurs in untreated patients with type I tyrosinemia during the first year of life in the acute form and during the first decade of life in the chronic form. Hepatocellular carcinoma may also be a cause of death. The introduction of 2-(2-nitro-4-trifluoromethylbenzyl)-1,3-cyclohexanedione (NTBC) has changed the outcome of this disorder dramatically.¹⁸² More than 90% of patients respond clinically to treatment with NTBC. The current indications for liver transplantation in type I tyrosinemia are nonresponsiveness to NTBC, risk of malignancy, and decreased quality of life related to dietary restriction and frequency of blood sampling. Successful liver transplantation can further reduce the mortality rate in nonresponders to 5%.¹⁸³ There is a strong decrease in the risk of early development of hepatocellular carcinoma in patients with effective, early therapy.

Screening

The BIA can be used to screen for tyrosinemia using dried blood spots. Abnormal concentrations of tyrosine are reported as more than 6 mg/dL. Newer methods include direct measurement of tyrosine by MS/MS. The

test is performed in the neonatal period, but the optimal time for study is unclear. Presumably, it is best if measurements are obtained 48 to 72 hours after milk feeding. The stability of tyrosine in specimens has not been determined specifically but should be similar to that of phenylalanine. The rate of false-negative results has not been determined. Data from the 1999 National Newborn Screening Report¹⁸⁴ showed an initial positive screening result in 136 of 407 118 newborn infants tested (1 in 3000), with 2 positive confirmed cases. Available data on second screenings performed between 1 and 4 weeks of age showed 2 positive results in 60 474 infants (1 in 30 000); no cases of tyrosinemia were confirmed among this group.

Follow-up and Diagnostic Testing

An increased tyrosine concentration on newborn screening requires confirmation and additional testing, because it may be caused by other metabolic disorders (eg, fructose and galactose enzyme deficiencies), giant cell hepatitis, neonatal hemochromatosis, and neonatal infections. The optimal approach is complex and requires determination of the concentrations of tyrosine and other amino acids and metabolites in the blood and urine. Type I tyrosinemia involves increased concentrations of urine succinylacetone and nonspecific aminoaciduria and requires tissue analysis (fibroblasts, erythrocytes, lymphocytes, or liver) for FAH activity. Type II tyrosinemia involves increased tyrosine concentrations only in blood and urine. Confirmation of neonatal tyrosinemia depends on the presence of increased concentrations of tyrosine and phenylalanine.

Brief Overview of Disease Management

Type I

Treatment options for tyrosinemia include dietary therapy, liver transplantation, and use of the pharmacologic agent NTBC. Clinical signs and symptoms improve with NBTC therapy and diet.¹⁸² Signs of improvement include a decrease in concentrations of metabolites, correction of the secondary abnormality in porphyrin synthesis, improved liver and renal function, and regression of hepatic abnormalities by computed tomography. Correction of porphyrin synthesis reduces the risk of porphyric crises.

Type II

Therapy with a diet low in tyrosine and phenylalanine is curative in type II tyrosinemia. Early diagnosis can help avoid the risk of mental retardation in these patients.

Neonatal

Most cases of neonatal tyrosinemia, especially those seen in small preterm infants, may be transient and controlled by reducing the protein intake to 2 to 3 g/kg per day or

by breastfeeding. Some patients respond to ascorbic acid supplementation.

Current Controversies

The incidence and pathogenetic mechanisms of specific disorders associated with increased concentrations of tyrosine require clarification. The consequences of early diagnosis and treatment for type I tyrosinemia (the most formidable disorder in this group) should be beneficial. NBTC therapy seems to be very effective. No marked adverse effects have been noted. Follow-up for long-term outcome is needed.

Special Issues/Concerns

Confirmation of the exact cause of increased concentrations of tyrosine requires referral and evaluation by an expert in the field. Outcome with treatment remains variable.

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Newborn Screening Fact Sheets

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Pediatrics 2006;118;e934

DOI: 10.1542/peds.2006-1783

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