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CLINICAL UTILITY GENE CARD

Clinical utility gene card for: Biotinidase deficiency

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1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)

Biotinidase deficiency (late-onset multiple carboxylase deficiency; late-onset biotin-responsive multiple carboxylase deficiency; juvenile-onset multiple carboxylase deficiency; BTD deficiency).

1.2 OMIM# of the disease

253260.

1.3 Name of the analysed genes or DNA/chromosome segments

BTD.[1]

1.4 OMIM# of the gene(s)

609019.

1.5 Mutational spectrum

Biotinidase deficiency is inherited as an autosomal recessive trait; as expected, the vast majority of mutations are homozygous or compound heterozygous. A total of 140 mutations have been reported so far. All types of alterations have been observed: missense, nonsense, splice-site, and frameshift mutations.[2] However, no large deletion has been identified to date. No hotspot mutation region has been observed and alterations have occurred throughout the coding sequence, except in exon 1 that may not contain the true initiation ATG codon. Five mutations account for about 60% of the genetic abnormalities encountered in individuals who are symptomatic or were identified by newborn screening: c.98_104del7ins3 (p.Cys33PhefsX36), c.1368A>C (p.Gln456His), c.1612C>T (p.Arg538Cys), c.1330G>C (p.Asp444His), and the combined allelic alteration c.[511G>A;1330G>C] (p.[Ala171Thr;Asp444His]).[3–5]

1.6 Analytical methods

Site-specific mutation analysis can focus on the five most frequent mutations cited above using real-time PCR.[6] Another widely used method is the bi-directional gene sequencing of all four *BTD* exons and their flanking intronic sequences, which increases the detection rate to 99%.[5] Alternatively, dHPLC has been proposed for the screening of *BTD* variants,[7] but its use is not yet generally used for diagnostic purposes, and the identification of variants by this method requires confirmation by sequencing. Screening of large rearrangements is also possible, but no such alteration has yet been reported.

1.7 Analytical validation

Using real-time PCR, the validation of the results is accomplished by comparing the results of positive and negative controls. Using direct gene sequencing, validation is performed by sequencing both DNA strands, thereby excluding possible artifacts. Finding known mutations can be correlated with the biochemical enzymatic results (see 3.1.2 for explanations on classification of biotinidase deficiency according to enzymatic activity):[8,9] severe mutations, such as c.98_104del7ins3 (p.Cys33PhefsX36), c.1368A>C (p.Gln456His), or c.1612C>T (p.Arg538Cys), are associated with profound biotinidase deficiency when they are present in the homozygous or the compound heterozygous state; in contrast, milder mutations, such as c.1330G>C (p.Asp444His), result in partial biotinidase deficiency, if they are homozygous or compound heterozygous. It is worth noting that the mutation c.1330G>C (p.Asp444His) causes partial biotinidase deficiency when in *trans* with a mutation with very low biotinidase activity or profound biotinidase deficiency, whereas it causes profound biotinidase deficiency when in *cis* with c.511G>A (p.Ala171Thr). When a suspected novel mutation is identified on both alleles, it can be validated by correlating the suspected mutation with the individual's enzymatic activity, the presence of the mutations in the parents and correlating with their respective enzymatic activities, and by comparing the alteration to mutations and polymorphisms found in previously confirmed individuals with the disorder often available in public databases (eg, dbSNP). Potential pathogenic effects of the mutation can be assessed using various prediction bioinformatic programmes. Correlation of the suspected mutation with enzymatic activity will exclude the possibility that the alteration is a polymorphism.

1.8 Estimated frequency of the disease (incidence at birth ('birth prevalence') or population prevalence)

In 2004, the incidence of biotinidase deficiency in Europe was estimated to 1:47 486, according to statistics data collected through newborn screening programmes in seven countries (Austria, Belgium, Germany, Italy, Spain, Sweden, and Switzerland).[10] In 1991, the worldwide incidence for combined profound and partial biotinidase deficiency was approximately 1:60 000 based on 8.5 million newborn infants identified by newborn screening in 14 countries of which the majority were of European descent.[11]

1.9 If applicable, prevalence in the ethnic group of investigated person

Approximately 1:60 000 for combined profound and partial biotinidase deficiency, according to extrapolations of prevalence made by the US Census Bureau in 2004.

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1.10 Diagnostic setting

	Yes	No
A. (Differential) diagnostics	☒	☐
B. Predictive testing	☒	☐
C. Risk assessment in relatives	☒	☐
D. Prenatal	☒	☐

Comment:
Biotinidase deficiency is an autosomal recessively inherited disorder that, if untreated, usually manifests in children from 1 week of age to adolescence, with most exhibiting symptoms between 3 and 6 months of age.[12] If undetected and untreated, children with profound biotinidase deficiency usually develop one or more of the following: neurological symptoms (myoclonic seizures, hypotonia, ataxia, developmental delay, vision problems, and/or hearing loss)[13] and dermatological symptoms (alopecia, eczema, and/or candidiasis). In a few cases, individuals with profound biotinidase deficiency may remain asymptomatic or develop symptoms after adolescence.[14] Asymptomatic individuals usually have partial biotinidase deficiency and stress appears to trigger the development of symptoms, including principally hypotonia, skin rash, and hair loss.

2. TEST CHARACTERISTICS

	Genotype or disease		A: True positives B: False positives	C: False negative D: True negative
	Present	Absent		
Test				
Positive	A	B	Sensitivity:	A/(A+C)
			Specificity:	D/(D+B)
Negative	C	D	Positive predictive value:	A/(A+B)
			Negative predictive value:	D/(C+D)

2.1 Analytical sensitivity
(proportion of positive tests if the genotype is present)
Close to 100% by direct gene sequencing and about 60% by targeted real-time PCR.

2.2 Analytical specificity
(proportion of negative tests if the genotype is not present)
100%.

2.3 Clinical sensitivity
(proportion of positive tests if the disease is present)
The clinical sensitivity can be dependent on variable factors, such as age or family history. In such cases, a general statement should be given, even if a quantitation can only be made case by case.

It is almost 100%. Failure to identify mutations on both alleles of the biotinidase (*BTD*) gene has usually resulted from incorrect evaluation of enzymatic activity. Yet, in a few rare cases, mutations may be located in unexplored regions of the *BTD* gene (introns, 5′UTR, 3′UTR, or upstream-regulating regions), as has been observed in the Austrian patients.[15]

2.4 Clinical specificity
(proportion of negative tests if the disease is not present)
The clinical specificity can be dependent on variable factors, such as age or family history. In such cases, a general statement should be given, even if a quantitation can only be made case by case.

100%.

2.5 Positive clinical predictive value
(life-time risk of developing the disease if the test is positive)
Almost 100% of the children identified with two alleles of profound biotinidase deficiency will develop symptoms or are considered at high risk of becoming symptomatic if they are not treated with biotin. Without biotin treatment, even adults with the enzyme deficiency who have never been symptomatic can develop clinical features during stress, such as a prolonged infection.[5]

2.6 Negative clinical predictive value
(probability of not developing the disease if the test is negative)
Assume an increased risk based on family history for a non-affected person. Allelic and locus heterogeneity may need to be considered.

Index case in that family had been tested:

When both alleles of the *BTD* gene are abnormal in an index case, the negative predictive value is 100% in relatives who are found non-carriers or heterozygous carriers. Individuals in both groups will remain asymptomatic.

Index case in that family had not been tested:

Not applicable.

3. CLINICAL UTILITY

3.1 (Differential) diagnosis: The tested person is clinically affected
(To be answered if in 1.10 'A' was marked)

3.1.1 Can a diagnosis be made other than through a genetic test?

No	☐ (continue with 3.1.4)	
Yes	☒	
	Clinically	☒
	Imaging	☐
	Endoscopy	☐
	Biochemistry	☒
	Electrophysiology	☐
	Other (please describe)	

3.1.2 Describe the burden of alternative diagnostic methods to the patient
Diagnosis can be established by the concomitant presence of characteristic clinical and biochemical features. Clinically, a child with untreated profound biotinidase deficiency can exhibit symptoms shared with other metabolic disorders, such as seizures, hypotonia, respiratory problems, developmental delay, and vision problems, and those more specific of biotinidase deficiency, such as eczematous skin rash, alopecia, conjunctivitis, candidiasis, and ataxia.[5] Symptoms occurring in older children or adolescents include limb weakness, paresis, and scotomata. Symptomatic-affected individuals usually exhibit biochemical features, such as ketolactic acidosis, organic aciduria, and hyperammonemia. A milder expression of the above symptoms occurs in individuals with partial biotinidase deficiency. All symptomatic-affected individuals improve with oral pharmacological doses of the vitamin, biotin. Biotin treatment prevents the development of symptoms in affected children identified before they have clinical findings or are identified by newborn screening. Biotin therapy is life-long.

The diagnostic biochemical finding of the disorder is decreased or undetectable biotinidase activity in serum or plasma. Biotinidase is

essential for recycling the vitamin, biotin (also known as vitamin H or B8). Biotinidase activity can be determined by colorimetric or fluorimetric enzymatic assays in serum or plasma. The degree of enzyme deficiency can distinguish individuals with profound biotinidase deficiency (activity less than 10% of mean normal serum activity) and those with partial biotinidase deficiency (activity between 10 and 30% of mean normal serum activity).[16] Clinical and biochemical findings may be sufficient to differentially diagnose nutritional biotin deficiency, other causes of sensorineural hearing loss or ataxia. Biochemical and/or mutational analysis of the *BTD* gene may be helpful to exclude other diagnostic possibilities, such as an isolated carboxylase deficiency, holocarboxylase synthetase deficiency, zinc deficiency (acrodermatitis enteropathica), or essential fatty acid deficiency.[5] Measuring plasma or urinary biotin concentrations is usually of little or no value in the diagnosis of biotinidase deficiency, but may be useful to confirm compliance of biotin treatment.

3.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?

In countries that perform newborn screening of biotinidase deficiency, almost all enzymatically-deficient children are identified. Yet, such a screening programme is not performed in all countries; some European countries have indicated that screening is not warranted for a variety of reasons. However, some of the clinical features of the disorder, once they occur, are not reversible, such as developmental delay, hearing loss, and optic atrophy. In addition, because the clinical features may be non-specific or gradual in onset, the diagnosis may not be readily made. Molecular testing is particularly useful for confirming or excluding the diagnosis of biotinidase deficiency, whenever the interpretation of enzymatic activity is ambiguous. For instance, mutation analysis can definitively differentiate individuals with profound biotinidase deficiency from those with partial biotinidase deficiency, or heterozygous carriers of an allele of profound deficiency from homozygous carriers of an allele of partial deficiency.[8]

3.1.4 Will disease management be influenced by the result of a genetic test?

No	☐	
Yes	☒	
	Therapy (please describe)	Medical care is most dependent on the biotinidase activity of the patient. Profound deficiency is usually treated with 5–10 mg of biotin per day, whereas partial deficiency is usually treated with 1–5 mg of biotin per day. In patients with obvious biotinidase deficiency as demonstrated by very low enzymatic activity (<10%), therapy is not affected by molecular testing. Yet, in ambiguous cases, molecular testing can have a major impact on the recommended dose of biotin to be administered. For example, biotin therapy is not needed in individuals who are heterozygous for an allele of profound biotinidase deficiency or homozygous for the c.1330G>C (p.Asp444His) mutation. However, there are some individuals who have the c.1330G>C (p.Asp444His) mutation in combination (allelic) for a second mutation, such as the c.511G>A (p.Ala171Thr) mutation; this combination acts like a mutation for profound biotinidase deficiency. If individuals are only heterozygous for this combined mutation and no mutation on the other allele, they do not require biotin treatment, however, if they are compound heterozygous for this combined mutation with either a mutation for profound on the other allele, they require 5–10 mg of biotin per day or for a mutation for partial deficiency, 1–5 mg of biotin per day. Enzyme-deficient relatives of an index case, even if currently asymptomatic, can be identified and, if deficient, will benefit from biotin therapy.
	Prognosis (please describe)	Patients correctly diagnosed with biotinidase deficiency using second-tier molecular testing can benefit from biotin treatment. Biotin therapy resolves or improves many clinical features in symptomatic individuals with biotinidase deficiency and prevents the development of symptoms in those detected immediately after birth (newborn screening or prenatally diagnosed) or in at-risk, asymptomatic affected relatives before they develop clinical findings.
	Management (please describe)	Asymptomatic individuals with biotinidase deficiency detected by enzymatic or molecular testing can be treated with oral biotin. These individuals should be followed regularly by a metabolic disease specialist. Biotin supplementation should also be considered during the pregnancy of a mother carrying an at-risk pregnancy for biotinidase deficiency.

3.2 Predictive setting: The tested person is clinically unaffected but carries an increased risk based on family history

(To be answered if in 1.10 'B' was marked)

3.2.1 Will the result of a genetic test influence lifestyle and prevention?

If the test result is positive (please describe)

In each case where molecular analysis confirmed the diagnosis of biotinidase deficiency, individuals should be treated with biotin.

If the test result is negative (please describe)

If molecular testing excluded the diagnosis of biotinidase deficiency, no biotin treatment is required.

3.2.2 Which options in view of lifestyle and prevention does a person at-risk have if no genetic test has been done (please describe)?

Biotin treatment can prevent the occurrence of irreversible symptoms, such as developmental delay, hearing loss, and optic atrophy, if they have not occurred in an individual with biotinidase deficiency. Confirmatory testing is recommended to avoid possible, as yet unknown, side effects of the treatment.

3.3 Genetic risk assessment in family members of a diseased person

(To be answered if in 1.10 'C' was marked)

3.3.1 Does the result of a genetic test resolve the genetic situation in that family?

Yes, because the disorder is inherited as an autosomal recessive trait, other asymptomatic relatives can be evaluated and subsequent children born to the family can be evaluated immediately after birth for the disorder, particularly in those locations that do not perform newborn screening for biotinidase deficiency.

3.3.2 Can a genetic test in the index patient save genetic or other tests in family members?

Yes, at-risk family members and possible mutation carriers can be screened for the enzyme deficiency or for causative *BTD* mutations, if both alleles have been identified in the index case.

3.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?

Yes.

3.4 Prenatal diagnosis

(To be answered if in 1.10 'D' was marked)

3.4.1 Does a positive genetic test result in the index patient enable a prenatal diagnosis?

It is possible, but it is not very common, because biotinidase deficiency is a readily treatable disease.

4. IF APPLICABLE, FURTHER CONSEQUENCES OF TESTING

Please assume that the result of a genetic test has no immediate medical consequences. Is there any evidence that a genetic test is nevertheless useful for the patient or his/her relatives? (Please describe)

Finding mutations in both of an index case's alleles is helpful in identifying at-risk individuals and those who are carriers in the family. Testing will have impact on genetic counselling and may consequently influence reproductive decisions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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