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ORIGINAL INVESTIGATION

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Partial biotinidase deficiency is usually due to the D444H mutation in the biotinidase gene

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Abstract Newborn screening for biotinidase deficiency has identified children with profound biotinidase deficiency (<10% of mean normal serum activity) and those with partial biotinidase deficiency (10%–30% of mean normal serum activity). Children with partial biotinidase deficiency and who are not treated with biotin do not usually exhibit symptoms unless they are stressed (i.e., prolonged infection). We found that 18 of 19 randomly selected individuals with partial deficiency have the transversion missense mutation G1330>C, which substitutes a histidine for aspartic acid444 (D444H) in one allele of the biotinidase gene. We have previously estimated that the D444H mutation results in 48% of normal enzyme activity for that allele and occurs with an estimated frequency of 0.039 in the general population. The D444H mutation in biotinidase deficiency is similar to the Duarte variant in galactosemia. The D444H mutation in one allele in combination with a mutation for profound deficiency in the other allele is the common cause of partial biotinidase deficiency.

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Introduction

Biotin is an essential water-soluble B vitamin. In humans, it is the coenzyme of four carboxylases that are involved in fatty acid synthesis, amino acid catabolism, and gluconeogenesis (Wolf and Feldman 1982). Degradation of these carboxylases results in the production of biocytin (ε-N-biotinyl-L-lysine). Biotinidase (EC 3.5.1.12) is responsible for cleaving biotin from biocytin and from dietary protein-bound sources, thereby recycling the vitamin (Pispa 1965). Because humans cannot synthesize biotin, they depend on dietary biotin and the recycling of endogenous biotin (Wolf et al. 1985a). This biotinyl-hydrolase activity is no longer thought to be the only function of biotinidase. Biotinidase also has biotinyl-transferase activity in which biotin is transferred from biocytin to histones at physiologic pH (Hymes et al. 1995).

Biotinidase deficiency is an inherited metabolic disorder caused by the deficient activity of this enzyme and is the primary biochemical defect in individuals with late-onset multiple carboxylase deficiency (Wolf et al. 1983a). Individuals with untreated profound biotinidase deficiency (less than 10% of mean normal serum activity) usually develop seizures, hypotonia, skin rash, alopecia, and developmental delay with ketolactic acidosis and organic aciduria (Wolf et al. 1985b). Other symptoms include ataxia, conjunctivitis, optic atrophy, progressive hearing loss, and occasionally coma (Wolf et al. 1983b). Individuals with profound biotinidase deficiency are successfully treated with pharmacological doses of biotin (Wolf et al. 1983a; Wolf 1995). Many symptoms can be reversed once biotin treatment is started; however, some symptoms, such as the eye and hearing problems or developmental delay, may be irreversible. Because the clinical features of biotinidase deficiency are preventable, it is important to identify individuals with the enzyme deficiency and initiate biotin therapy as early as possible. Therefore, newborn screening for biotinidase deficiency is currently performed in 25 states in the United States and in 25 countries. Newborn screening has identified not only those children with profound

biotinidase deficiency, but also another group of children with 10%–30% of mean normal serum biotinidase activity, which has been designated as partial biotinidase deficiency (Heard et al. 1986). The incidence of profound biotinidase deficiency has been estimated to be 1 in 137,000 live births and partial deficiency to be 1 in 110,000 live births (Wolf 1991).

Children with partial biotinidase deficiency and who are not treated with biotin rarely exhibit symptoms unless they are stressed by infection or poor nutrition (McVoy et al. 1990). Currently, there is no way to determine which children with partial biotinidase deficiency will develop symptoms. We now report that the major cause of partial biotinidase deficiency is the D444H mutation in one allele of the biotinidase gene in combination with a mutation that results in profound biotinidase deficiency in the other allele.

Materials and methods

Materials and reagents

Unless otherwise stated, all chemicals were purchased from Sigma (St. Louis, Mo.) and radioactive nucleotides (α-[^{32}P]-dTTP and γ-[^{32}P]-dATP) were purchased from NEN/Dupont (Boston, Mass.). Dideoxynucleotides, $MgCl_2$, 10×PCR (polymerase chain reaction) buffer and AmpliTaq DNA polymerase were purchased from Perkin Elmer (Foster City, Calif.). Primers and oligonucleotides were synthesized by the DNA Core Laboratory at the Medical College of Virginia/Virginia Commonwealth University.

Subjects

Individuals with partial biotinidase deficiency were identified by newborn screening conducted in the United States and in several foreign countries. Blood was obtained from these children and from their parents and siblings when possible. Individuals with partial biotinidase deficiency are designated by the letter P and the number of the individual.

Biotinidase activity

Biotinidase activity in the serum of the children with partial biotinidase deficiency and their parents, when available, was determined by the colorimetric assay which measures the formation of p-aminobenzoate from the artificial substrate, biotinyl-p-aminobenzoate, as previously described (Wolf et al. 1983a). Activity in a normal control was determined with each assay of the proband and/or parents to correct for day to day variation in the assay.

Isolation of nucleic acids

Genomic DNA was isolated from peripheral blood lymphocytes, lymphoblasts, or whole blood by using the Gentra Puragene DNA isolation kit (Research Triangle Park, N.C.) according to the manufacturer's recommendations. The concentration of DNA in each sample was calculated from the optical density at 260 nm and diluted to a working concentration of 0.2 µg/µl.

Polymerase chain reaction

Genomic DNA (1 µg) was used as the template in a total reaction volume of 50 µl (1×PCR buffer, 2.5 mM $MgCl_2$, 20 µM dATP, 20 µM dTTP, 20 µM dGTP, 20 µM dCTP, 25 pM each primer, and 2.5 U AmpliTaq DNA polymerase). Primers were designed to span the exonic sequences, including the intron/exon boundaries, of the entire biotinidase gene (Cole et al. 1994; Cole 1994). The PCR amplification procedure was identical for single-stranded conformation polymorphism analysis (SSCA), except only 5 µM dTTP was used and 2.5 µCi α-[^{32}P]-dTTP was added. Specifically, the primers used for amplification of the 561 bp region at the 3' end of the gene designated D7 were 1150.S and 1790.A as described previously (Norrgard et al. 1997a). Region D7 amplification was performed at an initial denaturation time of 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min, with a final extension interval at 72°C for 10 min.

Single-stranded conformation analysis

SSCA was used to screen for mutations by using multiple overlapping fragments that span the entire length of the biotinidase gene including the flanking intron-exon boundaries (Cole 1994; Pomponio et al. 1995). SSCA of PCR products of DNA from all 19 probands was performed as described (Pomponio et al. 1995), except that the products were run on 6% nondenaturing polyacrylamide gels (49:1 acrylamide:bis with 5% glycerol) at room temperature at 10 W constant power until the xylene cyanol band had migrated 75% of the length of the gel (approximately 20 h for a single 33×62 cm gel). Autoradiography was performed on undried gels at –80°C for a period of time dependent on the strength of the signal for each particular gel such that the migration pattern was visible at optimum clarity. PCR samples from normal individuals and individuals with known mutations were run adjacent to samples from children with partial biotinidase deficiency on each gel to ensure that the technique identified known aberrant migration patterns and polymorphisms in the region being studied.

Allele-specific oligonucleotide analysis

Allele-specific oligonucleotide (ASO) analysis of PCR products was performed as described (Norrgard et al. 1997a). Probes G1330NL and C1330ABN were used to screen for the D444H mutation.

DNA sequencing

Templates for sequence analysis were prepared in duplicate by nonradioactive PCR amplification by using the primers and conditions described above. The PCR products were then pooled and purified by using the QIAQuick gel extraction kit (QIAGEN, Chatsworth, Calif.) or Jetsorb (Genomed, Research Triangle Park, N.C.) according to manufacturer's instructions. Automated fluorescent sequencing reactions were performed as described (Pomponio et al. 1995). Sequence analysis was performed by using the Sequencher version 3.0 computer program (Gene Codes, Ann Arbor, Mich.).

Results

By using SSCA and direct sequencing, mutations were determined in all 19 children with partial biotinidase deficiency. Sequence analysis confirmed the G1330>C transversion that causes a substitution of His for Asp444 (D444H) in 18 of the 19 children (Table 1). Four of these children are homozygous for this mutation and 14 are het-

Table 1 Biochemical, molecular, and clinical characterization of children with partial biotinidase deficiency

ID	Percent normal activity[a]	Mutation allele no. 1	Result of mutation allele no. 1	Mutation allele no. 2	Result of mutation allele no. 2	Consanguinity	Ethnic background[b] Mother/Father
P285	26.8	G1330>C	D444H	G511>A + G1330>C	A171T+D444H	No	?/[b]?
P390	23.2	G1330>C	D444H	G511>A + G1330>C	A171T+D444H	No	Hungarian, French, Dutch/[b]German
P398	26.8	G1330>C	D444H	G511>A + G1330>C	A171T+D444H	?	[b]?/?
P545	20.6	G1330>C	D444H	G511>A + G1330>C	A171T+D444H	No	[b] Turkish/Turkish
P53	25.2	G1330>C	D444H	C1612>T	R538C	?	?/?
P212	30.6	G1330>C	D444H	C1612>T	R538C	No	[b]?/?
P325	16.3	G1330>C	D444H	A1368>C	Q456H	No	German, Italian/[b]Scottish, Irish, French
P384	23.2	G1330>C	D444H	A1368>C	Q456H	No	[b]German, Irish, Czech/German, Native American
P408	10.4	G1330>C	D444H	A1368>C	Q456H	No	German, Dutch/[b]German
P448	32.8	G1330>C	D444H	A1368>C	Q456H	?	Polish?/[b]Polish?
P519	29.2	G1330>C	D444H	A1368>C	Q456H	No	[b]German/Irish, German
P185	30.1	G1330>C	D444H	G98:d7i3	Frameshift	No	German, Irish, English/[b]Italian, Irish, German, English
P410	26.1	G1330>C	D444H	G98:d7i3	Frameshift	No	[b]Scottish, Swiss, German, Irish/Greek, English
P556	18.6	G1330>C	D444H	G98:d7i3	Frameshift	No	?/?
P334	25.4	G1330>C	D444H	T382>G	F128V	No	Italian/Lithuanian, Jewish
P375	18.9	G1330>C	D444H	delT933	Frameshift	No	German/[b]German
P503	24.4	G1330>C	D444H	G682>T	D228Y	No	[b]German/German
P505	21.7	G1330>C	D444H	C1595>T	T532 M	?	[b]German/Mixed European
P143	22.5	A968>G	H323R	G1352>A	G451D	No	Afghan/Afghan

[a]Percent of mean normal biotinyl-hydrolase activity. Mean normal serum biotinyl-hydrolase activity is 7.1 nmol/min per ml with a range of 3.5 to 12.0
[b]Parent contributing the D444H allele

erozygous. All four of the homozygotes (P285, P390, P398, P545) also have the A171T mutation in one allele. The A171T mutation in syntenic conjunction with the D444H mutation is a known double mutation that causes less than 10% of mean normal activity or profound biotinidase deficiency in that allele (Norrgard et al. 1997a). Of the 19 children, 18 have the D444H mutation in one allele and a mutation or the double mutation that causes profound biotinidase deficiency in the other allele (Table 1). Although four mutations that cause profound biotinidase deficiency (A171T+D444H, R538C, Q456H, and G98:d7i3) have been described previously (Pomponio et al. 1995, 1997; Norrgard et al. 1997a, b), four other mutations that cause profound biotinidase deficiency in the second allele have not been previously reported. These include a transversion mutation T382>G resulting in the substitution of a valine for phenylalanine128 (F128V) in P334, a single base deletion (dT933) causing a frameshift in P375, a child from Germany, a transversion mutation G682>T that substitutes a tyrosine for aspartic acid 228 (D228Y) in P503, and C1595>T that substitutes methionine for threonine532 (T532M) in P505. We have sequenced all the exon sequences of the biotinidase gene, including all exon/intron boundaries of DNA, from several of these children and have found no other abnormalities.

To determine the effect that the D444H mutation has on biotinidase activity, biotinyl-hydrolase activity was measured in sera from the children with partial deficiency, parents who were heterozygous for the mutation causing profound biotinidase deficiency, parents who were heterozygous for the D444H mutation, and normal individuals who were assayed at the same time as the parents. Children with partial biotinidase deficiency have a mean biotinidase activity of 1.69±0.38 nmol p-aminobenzoate formed per minute per milliliter serum (n=19) or 23.8%±5.4% of mean normal hydrolase activity (n=19). Parents who are heterozygous for a mutation causing profound biotinidase deficiency have a mean normal activity of 4.41±0.82 nmol/min per ml serum (n=13) or 62.6% of mean normal control activity (7.05±0.75 nmol/min per ml; n=16), and the mean activity of parents who are heterozygous for the D444H mutation is 5.40±0.71 nmol/min per ml (n=12) or 76.6% of the mean normal activity.

To determine whether imprinting plays a role in partial biotinidase deficiency, we compared the biotinyl-hydrolase activity of children who had inherited the D444H allele from their mothers with that from those who had inherited it from their fathers. Assuming that the contribution of the allele for profound deficiency is equal in all children with partial deficiency, we found that the mean percent of normal serum biotinidase activity of the children who had received the D444H allele from the mother (26.0%±3.2%; n=7) is not significantly different from that of the children who had obtained the mutation from their father (22.6%±8.0%; n=7).

In an attempt to attribute the D444H allele to a specific nationality or ethnic group, ASO analysis was used to determine which parent had the D444H allele (data not shown). In cases where both parents had the mutation, sequence analysis of the D1 region was used to determine which parent contributed the double mutation and which had the D444H mutation alone. It appears that the D444H mutation allele is frequently observed in parents of German descent. This suggests that a founder effect may be responsible for this mutation.

Discussion

The gene encoding human serum biotinidase has been cloned and sequenced by using a functional cloning technique and found to contain four exons designated A–D (Cole 1994). Two groups of children have been identified by newborn screening; those with profound biotinidase deficiency and those with partial biotinidase deficiency (Heard et al. 1986; Wolf 1991). We have previously reported approximately 40 mutations that result in profound biotinidase deficiency (Pomponio et al. 1995, 1997; Norrgard et al. 1997a, b). Partial biotinidase deficiency was originally predicted to represent heterozygosity for a mutation in the biotinidase gene causing profound biotinidase deficiency (Dunkel et al. 1989; McVoy et al. 1990). We have found that 18 of 19 children with partial biotinidase deficiency have a transversion mutation G1330>C in one allele at the 3’ end of exon D, substituting a histidine for aspartic acid444 (D444H). Partial biotinidase deficiency is, therefore, due to this mutation in one allele and a mutation that results in less than 10% of normal activity or profound enzyme deficiency in the other allele. We have previously reported that this mutation results in 48% of mean normal activity in this allele and has an estimated frequency of 0.039 in the general population (Norrgard et al. 1997a). Using the mean biotinidase activity in sera of children with partial biotinidase deficiency and who are compound heterozygotes for the D444H mutation and a mutation that results in less than 10% of mean normal activity, we estimate that the D444H mutation results in 48% of mean normal serum activity, whereas using the activities of the parents who are heterozygous for the D444H mutation, we estimate that the mutation results in 53% of mean normal activity. Both estimates are in good agreement with the previously reported estimate. Evaluation of the ethnic and/or nationalities of the parents having the D444H mutation suggests that they are commonly of German ancestry.

The D444H mutation is similar to the Duarte variant in galactosemia (Segal and Berry 1995). Biochemical phenotyping shows that individuals heterozygous for a normal allele and an allele with the Duarte variant (N/D) have about 75% of normal galactose-1-phosphate uridyltransferase activity, whereas compound heterozygotes for the Duarte variant and the mutation causing classic galactosemia (D/G) have 25% of normal activity. Children with D/G are usually asymptomatic, but it is still controversial whether these children should be treated with galactose-free diets (Segal and Berry 1995). The degree of enzyme deficiency in individuals with N/D and D/G galactosemia closely parallels that in individuals with partial biotinidase deficiency and who are heterozygous for D444H and in those who are compound heterozygous for the D444H mutation and a mutation that causes profound biotinidase deficiency, respectively.

We conclude that partial biotinidase deficiency usually occurs when an individual has one allele that results in nearly total loss of activity in combination with an allele having the D444H mutation. Further study is necessary to determine whether these children require biotin therapy.

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