Hum Genet (1997) 99 : 506–512

ORIGINAL INVESTIGATION

Robert J. Pomponio · Karen J. Norrgard
Jeanne Hymes · Thomas R. Reynolds · Gregory A. Buck
Regula Baumgartner · Terttu Suormala · Barry Wolf

Arg538 to Cys mutation in a CpG dinucleotide of the human biotinidase gene is the second most common cause of profound biotinidase deficiency in symptomatic children

Received: 13 August 1996 / Revised: 13 November 1996

Abstract Biotinidase deficiency is an autosomal recessively inherited disorder in the recycling of the vitamin biotin. The most common mutation that causes profound biotinidase deficiency in symptomatic individuals is a deletion/insertion (G_{98}:d7i3) that occurs in exon B of the biotinidase gene. We now report the second most common mutation, a C-to-T substitution (position 1612) in a CpG dinucleotide in exon D of the biotinidase gene. This mutation results in the substitution of a cysteine for arginine538 (designated R538C) and was found in 10 of 30 symptomatic children with profound biotinidase deficiency, 5 of whom also have the G_{98}:d7i3 mutation. This mutation was not found in DNA samples from 32 individuals with normal biotinidase activity, but was found in one individual with enzyme activity in the heterozygous range. This mutation was not detected in 371 randomly selected, normal individuals using allele-specific oligonucleotide hybridization analysis. Aberrant biotinidase protein was not detectable in extracts of fibroblasts from a child who is homozygous for the R538C mutation, but was present in less than normal concentration in identical extracts treated with β-mercaptoethanol. Because there is no detectable biotinidase protein in sera of children who are homozygous for the R538C mutation and in combination with the deletion/insertion mutation, the R538C mutation likely results in inappropriate intra- or intermolecular disulfide bond formation, more rapid degradation of the aberrant enzyme, and failure to secrete the residual aberrant enzyme from the cells into blood.

R. J. Pomponio · K. J. Norrgard · J. Hymes · B. Wolf (✉)
Department of Human Genetics, Medical College of Virginia,
P.O. 980033, Richmond, VA 23298, USA
Tel.: +1-804-828-9632; Fax: +1-804-828-3760

T. R. Reynolds · G. A. Buck
Department of Microbiology and Immunology,
Medical College of Virginia/Virginia Commonwealth University,
Richmond, VA 23298, USA

R. Baumgartner · T. Suormala
Metabolic Unit, Basler Kinderspital,
Universitat-Kinderkliniken und Poliklinken, Romergasse 8,
Basel, Switzerland

B. Wolf
Departments of Pediatrics and Biochemistry
and Molecular Biophysics,
Medical College of Virginia Commonwealth University,
Richmond, VA 23298, USA

Introduction

Biotin is an essential water-soluble B vitamin and is the coenzyme for four carboxylases in humans, propionyl-CoA carboxylase, β-methylcrotonyl-CoA carboxylase, acetyl-CoA carboxylase, and pyruvate carboxylase (Bonjour 1977; Wolf and Feldman 1982). These carboxylases are involved in amino acid catabolism, fatty acid synthesis, and gluconeogenesis. The carboxyl group of biotin is covalently attached through an amide bond to an ε-amino group of a lysyl residue of the apocarboxylases by holocarboxylase synthetase, thereby forming the active holocarboxylases (Wolf 1995). The holocarboxylases are eventually degraded to biocytin (biotin-ε-lysine), which is then cleaved by biotinidase (EC 3.5.1.12), forming biotin for reutilization (Pispa 1965; Wolf 1995).

Biotinidase deficiency is an autosomal recessively inherited disorder (Wolf et al. 1983a; Wolf 1995). Children with biotinidase deficiency cannot cleave biocytin and, therefore, cannot recycle biotin. If not treated with biotin, these children become secondarily biotin deficient resulting in decreased activities of the biotin-dependent carboxylases, accumulation of toxic metabolites, and development of clinical symptoms (Wolf et al. 1985a). Untreated individuals with biotinidase deficiency may exhibit seizures, hypotonia, ataxia, developmental delay, hearing loss, optic abnormalities, alopecia, skin rash, ketolactic acidosis and organic aciduria (Wolf et al. 1983b, 1985b). Some enzyme-deficient children have become comatose and died before they were diagnosed and treated. There is considerable variability in the age of onset of symptoms and in the severity of the clinical features of the disorder (Wolf et al. 1983b). Pharmacologic doses of bio-

tin can resolve and reverse many of the clinical symptoms (Wolf 1995). If treatment is delayed, deficits in hearing, sight or development may be irreversible, even after biotin therapy (Wolf et al. 1983c; Salbert et al. 1993a,b). Therefore, it is important that biotinidase deficiency is diagnosed early so that biotin treatment can be initiated before permanent neurological damage occurs. Many states and countries perform newborn screening for biotinidase deficiency (Wolf 1991).

We have isolated and characterized the cDNA for normal human serum biotinidase (BTD; Cole et al. 1994a) and localized the gene to chromosome 3p25 (Cole et al. 1994b). We have previously identified the most common mutation in the biotinidase gene that causes profound biotinidase activity (<10% of mean normal serum enzyme activity; mean normal activity is 7.1 nmol/min/ml serum, range 4.5–11) in symptomatic children (Pomponio et al. 1995). This deletion/insertion mutation, designated del G_{98}-G_{104}:ins TCC or G_{98}:d7i3, occurred in half of the symptomatic children studied. We now describe a mutation in a CpG dinucleotide in exon D of the biotinidase gene that results in the substitution of cysteine for arginine538. This mutation is the second most common cause of profound biotinidase deficiency in symptomatic children.

Materials and methods

Subjects

Whole blood was obtained from 34 symptomatic children (30 probands and 4 affected siblings) with profound biotinidase deficiency, their parents (when available), 32 individuals with normal serum biotinidase activity, and one "control" with activity in the heterozygous range. Lymphoblast cultures were established from the blood as described below. Cultured fibroblasts were established as described previously (Wolf et al. 1981) from two normal individuals, a child with profound biotinidase deficiency (P2), who is a compound heterozygote for the common deletion/insertion mutation (G_{98}:d7i3) (Pomponio et al. 1996) and a 12-bp deletion mutation, both resulting in null mutations, and a child (P218) who is homozygous for the R538C mutation. Randomly selected, anonymous blood-soaked filter paper spots from 371 newborns were obtained from Consolidated Laboratories (Richmond, Va.) where newborn screening in Virginia is performed. Of these newborns, 73% were Caucasian, 20% were African Americans, and 7% were Hispanic, Asian, and American Indian. DNA was isolated from the lymphoblastic cells and blood spots as described below. This study was approved by the institutional review board at the Medical College of Virginia/Virginia Commonwealth University.

Biotinyl-hydrolase activity, biotinyl-transferase activity and determination of crossreacting material to antibody to biotinidase in serum and in cultured fibroblasts

Biotinyl-hydrolase activity was determined quantitatively in serum by a colorimetric assay that uses biotinyl-*p*-aminobenzoate as substrate (Wolf et al. 1983a). Biotinyl-transferase was determined semiquantitatively in these samples. In this assay, sera were incubated with and without calf thymus histones, subjected to electrophoresis on sodium dodecyl sulfate (SDS)-polyacrylamide gels, blotted onto nitrocellulose and detected by avidin transblot as described previously (Hymes et al. 1995). The intensity of the bands corresponding to the avidin-biotinylated histones was assessed from absent to +4 (normal). Crossreacting material to antibody prepared against normal serum biotinidase, which was purified to homogeneity, was determined in serum as described previously (Hart et al. 1992). In this assay, the serum samples were denatured and reduced by treatment with SDS, β-mercaptoethanol and boiling. The degree of crossreacting material was assessed semiquantitatively from absent to +4 (normal).

Equal aliquots of fibroblast extracts from two normal individuals and enzyme-deficient children, P2 and P218, were diluted with sample buffer with and without 1% β-mercaptoethanol. Samples (10 μl) of the nonreduced and reduced extracts were subjected to SDS-polyacrylamide electrophoresis (Hymes et al. 1995). The gels were blotted, and crossreacting material (CRM) to antibodies to purified normal biotinidase was detected as described previously (Hart et al. 1992).

Lymphoblast cultures and nucleic acid isolation

Lymphoblasts were propagated as described previously (Pomponio et al. 1995). Genomic DNA was isolated from lymphoblast cultures and whole blood samples using the Gentra (Minneapolis, Minn.) Pure Gene DNA isolation kit, according to the manufacturer's recommendations. DNA was isolated in situ from each 1/8″ diameter blood spot disk in a 96-well microtiter plate using the Gentra Generations blood spot DNA isolation solution, according to the manufacturer's recommendations, with the aid of a Beckman Biomek 1000 robotic workstation. The concentration of DNA in samples of whole blood and lymphoblasts was calculated from the optical density at 260 nm and diluted to a concentration of 0.2 μg/μl.

Amplification of genomic BTD DNA fragments

A 1-μg sample of genomic DNA from each subject was amplified in 50 μl of reaction volume by polymerase chain reaction (PCR; Saiki et al. 1985) using previously described amplification parameters and 21-mer oligonucleotide primers 267.S (5′-CACAGTACATGGCACTTAC-3′), located within exon D, and 1790.A (5′-TCAACATGATGGCCAGAGTC-3′), located in the noncoding region of the 3′ end of the gene in exon D of the human biotinidase gene (Pomponio et al. 1995; Cole et al. 1994a). The PCR-amplified product is a 300-bp fragment (designated fragment D5).

DNA was isolated from blood spots that were rehydrated by incubating them in 10 μl of distilled water for 1 h at room temperature. Nonradioactive PCR of a 561-bp region (designated fragment D7, which includes D5) encompassing this mutation was performed in a 50-μl reaction volume. The primers used were 1150.S (5′-AGAAGGATGCTCAGGAAGTC-3′) and 1790.A. PCR was performed with an initial denaturation of 5 min at 94°C, followed by 40 cycles (94°C for 30 s, 60°C for 30 s, and 72°C for 1 min), and a final extension of 72°C for 10 min.

Screening for mutations using single-stranded conformation analysis (SSCA)

SSCA was performed using the D5 PCR-amplified products from patients, their relatives, and individuals with normal biotinidase activity as described previously (Orita et al. 1989a,b; Pomponio et al. 1995), except that the denatured PCR products were subjected to electrophoresis on a 6% nondenaturing polyacrylamide gel (37:1 acryl:bis) run at 60 W at 4°C.

DNA sequence analysis

Templates for sequence analysis of patient and normal genomic DNA were prepared by nonradioactive PCR amplification of this region of exon D using primers 267.S and 1790.A as described previously (Pomponio et al. 1995) with the following modifications. Three separate 50-μl PCR reactions were prepared for each

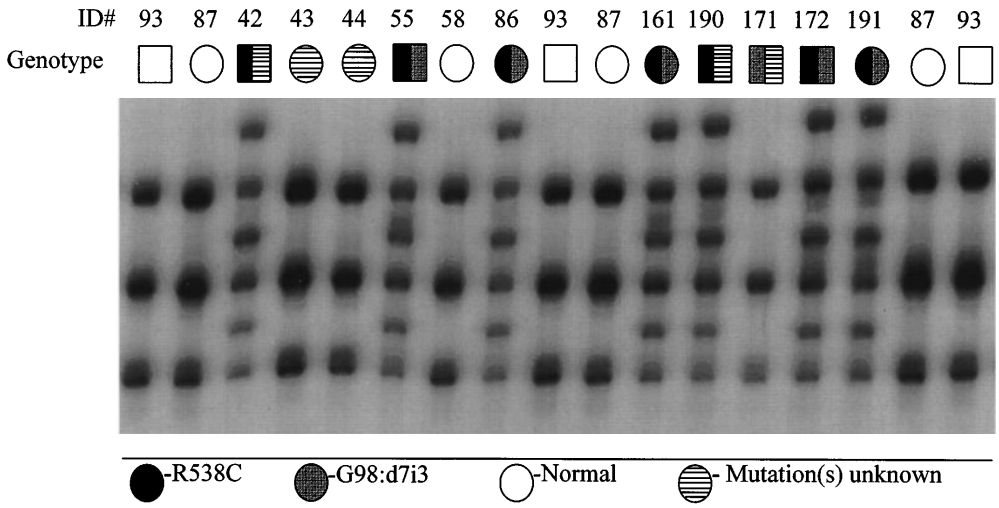

Fig. 1 Single-stranded conformation analysis of PCR-amplified DNA. *ID numbers* refer to the individuals tested. PCR-amplified allelic conformations are from patients P42, P43, P44, P55, P86, P87, P161, P190, P171, P172, and P191. The genotype symbols for individuals with profound biotinidase deficiency are coded as follows: *blackened symbol*, R538C mutation; *diagonal crosshatch*, G98:d7i3 mutation; *horizontal bars*, mutation unknown; and *open symbol*, normal individuals P93, P87 and P58

individual. A negative control lacking DNA template was prepared for each set of samples to be amplified. The three PCR reactions from each subject were pooled and gel purified on a 1% Seakem GTG agarose gel (FMC Bioproducts, Rockport, Me.) containing ethidium bromide. The PCR product band was excised from the gel and purified using the JetSorb Gel Extraction Kit (Genomed, Research Triangle Park, N.C.) to recover the product. The purified samples were lyophilized and dissolved in 30 µl of distilled water. Samples (5 µl) of this purified product were subjected to electrophoresis in a 1% agarose gel in parallel with standards to estimate yields. Sequencing reactions were performed using at least 200 ng of purified PCR product, 20 ng of primer (either 267.S or 1790.A), and fluorescence-labeled *Taq* DyeDeoxy terminator reaction mix (Applied Biosystems, Foster City, Calif.) according to the manufacturer's instructions. Reactions were performed in a Perkin Elmer 9600 thermocycler and DNA sequence was determined using either a 373A or 377 Automated DNA Sequencer (Applied Biosystems). Sequence editing was performed using Sequencher software, version 3.0 (Gene Codes Corporation, Ann Arbor, Mich.).

Allele-specific oligonucleotide (ASO) hybridization analysis

Samples (5 µl) of each PCR reaction product were run on a 1% SeaKem agarose gel containing ethidium bromide and photographed using Polaroid type 55 pos/neg film. Negatives were scanned using a PDSI 486 (Molecular Dynamics, Sunnyvale, Calif.) laser densitometer and analyzed with ImageQuaNT software (Molecular Dynamics) to determine the relative intensity of each band. The remaining PCR product (45 µl) was denatured by adding 225 µl of sample denaturation solution (500 mM NaOH, 2 M NaCl, 25 mM EDTA, and 0.0001% (w/v) bromophenol blue (Handelin and Shuber 1994)). Using the densitometry data, approximately equal amounts of denatured PCR products were replicate blotted onto Nytran Plus nylon membranes (Schleicher and Schuell, Keene, N.H.) using a 96-well dot-blot manifold (BRL Life Technologies, Gaithersburg, Md.). After DNA transfer, membranes were neutralized by laying them on top of pieces of 2X SSC-soaked Whatman 3 M paper for 2 min. Membranes were then baked at 80°C for 1 h under vacuum.

ASO probes, ASO C1612NL (5′-ATGGGCGCTTGTATGAG-3′) and ASO T1612ABN (5′-ATGGGTGCTTGTATGAG-3′), were synthesized to detect normal and mutant sequences, respectively, in genomic DNA. Each primer was 5′ end-labeled with γ-^{32}P dATP (800 Ci/mM) (DuPont New England Nuclear, Boston, Mass.) and hybridization was performed by incubation in tetramethylammonium chloride (Handelin and Shuber 1994) for 4 h at 55°C, followed by washing conditions described previously (Pomponio et al. 1995). Membranes were then subjected to autoradiography at –70°C from 30 min to overnight.

Results

Analysis of genomic DNA of children with biotinidase deficiency and normal individuals

PCR amplification of the entire 300-bp product did not reveal any differences in the size of the PCR product of the patients when compared to that of individuals with normal biotinidase activity as determined by nondenaturing polyacrylamide gel electrophoresis (data not shown).

SSCA of the 300-bp product from 32 of 33 normal individuals, as exemplified by individuals P58, P87, and P93, exhibited three band conformations (Fig. 1). SSCA of PCR products of patients P14, P42, P55, P86, P161, P172, P190, P191, and P237 revealed three aberrant conformations in addition to the normal conformations. Identical patterns were observed for patients P8, P9, and P13 (sibling of patient P14; data not shown). One of our volunteer controls, P237 (data not shown), who had biotinidase activity in the heterozygous range (3.07 nmol/min/ml serum), also had three aberrantly migrating bands in addition to the normal conformations. P218 and P253 only had aberrant conformations (data not shown).

Automated sequence analysis of exon D

Automated sequence analysis of PCR products of this region revealed a C-to-T transition at nucleotide position 1612 [based on the cDNA sequence (Cole et al. 1994a)] in one allele from each of the patients with the aberrantly migrating conformations and in individual P237 (Fig. 2a, b). Two individuals, P218 and P253, were found to be homozygous for this mutation (Fig. 2c). The results are shown in Table 1.

To determine whether the C_{1612}-to-T mutation represents a spontaneous mutation in these patients, DNA from the parents of each child, when available, was sequenced for this exon D region of the biotinidase gene.

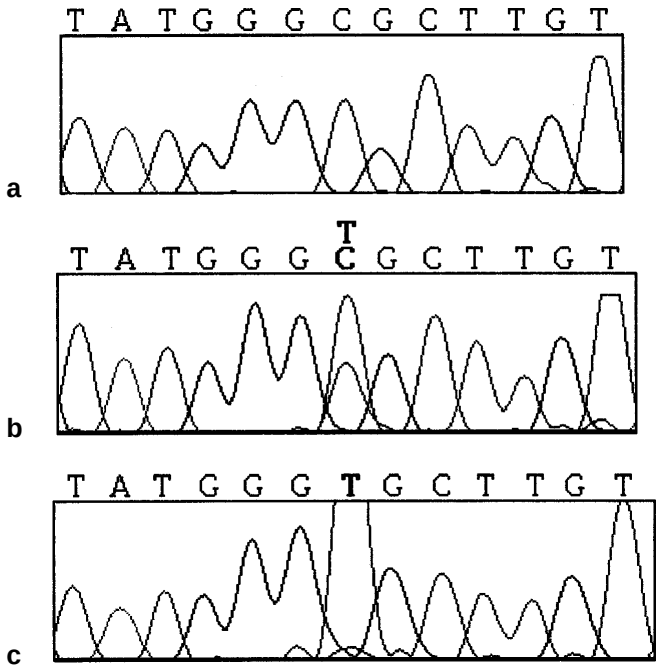

Fig. 2a–c Automated sequencing tracings for the sequence flanking the C_{1612}>T point mutation from genomic DNA samples. All sequences are shown in the 5′→3′ orientation. **a** Sequence from an individual with normal biotinidase activity; **b** sequence from an individual with an aberrant SSCA conformation for this region of exon D who has profound biotinidase deficiency. The *C* and *T* sequenced at nucleotide position 1612 from each amplified allele is depicted above the sequence tracings (based on the assignment of the cDNA, GenBank accession U03274). This individual and the other individuals with this mutation are heterozygous for the R538C mutation. **c** Sequence from an individual who is homozygous for the R538C mutation

ASO hybridization analysis

ASO hybridization analysis using ASO T1612ABN confirmed the presence of the C_{1612}-to-T mutation on one allele of individuals P8, P9, P13, P14, P42, P55, P86, P161, P172, P190, P191 (Fig. 3) and P237 (data not shown). Additionally, this mutation was shown to be present in at least one parent from each of the six parental pairs available for study (Fig. 3). ASO hybridization analysis was used to screen for the occurrence of this mutation in the general population using PCR-amplified DNA isolated from blood spots obtained from an anonymous, random sampling of 371 children. ASO T1612ABN did not hybridize to DNA from any of the 371 blood spot, PCR-amplified DNA, but did hybridize to a positive control on each blot. ASO C1612NL hybridized to DNA from all the normal individuals, indicating the absence of this mutation in these children (data not shown).

Biotinyl-hydrolase activity, biotinyl-transferase activity and crossreacting material to antibody to biotinidase in serum and in cultured fibroblasts

All patients with the R538C mutation studied have profound biotinidase deficiency (less than 10% of mean normal biotinyl-hydrolase activity; Table 1). In addition, all patients have no biotinyl-transferase activity in their serum (Table 1). Trace quantities of crossreacting material to the antibody prepared against the purified normal serum enzyme were found in serum of four of five patients in whom the second mutation is as yet unidentified (Table 1). There was no crossreacting material (CRM) detected in sera from all five patients who have both the R538C and G_{98}:d7i3 deletion/insertion mutation. There was no biotinyl-hydrolase or biotinyl-transferase activity in the serum of P218.

CRM to anti-biotinidase was detected in extracts of fibroblasts from the two normal individuals and was identical in size and concentration under reduced and unreduced conditions (Fig. 4). In addition, there is no indication that the normal enzyme is polymerized in normal cell extracts. CRM was not detected in fibroblast extracts from the child who was heterozygous for the two null mutations under reduced and unreduced conditions. CRM was not detected in the fibroblast extracts of the child who is homozygous for the R538C mutation (P218), but was present at less than normal concentration in the reduced fibroblast extracts.

Table 1 Summary of information on individuals with profound biotinidase deficiency having the R538C mutation

ID	Biotinyl-hydrolase activity[a]	Biotinyl-transferase activity[b]	CRM status[c]	Mutations	Ethnic background Mother/Father
8	0	–	Trace	R538C/?	Am. Indian, Engl., French/English
9	0.19	–	Trace	R538C/?	English, German/Am. Indian, German*
13, 14	0.02, 0	–	Trace	R538C/?	German/German*
42	0.4	–	2+	R538C/?	German, Romanian, Polish/Irish
190	0	–	Trace	R538C/?	Dutch, English, German/Irish, English
218	0.2	–	–	R538C/R538C	Irish*/Irish*
253	0	–	–	R538C/R538C	Italian/Italian
55	0.7	–	–	R538C/G98:d7i3	English, Irish*/? (Adopted)
86	0.4	–	–	R538C/G98:d7i3	Am. Indian, German/Unknown
161	0.14	–	–	R538C/G98:d7i3	French, German, English, Welsh, Indian*/German
172	0.02	–	–	R538C/G98:d7i3	German, Dutch/Scandanavian
191	0	–	–	R538C/G98:d7i3	French, German/German*

? indicates second mutation is unknown; * indicates the parent who has the R538C mutation

[a] Mean normal biotinyl-hydrolase activity is 7.1 nmol/min/ml serum; range 4.4 to 11

[b] Biotinyl-transferase activity ranges from absent (–) to +4 (normal)

[c] CRM is crossreacting material to antibodies prepare against biotinidase that has been purified to homogeneity; ranges from absent (–) to +4 (normal)

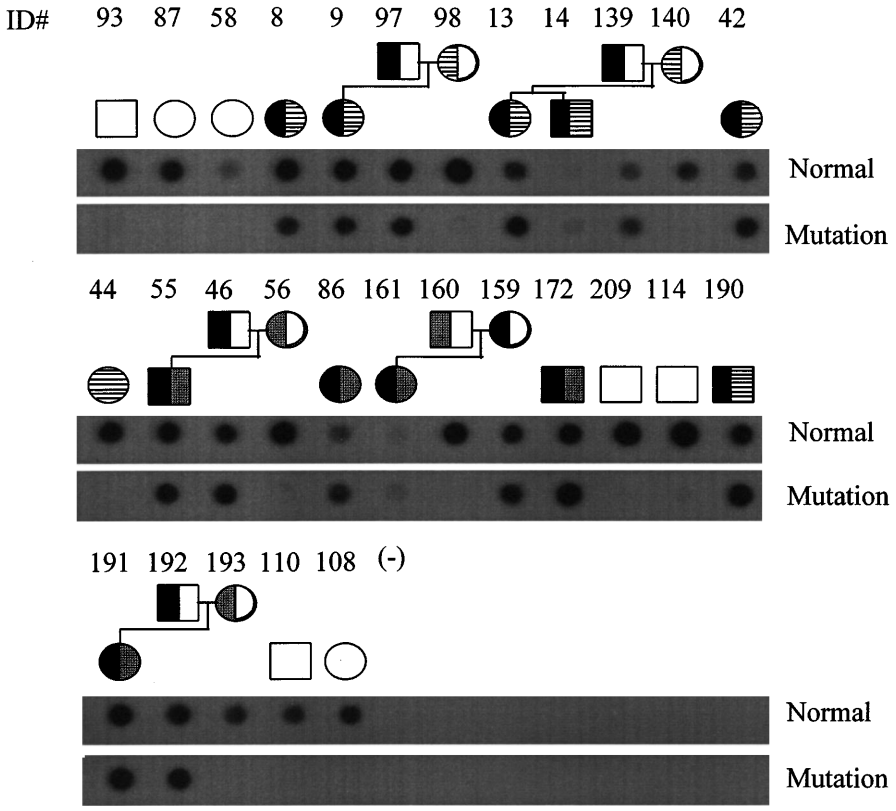

Fig. 3 Allele-specific oligonucleotide (ASO) hybridization analyses of children with biotinidase deficiency and their parents. The *ID number* represents each individual studied and is located above the genotype symbol and ASO blots for each person. The symbols are coded as in Fig. 1. *Normal* refers to the panel of blotted PCR products hybridized with ASO C1612NL, which only hybridizes to normal sequence in this region. *Mutation* refers to a replicate panel hybridized with ASO T1612ABN, which only hybridizes to the R538C mutation. Individuals P93, P87, P58, P209, P114, P110 and P108 are normal controls. The negative control for the PCR reaction is located by (–)

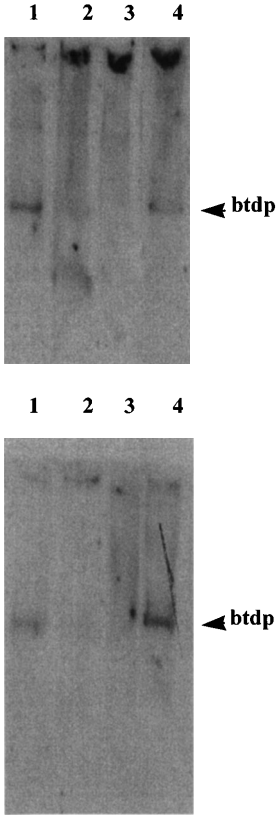

Fig. 4a, b Crossreacting material (CRM) to antibodies to normal biotinidase was determined in extracts of fibroblasts from two normal individuals (*lanes 1* and *4*), a child (P253) who is homozygous for the R538C mutation (*lane 2*), and a child with profound biotinidase deficiency (P2), who is a compound heterozygote for the common deletion/insertion mutation (G_{98}:d7i3) and a 12-bp deletion mutation both resulting in null mutations (*lane 3*). Ten µl of fibroblast extract, unreduced (**b**) and treated with 1% β-mercaptoethanol (**a**), from each individual was run on sodium dodecyl sulfate-polyacrylamide electrophoresis. The gels were blotted and CRM was detected using primary antibody to purified biotinidase and secondary peroxidase-labeled antibody to immunoglobulin. The location of the biotinidase protein is indicated by *btdp*

Discussion

We have previously reported the most common mutation (G_{98}:d7i3) in symptomatic children with profound biotinidase deficiency (Pomponio et al. 1995). This deletion/insertion mutation in the putative secretory signal peptide sequence of the BTD gene causes a frame shift and results in the premature termination of translation of BTD mRNA. We now report another mutation, a C-to-T transition at nucleotide position 1612 (C_{1612} > T) of the BTD gene. This mutation occurred in 10 of 30 symptomatic children with profound biotinidase deficiency.

Since the initial report of the common G98:d7i3 mutation, we have identified seven other patients (P86, P161, P171, P172, P191, P277, P287) with the deletion/insertion mutation on at least one allele. Of these, four (P86, P161, P172, P191) were found to have the R538C mutation and were included in this study.

ASO hybridization confirmed the presence of this mutation in one allele of the 10 patients as well as in one "control" individual, P237. This latter individual was shown to have serum biotinidase activity in the heterozygous range, consistent with having the R538C mutation on one allele. This mutation was not observed in DNA from 32 individuals with normal serum biotinidase activity and in DNA isolated from blood spots from 371 anonymous, randomly selected newborns.

The C_{1612} > T transition occurs within a CpG dinucleotide. Spontaneous mutations at CpG dinucleotides have frequently been observed to result in human genetic disease (Cooper and Youssoufian 1988; Tanaka et al.

1996; Maliaka et al. 1996; Leibundgut et al. 1995; Santisteban et al. 1995). In these cases, the cysteine is methylated to 5-methylcytosine, which can then be deaminated to thymidine (Rideout et al. 1990). To determine if the mutation occurred spontaneously or was inherited in the children with biotinidase deficiency, PCR products of the region of exon D from the available parents were subjected to ASO hybridization analysis and sequenced. The mutation was found in one parent of each parental pair by both of these methods. These results indicate that the mutation in these patients is a stable, inherited trait rather than the result of a spontaneous mutation. Interestingly, most of the patients with the R538C mutation have German ancestry. Five of the six parental pairs who were available for study had German ancestry. Of these, in each case the parent who had German background had the R538C mutation. These results suggest that this mutation may have arisen from a single founder.

The R538C mutation predicts a change of an arginine to cysteine at amino acid position 538, which is located only five amino acids from the carboxy-terminus of the enzyme. There are many reports of arginine-to-cystine mutations that decrease or abolish activity of enzymes. Initially, one would speculate that arginine-to-cysteine mutation is in the active site or important for normal enzyme conformation, thereby causing enzyme deficiency. However, the position of the mutation in the biotinidase molecule probably allows the thiol group of the substituted cysteine to react readily with other cysteines either within the protein, perhaps as it is folded, or with other protein molecules.

A mutation that causes a single amino acid change in an enzyme, especially in the carboxy-terminus, should be detectable with antibody prepared to the purified normal enzyme. No crossreacting material to anti-biotinidase was detected by Western blot analysis under denaturing and reducing conditions in sera of patients who are compound heterozygotes for the R538C and the deletion/insertion mutations. The latter mutation is predicted to produce a protein that does not resemble biotinidase. Therefore, the R538C mutation must result in the absence of an aberrant biotinidase protein in serum.

Biotinidase is present and exists as a monomer in normal fibroblasts. There is no CRM, under reduced and unreduced conditions, in the fibroblasts of the child with two null mutations (P253). The serum of this child also has no CRM. CRM is absent from the fibroblast extracts of this child under unreduced conditions, but is present at a lower concentration in identical extracts treated with the reducing agent. This indicates that the enzyme is present in the cell, but is probably being degraded more rapidly. Therefore, the mutation likely results in abnormal intramolecular folding or intermolecular binding of the aberrant enzyme and its subsequent degradation. Regardless of the exact mechanism, the residual aberrant enzyme is not secreted from the cell.

Serum biotinidase is a glycosylated monomeric enzyme (Craft et al. 1985; Chauhan and Dakshinamurti 1986) that is synthesized mainly in the liver and is then secreted into the blood (Pispa 1965; Weiner et al. 1983). The results of the immunoblot analysis of the fibroblast extracts suggest that the aberrant enzyme is involved in cellular intra- or intermolecular disulfide bond formation, which causes increased degradation and prevents its secretion from liver and other tissues into the blood.

In conclusion, we have described a point mutation (C_{1612}-to-T) in the human biotinidase gene that results in the substitution of a cysteine for arginine538 in the enzyme. This mutation occurred in one-third of symptomatic children with profound biotinidase deficiency and appears to be the second most common mutation that causes profound biotinidase deficiency in symptomatic children.

Acknowledgements This work was supported by NIH grant HD48258 to B.W. We thank Kristin Fleischhauer, Katie Swango, and Greg Meyers for technical assistance and Wanda Andrews for supplying us with the newborn screening samples. We thank the children, their parents, and their physicians, Drs. M. Batshaw, G. Breningstal, D. Crisp, S. Goodman, B. Hainline, R. Kennedy, S. Packman, J. Thoene, and A. Tsao, for the information and samples.

References

Bonjour J-P (1977) Biotin in man's nutrition and therapy: a review. Int J Vitam Nutr Res 47:107

Chauhan J, Dakshinamurti J (1986) Purification and characterization of human serum biotinidase. J Biol Chem 261:4268–4274

Cole H, Reynolds TR, Buck GB, Lockyer JM, Denson T, Spence JE, Hymes J, Wolf B (1994a) Human serum biotinidase: cDNA cloning, sequence and characterization. J Biol Chem 269: 6566–6579

Cole H, Weremowicz H, Morton CC, Wolf B (1994b) Localization of serum biotinidase (BTD) to human chromosome 3 in band p25. Genomics 22:662–663

Cooper DN, Youssoufian H (1988) The CpG dinucleotide and human genetic disease. Hum Genet 78:151–155

Craft DV, Goss NH, Chandramouli N, Wood HG (1985) Purification of biotinidase from human plasma and its activity on biotinyl peptides. Biochemistry 24:2471–2476

Handelin B, Shuber AP (1994) Simultaneous detection of multiple point mutations using allele-specific oligonucleotides. In: Dracapoli NC, Haines JL, Korf BR, Moir DT, Morton CC, Seidman CE, Seidman JG, Smith DR (eds) Current protocols in human genetics. Wiley, New York, pp 4.1–4.8

Hart PS, Hymes J, Wolf B (1992) Biochemical and immunological characterization of serum biotinidase in profound biotinidase deficiency. Am J Hum Genet 50:126–136

Hymes J, Fleischhauer K, Wolf B (1995) Biotinylation of histones by human serum biotinidase: assessment of biotinyl-transferase activity in sera from normal individuals and children with biotinidase deficiency. Biochem Mol Med 56:76–83

Leibundgut EO, Liechti-Gallati S, Columbo J-P, Wermuth B (1995) Ornithine transcarbamylase deficiency: new sites with increased probability of mutation. Hum Genet 95:191–196

Maliaka YK, Chudina AP, Belev NF, Alday P, Bochkov NP, Buerstedde J-M (1996) CpG dinucleotides in the hMSH2 and hMLH1 genes are hotspots for HNPCC mutations. Hum Genet 97:251–255

Orita M, Iwahana H, Kanazawa H, Hayashi K (1989a) Detection of polymorphisms of human DNA by gel electrophoresis as single-stranded conformation polymorphisms. Proc Natl Acad Sci USA 86:2766–2770

Orita M, Suzuki Y, Sekiya T, Hayashi K (1989b) Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 5:874–879

Pispa J (1965) Animal biotinidase. Ann Med Exp Biol Fenn 43 [Suppl 5]:1–39

Pomponio RJ, Reynolds TR, Cole H, Buck GA, Wolf B (1995) Mutational "hotspot" in the human biotinidase gene as a cause of biotinidase deficiency. Nat Genet 11:96–98

Pomponio RJ, Narasimhan V, Reynolds TR, Buck GA, Povirk LF, Wolf B (1996) Deletion/insertion mutation that causes biotinidase deficiency may result from the formation of a quasi-palindromic structure. Hum Mol Genet 5:1657–1661

Rideout WM, Coetsee GA, Olumi AF, Jones PA (1990) 5-Methylcytosine as an endogenous mutagen in the human LDL receptor and p53 genes. Science 249:1288–1290

Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N (1985) Enzymatic amplification of beta-globin genomic sequences and restriction site analysis diagnosis for sickle cell anemia. Science 230:1350–1354

Salbert BA, Astruc J, Wolf B (1993a) Ophthalmological findings in biotinidase deficiency. Ophthalmologica 206:177–181

Salbert BA, Pellock JM, Wolf B (1993b) Characterization of seizures associated with biotinidase deficiency. Neurology 43: 1351–1354

Santisteban I, Arredonto-Vega FX, Kelly S, Loubser M, Meydan N, Roifman C, Howell PL (1995) Three new adenosine deaminase mutations that define a splicing enhancer and cause severe partial phenotypes: implications for evolution of a CpG hotspot and expression of a transduced ADA cDNA. Hum Mol Genet 4:2081–2087

Tanaka H, Shibagaki I, Shimada Y, Wagata T, Imamura M, Ishizaki K (1996) Characterization of p53 gene mutations in esophageal squamous cell carcinoma cell lines: increased frequency and different spectrum of mutations from primary tumors. Int J Cancer 65:372–376

Weiner DL, Grier RE, Watkins P, Heard GS, Wolf B (1983) Tissue origin of serum biotinidase: implication in biotinidase deficiency. Am J Hum Genet 34:56A

Wolf B (1991) Worldwide survey of neonatal screening for biotinidase deficiency. J Inherit Metab Dis 14:923–927

Wolf B, Feldman GL (1982) The biotin-dependent carboxylase deficiencies. Am J Hum Genet 34:699–716

Wolf B, Hsia YE, Sweetman L, Feldman G, Boychuk RB, Bart RD, Crowell DH, Di Mauro RM, Nyhan WL (1981) Multiple carboxylase deficiency: cinical and biochemical improvement following neonatal biotin treatment. Pediatrics 68:113–118

Wolf B, Grier RE, Allen RJ, Goodman SI, Kien CL (1983a) Biotinidase deficiency: the enzymatic defect in late-onset multiple carboxylase deficiency. Clin Chim Acta 131:273–281

Wolf B, Grier RE, Allen RJ, Goodman SI, Kien CL, Parker WD, Howell DM, Hurst DL (1983b) Phenotypic variation in biotinidase deficiency. J Pediatr 103:233–237

Wolf B, Grier RE, Heard GS (1983c) Hearing loss in biotinidase deficiency. Lancet II:1365

Wolf B, Grier RE, Secor McVoy Jr, Heard GS (1985a) Biotinidase deficiency: a novel vitamin recycling defect. J Inherit Metab Dis 8 [Suppl 1]:53–58

Wolf B, Heard GS, Weissbecker KA, Secor McVoy Jr, Grier RE, Leshner RT (1985b) Biotinidase deficiency: initial clinical features and rapid diagnosis. Ann Neurol 18:614–617

Wolf B (1995) Disorders of biotin metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease, 7th edn. McGraw-Hill, New York, pp 3151–3180