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Original Article

Comprehensive analysis of genotypic and phenotypic characteristics of biotinidase deficiency patients in the eastern region of Türkiye

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ABSTRACT

Background. Biotin is a water-soluble vitamin that plays a key role in carboxylation. The formation of free biotin is impaired in biotinidase deficiency (BD), resulting in impaired biotin-dependent carboxylase functions. Based on the percentage of residual serum enzyme activity, BD is classified as partial and profound.

Methods. Retrospective data including gender, age, parental consanguinity, family history, biotinidase activity analyses, type of deficiency (partial-profound), physical examination, treatment, and genotypes were evaluated in patients diagnosed with biotinidase deficiency in a single center in the eastern region of Türkiye. Patients whose biotinidase enzyme activity was below 30% with biallelic variants in the *BTD* gene were diagnosed as BD.

Results. A total of 302 patients were included in the study. Parental consanguinity was present in 135 (44.7%) of them. Two hundred eighty-six (94.7%) were diagnosed by neonatal screening, 14 (4.6%) by family screening and two (0.06%) by clinical symptoms. Ninety-two (30.5%) of the patients were followed-up with profound deficiency and 210 (69.5%) with partial deficiency. A total of 306 variants were detected. Twenty different variants (3 novel - 3 rare) and 31 different genotypes were detected. The 3 most frequently detected variants were c.410G>A (p.Arg137His; 47.3%), c.1270G>C (p.Asp424His; 29.7%), and c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36; 15.3%). The 3 most frequently identified genotypes were c.410G>A (p.Arg137His) / c.1270G>C (p.Asp424His) compound heterozygous (32.4%), c.410G>A (p.Arg137His) homozygous (24.8%), and c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.1270G>C (p.Asp424His) compound heterozygous (12.2%). Patients with c.410G>A (p.Arg137His) homozygous variant, c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) homozygous variant and c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.410G>A (p.Arg137His) compound heterozygous variant were statistically significantly associated with profound deficiency. Compound heterozygosity of c.410G>A (p.Arg137His) / c.1270G>C (p.Asp424His) variants were significantly associated with partial deficiency.

Conclusions. The association between the *BTD* genotype and biochemical phenotype is not always consistent. Our study provides valuable data by adding variants with genotype-phenotype correlations to the literature and three novel variants, which can provide significant guidance in clinical follow-up.

Key words: biotin, biotinidase deficiency, *BTD*, genotype–phenotype correlation, partial, profound.

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Biotin, also known as vitamin B7 or Vitamin H, is a water-soluble vitamin that plays a key role in carboxylation reactions such as pyruvate carboxylase, propionyl-CoA carboxylase, methylcrotonyl-CoA carboxylase, and acetyl-CoA carboxylase. Therefore, biotin is required for gluconeogenesis, fatty acids biosynthesis, branched-chain amino acids catabolism and tricarboxylic acid cycle in which these carboxylase enzymes are involved. Biotin is released from these carboxylase enzymes by the enzyme biotinidase. Free biotin is then available for further carboxylation reactions. The formation of the free biotin is impaired in biotinidase deficiency (BD), resulting in impaired biotin-dependent carboxylase functions.[1,2]

Based on the percentage of residual serum enzyme activity, BD is classified as partial (enzyme activity 10–30%) and profound (enzyme activity <10%) deficiency. BD can occur at any age and may be asymptomatic for a long time. Symptoms include feeding difficulties, laryngeal stridor, apnea, alopecia, eczematous rash, conjunctivitis, lethargy, hypotonia, seizures, ataxia, muscle weakness, spastic paraparesis, global developmental delay, intellectual disability, optic atrophy, hearing loss, lactic acidosis, ketosis, hyperammonemia even coma, and death. The diagnosis is confirmed with the enzyme activity and the *BTD* gene analysis. The *BTD* gene is located at chromosome 3p25. The gene has four exons with sizes of 79, 265, 150, and 1,502 bp. More than 200 variants have been identified in the *BTD* gene so far. Genotype-phenotype correlation is still not well established.[1-4]

Biotin 5-20 mg/day is recommended for treatment. The onset of symptoms and the progression of the disease is mostly prevented with early treatment in various variants. However, optic atrophy, hearing loss and global developmental delay may be irreversible even if treatment is started or the treatment dose is increased. There is still no consensus on the follow-up and treatment protocol of the disease. The aim of this study was to investigate the clinical and genetic characteristics of our patients.[1-4]

Materials and Methods

Patients

Patients who were diagnosed with BD by low biotinidase activity (below 30%) and biallelic pathogenic / likely pathogenic variants in the BTD gene at the Pediatric Metabolism Department of Van Research and Training Hospital between January 2016 and December 2023 were enrolled in this study. This also included newborns screened in the Turkish newborn screening program and referred to the Nutrition and Metabolism unit for further evaluation with a biotinidase enzyme activity ≤ 65 microplate response units (MRU) in their dried blood spots. Enzyme activity and genetic analysis were performed in all patients. Gender, age, method of diagnosis (newborn screening, family screening or symptomatic), parental consanguinity, family history, enzyme levels, type of deficiency (partial vs. profound), clinical symptoms, treatment dose and genotypes were evaluated retrospectively.

Biotinidase activity measurement

Biotinidase activity was measured by the modified method of Wolf in dried blood spots using microtiter plates. Following incubation of 3 mm punch with N-biotinyl p-aminobenzoate (Sigma-Aldrich Co., St. Louis, MO, USA) for 16 hours, enzymatic reaction was stopped by the gradual addition of trichloroacetic acid (Merck, Darmstadt, Germany). The clear solution free from blood spot and debris was mixed with sodium nitrite (Merck), ammonium sulfamate (Fluka Chemica GmbH, Buchs, Switzerland), and N-1-napthylethylenediamine dihydrochloride (Fluka analytical) in order; the absorbance of the developed color was measured using microplate reader named Stat Fax 3200; Awareness Technology INC PO

Drawer, Palm City, FL, USA, by 580/690 dual wavelength measurement using the software of the reader linear regression analysis: Y=Abs, X=Conc, against a blank with a six-point calibration curve with the enzyme unit=eu between the range 11.2–360 eu (median 254 eu).[5]

Molecular analysis

After receiving written informed consent from the patients and their parents, genomic DNA was extracted from peripheral blood leukocytes using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. One hundred nanograms of total genomic DNA was used for library preparation with the Nextera DNA Library Preparation Kit (Illumina, San Diego, CA, USA). Next-generation sequencing (NGS) was performed via the MiSeq platform (Illumina, San Diego, CA, USA). The reference sequence NM_001370658.1 was used for the *BTD* gene. Variants were interpreted according to the American College of Medical Genetics and Genomics (ACMG) guidelines.[6] Verification of whether variants had been previously reported in the literature were assessed via ClinVar, or the Human Gene Variant Database (HGMD). Segregation analyses were also performed using the Miseq platform (San Diego, CA, USA).

Statistical analysis

The data were analyzed using IBM SPSS 25 (IBM Inc., Armonk, NY, USA) program. Descriptive statistics (mean, standard deviation, median) were provided for numerical variables. The distribution of pathogenic variants among *BTD* genotypes was analyzed. If the p-value was <0.01, it was considered statistically significant.

Ethical approval

The study was approved by the Ethics Committee of Van Research and Training Hospital on October 18th, 2023 (Approval No:2023/22-01). Informed consent was obtained from the legal guardians of the patients for the genetic analyses.

Results

A total of 302 patients with a diagnosis of BD were included in the study. Of the patients, 141 (46.7%) were female and 161 (53.3%) were male. The mean age was 6.05 years (median: 4.73 years). Parental consanguinity was presented in 135 (44.7%) patients. Seventy-four patients (24.6%) had at least one member of their family with a BD. Two hundred eighty-six (94.7%) patients were diagnosed by neonatal screening, 14 (4.6%) by family screening and 2 (0.06%) by clinical symptoms. According to the percentages of biotinidase enzyme activity, 92 (30.5%) of the patients were followed up with profound deficiency and 210 (69.5%) with partial deficiency. The percentage of biotinidase enzyme activity was between 0-30 % (mean: 17.5%; median: 20%). One hundred seventy-eight patients (59%) received 5 mg, 113 patients 10 mg (37.4%), 8 patients 15 mg (2.6%), 1 patient 20 mg (0.3%) and 2 patients 25 mg biotin (0.7%) daily. Patients with clinical symptoms received high-dose therapy (usually >10 mg/day biotin).

Two patients who presented with clinical findings of BD were born before biotinidase deficiency screening was included in the Turkish newborn screening programme. Biotinidase enzyme activity were found to be 0, and a homozygous variant, c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36), was revealed in both patients through *BTD* gene analysis. One of the patients presented with seizures in the neonatal period, while the other exhibited developmental delay, seizures and hearing loss. In total, 16 patients (5%) had clinical symptoms. Two were symptomatic at the time of diagnosis whereas 14 patients developed symptoms during follow-up. Seven patients had seizures; four patients had dermatitis; four patients had hearing loss; three patients had global developmental delay; two patients had autism and one patient had optic atrophy. The most frequent variants revealed in symptomatic patients were c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36; 36%), c.410G>A (p.Arg137His; 30.5%) and

c.1270G>C (p.Asp424His; 13.8%). Clinical and laboratory findings of the symptomatic patients are given in Table I.

Fourteen patients (9 siblings and 5 parents) were diagnosed during family screening. Eight patients had profound deficiency, and 6 patients had partial deficiency. None of these patients presented with symptoms or metabolic decompensation. Subsequently, biotin treatment was initiated, and all patients are currently in routine follow-up.

BTD gene analysis was performed in all patients. Six hundred six variants were revealed in 302 patients. Twenty different variants (3 novel-3 rare) and 31 different genotypes were revealed. While 110 patients had homozygous variants, 192 patients had compound heterozygous variants. The 3 most

Table I. Clinical and laboratory findings of symptomatic patients with biotinidase deficiency.

No	Sex	Age (yr)	Biotinidase enzyme activity, %	Clinical findings	*BTD* genotype
1	Male	3.85	0	Dermatitis	c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.896C>T (p.Ser299Phe) compound heterozygous
2	Male	8.35	0	Seizure	c.534_536delCGT (p.Val179del) homozygous
3	Female	11.57	0	Hearing loss, optic atrophy	c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) homozygous
4	Female	15.42	0	Dermatitis	c.410G>A (p.Arg137His) homozygous
5	Male	15.69	0	Seizure	c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) homozygous
6	Male	16.75	0	Global developmental delay, hearing loss, seizure	c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) homozygous
7	Male	2.93	2	Seizure	c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.410G>A (p.Arg137His) compound heterozygous
8	Male	9.19	3	Hearing loss, seizure	c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) homozygous
9	Male	2.83	9	Hearing loss, optic atrophy	c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.1270G>C (p.Asp424His) compound heterozygous
10	Female	7.61	9	Dermatitis	c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.410G>A (p.Arg137His) compound heterozygous
11	Male	11.78	9	Autism	c.410G>A (p.Arg137His) homozygous
12	Male	4.00	12	Autism	c.410G>A (p.Arg137His) homozygous
13	Female	3.93	22	Dermatitis	c.410G>A (p.Arg137His) / c.1270G>C (p.Asp424His) compound heterozygous
14	Female	5.86	22	Seizure	c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.1270G>C (p.Asp424His) compound heterozygous
15	Male	3.68	28	Global developmental delay	c.410G>A (p.Arg137His) / c.1270G>C (p.Asp424His) compound heterozygous
16	Male	4.24	29	Global developmental delay	c.410G>A (p.Arg137His) / c.1270G>C (p.Asp424His) compound heterozygous

frequently revealed variants were c.410G>A (p.Arg137His; 47.3%), c.1270G>C (p.Asp424His; 29.7%) and c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36; 15.3%). The 3 most frequently revealed genotypes were c.410G>A (p.Arg137His) / c.1270G>C (p.Asp424His) compound heterozygous (32.4%), c.410 G>A (p.Arg137His) homozygous (24.8%), and c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.1270G>C (p.Asp424His) compound heterozygous (12.2%). Details of variants and genotype frequencies are given in Tables II and III. A statistical significance was found in patients with profound deficiency and c.410G>A (p.Arg137His) homozygous variant, c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) homozygous variant and c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.410G>A (p.Arg137His) compound heterozygous variants. Patients with c.410G>A (p.Arg137His) / c.1270G>C (p.Asp424His) compound heterozygous variant were significantly associated with partial deficiency.

Discussion

BD, first described in 1983 by Wolf et al, is an autosomal recessive disorder.[1,7] Newborn screening for BD has been implemented in the USA since 1984, and in Türkiye since 2008.[8,9] The incidence of biotinidase deficiency varies between populations and is approximately 1/40,000 to 1/60,000 worldwide.[1] Türkiye is one of the countries with the highest incidence of BD and the incidence is approximately 1/7,116.[10] It was previously reported that the incidence of BD in southeastern provinces such as Diyarbakır and Şanlıurfa, where consanguineous marriages are common, was 1 in 2,359 and 1 in 1,177, respectively.[11] Van is a city located in the east of Türkiye. Our patients are from Van and neighbouring provinces such as Ağrı/Doğubeyazıt and Iğdır which

Table II. *BTD* variant analysis of patients.

Variant	Total number	Partial BD	Profound BD
c.410G>A (p.Arg137His)	287	176	111
c.1270G>C (p.Asp424His)	180	177	3
c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36)	93	35	58
c.175C>T (p.Arg59Cys)	6	4	2
c.333delC (p.Phe111Leufs*28)	5	1	4
c.534_536del (p.Val179del)	5	3	2
c.581A>G (p.Asn194Ser)	5	5	0
c.1535C>T (p.Thr512Met)	4	4	0
c.565C>T (p.Arg189Cys)	3	3	0
c.1273G>C (p.Gly425Arg)*	3	1	2
c.329A>C (p.Asp110Ala)*	2	2	0
c.497G>A (p.Cys166Tyr)	2	0	2
c.625C>T (p.Arg209Cys)**	2	2	0
c.896C>T (p.Ser299Phe)	2	1	1
c.1368A>C (p.Gln456His)**	2	1	1
c.908A>G (p.His303Arg)	1	1	0
c.1361A>G (p.Tyr454Cys)**	1	0	1
c.1350dupC (p.Cys451Leufs*13)	1	1	0
c.1466del p.(Pro489Leufs*13)*	1	0	1
c.1550G>A (p.Gly517Glu)	1	0	1

BD, biotinidase deficiency.
* Novel variants likely pathogenic according to the ACMG classification.[6] ** Rare variants in the literature.

Table III. *BTD* genotypes identified in patients and their phenotypic characteristics.

BTD genotype	Total number (%)	Partial BD (%)	Profound BD (%)	p
c.410G>A (p.Arg137His) / c.1270G>C (p.Asp424His) compound heterozygous	98 (32.4%)	98 (32.4%)	0 (0%)	<0.001
c.410G>A (p.Arg137His) homozygous	75 (24.8%)	35 (11.6%)	40 (13.2%)	<0.001
c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.1270G>C (p.Asp424His) compound heterozygous	37 (12.2%)	34 (11.2%)	3 (1%)	0.001
c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.410G>A (p.Arg137His) compound heterozygous	27 (8.9%)	1 (0.3%)	26 (8.6%)	<0.001
c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) homozygous	14 (4.6%)	0 (0%)	14 (4.6%)	<0.001
c.1270G>C (p.Asp424His) homozygous	14 (4.6%)	14 (4.6%)	0 (0%)	0.010
c.175C>T (p.Arg59Cys) / c.1270G>C (p.Asp424His) compound heterozygous	4 (1.3%)	4 (1.3%)	0 (0%)	0.175
c.534_536del (p.Val179del) / c.1270G>C (p.Asp424His) compound heterozygous	3 (1%)	3 (1%)	0 (0%)	0.241
c.565C>T (p.Arg189Cys) / c.1270G>C (p.Asp424His) compound heterozygous	3 (1%)	3 (1%)	0 (0%)	0.241
c.410G>A (p.Arg137His) / c.1535C>T (p.Thr512Met) compound heterozygous	3 (1%)	3 (1%)	0 (0%)	0.241
c.333delC (p.Phe111Leufs*28) homozygous	2 (0.6%)	0 (0%)	2 (0,6%)	0.034
c.581A>G (p.Asn194Ser) homozygous	2 (0.6%)	2 (0.6%)	0 (%0)	0.340
c.410G>A (p.Arg137His) / c.497G>A (p.Cys166Tyr) compound heterozygous	2 (0.6%)	0 (0%)	2 (0.6%)	0.034
c.175C>T (p.Arg59Cys) / c.1361A>G (p.Tyr454Cys)/c.1368A>C (p.Gln456His) compound heterozygous	1 (0.3%)	0 (0%)	1 (0.3%)	0.136
c.175C>T (p.Arg59Cys) / c.410G>A (p.Arg137His) compound heterozygous	1 (0.3%)	0 (%0)	1 (0.3%)	0.136
c.393del (p.Phe131LeufsTer28) / c.1270G>C (p.Asp424His) compound heterozygous	1 (0.3%)	1 (0.3%)	0 (0%)	0.500
c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.896C>T (p.Ser299Phe) compound heterozygous	1 (0.3%)	0 (0%)	1 (0.3%)	0.136
c.329A>C (p.Asp110Ala) homozygous	1 (0.3%)	1 (0.3%)	0 (0%)	0.500
c.534_536delCGT (p.Val179del) homozygous	1 (0.3%)	0 (0%)	1 (0.3%)	0.136
c.410G>A (p.Arg137His) / c.625C>T (p.Arg209Cys) compound heterozygous	1 (0.3%)	1 (0.3%)	0 (0%)	0.500
c.410G>A (p.Arg137His) / c.908A>G (p.His303Arg) compound heterozygous	1 (0.3%)	1 (0.3%)	0 (0%)	0.500
c.410G>A (p.Arg137His) / c.1270G>C (p.Asp424His)/c.641A>G (p.Asn214Ser) compound heterozygous	1 (0.3%)	1 (0.3%)	0 (0%)	0.500
c.410G>A (p.Arg137His) / c.1368A>C (p.Gln456His) compound heterozygous	1 (0.3%)	1 (0.3%)	0 (0%)	0.500
c.410G>A (p.Arg137His) / c.1466del p.(Pro489Leufs*13) compound heterozygous	1 (0.3%)	0 (0%)	1 (0.3%)	0.136
c.410G>A (p.Arg137His) / c.1550G>A (p.Gly517Glu) compound heterozygous	1 (0.3%)	0 (0%)	1 (0.3%)	0.136
c.625C>T (p.Arg209Cys) / c.1270G>C (p.Asp424His) compound heterozygous	1 (0.3%)	1 (0.3%)	0 (0%)	0.500
c.896C>T (p.Ser299Phe) / c.1270G>C (p.Asp424His) compound heterozygous	1 (0.3%)	1 (0.3%)	0 (0%)	0.500
c.1270G>C (p.Asp424His)/c.1273G>C (p.Gly425Arg) compound heterozygous	1 (0.3%)	1 (0.3%)	0 (0%)	0.500
c.1270G>C (p.Asp424His) / 1350dupC (p.Cys451Leufs*13) compound heterozygous	1 (0.3%)	1 (0.3%)	0 (0%)	0.500
c.1270G>C (p.Asp424His) / c.1535C>T (p.Thr512Met) compound heterozygous	1 (0.3%)	1 (0.3%)	0 (0%)	0.500
c.1273G>C (p.Gly425Arg) homozygous	1 (0.3%)	0 (0%)	1 (0.3%)	0.136

BD, biotinidase deficiency.

have a founder effect for BD. Unfortunately, consanguineous marriages are also common in the eastern provinces of Türkiye, similar to the cities in the south- eastern part of the country. Therefore, autosomal recessive diseases are more common in these regions.

Our study has the largest series of BD patients evaluated biochemically and genotypically in Türkiye including 302 patients. A significant proportion of our patients were diagnosed by neonatal screening (94.7%) similar to the literature.[2] In the study by Karaca et al., the majority of patients had profound deficiency.[9] In the study conducted by Kasapkara et al., equal numbers of partial and complete deficiency patients were seen.[12] Partial deficiency is more common in other studies reported from Türkiye similar to our study.[13-19] All patients (14 patients) diagnosed by family screening were asymptomatic which exhibits the importance of family screening even during adulthood. Diagnosis is essential to prevent severe deterioration in every stage of life. In the literature, in adult patients aged 19-63 years, presentation with impaired consciousness, oppositional paratonia (resistance to passive movement), bilateral optic atrophy and sensorineural hearing loss, scaly and erythematous diffuse rashes, bilateral horizontal nystagmus, ataxia, especially tetraparesis, spastic paraparesis, diplegia and peripheral neuropathy have been reported.[2] It is reasonable to start biotin treatment in patients with BD diagnosed by family screening, even if they are asymptomatic, to prevent these possible complications.

A meta-analysis published in 2023 reported that the most common finding in BD was neurological involvement, and the second most common was dermatological findings.[2] In Türkiye, studies have reported frequencies of clinical findings between 0.4% -15.4%.[9,12-19] In our study, the frequency of clinical findings was 5.2%. Neurological findings were common in our study. While neurological findings were reported to be more common in the studies by Karaca et al. and Sürücü Kara et al., dermatological findings were reported to be more common in the study by Öz et al.[9,13,17,19] The reason for this clinical difference is related to variants in the *BTD* gene. In our study, the c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) variant was more frequent compared to other studies. This variant is also associated with neurological findings.[9,17]

The most common variants reported in Turkish studies were c.1270G>C (p.Asp424His), c.410G>A (p.Arg137His), c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36), c.175C>T (p.Arg59Cys) and c.1535C>T (p.Thr512Met).[1,9,12-19] In our study, these variants constituted 93.8% of all detected variants.

Biotinidase is a very labile enzyme. For this reason, prematurity and cholestasis may cause false positivity. The time duration between the sample collection and the onset of the biochemical analyses, as well as inappropriate transportation conditions and temperature may affect the level of biotinidase enzyme activity.[20,21] Some research demonstrated that biotinidase activity may increase with age.[19,21] This is why results of the enzymatic and genetic tests should be evaluated together. Forny et al. recommended biotinidase activity should be re-performed at the age of 5 years in patients with partial BD, especially in those with the c.1270G>C (p.Asp424His) variant.[22]

The association between the *BTD* genotype and biochemical phenotype is not always consistent. Severe variants (deletions, insertions, or nonsense pathogenic variants) in homozygous or compound heterozygous individuals are associated with profound BD. Compound heterozygosity of c.1270G>C (p.Asp424His) with a severe variant is associated with partial BD.[3,20,23] However, in our study, the enzyme activity of 3 patients who had the compound heterozygous genotype c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.1270G>C (p.Asp424His) was consistent with profound deficiency. Furthermore, in our study, the findings of some patients with the c.410G>A (p.Arg137His) homozygous variant were found to be

compatible with partial deficiency, while others were compatible with profound deficiency.

Patients with homozygous c.1270G>C (p.Asp424His) variant were reported to have 40-50% enzyme activity like carrier individuals. Moreover, it has been reported that these patients do not require treatment.[3,20] In our study, enzyme activities of 14 patients with c.1270G>C (p.Asp424His) homozygous variant were compatible with partial deficiency. In this study, none of the patients with profound deficiency were homozygous for the c.1270G>C (p.Asp424His) variant. Studies in the literature demonstrate that the c.1270G>C (p.Asp424His) variant is associated with partial deficiency. Although patients in the current study with the c.1270G>C (p.Asp424His) homozygous variant did not exhibit any symptoms, other studies with the same genotype have reported a varied clinical phenotype encompassing dermatitis, alopecia, hypotonia, seizure, hearing loss, speech delay, global developmental delay and autism.[13,15,17,19] Therefore, it is more appropriate to decide according to the biotinidase enzyme activity in patients with this genotype. According to the literature, the c.410G>A (p.Arg137His) and c.1270G>C (p.Asp424His) variants present mainly with cutaneous findings, whereas the c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) variant is mostly related to neurological findings which is an important demonstration of a severe clinical phenotype.[9,15,17] In our study, the most common variant in patients with neurological findings was c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) in accordance with the literature.

As a result of this study, the genotypic spectrum was expanded by adding three novel variants to the literature, namely c.1273G>C (p.Gly425Arg), c.329A>C (p.Asp110Ala), and c.1466del (p.Pro489Leufs*13). We also detected three variants rarely reported previously c.625C>T (p.Arg209Cys), c.1368A>C (p.Gln456His) and c.1361A>G (p.Tyr454Cys) (Tables II and III).[15,24,25]

In BD, 5-10 mg/day biotin is generally recommended. In symptomatic patients, dose escalation has been shown to improve symptoms or are associated with slow progression.[1,26] In our study, 11 patients received biotin treatment above 10 mg/day.

Wolf had previously drawn attention to the simplicity of this treatable disorder compared to other inherited metabolic disorders with the following sentence: "if you have to have an inherited metabolic disease, this is the one to have."[27] However, we believe this relatively simple disease is often a challenge for clinicians due to biochemical and genotypic discordance. Clinicians face challenges such as concerns about missed patients, overdiagnosis and unnecessary treatment. We presented biochemical and genetic results of a large cohort, and reported novel and rare variants related to BD in this study. To conclude, biochemistry and genotype may not always be compatible. Some patients with the same genotype may have a different biochemical phenotype. Unknown modified genes, environmental and hormonal factors may be the cause of this incompatibility. We recommend performing more than one measurement of biotinidase activity. The recurrent measurement of biotinidase activity and the genotype should be evaluated together and a decision should made based on the whole picture. Treatment is essential in patients diagnosed through family screening due to the possibility of variability of symptoms at any age. Ophthalmological and auditory examinations should be performed periodically especially for optic atrophy and hearing loss (yearly in profound BD, every two years in partial BD).[1] Patients with the c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) variant might have a higher risk of developing severe symptoms.

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Ethical approval

Study was approved by the Ethics Committee of Van Research and Training Hospital on October 18th, 2023 (Approval No: 2023/22-01).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: KÇ, CA; data collection: KÇ, CA; analysis and interpretation of results: KÇ, CA, EİC, TT, SK; draft manuscript preparation: KÇ, CA, EİC, TT, SK. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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