Technical standards and guidelines for the diagnosis of biotinidase deficiency

Tina M. Cowan, PhD1, Miriam G. Blitzer, PhD2, and Barry Wolf, MD, PhD3; A Working Group of the American College of Medical Genetics (ACMG) Laboratory Quality Assurance Committee

Abstract: Biotinidase deficiency is an autosomal recessively inherited disorder of biotin recycling that is associated with neurologic and cutaneous consequences if untreated. Fortunately, the clinical features of the disorder can be ameliorated or prevented by administering pharmacological doses of the vitamin biotin. Newborn screening and confirmatory diagnosis of biotinidase deficiency encompasses both enzymatic and molecular testing approaches. These guidelines were developed to define and standardize laboratory procedures for enzymatic biotinidase testing, to delineate situations for which follow-up molecular testing is warranted, and to characterize variables that can influence test performance and interpretation of results. Genet Med 2010;12(7):464–470.

Key Words: clinical genetic testing, technical standards and guidelines, biotinidase deficiency, multiple carboxylase deficiency, newborn screening

From the 1Department of Pathology, Stanford University Medical Center, Stanford, California; 2Division of Human Genetics, Department of Pediatrics, University of Maryland School of Medicine, Baltimore, Maryland; and 3Department of Medical Genetics, Henry Ford Hospital, and Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, Michigan.

Disclosure: The authors declare no conflict of interest.

Technical standards and guidelines for the diagnosis of biotinidase deficiency

Disclaimers: These standards and guidelines are designed primarily as an educational resource for clinical laboratory geneticists to help them provide quality laboratory genetic services. Adherence to these standards and guidelines does not necessarily ensure a successful medical outcome. These standards and guidelines should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the clinical laboratory geneticists should apply their own professional judgment to the specific clinical circumstance presented by the individual patient or specimen. It may be prudent, however, to document in the laboratory record the rationale for any significant deviation from these standards and guidelines.

Biotinidase deficiency, also known as late-onset multiple carboxylase deficiency, is an autosomal recessively inherited disorder of biotin recycling associated with secondary alterations in amino acid, carbohydrate, and fatty acid metabolism. The disorder is caused by absent or markedly deficient activity of biotinidase, a cytosolic enzyme that liberates free biotin from biocytin during the normal proteolytic turnover of holocarboxylases and other biotinylated proteins. Biotinidase may also play a role in the biotinylation of specific proteins, such as histones. The diagnosis of biotinidase deficiency is based on demonstration of deficient enzyme activity in serum or plasma. Patients with profound biotinidase deficiency have <10% mean normal serum activity, whereas patients with the partial biotinidase deficiency variant have 10–30% of mean normal serum activity and are largely asymptomatic. Confirmation of biotinidase deficiency by DNA analysis, by either allele-targeted methods or full gene sequencing, may be useful in some cases. Biotinidase testing may be part of a larger workup in a child who exhibits clinical features suggestive of the disease but more frequently is performed on asymptomatic infants after abnormal newborn screening results. Because all newborn screening programs in the United States and many other countries worldwide include biotinidase deficiency in their panel of screened conditions, there is an increased need to define and standardize laboratory approaches for enzymatic biotinidase testing, to delineate situations for which follow-up molecular testing is warranted, and to characterize variables that can influence test performance and interpretation of results. These guidelines describe best laboratory practices for biotinidase deficiency testing, including preanalytical considerations, test selection and performance, results interpretation, and appropriate follow-up testing.

BACKGROUND

Biochemical and molecular characteristics of biotinidase

Biotinidase (E.C. 3.5.1.12) is a 70–80 kD glycoprotein that is ubiquitously expressed and detectable in multiple sample types including serum, leukocytes, fibroblasts, and liver. The enzyme functions in the recycling of biotin, a water-soluble cofactor necessary for the function of four carboxylases: propionyl-CoA carboxylase (E.C. 6.4.1.3), 3-methylcrotonyl-CoA carboxylase (E.C. 6.4.1.4), pyruvate carboxylase (E.C. 6.4.1.1), and acetyl-CoA carboxylase (E.C. 6.4.1.2). An inherited disorder of biotin recycling or utilization, from a deficiency of biotinidase (OMIM 253260) or holocarboxylase synthetase (OMIM 253270), leads to secondary disruptions in the activities of the aforementioned carboxylases which, in turn, cause impairments in the metabolic pathways of amino acids, carbohydrates, and lipids.
Biotinidase is a monomeric enzyme encoded by a single gene (BTD) located on chromosome 3p25 and comprised 543 amino acid residues including 41 amino acids of a potential signal peptide. The gene spans at least 23 kb of genomic DNA and consists of four exons and three introns, reflecting a simple structural organization that facilitates gene sequencing for the genotyping of patients. Two putative translation initiation codons exist, one in exon 1 and the other in exon 2, with the second containing the N-terminal methionine of the mature enzyme and also containing features of housekeeping genes. The sequences upstream of exons 1 and 2 contain promoter elements consistent with the ubiquitous expression of biotinidase. The presence of an intron between the two possible initiation codons could allow for alternative splicing, raising the possibility of tissue-specific expression of different BTD transcripts. In support of this idea, a consensus sequence for the liver-specific transcription factor HNF-5 is present at nucleotide position -352, although the physiologic significance of this is unknown.

More than 100 disease-causing mutations in the BTD gene have been reported to date. Four common mutations cause profound biotinidase deficiency. Among children ascertained because of clinical symptoms, the two most commonly reported mutations are a seven-base deletion/three-base insertion (c.98-104del7ins3 [G98d7i3]) in exon 2, occurring in at least 50% of patients, and R538C in exon 4, occurring at least 30% of children. Both of these mutations result in a complete absence of biotinidase protein. Other relatively common mutations discovered by newborn screening are Q456H, associated with profound deficiency, and D444H, a mild mutation that reduces enzymatic activity by ~50%. Another mutation is associated with partial biotinidase deficiency, whereas in cis with A171T (i.e., as the double mutant A171T/D444H), results in profound biotinidase deficiency allele. A compilation of disease-causing BTD mutations has been reported.

Initially, most symptomatic children with biotinidase deficiency were found to have 3% of mean serum biotinidase activity of normal individuals. Three standard deviations above this mean, corresponding to 10% of mean normal activity, was taken as the threshold below which individuals were considered to have profound biotinidase deficiency. After newborn screening for biotinidase deficiency was introduced, babies were identified with ~25% of mean normal activity. Ultimately, it was determined that essentially all these children had the D444H mutation as one of their alleles, with the resultant aberrant enzyme contributing ~50% of normal activity. This mutation, together with a mutation for profound deficiency on the other allele, results in profound biotinidase deficiency. Because biotinidase deficiency is not due to a continuous spectrum of residual activity but rather a biphasic distribution of activity, the terms profound biotinidase deficiency and partial biotinidase deficiency are useful functional descriptions.

Clinical description of disease

The initial clinical presentation and ultimate expression of profound biotinidase deficiency are quite variable, even within the same family. Symptoms in untreated patients usually appear between 2 and 5 months of age but may not be evident until several years of age. Abnormalities involving the central nervous system are frequently the first features to occur. More than 70% of clinically ascertained children exhibit seizures, hypotonia, skin rash, or alopecia at some time before diagnosis and treatment. About half of the children have ataxia, developmental delay, conjunctivitis, and visual problems, including optic atrophy. More than three quarters of symptomatic children develop hearing loss. The hearing loss, visual abnormalities, and degrees of developmental delay do not seem to be reversible once they occur, even after initiation of biotin therapy. Affected individuals may have features ranging from multiple mild episodes of seizures and ataxia, to severe metabolic compromise, which can result in coma or death. Some children with profound biotinidase deficiency developed symptoms only later in childhood or during adolescence. They exhibited motor limb weakness, spastic paresis, and eye problems, such as loss of visual acuity and scotomata, rather than the more characteristic symptoms observed in young untreated children with the disorder. There are several reports of adults with profound biotinidase deficiency who were ascertained because they had children with profound biotinidase deficiency identified by newborn screening. These adults were asymptomatic although they had never been treated with biotin. Several of these parents had extensive evaluations, including rigorous dietary histories, but they failed to demonstrate any clinical or biochemical features. None of these adults had null mutations, but all had enzymatic activities in the profoundly deficient range with some residual activity. The explanation as to why they have remained asymptomatic is not known.

Biochemically, untreated individuals may exhibit metabolic ketoacidosis, lactic acidosis, and/or hyperammonemia. Other metabolic abnormalities are more variable and may include elevated excretion of 3-hydroxyisovalerlic, lactic and 3-hydroxypropionic acids, and 3-methylcrotonylglycine by urine organic acid analysis, as well as mildly elevated 3-hydroxyisovalerylcarnitine (C5-OH) by plasma acylcarnitine analysis. These metabolic abnormalities are variable, and affected children, whether symptomatic or asymptomatic, do not always exhibit ketoacidosis or organic aciduria.

Biotin supplementation seems to prevent the development of symptoms in asymptomatic children with profound biotinidase deficiency. Furthermore, all symptomatic children with profound deficiency who have been treated with pharmacological doses of biotin (5–20 mg daily) have shown clinical improvement. Seizures and ataxia resolve within hours to days, and the cutaneous manifestations usually resolve within weeks. Depending on the severity and frequency of episodes of metabolic and neurologic compromise, many children with developmental delay rapidly achieve new milestones or regain those that were lost. Children with biotinidase deficiency who have failed to comply with their biotin therapy, either unintentionally or deliberately, developed symptoms within several weeks to months. These cases illustrate the importance of early diagnosis and initiation of lifelong treatment.

Partially biotinidase deficiency, defined as 10–30% of mean normal serum activity, initially was considered a variant without clinical consequences. Later, several reports described children with partial biotinidase deficiency who developed symptoms when under stress, such as an infection or starvation. The symptoms resolved with biotin therapy.

Newborn screening for biotinidase deficiency

Methods for the semiquantitative determination of biotinidase activity on dried blood spots were developed in 1984 and first used that same year in a pilot newborn screening program in Virginia. Biotinidase deficiency meets many of the criteria for inclusion in state newborn screening programs: there is an inexpensive and reliable screening test, the disorder is associated with high morbidity and mortality if untreated, an easy and
effective treatment exists, and the population incidence is comparable with that of other disorders screened.34 Currently, all newborn screening programs in the United States and more than 30 other countries screen their newborns for biotinidase deficiency.35,36 The screening method used by almost all programs is based on a colorimetric assay of biotinidase activity in dried blood spots,33 although states have individually established their own screening cutoffs and rescreening or follow-up protocols. Some states report qualitative screening results as either positive or negative, whereas others report quantitative enzyme activities with a screening cutoff value.

Because biotinidase deficiency screening is performed by direct enzyme assay, results are not influenced by dietary protein intake as is the case for phenylketonuria or other amino acidopathies. Approximately 50% of false-positive results (i.e., low estimates of enzyme activity) are due to prematurity and most others to mishandling of samples and possibly their exposure to excessive heat and/or humidity.37,38 The effect of transfusion on biotinidase screening has not been established. Because red blood cells do not express biotinidase activity, it may be helpful to determine whether the patient was transfused with whole blood or packed red blood cells. However, variable, but possibly significant, quantities of plasma/serum are retained in the preparations of packed red blood cells, and the “circulating life” of biotinidase in these preparations is unknown. One approach for determining the child’s actual enzyme status after transfusion is to measure biotinidase activities in the parents. If both parents are clearly in the normal range, then it is unlikely that the child has profound or partial deficiency. Mutation analysis may also be used to resolve the issue. It is also possible to provide the infant with biotin supplementation for several months before repeating the confirmatory testing, because this treatment does not affect performance of the biotinidase assay. In general, the parental enzymatic results are reassuring.

Many of the clinical issues and frequently asked questions about biotinidase deficiency have been addressed recently in an article by one of the authors.39

**Conditions identified by enzymatic biotinidase testing**

The enzymatic determination of biotinidase activity identifies individuals with profound biotinidase deficiency (<10% mean normal serum activity) and those with partial biotinidase deficiency (10–30% of mean normal serum activity).19 Heterozygous individuals may also be identified by this test as having ~50% of mean normal activity, although there may be significant overlap among the various groups. At least some of the variability arises from clinical status, assay interferents, and sample handling artifacts (see later). Before a diagnostic category can definitively be assigned, the laboratory must establish ranges from known affected individuals and obligate heterozygotes. In addition, all preanalytical and analytical variables must be carefully controlled before a measured decrease in activity can be attributed to an inherited defect in biotinidase.

**Prevalence**

Based on newborn screening outcome data from 2007 to 2008, the incidence of profound biotinidase deficiency in the United States is estimated at ~1 of 80,000 births and that of partial biotinidase deficiency between 1 of 31,000 and 1 of 40,000 (available at: http://www2.uthscsa.edu/mnis/s/), although frequencies may vary within and between states by local population. Earlier studies have indicated similar findings, with the overall incidence of biotinidase deficiency estimated at ~1 in 60,000 newborns worldwide, equally divided between profound and partial biotinidase deficiency.35 The majority of reported patients who were ascertained clinically have been of European descent.36 The disease incidence varies between countries, with incidences in Brazil as high as 1 in 9,00040 and possibly higher incidences in countries with a high degree of consanguinity, such as Turkey and Saudi Arabia.41–43

**Mode of inheritance**

Biotinidase deficiency is inherited as an autosomal recessive trait.4

**PREANALYTICAL REQUIREMENTS**

**Sample types**

Biotinidase activity is reliably measured in serum and plasma; activity can less readily be determined in fibroblasts and leukocytes and other tissue extracts.44 Biotinidase activity can also be measured in cultured amniocytes,45 although in practice this is seldom performed because of the treatability of the disorder. Plasma or serum samples may not be suitable for individuals who have undergone transfusion; in such cases, determining the carrier status of the parents may be an alternate strategy (see “Newborn screening for biotinidase deficiency”). Samples collected postmortem are not suitable for enzyme assay; in such cases, the diagnosis may be established by molecular testing or by enzymatically determining the carrier status of the parents. Molecular studies for BTD mutations are performed on DNA isolated from leukocytes, fibroblasts, or dried blood spots.

**Sample volumes**

Specific requirements of sample type, collection volumes, and conditions of sample shipping and handling must be established by the laboratory and made available to referring physicians on request. As a general guideline, 1–2 mL of serum or plasma is required for biotinidase testing. Approximately 2–3 mL of whole blood should be collected by venipuncture in a red-top or green-top (sodium or lithium heparin) tube, and the cells separated from serum/plasma by centrifugation within 1 hour of sample collection. Samples should be immediately frozen at ~80°C, and should remain frozen at this temperature until the time of testing. When testing is being performed after an abnormal newborn screening result, blood should also be collected from the parents and sent together with the patient’s sample whenever possible to aid in the interpretation of results. Similarly, a sample obtained from an unrelated control is useful for distinguishing true biotinidase deficiency from decreased activity because of sample handling artifacts. DNA testing typically requires 1–2 mL whole blood collected in a lavender-top (EDTA) tube.

**Conditions of sample shipping, handling, and storage**

Blood spots collected for newborn screening must be dried completely before shipping to the laboratory, because humidity or wet samples result in significant loss of enzyme activity. Serum or plasma samples for diagnostic assays must be frozen at ~80°C and shipped to the laboratory on dry ice. Long-term storage of samples at ~20°C has been shown to lead to loss of enzyme activity and artifically low values46 (Wolf, unpublished data). As noted earlier, the use of parental and/or unrelated controls is recommended whenever possible, although this may not be operationally feasible in all settings. Samples for DNA testing should be shipped to the laboratory at room...
### Table 1: Biotinidase activities of unaffected individuals, carriers, patients with biotinidase deficiency ascertained either by clinical symptoms or newborn screening, and patients with partial biotinidase deficiency

<table>
<thead>
<tr>
<th>Category</th>
<th>Biotinidase activity ± SD (nmol/min/ml serum), (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unaffected individuals</td>
<td>7.57 ± 1.41 (100)</td>
</tr>
<tr>
<td>Parents of children with profound biotinidase deficiency</td>
<td>3.49 ± 0.72 (21)</td>
</tr>
<tr>
<td>Children with profound biotinidase deficiency ascertained by clinical symptoms</td>
<td>0.12 ± 0.18 (23)</td>
</tr>
<tr>
<td>Children with profound biotinidase deficiency ascertained by newborn screening</td>
<td>0.19 ± 0.16 (41)</td>
</tr>
<tr>
<td>Individuals with partial biotinidase deficiency</td>
<td>1.47 ± 0.41 (23)</td>
</tr>
</tbody>
</table>

Data adapted from references 67 and 68.

### METHOD VALIDATION

**Calibration and quantitation**

The most widespread method for the diagnosis of biotinidase deficiency is based on enzymatic assay using the artificial substrate biotin-4-amidobenzoic acid.\(^4\)\(^{,}\)\(^{33}\)\(^{,}\)\(^{47}\) This substrate and all other reagents used in the assay are commercially available but must be individually prepared and validated by each laboratory before being put into use. Validation of assay performance should be established by assessing inter- and intraday variability, analytical measurement range, and effects of interference. In particular, sulfa drugs are known to interfere and can lead to falsely elevated estimates of activity if not properly controlled (see later). Performance characteristics should be verified on a regular basis as specified by the Clinical Laboratory Improvement Amendments.

**Reference ranges**

Biotinidase activity reference ranges for normal, affected, and obligate carrier individuals have been published and are summarized in Table 1. Before clinical enzymatic testing is implemented, a laboratory should determine the mean biotinidase activity of a cohort of normal individuals (e.g., 20–50) and obligate carrier individuals have been published and are periodically verified and updated as specified by the Clinical Laboratory Improvement Amendments.

**Testing personnel**

Appropriate training and ongoing competency requirements for laboratory personnel performing biotinidase deficiency testing must be established and documented. Testing personnel must satisfy CLIA requirements for high-complexity testing and have, at a minimum, an associate degree in a laboratory science or medical laboratory technology from an accredited institution. Stricter requirements apply in some states.

### TESTING FOR BIOTINIDASE DEFICIENCY

**Sample preparation**

For enzymatic testing, serum or plasma samples should be thawed immediately before analysis with no other sample preparation steps required. Information pertaining to the collection, analysis, interpretation, and quality control of molecular studies is given by the American College of Medical Genetics Standards and Guidelines for Clinical Genetics Laboratories, Part G (Molecular Genetics), available at: [http://www.acmg.net/AM/Template.cfm?Section=Laboratory_Standards_and_Guidelines&Template=/CM/HTMLDisplay.cfm&ContentID=3735](http://www.acmg.net/AM/Template.cfm?Section=Laboratory_Standards_and_Guidelines&Template=/CM/HTMLDisplay.cfm&ContentID=3735).

**Analytical methods**

The most widespread method for the diagnosis of biotinidase deficiency is based on enzymatic assay using the artificial substrate biotin-4-amidobenzoic acid.\(^4\)\(^{,}\)\(^{33}\)\(^{,}\)\(^{47}\) In a typical assay, serum is incubated with a substrate buffer at 37°C for 30 minutes and the reaction stopped with trichloroacetic acid. During the reaction, biotinidase in patient serum acts to liberate free p-amidobenzoic acid (PABA) from the artificial substrate. The released PABA is diazotized with sodium nitrite and the excess nitrite removed by the addition of ammonium sulfamate. Finally, the diazotized PABA reacts with N-1-naphthyl-ethyl-ene-diamine dihydrochloride to yield a mauve-colored product, which is measured spectrophotometrically at 546 nm. The net absorbance is directly proportional to the amount of PABA released, which, in turn, is directly proportional to biotinidase activity in the sample. This method also forms the basis for newborn screening for biotinidase deficiency\(^33\) and had been adapted for use with microtiter plates.\(^49\)

Biotinidase activity can also be measured using a fluorescent technique with biotinyl-6-aminocoumarine as an artificial substrate.\(^50\) This substrate is more expensive than that for the colorimetric method but has also been adapted to newborn screening and the testing of dried blood spots.\(^51\)\(^{,}\)\(^{52}\) There are other methods for determining biotinidase activity, such as measuring the release of biotin from biocytin,\(^52\)\(^{,}\)\(^{53}\)\(^{,}\)\(^{54}\) using other fluorescent biotinylated derivatives\(^4\) and radioisotopic biotinylated analogs,\(^4\)\(^{,}\)\(^{45}\) but they are more expensive, require more time, are difficult to perform, and often are not readily adaptable to enzymatic determinations using dried blood spots.\(^51\)\(^{,}\)\(^{55}\)

Analysis of urine organic acids by gas chromatography/mass spectrometry or plasma acylcarnitines by liquid chromatography-tandem mass spectrometry sometimes may reveal characteristic abnormalities in patients with biotinidase deficiency. However, this approach is never appropriate as the sole testing modality in individuals suspected of having the disorder because many biotinidase deficient patients will have normal results by analyte testing and, therefore, be missed. Conversely, metabolic abnormalities that may be compatible with biotinidase deficiency, including elevations of 3-hydroxyisovalerate, 3-methylcrotonylglycine, and 3-hydroxyisovaleryl carnitine, can also be a characteristic of other conditions and disease states and, if not confirmed by enzyme assay, can lead to an incorrect diagnosis of biotinidase deficiency. Therefore, if biotinidase deficiency is excluded by enzyme testing, other conditions must be considered.
Because of the simple organization of the BTD gene, follow-up molecular testing by either targeted mutation analysis or complete sequencing is possible. Molecular testing is particularly useful for differentiating individuals with profound biotinidase deficiency from those who have partial deficiency and those with partial deficiency from those who are heterozygous for profound deficiency, because almost all children with partial deficiency have the D444H mutation. Common mutations, including D444H, can be readily determined by targeted analysis using DNA extracted from a dried blood spot. If results are not conclusive after performing this testing, complete sequencing and, when necessary, deletion/duplication analysis, is also clinically available.

**Quality control**

A positive (abnormal) and normal control should be included with each set of patient samples. The positive control, representing decreased biotinidase activity, may be prepared by heat inactivating a large pool of normal serum at 80°C for 1 hour. Both sets of controls should be stored as aliquots at −80°C and thoroughly evaluated before being used in clinical testing. Tolerance limits for acceptance or rejection of quality control (QC) performance should be established, and procedures should be developed for instances where QC criteria are not met. A QC designee should perform an ongoing (at least monthly) compilation, review, and sign off of control values.

For diagnostic evaluations, all samples should be tested for the presence of interfering substances, such as sulfa drugs, which may cause false-negative results. This is readily performed by running a substrate-free blank for each patient sample tested (i.e., by testing the serum/plasma for positive color development in the absence of substrate). This control is typically not performed for newborn screening assays and, fortunately, sulfa drugs are contraindicated during pregnancy and in neonates.

**Proficiency testing**

An ongoing proficiency testing (PT) program for biotinidase testing must be implemented and documented. An external PT program for biotinidase deficiency newborn screening is offered through the Newborn Screening Quality Assurance Program of the Centers of Disease Control and Prevention.60 No external PT programs for diagnostic testing are currently available. A number of approaches given by the Clinical and Laboratory Standards Institute may be taken, including split sample analysis with a reference or other laboratory and storing aliquots of samples from affected individuals (at −80°C) and rerunning these samples periodically.61

**TEST INTERPRETATION AND REPORTING**

**Interpretation**

For tests initiated because of abnormal newborn screening, biotinidase activity <10% of mean normal activity is indicative of profound deficiency, and activity between 10 and 30% of mean normal activity is indicative of partial deficiency. Laboratories should apply these guidelines by comparing a child’s activity with the mean normal biotinidase activity established in their laboratory (see “Reference ranges”). Decreased enzymatic activity can also be due to prematurity or liver dysfunction.62,63 Although, if all preanalytical variables are properly controlled, this typically does not lead to misdiagnosis of biotinidase deficiency. Biotinidase activity in newborns usually increases during the first days to weeks of life, and those with activities between 50 and 70% of mean normal activity do not usually require retesting at a later date. The most common cause of decreased enzymatic activity is sample mishandling. Therefore, a definitive diagnosis of biotinidase deficiency based on low enzyme activity cannot be made unless these variables are properly controlled. The optimal approach is to determine enzyme activities on samples from an unrelated individual (normal control) and both parents (if available). These samples should be collected simultaneously and sent with the patient’s sample for testing. The authors are aware of multiple instances where children were diagnosed with profound biotinidase deficiency by reputable laboratories but did not have appropriate unrelated or parental control samples for comparison. Several of these children were treated for extended periods with biotin before repeat enzyme testing, initiated as part of a later family study or because clinical symptoms failed to improve, revealed normal biotinidase activities. These examples emphasize the importance of obtaining appropriate control samples to avoid misdiagnosis of enzyme deficiency. If there is any question of interpretation, gene sequencing can be used to confirm or exclude the diagnosis (targeted mutation analysis will not identify all the possible mutations).

Biotinidase activity in the range of 10–30% of mean normal activity is suggestive of partial biotinidase deficiency. Typically, when testing the parents, one has ~50% of mean normal activity, indicative of being a carrier for a profound deficiency allele, and the other parent has activity that is ~75% of mean normal activity, indicative of being a carrier for the D444H mutation. It is not uncommon that one or both parents have activities that are not clearly interpretable, such as clearly in the normal range. These cases can be resolved by mutation analysis. Occasionally, a parent has enzymatic activity in the heterozygous range and is ultimately found to be homozygous for the D444H allele by mutation analysis or a parent will be found to have partial biotinidase deficiency.

Intermediate biotinidase activity (i.e., 50% of normal) could be compatible with either heterozygosity for profound biotinidase deficiency or homozygosity for a variant allele, most often D444H. Again, evaluation of biotinidase activity in the parents is useful in distinguishing between these possibilities but still may require mutation analysis, possibly targeted analysis for the D444H mutation, to definitively determine their status. When interpreting results for possible carrier or variant status, the laboratories must have sufficient experience with the enzymatic ranges of these genotypic groups and must have proper controls in place.

For patients with clinical symptoms suggestive of biotinidase deficiency, the disorder is excluded by enzyme activity >10% of normal. Enzyme activity <10% of normal is consistent with profound deficiency, providing that the proper controls were in place to exclude sample mishandling as the source of decreased activity.57 Confirmation by molecular testing is often useful but may not be necessary in all cases.

Mutation analysis for biotinidase deficiency is readily available. The BTD gene is composed of four exons, permitting rapid sequencing of the entire complementary DNA and intron-exon junctions.8 Mutation analysis is not suitable for newborn screening but is useful for confirmatory testing to help differentiate between individuals with profound and partial biotinidase deficiency, and individuals who are carriers for profound deficiency and those homozygous for a partial deficiency allele. It is also useful for family studies and prenatal diagnosis. Optimal sample preparation, handling, and shipping together with the inclusion of appropriate control samples often makes molecular analysis unnecessary and can eliminate the expense of repeated confirmational testing.40,61
There is still little known about phenotype/genotype correlations. There is one report of such a relationship between genotype and hearing loss. In addition, it has been proposed that children with some residual enzyme activity may have a milder clinical course than those with no measurable biotinidase activity and may have a lower requirement for biotin supplementation for treatment. However, it is important that all children with profound biotinidase deficiency be treated with adequate biotin therapy.

Reporting

Patient reports must contain appropriate patient and specimen information as given by the American College of Medical Genetics Standards and Guidelines for Clinical Genetics Laboratories, Sections 2.4, 2.41 and 2.42 (http://www.acmg.net/AM/Laboratory_Standards_and_Guidelines/Laboratory_Standards_and_Guidelines.cfm?Section=ratories, Sections 2.4, 2.41 and 2.42 (http://www.acmg.net/AM/Laboratory_Standards_and_Guidelines/Laboratory_Standards_and_Guidelines.cfm?Section=ratories, Sections 2.4, 2.41 and 2.42 (http://www.acmg.net/AM/Laboratory_Standards_and_Guidelines/Laboratory_Standards_and_Guidelines.cfm?Section=ratories, Sections 2.4, 2.41 and 2.42). Written reports should contain biotinidase activity, units of measure, and an appropriate reference range, together with an interpretation (see “Interpretation”). Recommendations for follow-up, including referral to a metabolic specialist, should also be included when appropriate.

ACKNOWLEDGMENTS

This work is supported in part by the Safran Research Fund (B.W.). The authors thank Drs. Jerry Feldman, Maria Descartes, and Robert Steiner for their constructive comments and suggestions. The revised document was reviewed and endorsed by the Biochemical Genetics Working Group of the ACMG Quality Assurance Committee.

REFERENCES


4. Wolf B, Grier RE, Allen RJ, Goodman SI, Kien CL. Biotinidase deficiency: activity, units of measure, and an appropriate reference range, specified by CLIA. Written reports should contain biotinidase activity, units of measure, and an appropriate reference range, together with an interpretation (see “Interpretation”). Recommendations for follow-up, including referral to a metabolic specialist, should also be included when appropriate.


