

# A Rare Biotinidase Deficiency in the Pediatrics Population: Genotype–Phenotype Analysis

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## Abstract

Biotinidase (BTD) deficiency is a rare autosomal recessive metabolic disorder caused by insufficient biotin metabolism, where it cannot recycle the vitamin biotin. When this deficiency is not treated with supplements, it can lead to severe neurological conditions. Approximately 1 in 60,000 newborns are affected by BTD deficiency. The BTD deficiency causes late-onset biotin-responsive multiple carboxylase deficiency, which leads to acidosis or lactic acidosis, hypoglycemia, and abnormal catabolism. BTD deficiency is of two types based on the amount of BTD Enzyme present in the serum. A wide range of pathogenic mutations in the *BTD* gene are reported worldwide. Mutations in the *BTD* gene lead to profound and partial BTD deficiency. Profound BTD deficiency results in a severe pathogenic condition. A high frequency of newborns are affected with the partial deficiency worldwide. They are mostly asymptomatic, but symptoms may appear during stressful conditions such as fasting or viral infections. Several pathogenic mutations are significantly associated with neurological, ophthalmological, and skin problems along with several other clinical features. This review discusses the *BTD* gene mutation in multiple populations detected with phenotypic features. The molecular-based biomarker screening is necessary for the disease during pregnancy, as it could be helpful for the early identification of BTD deficiency, providing a better treatment strategy. Moreover, implementing newborn screening for the BTD deficiency helps patients prevent several diseases.

## Keywords

- ▶ biotinidase deficiency
- ▶ BTD gene mutation
- ▶ multiple carboxylase deficiency
- ▶ neurological problems

## Introduction

Biotinidase (BTD) is an enzyme responsible for cleaving and recycling the biotin from biocytin and protein-bound sources.<sup>1,2</sup> The biocytin is subsequently cleaved by BTD, which results in the release of biotin and lysine.<sup>3</sup> In humans, biotin, also known as vitamin B7 or Vitamin H, is water-soluble and is required for the coenzyme of five carboxylases, including

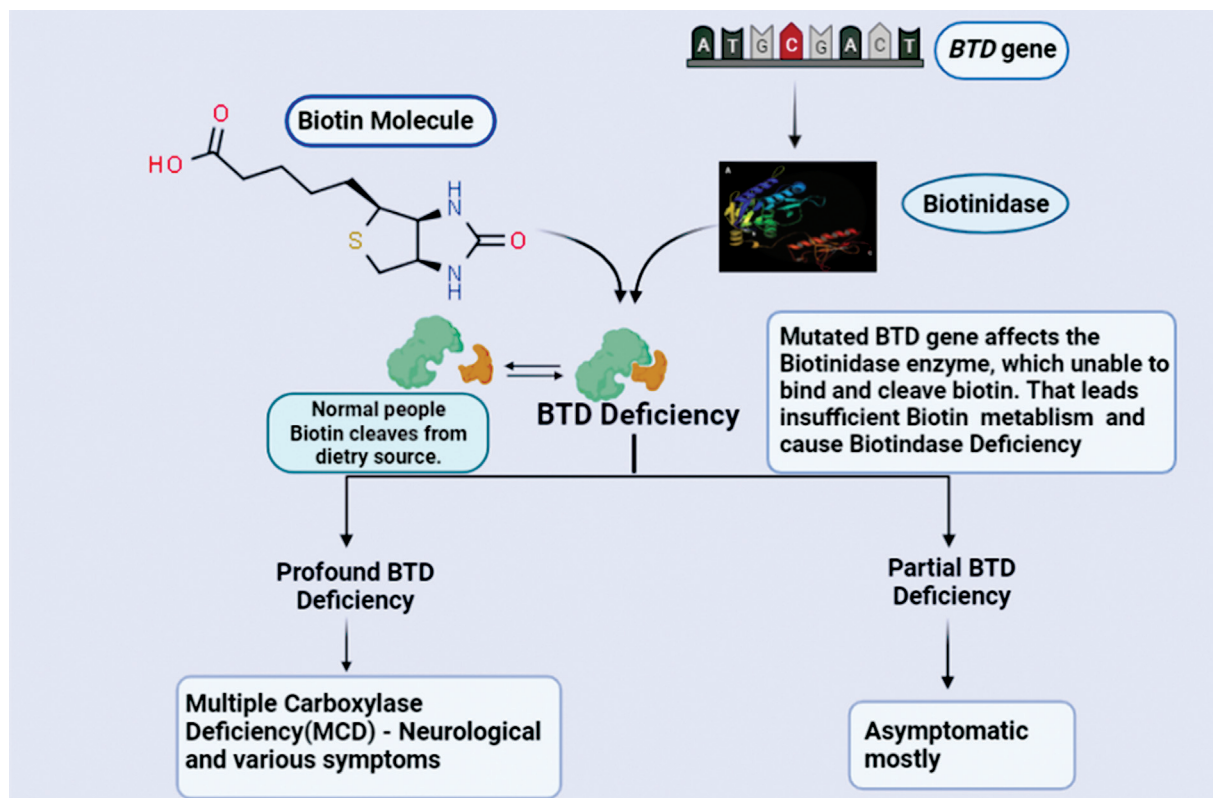
amino acid metabolism, fatty acid metabolism, and gluconeogenesis. It is a proteolytic digestion product of holocarboxylase. BTD has biotinyl-transferase activity, where the biotin is transferred to histone from biocytin under physiological conditions.<sup>4</sup> BTD is a mammalian enzyme found primarily in serum, kidney, and liver.

BTD deficiency is a rare autosomal recessive metabolic disorder caused by insufficient biotin metabolism. In this

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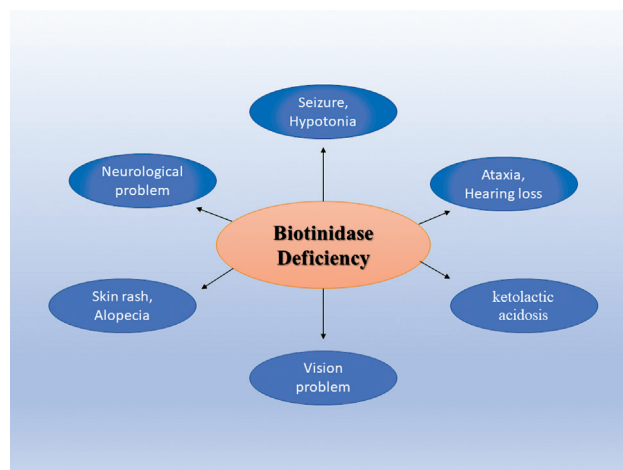
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**Fig. 1** Mutation in the BTD gene affects the biotinidase enzymes that lead to insufficient biotin metabolism and causes BTD deficiency, which is classified into two types, such as profound BTD deficiency and partial BTD deficiency, based on the biotinidase level in the serum. Profound BTD deficiency means less than 10% of normal serum activity. This type of child cannot recycle their endogenous biotin by cleaving it from biocytin, which also consequently causes multiple carboxylase deficiency (MCD). Partial BTD deficiency means 10 to 30% of biotinidase in the normal serum activity, they are mostly asymptomatic, but symptoms may appear during stressful conditions such as fasting or viral infections. BTD, biotinidase.

condition, the body cannot appropriately recycle vitamin biotin. If it's not treated with supplements, it can lead to severe neurological conditions. BTD deficiency causes late-onset biotin-responsive multiple carboxylase deficiency (MCD), leading to acidosis or lactic acidosis, hypoglycemia, and abnormal catabolism.<sup>5-7</sup> The BTD deficiency was first reported by Wolf et al in 1981.<sup>8</sup> Approximately 1 in 60,000 newborns are affected with BTD deficiency.<sup>9</sup> The symptoms may occur after a few weeks of birth or in late childhood. In some conditions, the patient may be asymptomatic. The biotin supplement will reduce the symptoms. BTD deficiency is classified into two types: profound BTD deficiency and partial BTD deficiency, based on the BTD level present in the serum. The average amount of BTD in the human serum range is 4.4 to 10 nmol/min/mL, with a mean activity of 7.1 nmol/min/mL. Profound BTD deficiency means less than 10% of regular serum activity. A child with this type cannot recycle their endogenous biotin by cleaving it from biocytin, which causes MCD (→ Fig. 1). Untreated children have several neurological symptoms and clinical signs such as seizures, ataxia, hypotonia, hearing loss, vision problems, and developmental delay (→ Fig. 2). Unfortunately, they are irreversible.<sup>2,10,11</sup> Symptoms, including skin rashes, alopecia, conjunctivitis, ketolactic acidosis, and organic aciduria can be normalized when treated with supplements. Early treatment may help to prevent expression of symptoms. If un-

treated, the patient might die. Most symptoms of BTD deficiency appear between the ages of 1 week and 10 years with a mean age of 3.5 months.<sup>8</sup> Many countries are screening newborns at birth, to learn about prevalence of the BTD disorder and treat them with biotin supplementation. Partial



**Fig. 2** Untreated BTD children have several neurological symptoms and clinical signs, which includes seizures, ataxia, hypotonia, hearing loss, vision problems, skin rash, alopecia, conjunctivitis, ketolactic acidosis, organic aciduria, and developmental delay. Unfortunately, some symptoms are irreversible. BTD, biotinidase.

BTD deficiency means 10 to 30% of BTD in the average serum activity in many populations. The patient with partial BTD deficiency is mostly asymptomatic (►Fig. 1), symptoms may appear during stressful conditions such as fasting or viral infections.<sup>12</sup> A high level of BTD activity has been reported in children with glycogen storage disease type 1a, but the reason for the BTD level in this disease is unknown.<sup>13</sup>

BTD deficiency fails to release biotin from dietary proteins, or endogenously synthesized carboxylase, which decreases bioavailability. Furthermore, urinary biotin excretion may increase due to renal filtration of free band impaired recycling of biotin from breakdown products of biotinylated carboxylases, such as biocytin.<sup>14</sup> It has a vital role in developing the central nervous system in humans. BTD is present in brain parts such as the red nucleus, lower auditory brainstem nuclei, and cerebellar Purkinje cells.<sup>15</sup> These are the reasons that can cause neurological problems among BTD deficiency patients. In addition, electrophysiological study helps identify the role of biotin deficiency in hearing loss. Studies show that a biotin-deficient diet results in prolonged latency on the auditory brainstem response test (ABR) or increased wave I-IV interpeak intervals as an evidence of a neurological finding.<sup>16</sup> The BTD deficiency patients' clinical symptoms varied, which are based on the severity of mutation.<sup>17,18</sup>

Interestingly, United States and certain countries have mandatory newborn screening (NBS) programs that successfully help prevent or treat BTD deficiency symptoms by giving pharmacological doses of biotin supplements.<sup>7,17</sup> The BTD deficiency is diagnosed among neonates. Enzymatic activity is measured by a colorimetric assay that uses biotinyl-*p*-aminobenzoate as a substrate.<sup>19</sup> Currently, BTD deficiency is primarily diagnosed by biochemical assay. The semiquantitative fluoroimmunoassay determines the BTD serum activity in the dried blood spots of neonates, which use biotinyl-6-aminoquinoline as a substrate.<sup>20</sup> Moreover, commercial ELISA kits are available to screen for BTD deficiency in newborns. If a positive result comes from the biochemical assay, we should further analyze the molecular test for confirmation. The mutations of the *BTD* gene have been detected by sequencing the gene. *BTD* gene mutation causes both profound and partial BTD deficiency in patients. The amino acid effect from *BTD* mutations determines the severity of the disease, either profound or partial BTD deficiency. Moreover, the D444H variant is the most common mutation in the *BTD* gene. This review discusses the *BTD* mutations in both profound and partial BTD deficiency among various populations with clinical signs, the prevalence of the *BTD* mutation, prevention of BTD deficiency through a NBS program, and significance of prenatal diagnosis.

## BTD Gene

The *BTD* gene of humans (OMIM 609019) has been isolated and characterized.<sup>21,22</sup> The *BTD* gene regulates the synthesis of BTD enzyme and is the only gene associated with BTD deficiency. It is located on chromosome 3q25.<sup>21</sup> The human

*BTD* cDNA is from the cDNA hepatic library. The *BTD* cDNA encodes 543 amino acids, and their molecular weight is 61,133 Da. It has highly putative *N*-glycosylation sites and resultant total masses are approximately 74 and 80 kDa. In BTD, there are two potential AUG start codons and an open reading frame of 1629bp relative to the first AUG. The presence of an intron between the two possible start codons could allow alternative splicing. Both AUG start codons are in the same reading frame. Two start codons encode the two putative signal peptides, and the first peptide consists of 21 amino acids and the second of 41 amino acids. The BTD contains 13 cysteine residues, one of which is active and connects the biotinyl carboxyl group by a thiol ester before amide cleavage or biotinyl transfer.

Moreover, the human *BTD* gene contains four exons and three introns. The sizes of the exon 1, 2, 3, and 4 measure around 79 bp, 265 bp, 150 bp, and 1502 bp, respectively and the sizes of the respective intron regions are >12.5 kb, 6.2 kb, and 0.7 kb.<sup>22</sup> The first potential translation initiation codons are encoded in exon-1, while the second is encoded in exon-2. The nucleotide sequence upstream of exons 1 and 2 was examined for putative promoter elements. The promoter features identified from -600 to +400 are consistent with the ubiquitous expression of BTD, which has CpG island characteristics but lacks a TATA element. The six consensus methylation sites and three initiators (INR) sequences are considered necessary in the transcription initiation of TATA-less promoters. The consensus sequence for the HNF-5 liver-specific transcription factor is found at -352. The nucleotide sequence 5' of exon 2, which contains the second putative ATG initiation codon, has features associated with house-keeping genes but does contain a consensus sequence for the liver-specific transcription factor C/EBP within 300 bp of the 5' ends of exon 2.<sup>11</sup>

On a related note, when the human BTD amino acid sequence was compared with bacterial amidases and nitro-lases, the result indicated that certain regions are highly conserved.<sup>23</sup> The conserved regions consist of active sites of cysteine of amidases and nitro-lases that likely indicate the site of BTD involved in the thioester binding of biotin upon their cleavage from biocytin. *In silico* prediction tools and empiric data from the BTD enzyme activity help predict the pathogenicity of mutations, but sometimes the results are discordant. *BTD* gene sequencing is an essential tool for understanding the correlation between the genotype and biochemical phenotype of the patient.<sup>23,24</sup>

## BTD Mutation

Mutations in the *BTD* gene cause profound and partial BTD deficiency. More than 300 pathogenic mutations in the *BTD* gene have been identified, possibly causing BTD deficiency. Direct sequencing of some of these overlapping regions of the BTD gene, including intron and exon junctions, helps to perform mutation analysis. In *BTD* gene mutations, all types of mutations have been identified that can cause BTD deficiency. The mutations include missense, nonsense, cryptic splice site mutations, compound allelic mutations, single and

multiple nucleotide insertions, single and multiple nucleotide deletions, and point mutations, which result from either premature stop codon formation or single amino acid substitution. Profound *BTD* deficiency arises in the homozygous or heterozygous state.<sup>25,26</sup>

In addition, mutations have been identified in the entire coding sequence; however, none has been reported in exon 1. While several published studies have sequenced the entire *BTD* gene, including exon 1<sup>25–28</sup> failure to find a mutation in exon 1 is most likely explained by the fact that exon 1 contains the first in-frame ATG, but not the highly conserved second ATG, which is the preferred or only actually used initiation codon. If so, we could not expect exon 1 changes to influence the *BTD* synthesis or secretion when the second ATG is the primary or only beginning site encoding the signal peptide sequence.<sup>29</sup> In some patients with *BTD* deficiency, there is no correlation between genotype and phenotype in *BTD* deficiency,<sup>30</sup> and *BTD* deficiency is primarily characterized based on skin and neurological abnormalities. The C-terminus of the protein mutation results in the severe loss of *BTD* activity.<sup>25</sup> Mutations in the carboxy-terminus of the *BTD* gene cause profound deficiency, and the site is affected by several missense-mutations.<sup>23</sup>

A high level of *BTD* gene mutations was identified in the U.S. population. Several unique mutations were reported in Turkey, France, United Kingdom, Saudi Arabia, Austria, Hungary, Italy, Brazil, and China. The *BTD* gene mutations are reported worldwide, including India, Germany, Pakistan, Sri Lanka, Afghanistan, Iraq, Poland, Nigeria, Iran, Spain, Sweden, Egypt, Syria, and Ethiopian populations, all listed in **Table 1**. According to the analysis of various studies and reports on *BTD* deficiency, a novel mutation in the *BTD* gene arises continuously every decade.

### Mutation in the U.S. Population

In 2016, Procter et al discovered 48 novel mutations in a patient with *BTD* deficiency in the U.S. population. Their study analyzed 300 samples to determine the genotype to confirm the degree of *BTD* deficiency. The heterozygous novel alteration was found in all cases, and almost all of them have a second heterozygous mutation; such alterations are determined as mutations and classified as pathogenic. A total of 32 of 48 individuals had a second mutation with D444H in another allele. Children with partial *BTD* deficiency mostly had a D444H second mutation in another allele. One of the individuals had a missense alteration in one allele and an alteration within an intron in another allele. The alteration in the intron does not affect the enzymatic activity.<sup>31</sup>

A transversion of the 1368A > C cDNA mutation causes the substitution of histidine for glutamine at 456 positions. The Q456H amino acid substitution is a simple polymorphism and is the most common mutation in *BTD* deficiency in children identified in NBS.<sup>32</sup> The partial *BTD* 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. The D444H variant of *BTD* deficiency is similar to the Duarte variant in galactosemia.<sup>33</sup>

Cowan et al studied the *BTD* deficiency in California from July 2007 to June 2011. The population had profound *BTD* deficiency being estimated at one in 73,629 and profound plus partial variant cases at one in 31,717. Compared with global incidence, California has demonstrated a higher ratio of patients with one in 112,271 and one in 60,089 with profound variant and combined cases, respectively.<sup>34</sup>

Fascinatingly, Li et al identified the first intronic mutation in the *BTD* gene c.310–15delT in the U.S. population. The homozygous mutation p.T234I found in a child, which causes profound deficiency, also carries a single benign polymorphism, p.C471C. The combination of p.M86R with p.Q456H mutation is pathogenic. The p.Q456H is the other most common mutation that is combined with another mutation in the second allele.<sup>5</sup>

However, partial *BTD* deficiency in patients with spinal cord disease had a novel missense mutation, H447Y, which also caused the profound *BTD* deficiency. The individual also had mild alopecia, rapidly progressive symptoms, atypical neurological features, and an absence of cutaneous manifestations that initially led to an erroneous diagnosis and treatment. In the United States, the four mutations most commonly linked with complete *BTD* deficiency are C33Fs\*36, Q456H, R538C, and the double mutation D444H: A171T. Partial *BTD* deficiency is almost universally accredited to the D444H mutation.<sup>11</sup>

### Mutation in the Turkish Population

*BTD* deficiency is higher in Turkey compared with the rest of the world. The recently published data from the Ministry of Health, Turkey, reported the incidence at approximately one in 7,116, compared with worldwide incidence of one in 60,000. Karaca et al study of mutations in NBS found 26 mutations in 192 patients, where 91% of the *BTD* patients were affected with two mutations in two different alleles. The p.R157H, p.D444H, c.98–104del7ins3, and p.T532M were commonly found in 72.3% of the mutant alleles.<sup>35</sup> Mutations R79C, Y93C, and R211F create a new abnormal cysteine, while C143F, C186Y, and C418S mutations reveal a loss of cysteine residues in the *BTD* gene. There are 13 functionally important cysteine residues present in the matured *BTD* enzyme.<sup>21</sup>

Various studies suggest that the mutations T196R, V199M, G310E, P368L, Y454C, R538C, D543H, and 3' splice site mutation with 100G > A are the most common mutations in the Turkish population.<sup>6,26,36–38</sup> D444H, H447Y, Q456H, V457L, V457M, G480R, P497S, and T532M mutations are located in a conserved region that corresponds to the protein's C-terminus and may impact its biotin-binding properties.<sup>29,36,39</sup>

In Turkey, NBS program is mandatory to detect *BTD* deficiency. Interestingly, mutation H447Y is associated with myelopathy, whose amino acid effect disrupts the nearby disulfide bond formation.<sup>40</sup> In the southeastern part of Turkey, there is a high rate of consanguineous marriages. The children of these parents were affected with homozygous or compound heterozygous mutations (c.235C > T in exon 2 and c.470G > A, c.557G > A, c.1330G >

**Table 1** BTD gene mutation among the various population in the world. Location of the mutation in BTD gene DNA sequence, the effect of amino acid, and clinical symptoms in BTD deficiency patient

S. no	cDNA	Location	Amino acid effect	Phenotype	Clinical symptoms	Population	Reference
1	98–104del7ins3	Exon 2	Frameshift	Patient with BTD	N/A	British	54
2	98_104del7ins3; 212T > C	Exon 2; Exon 2	Frameshift	Patient with BTD	None	Spain	25
3	99C > T	Exon 2	C33C (PM)	Patient with BTD	None	N/A	38
4	100G > A	Exon 2	G34A	Patient with BTD	Neurological problems	Spain	25
5	128A > G;1330G > C	Exon 2; Exon 4	H43R	Patient with BTD	N/A	United States	31
6	133G > A	Exon 2	G45R	Patient with BTD	N/A	Brazil	78
7	133G > A;865G > C	Exon 2, Exon 4	G45R; A289P	Patient with BTD	N/A	United States	30
8	133G > A;1271G > c	Exon 2; Exon 4	G45R; C424S	Patient with BTD	Tachypnoea, eczema	Scottish	38
9	133C > T	Exon 2	H447Y	Myelopathy (7-y-old boy)		India	58
10	136G > T	Exon 2	E46X	Patient with BTD	N/A	Hungarian	56
11	159C > A; 160G > T	Exon 2	H53Q; E54X	Patient with BTD	None	Nigerian	38
12	171T > G	Exon 2	Y57X	Patient with BTD	N/A	Turkish	47
13	184G > T	Exon 2	V62L	Patient with BTD	N/A	United States	30
14	184G > A	Exon 2	V62M	Patient with BTD	None	Austria	26
15	190G > A	Exon 2	E64K	Patient with BTD	N/A	Spanish	28
16	192G > C;1330G > C	Exon 2; Exon 4	E64D;D444H	Patient with BTD	N/A	United States	31
17	192–193ins5	Exon 4	L69H	Patient with BTD	N/A	Turkish	43
18	194ins4	Exon 2	Frameshift	Patient with BTD	Hearing loss	Turkish	37
19	194A > G	Exon 2	H65R	Patient with partial deficiency	N/A	Caucasian	52
20	203–206dup	Exon 2	Frameshift	Patient with BTD	N/A	Italy	62
21	211C > T	Exon 2	L71L (PM)	Patient with BTD	N/A	N/A	68
22	212T > C	Exon 2	L71P	Patient with BTD	Asymptomatic	N/A	38
23	212T > C;236G > A	Exon 2	L71P; R79H	Patient with BTD	N/A	Hungarian	57
24	235C > T	Exon 2	R79C	Patient with BTD	N/A	Turkish	47
25	235C > T;1361A > C	Exon 2	R79C; Y454C	Patient with BTD	Sparse hair	Turkish	38
26	235C > T;470G > A	Exon 2; Exon 4	R79C; R157H	Patient with BTD	N/A	United States	42
27	236G > A	Exon 2	R79H	Patient with BTD	N/A	Brazil	78
28	245C > A	Exon 2	A82D	Patient with BTD	N/A	Hungarian	57
29	245C > T;1330G > C	Exon 2; Exon 4	A82V; D444H	Patient with BTD	N/A	United States	31
30	245C > G	Exon 2	A82G	Patient with BTD	N/A	Turkish	79
31	246–254del9	Exon 2	L83-L85del	Patient with BTD	N/A	N/A	54
32	248T > C	Exon 2	L83S	Patient with BTD	N/A	Egyptian	68
33	250G > C; 878dupT	Exon3	Splice site; H294T	Patient with profound deficiency	Seizure, motor delay, dermatitis, and hearing loss	Chinese	49
34	257T > G;1368A > c	Exon 2; Exon 4	M86R; Q456H	Patient with partial deficiency	Mild sensorineural hearing loss.	United States	5
35	262C > T	Exon 2	Q88X	Patient with BTD	N/A	N/A	54
36	278A > G	Exon 2	Y93C	Patient with BTD	N/A	Turkish	28
37	283C > T;1330G > C	Exon 2; Exon 4	Q95X; D444H	Patient with BTD	None	Caucasian	38
38	298G > A	Exon 2	A100T	Patient with BTD	N/A	Turkish	28
39	299C > T;1330G > C	Exon 2; Exon 4	A100V; D444H	Patient with BTD	N/A	United States	31
40	310G > T	Exon 3	D104Y	Patient with BTD	N/A	United States	30
41	321T > G;1330G > C	Exon 3; Exon 4	I107M; D444H	Patient with BTD	N/A	United States	31

(Continued)

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**Table 1** (Continued)

S. no	cDNA	Location	Amino acid effect	Phenotype	Clinical symptoms	Population	Reference
42	326T > G	Exon 3	V109G	Patient with BTD	N/A	Austria	26
43	326dupT	Exon 3	Frameshift	Patient with BTD	N/A	United States	31
44	334G > C	Exon 3	E112Q	Patient with BTD	N/A	British	54
45	334G > A	Exon 3	E112K	Patient with BTD	N/A	Hungarian	57
46	341G > T	Exon 3	G114V	Patient with BTD	UTI, hearing loss	Syrian	38
47	356A > G;1459delT	Exon 3	N119S	Patient with BTD	N/A	United States	31
48	364A > G	Exon 3	R122G	Patient with BTD	N/A	United States	30
49	382T > G	Exon 3	F128V	Patient with BTD	N/A	Italy	33
50	383T > C	Exon 3	F128S	Patient with BTD	N/A	Turkish	79
51	386dupT	Exon 3	Frameshift	Patient with BTD	N/A	Italy	62
52	393delC	Exon 3	Frameshift	Patient with BTD	N/A	Palestinian	28
53	395T > G;637delC	Exon 3; Exon 4	M132W; Frameshift	Patient with BTD	Hypotonia, fatigue, respiratory problems	China	44
54	406delC	Exon 3	Frameshift	Neonates with BTD	N/A	Hungarian	55
55	419G > A	Exon 3	W140	Patient with BTD	N/A	Turkish	43
56	420G > A;637delC	Exon 3; Exon 4	W140X	Patient with BTD	Seizure, hypotonia	China	44
57	424C > A	Exon 3	P142T	Patient with profound deficiency	N/A	Somalian	52
58	428G > T	Exon 3	C143F	Patient with BTD	N/A	Turkish	35
59	443G > A	Exon 3	R148H	Patient with BTD	N/A	N/A	68
60	444C > A	Exon 3	A148A (PM)	Patient with BTD	N/A	Spanish	80
61	445T > C	Exon 3	F149L	Patient with BTD	N/A	United States	30
62	449T > A	Exon 4	V150G	Patient with BTD	Seizures, hypotonia	Jordan	67
63	454A > C	Exon 3	T152P	Patient with BTD	N/A	Hungarian	56
64	455C > G;1330G > c	Exon 3; Exon 4	T152R; D444H	Patient with partial deficiency	N/A	United States	5
65	459G > A	Exon 4	Splice site	Patient with BTD	N/A	United States	54
66	460-1G > T	Exon 4	V461D	Patient with BTD	Developmental delay	China	81
67	464T > C;637delC	Exon 4	L155P	Patient with BTD	Seizure, hypotonia	China	44
68	466C > T	Exon 4	Q156X	Patient with BTD	N/A	Turkish	47
69	469C > T	Exon 4	R157C	Patient with BTD	N/A	Hungarian	56
70	470G > A	Exon 4	R157H	Patient with BTD	N/A	British	54
71	470G > A;1330G > C	Exon 4	R157H; D444H	Patient with BTD	N/A	United States	30
72	485C > T	Exon 4	A162V	Patient with BTD	N/A	United States	30
73	490-491del2	Exon 4	Frameshift	Patient with BTD	N/A	Turkish	47
74	508G > A	Exon4	V170M	Patient with profound deficiency	None	Italy	63
75	511G > A;1330G > C	Exon 4	A171T; D444H	Patient with BTD	N/A	United States	30
76	515A > G	Exon 4	N172S	Patient with BTD	None	Austria	26
77	528G > T	Exon 4	K176N	Patient with BTD	N/A	United States	30
78	528-542del15	Exon 4	A197-S201	Patient with profound deficiency	Seizures, hearing loss, hypotonia	Iran	64
79	544delA	Exon 4	Frameshift	Patient with BTD	N/A	Turkish	47
80	557G > A	Exon 4	C186Y	Patient with BTD	N/A	Turkish	47
81	559C > T	Exon 4	P187S	Patient with BTD	None	Austria	25
82	566A > G; -34C > T	Exon 4	D189G	Patient with BTD	N/A	United States	31
83	582C > G;1330G > C	Exon 4	F194L; D444H	Patient with BTD	N/A	United States	31

**Table 1** (Continued)

S. no	cDNA	Location	Amino acid effect	Phenotype	Clinical symptoms	Population	Reference
84	583A > G	Exon 4	N195D	Patient with BTD	N/A	N/A	54
85	584A > G	Exon 4	N195S	Patient with BTD	N/A	Hungarian	56
86	587C > g	Exon 4	196R	Patient with BTD	N/A	Turkish	47
87	594-596del3	Exon 4	V199del	Patient with BTD	N/A	N/A	54
88	594delC	Exon 4	Frameshift	Patient with BTD	N/A	Italy	62
89	595G > A	Exon 4	V199M	Patient with BTD	N/A	Brazil	78
90	605A > T	Exon 4	N202I	Patient with BTD	N/A	Ethiopian	68
91	617-619del/TTG	Exon 4	V207del	Patient with BTD	N/A	Turkish	79
92	625C > T;1368A > C	Exon 4	R209C; Q456H	Patient with BTD	N/A	United States	31
93	626G > A;1368A > C	Exon 4	R209H; Q456H	Patient with profound deficiency	N/A	United States	51
94	629A > G	Exon 4	Y210C	Patient with BTD	N/A	British	54
95	631C > T	Exon 4	R211C	Patient with BTD	N/A	United States	30
96	631C > A	Exon 4	R211S	Patient with BTD	NA	Brazil	24
97	632G > A	Exon 4	Splice site	Neonates with BTD	N/A	Greek	18
98	632G > T	Exon 4	R211L	Patient with BTD	N/A	Brazil	24
99	641A > G;1330G > A	Exon 4	N21AS; D444H	Patient with BTD	None	Caucasian	38
100	643C > T	Exon 4	L215F	Patient with BTD	N/A	British	54
101	644T > A;637delC	Exon 4	L215H	Patient with BTD	Fatigue, ataxia	China	44
102	645C > T	Exon 4	L215L (PM)	Patient with BTD	N/A	N/A	68
103	646T > A;1330A > C	Exon 4	Y216N; D444H	Patient with BTD	N/A	United States	31
104	652G > C	Exon 4	E218Q	Patient with BTD	N/A	Hungarian	57
105	654G > C	Exon 4	E218D	Patient with BTD	Respiratory problem, Hypotonia	Caucasian	38
106	664G > C;1330G > C	Exon 4	D222H; D444H	Patient with BTD	N/A	United States	31
107	682G > T	Exon 4	D228Y	Patient with BTD	N/A	German	33
108	683A > G;1330G > C	Exon 4	D228G; D444H	Patient with partial deficiency	N/A	United States	5
109	692delC	Exon 4	Frameshift	Patient with BTD	N/A	United States	31
110	695T > C;1330G > C	Exon 4	F232S; D444H	Patient with BTD	N/A	United States	31
111	701C > T;1330A > G	Exon 4	T234I; D444H	Patient with partial deficiency	N/A	United States	5
112	709G > A;1330G > C	Exon 4	A237T; D444H	Patient with BTD	N/A	United States	31
113	731C > T	Exon 4	T244I	Patient with BTD	N/A	Turkish	35
114	734G > A	Exon 4	C245Y	Patient with BTD	None	Caucasian	38
115	743T > C;528G > T	Exon 4	I248T; K176N	Patient with BTD	N/A	United States	31
116	755A > G	Exon 4	D262G	Patient with BTD	N/A	United States	30
117	757C > T	Exon 4	P253S	Patient with BTD	None	Caucasian	38
118	758C > T;1489C > T	Exon 4	P253L; P497S	Patient with BTD	N/A	United States	31
119	764T > C	Exon 4	I255T	Patient with BTD	N/A	Swedish	68
120	770T > A;1330G > C	Exon 4	V257D; D444H	Patient with BTD	N/A	United States	31
121	783T > C;1330G > c	Exon 4	Y261X; D444H	Patient with BTD	N/A	United States	31
122	794A > T	Exon 4	H265L	Patient with BTD	N/A	Spanish	28
123	794A > T;933T > G	Exon 4	H265L; S311R	Patient with BTD	N/A	N/A	25
124	814T > G;1330G > C	Exon 4	W272G; D444H	Patient with BTD	N/A	United States	31
125	815G > A;1330G > C	Exon 4	W272T; D444H	Patient with BTD	N/A	United States	31
126	832C > G	Exon 4	L278V	Patient with BTD	N/A	Hungarian	57
127	833T > C	Exon 4	L278P	Patient with BTD	N/A	British	54

(Continued)

**Table 1** (Continued)

S. no	cDNA	Location	Amino acid effect	Phenotype	Clinical symptoms	Population	Reference
128	836T > G	Exon 4	L279W	Patient with BTD	None	Austria	26
129	836T > A;310–15delT	Exon 4	L279X	Patient with BTD	N/A	United States	31
130	856A > G;1330G > C	Exon 4	K286E; D444H	Patient with BTD	N/A	United States	31
131	858delA	Exon 4	Frameshift	Patient with BTD	N/A	Brazil	24
132	859G > A	Exon 4	A287T	Patient with BTD	N/A	Turkish	79
133	865G > C;1330G > C	Exon 4	A289P; D444H	Patient with BTD	N/A	United States	31
134	880A > G	Exon 4	I294V	Patient with BTD	N/A	United States	31
135	887T > G	Exon 4	V296G	Patient with BTD	N/A	German	28
136	895G > C;1413T > C	Exon 4	A299P; C471C	Patient with BTD	N/A	United States	31
137	896C > T	Exon 4	A299V	Patient with BTD	N/A	Spanish	80
138	898A > C;1330G > C	Exon 4	N300H; D444H	Patient with partial deficiency	N/A	United States	5
139	929G > A	Exon 4	G310E	Patient with BTD	N/A	Turkish	47
140	932G > A	Exon 4	S311N	Patient with BTD	N/A	United States	30
141	932G > C;1314T > A	Exon 4	S311T; Y438X	Patient with BTD	N/A	Brazil	24
142	933delT	Exon 4	Frameshift	Patient with BTD	N/A	French	54
143	933T > G; 933T > G	Exon 4	S311R	Patient with BTD	N/A	N/A	25
144	934G > A	Exon 4	G313S	Patient with BTD	N/A	Poland	28
145	935G > A	Exon 4	G312D	Patient with BTD	N/A	N/A	54
146	956C > T	Exon 4	S319F	Patient with BTD	N/A	Turkish	43
147	968A > G	Exon 4	H323R	Patient with BTD	N/A	Afghan	33
148	1001T > A;1330G > c	Exon 4	I334N; D444H	Patient with BTD	N/A	United States	31
149	1046A > C;1330G > C	Exon 4	N349T; D444H	Patient with BTD	N/A	United States	31
150	1049delC	Exon 4	Frameshift	Patient with BTD	N/A	Morocco	28
151	1052delC	Exon 4	Frameshift	Patient with BTD	None	Spain	25
152	1081T > G	Exon 4	F361V	Patient with BTD	N/A	Brazil	24
153	1096T > C	Exon 4	S366P	Patient with BTD	N/A	N/A	53
154	1096–1097dupTC	Exon 4	Frameshift	Patient with BTD	N/A	United States	31
155	1106C > T	Exon 4	P368L	Patient with BTD	N/A	Turkish	28
156	1126C > T;1612C > T	Exon 4	Q376X; R538	Patient with BTD	N/A	United States	31
157	1157G > A	Exon 4	W386X	Patient with BTD	N/A	N/A	38
158	1158G > A	Exon 4	W386X	Patient with BTD	N/A	United States	30
159	1171C > T;1334G > T	Exon 4; Exon 4	P391S (PM); G445V	Patient with BTD	Asymptomatic	Caucasian	38
160	1191–1192del2	Exon 4	Frameshift	Patient with BTD	N/A	N/A	28
161	1201G > A	Exon 4	D401N	Patient with BTD	N/A	Turkish	79
162	1205A > G	Exon 4	N402S	Patient with BTD	N/A	N/A	68
163	1207T > G;1330G > C	Exon 4	F403V; D444H	Patient with BTD	N/A	United States	30
164	1211C > T	Exon 4	T404I	Patient with BTD	N/A	Italy	62
165	1212–1222 del11	Exon 4	Frameshift	Patient with BTD	N/A	Turkish	35
166	1214T > C	Exon 4	L405P	Patient with BTD	N/A	Swedish	68
167	1227–1241del15ins11	Exon 4	Frameshift	Patient with BTD	N/A	United States	30
168	1239delC	Exon 4	Frameshift	Patient with BTD	N/A	N/A	28
169	1239del12	Exon 4	Frameshift	Patient with BTD	N/A	N/A	28
170	1240–1251del12	Exon 4	414-V417del	Patient with BTD	N/A	N/A	54
171	1241–1252del12bp	Exon 4	Y414-V471del	Patient with BTD	N/A	United States	31
172	1249G > T	Exon 4	V417F	Patient with partial deficiency	N/A	Caucasian	52



**Table 1** (Continued)

S. no	cDNA	Location	Amino acid effect	Phenotype	Clinical symptoms	Population	Reference
173	1250–1251TC > AG	Exon 4	V417E	Patient with BTD	Asymptomatic	China	81
174	1252T > C;1330G > C	Exon 4	C418R; D444H	Patient with BTD	N/A	United States	31
175	1253G > C	Exon 4	C418S	Patient with BTD	N/A	Hungarian	57
176	1264–1265insC	Exon 4	Frameshift	Patient with BTD	Neurological problems	Morocco	25
177	1267T > C	Exon 4	C423R	Patient with BTD	N/A	British	54
178	1268G > C	Exon 4	C423W	Patient with BTD	None	Austria	26
179	1271G > A	Exon 4	C424Y	Patient with BTD	The respiratory problem, seizure	Hispanic	38
180	1275T > G	Exon 4	Y425X	Patient with BTD	None	Austria	25
181	1284C > T;1489C > T	Exon 4; Exon 4	Y4228Y(PM); P497S	Patient with BTD	Asymptomatic	Nigerian	38
182	1284C > A	Exon 4	Y428X	Patient with BTD	N/A	China	27
183	1306G > A	Exon 4	V471E	Patient with BTD	Asymptomatic	China	81
184	1309C > G;1330G > C	Exon 4	L437V; D444H	Patient with BTD	N/A	United States	31
185	1313A > G	Exon 4	Y438C	Patient with BTD	N/A	Poland	28
186	1314T > A	Exon 4	Y438X	Patient with BTD	N/A	Brazil	78
187	1316T > C;1413T > C	Exon 4	A439D; C471C	Patient with profound deficiency	Global developmental delay	Sri Lankan	66
188	1320delG	Exon 4	Frameshift	Patient with BTD	N/A	Turkish	35
189	1328T > C	Exon 4	F443S	Patient with BTD	N/A	United States	31
190	1330G > C	Exon 4	D444H	Patient with BTD	N/A	Hungarian	33
191	1333G > A	Exon 4	G445R	Patient with BTD	N/A	Iraq	68
192	1334G > T	Exon 4	G445V	Patient with BTD	N/A	British	54
193	1134G > A;1330G > C	Exon 4	G445E; D444H	Patient with BTD	N/A	United States	31
194	1339C > T	Exon 4	H447Y	Spinal cord disease	Seizures, Hypotonia	United States	40
195	1352G > A	Exon 4	G451D	Patient with BTD	N/A	Afghan	33
196	1352–1353delGC	Exon 4	Frameshift	Patient with BTD	N/A	United States	31
197	1368A > C	Exon 4	Q456H	Patient with BTD	N/A	United States	30
198	1369G > A	Exon 4	V457M	Patient with BTD	N/A	Latin	54
199	1369G > C	Exon 4	V457C	Patient with BTD	N/A	Turkish	35
200	1372–1373insT	Exon 4	C458fs	Patient with partial deficiency		United States	5
201	1382T > A;460–1G > T	Exon 4	V461D	Patient with BTD	Developmental delay	China	81
202	1384delA	Exon 4	Frameshift	Patient with BTD	N/A	China	27
203	1388G > A	Exon 4	C463Y	Patient with BTD	N/A	German	28
204	1413T > C	Exon 4	C471C(PM)	Patient with BTD	N/A	N/A	53
205	1432G > C	Exon 4	A478P	Patient with profound deficiency	N/A	Pakistan, India	52
206	1432G > A;755A > G	Exon 4	A478T; D252G	Patient with BTD	N/A	United States	31
207	1438G > A	Exon 4	G480R	Patient with BTD	N/A	Turkish	35
208	1455C > G;1330G > C	Exon 4	H485Q; D444H	Patient with BTD	N/A	United States	31
209	1457T > A	Exon 4	L486Q	Hyperkeratosis	Eczematous lesions, hyperkeratotic erythema	China	48
210	1458delG;278A > G	Exon 4	Frameshift; Y93C	Patient with BTD	N/A	United States	31
211	1459T > C	Exon 4	W487R	Patient with BTD	N/A	Turkish	47
212	1459delT	Exon 4	Frameshift	Patient with BTD	N/A	United States	31
213	1463G > A	Exon 4	G488D	Patient with BTD	N/A	Poland	54
214	1466A > C	Exon 4	N489T	Patient with BTD	Asymptomatic	Japan	53

(Continued)

**Table 1** (Continued)

S. no	cDNA	Location	Amino acid effect	Phenotype	Clinical symptoms	Population	Reference
215	1471A > G	Exon 4	S491H	Patient with BTD	N/A	Turkish	79
216	1475C > T	Exon 4	T492I	Patient with BTD	Mild hypotonia	Italy	60
217	1481A > G	Exon 4	Y494C	Patient with BTD	N/A	United States	31
218	1489C > T	Exon 4	P497S	Patient with BTD	N/A	United States	30
219	1493dupT;235C > T	Exon 4; Exon 2	Frameshift; R79C	Patient with BTD	Seizures, fatigue, rash	China	44
220	1493–1494insT	Exon 4	Frameshift	Patient with BTD	N/A	China	27
221	1511T > A	Exon 4	M504K	Patient with BTD	N/A	Hungarian	57
222	1526C > G;1330G > C	Exon 4	P509R; D444H	Patient with BTD	N/A	United States	31
223	1531G > A	Exon 4	Q511E	Patient with BTD	None	Caucasian	38
224	1595C > T	Exon 4	T532M	Patient with BTD	N/A	United States	30
225	1601C > T	Exon 4	A534V	Patient with BTD	N/A	Brazil	24
226	1610C > A	Exon 4	G537V	Patient with BTD	N/A	French	28
227	1612C > T	Exon 4	R538C	Patient with BTD	N/A	United States	54
228	1612C > A	Exon 4	R538S	Patient with BTD	N/A	United States	31
229	1613G > A;1062G > A	Exon 4	R538H; T354T	Patient with BTD	N/A	United States	31
230	1616–1617insT	Exon 4	Frameshift	Patient with BTD	N/A	United States	30
231	1619A > G	Exon 4	Y540C	Patient with BTD	N/A	United States	30
232	1627G > C	Exon 4	D543H	Patient with BTD	None	Turkish	26
233	1628A > T	Exon 4	D543V	Patient with BTD	N/A	United States	31
234	1629C > A;1330G > C	Exon 4	D543E; D444H	Patient with BTD	N/A	United States	31
235	44 + 1G > A	Intron 1	Splice variant	Patient with partial deficiency	Asymptomatic	United States	82
236	310–15delT	Intron 2	mRNA expression	Patient with partial deficiency	N/A	United States	5
237	12236G > A	Intron	Intronic	Patient with BTD	None	Turkish	26
238	Entire Exon 1 Deletion	Exon 1	N/A	Patient with profound deficiency	Seizure, Global developmental delay	Sri Lanka	66
239			D444Y	Patient with BTD	N/A	Italy	62
240			W487X	Patient with BTD	N/A	Italy	62

Abbreviation: N/A, not available.

C, c1368A > C, c1489C > T, and c1595C > T mutations in exon 4), which are known to cause profound BTD deficiency.<sup>41</sup> These results were gathered from the study of BTD-deficient families. *BTD* mutation affected around 85% of the families screened. The father, mother, and siblings with BTD deficiency are studied in this family study. The family studies of the BTD patients are also important to detect this disorder. Most family members might be asymptomatic and transfer this disorder to their children.<sup>42</sup>

In one of the studies, a patient under biotin supplementation in Turkey had a clinical presentation of metabolic acidosis and ketosis during an acute gastroenteritis episode. The above patient had a homozygous mutation of W140X.<sup>43</sup> and a 16-year-old girl was diagnosed with partial BTD insufficiency. She suffered from recurring hypoglycemia and ketoacidosis crises, most frequently during upper respiratory tract illnesses. She had c98–104delinsTCC and D444H compound heterozygous mutations. Biotin therapy helped her to become asymptomatic. One of the patients with

profound deficiency had sudden vision loss and muscular weakness, and he was treated with steroids before the diagnosis.<sup>43</sup> The symptoms started getting better post the oral supplementation of biotin.

### Mutation in the Chinese Population

The *BTD* gene mutations are rarely reported among the Chinese population. In southern China, the *BTD* mutation is analyzed in BTD patients by Liu et al. They detected 10 different mutations; five were previously reported, such as R79C, C424S, C471Y, R538H, and H213TfsTer51. They found another five novel mutations such as M132W, L155P, L215H, W140X, and L498FfsTer13. Approximately, 68.75% of these mutations were localized in exon 4, which contains the enzyme active sites and is a hot spot for mutations.<sup>36</sup> Chinese patients with BTD deficiency were misdiagnosed as having encephalopathy, myelitis, or dermatitis at the onset.<sup>44</sup> Spinal cord impairment is a normal manifestation of delayed-onset BTD deficiency and is uncommon to recognize.<sup>45,46</sup>

Therefore, earlier detection is vital to prevent those severe illnesses and saving a patient's life.

In the Chinese population G98: d7i3, R538C, and Q456H are the most common mutations that appear to be the hot spot mutations for profound BTD variants reported in other countries.<sup>47</sup> Ye et al reported five different mutations in four patients with BTD deficiency from northern China,<sup>27</sup> of which only c.1493dupT was also detected in Liu et al study. The results indicate that the mutation spectrum of BTD deficiency may differ in different countries, even different districts in China. In China, the patient with profound BTD deficiency had several common clinical symptoms such as hypotonia, fatigue, hearing deficits, skin rash, proximal muscle weakness, respiratory problems, and seizures. In vitro study of the D444H mutation reduces the protein expression and does not affect BTD enzyme activity.<sup>44</sup> The incidence of BTD is unknown in China; only large city hospitals have NBS programs for BTD. A girl in China had clinical symptoms in skin and hair associated with BTD deficiency. She has been affected by hyperkeratosis in her hands, feet, and blonde hair, and her grandparent was consanguineous. The proband's biotin level was 0.048 pmol/min mm<sup>3</sup> by dry filter paper blood smear.<sup>48</sup> The consanguineous marriage might influence this inherited disorder.

Recently, a 17-year-old female with a profound deficiency in China exhibited novel *BTD* gene heterozygous variants, c.250-1G > C and c.878dupT, were identified. The mutation c.250-1G > C was inherited from her father, and c.878dupT was inherited from her mother. The patient showed various phenotypic characteristics, such as eczema-like rash, hair loss, hearing loss, hypotonia, and spontaneous recurrent epilepsy.<sup>49</sup>

### Mutations in Other Populations

In BTD patients, the alopecia is a common manifestation among Iranian and Indian populations (eight in 16 and nine in 10, respectively).<sup>50,51</sup> Minnesota is a U.S. state, where 40,000 Somalia immigrant people and their children live. Minnesota has a high incidence of combined profound and partial BTD deficiency of one in 8,540, and the profound incidence is one in 52,945, which is unusually high compared with a report worldwide. Homozygous mutation P497S was found in Somalia patients whose parents are consanguineous married<sup>52</sup>; that same mutation is also reported in the Caucasian population.<sup>29</sup> The mutation A478P was found in individuals of Asian ethnic background, affected with profound BTD deficiency.

Four recurrent mutations most frequently cause profound deficiency. In symptomatic patients, mutation G98: d7i3 and R538 are found with high frequency, while in asymptomatic patients, Q456H missense mutation and A171T were frequent. D444H mutation with combined variant was found with high frequency during NBS.<sup>32,53,54</sup> A study in western Hungary found that Q456H (14.2%), A171T (?), D444H (10%), c.98\_104del7insTCC (7.5%), and R538C (5%) mutations were common among children with BTD deficiency.<sup>55</sup> A newborn screening in western Hungary population found BTD defi-

ciency in 57 patients among 1,070,000 neonates from the year 1989 to 2008. The incidence of the disorder is one in 18,700, which was three times higher than the worldwide incidence.<sup>9</sup> Several unique mutations were found in western Hungary, and the study revealed that 10 out of 21 different mutations were unique to the Hungarian population.<sup>56</sup> The mutations identified in the Hungarian population in NBS program effectively helped to detect the patients with BTD deficiency. The mutation T532M is a common mutation among the Romanian Gypsy population.<sup>57</sup>

In India, a 7-year-old boy with recurrent myelopathy had a BTD deficiency. He had a lot of clinical significance, including progressive weakness in four limbs and difficulties in swallowing and breathing. He didn't have manifestation of hearing impairment, alopecia, and skin rashes. At the age of 5 years, he had a respiratory problem, but tomography of the chest and bronchoscopy showed negative results. His illness did not respond to steroids and immunotherapy such as intravenous immunoglobulin. A oral supplementation of biotin, thiamine, and multivitamins helped him recover from this illness in 1 month, after which medication was discontinued. At the age of 7 years, he was examined by various clinical analyses, but most of them were normal. However, the cerebrospinal fluid lactate level was slightly elevated, and the BTD level was significantly lower than normal. The molecular analysis revealed an H447Y homozygous mutation in his *BTD* gene, and he was not born to a consanguineous parent.<sup>58</sup> Therefore, NBS program is very important, which may help in early detection of this deficiency. In cases with the positive results, the biotin supplement helps to avoid those clinical signs and maintain the proper biotin metabolic cycle in the human body.

The homozygous mutation L215F is frequently found in the Northeastern region of Poland. There were three out of four patients affected by this L215F mutation, and the patients had symptoms such as hearing loss or vision problems.<sup>59</sup> In Italy, a patient showed a new variant of T492I with a combination of D444H, and the mother didn't have a similar mutation in her genomic DNA, which suggests the mutation is due to a de novo origin of maternal germline mosaicism.<sup>60</sup> The prevalence of BTD deficiency in the European population is estimated at one in 61,000.<sup>61</sup> A 10-year long NBS program of BTD deficiency revealed that 75 neonates were affected among 579,812 newborns in the regions of Tuscany and Umbria in Italy. The incidence is approximately one in 6,300 births, which is 10 times higher than worldwide.<sup>62</sup> In Italy, NBS by the Regional Screening Centre of Verona from 2014 until the end of 2020 found 49 BTD patients among 293,784 newborns screened. Among 49 BTD patients, five were affected with profound deficiency, and the other 44 were affected with partial BTD deficiency. They were treated with oral biotin supplement. The patients with profound deficiency were given a 10 mg/d dosage, and the partial BTD deficiency patients with 5 mg/d. None of them has shown clinical signs or symptoms, including patients with profound BTD deficiency during diagnosis and biotin treatment follow-up. The newborn screening program is a major help to those patients trying to prevent severe

neurological and other problems. The mutation c.1330G > C (D444H) is found in all partial BTD patients, its pathogenic variant in compound heterozygosity. This mutation has a high prevalence among the European population and was also reported in other regions of the world.<sup>63</sup> These results indicate, that D444H is the common variant in BTD gene and that may be used as a biomarker for the purpose of prenatal diagnosis.

Analysis of *BTD* mutations in the Brazilian population, combined with the NBS, revealed that there were 119 neonates affected by BTD deficiency from June 2013 to December 2017 in Minas Gerais, Brazil. In 2013, BTD deficiency testing was made mandatory in NBS, which successfully helped detect people born with BTD deficiency.<sup>24</sup> Because of the higher rate of consanguineous marriages, Iran has a high prevalence of BTD deficiency, which affects the BTD homozygous mutations in the Iranian population.<sup>64</sup>

The BTD deficiency test is recommended for all newborns, especially if their sibling is affected with BTD deficiency, which helps prevent clinical symptoms. Several mutations of the *BTD* gene have been found in the Malaysian population. A study demonstrated screening of 1,434 patients, nine of whom were affected by BTD deficiency. One of the patients had been affected by encephalopathy and had several symptoms such as developmental delay, alopecia, and sparse eyebrows. In Malaysia, BTD deficiency screening is not mandatory for NBS, but neonates are at high risk of BTD deficiency.<sup>65</sup>

Senanayake et al discovered the novel exon 1 deletion in *BTD* gene in Sri Lankan child, the homozygous contiguous deletion of entire exon1 in *BTD* gene has failed to produce active BTD enzyme. Exon-1 is the starting point of the BTD enzyme and the leading signal sequence. The child also had a deletion in the *HACL1* gene and an exon 1 deletion in the *COLQ* gene.<sup>66</sup> In Jordanian study, several mutations of the *BTD* gene have been identified, and the novel mutation V150E has been affected in one patient in the Jordanian population. In addition, the endogamy rate is higher in Jordanian and Middle Eastern countries, which has led to the incidence of autosomal inherited disorders. Early childhood or NBS helps to detect such a problem and is easily treated to avoid severe consequences.<sup>67</sup> Mutation Q456H has been found in the Austrian population. This mutation causing the profound deficiency is common in NBS in the United States.<sup>26</sup> BTD deficiency is rare among the Swedish population, but due to immigration and consanguineous marriages, some of them are affected with BTD deficiency. Newborn screening is mandatory in Sweden, which earlier detects the deficiency and treats them with biotin supplements. The BTD deficiency among the Swedish population demonstrates the disease's heterogeneity.<sup>68</sup>

## Clinical Significance

Patients with BTD deficiency experience a variety of symptoms. The profound BTD patients are caused by severe symptoms such as neurological disorders, vision problems, cutaneous manifestations, and hearing loss. The hearing and vision loss and neurological problems are not reversible. The

cutaneous symptoms are reversible when treated with oral supplementation. Epilepsy, alopecia, and seizures are common among BTD patients. Urinary organic acid levels are abnormal in BTD patients, such as propionic acid, lactic acid, alanine, and pyruvate acid. BTD deficiency also affects the patient's immunity, which declines cellular and humoral immunity. Sometimes children with BTD are caused by both *Candida* and bacterial infections.<sup>69</sup> Additionally, BTD deficiency can cause fetal malformation.<sup>70</sup>

Approximately, 76% of the symptomatic BTD patients are affected by sensory hearing loss. These hearing losses are not reversible by biotin supplementation. In the United States, a study on 33 children revealed that two-thirds of the children of profound symptomatic patients are affected by hearing loss.<sup>7</sup> Acrodermatitis dysmetabolic was found in BTD patients in India. After taking oral biotin supplements, the lesion disappeared in 2 weeks, and he was advised to take supplements lifelong.<sup>71</sup> In India, a patient has Ohtahara syndrome, which is associated with BTD deficiency. The syndrome is a rare epileptic encephalopathy condition.<sup>72</sup> Spastic paraparesis has rarely been reported in a patient with BTD deficiency, which is involved in the spinal cord.<sup>73,74</sup>

## Efficacy of Biotin Treatment

A symptomatic BTD patient was initially given a 20 mg biotin dose once a day, gradually reducing it to 5 mg based on the patient's clinical symptoms. In addition, asymptomatic BTD patients take 5 mg of oral biotin daily. A study of 22 Polish pediatric patients was screened for biotin follow-up for 20 years by Szymanska et al.<sup>59</sup> They routinely monitored the patient's urine organic acid (3-hydroxyisovaleric acid) by gas chromatography mass spectrometry. Once a year, ophthalmological, audiological, and neurological evaluations were also conducted. A rapid improvement in psychomotor skills was observed in most of the symptomatic patients after the initiation of biotin supplementation. The cutaneous symptoms of skin rash disappeared, and progressive optic nerve atrophy was observed before starting treatment. During the treatment, there was no further deterioration in the optic nerve. Sensorial hearing loss in all patients was not reversed, but no progression was observed.

Most profound BTD patients need to take oral biotin supplementation for life, which is the most effective and safest treatment. Partial patients are advised to take a low dose of oral biotin supplements.<sup>75</sup> Oral biotin supplementation at the pre-symptomatic stage helps to avoid the symptoms, including optic atrophy. The antiepileptic medication and some medications are not responding to the BTD patient.<sup>66</sup> For a child with severe BTD deficiency such as hyperammonemia or metabolic acidosis, it's necessary to limit protein, correct acidosis, and supplement glucose.<sup>75</sup> In India, a child with BTD deficiency has been treated with a biotin supplement, and his perioral lesions, alopecia, and seborrheic have disappeared after high doses of biotin treatment.<sup>76</sup> Intake of raw eggs must be avoided during the oral supplementation of biotin, which contains avidin that binds to the biotin. A follow-up study revealed that after taking a biotin supplement in two

patients with encephalopathy, it was found that the cerebral volume had been reversed in image findings.<sup>73</sup> Newborn screening programs have the potential to detect BTD deficiency in all infants and provide early diagnosis. Pre-symptomatic treatments help prevent this disorder's consequences, particularly neurological problems, and hearing and vision loss.<sup>77</sup>

## Conclusion

Worldwide, the estimated carrier frequency of the *BTD* mutation is approximately one in 120, and the combined incidence of profound and partial BTD deficiency cases is one in 60,000. The NBS programs are life-saving for patients with BTD deficiency. Developing and under developing countries do not have this system because of their economic conditions and insufficient hospitals. In the United States, Turkey, Hungary, Brazil, and European countries, screening of newborns for BTD and other disorders is mandatory, which helps to prevent or treat them earlier. Therefore, it is essential to implement the NBS program in developing and low-income countries to overcome the BTD deficiency, which is life-saving for newborns from severe disorders. In addition, there is a need for identification of biomarkers (molecular screening) for the BTD deficiency to provide a better treatment strategy. Treatment for partial BTD patients is still being debated because the biotin dosage and treatment period for this disease remain unclear. Moreover, for families having a history of BTD deficiency, molecular detection of the *BTD* gene in the father and mother during the pregnancy period could be helpful for the early detection of BTD deficiency in a newborn and provide a better treatment strategy.

### Authors' Contribution

B.K. undertook literature mining from various reputed databases, drafted the manuscript, and prepared illustrations. P.A. gave the concept for this article and is responsible for manuscript proof reading and validated the entire manuscript. H.K.N. and V.P.J. corrected the final manuscript draft.

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### Conflict of Interest

None declared.

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