

BTD Gene Mutations in Biotinidase Deficiency: Genotype-Phenotype Correlation

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ABSTRACT

Objective: To identify the biotinidase (BTD) gene mutations in patients with biotinidase deficiency in our region; and to determine the phenotype-genotype correlations in the presence of clinical findings.

Study Design: Descriptive study.

Place and Duration of Study: Department of Medical Genetics and Pediatric Metabolism Outpatient Clinic, Faculty of Medicine, Harran University, between January 2018 and June 2020.

Methodology: Two hundred and nine patients, who were found positive for biotinidase deficiency in heel blood screening, were included. Genomic DNA was isolated from peripheral blood. Next-generation DNA sequencing analysis was performed using primers covering the exon regions of the BTD gene. The results were analysed by the mutation surveyor programme.

Results: The most common mutation was c.1330 G>C (p.D444H) and the second most common mutation was c.470 G>A (p.R157H). The majority of the mutations are missense; and they are especially located in the exon 4. The most frequent mutations were found to be D444H and R157H with a rate of 66.66% in symptomatic patients.

Conclusion: Common mutations in BTD deficiencies were identified. Associating them with phenotype-genotype data will assist clinicians in better genetic counselling and management in the future by implementing prevention programmes.

Key Words: Biotinidase deficiency, BTD gene, Newborn screening, Inherited metabolic disease, Newborn screening programme.

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INTRODUCTION

Biotinidase deficiency is an autosomal recessive metabolic disease characterised by neurocutaneous findings.¹ Biotin is a water-soluble vitamin, a cofactor of carboxylase enzymes, involved in gluconeogenesis, fatty acid synthesis, and branched chain amino acid catabolism. Biotinidase is an enzyme involved in the biotin cycle and proteolytic degradation of biotin-dependent carboxylases.² In biotinidase deficiency, there is a disruption in the separation of biotin, which cannot be released from biocide and biotinyl peptides, and endogenous biotin cannot rejoin the cycle. Therefore, dietary protein-bound biotin cannot be used. Biotin is excreted in the form of biocide in the urine and a progressive level of biotin deficiency develops gradually.³

Biotinidase deficiency has been included in newborn screening programmes, which include phenylketonuria, congenital hypothyroidism and cystic fibrosis diseases in Turkey since 2008; and thus, increased the number of patients are being detected and treated. The incidence of disease is 1: 60.000 around the world, while the incidence is higher in Turkey with a 1:11.000 ratio.⁴ The disease is also diagnosed by biotinidase enzyme measurement with fluometric method and genetic mutation analysis with clinical suspicion.⁵ Biotinidase deficiency occurs as a result of mutations in the biotinidase (BTD) gene. The BTD gene is located in the 3q25.1 locus, consisting of four exons, and encoding 543 amino acids. It is expressed in lung, liver, skeletal muscle, kidney, pancreas, heart, brain and placenta in humans.⁶ To date, more than 150 different mutations have been identified in the BTD gene.⁷ The mutations that cause premature stop codon or cause amino acid changes, such as deletion, insertion, cryptic adhesion formation mutation, single nucleotide insertion and point mutations cause the disease.⁸

The neurologic and cutaneous symptoms of the condition include convulsions, hypotonia, alopecia, erythematous skin lesions, developmental retardation, conjunctivitis, and visual and hearing loss. If patients are treated after clinical manifesta-

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tions occur, findings such as hearing loss, optic atrophy, mental retardation; developmental retardation become evident, and it is not possible to improve the symptoms. If the disease is diagnosed and treated early, neurological findings would be prevented, and consequently morbidity and mortality can be significantly reduced.⁹

Because of common consanguineous marriages and high birth rates in Turkey, it is important to specify the different mutations defined in our country and other countries.

The aim of this study was to identify the BTD gene mutations in patients with biotinidase deficiency in Turkey; and to determine the phenotype-genotype correlation in the presence of clinical findings.

METHODOLOGY

This study was conducted at Department of Medical Genetics and Pediatric Metabolism Outpatient Clinic, Faculty of Medicine, Harran University, between January 2018 and June 2020 in order to identify the BTD gene mutations in patients and to determine the phenotype-genotype correlations in the presence of clinical findings. The results of the 209 patients' genetic analyses were investigated retrospectively. This study was conducted by considering ethical responsibilities according to the World Medical Association and the Declaration of Helsinki; and was approved by the independent Clinical Research Ethics Committee.

Patients who were found positive for BTD deficiency in heel blood screening in Medical Faculty Pediatric Metabolism Outpatient Clinic, were included in the study. Blood samples for the measurement of biotinidase activity included in the neonatal screening programme in Turkey were collected by the nurses with a heel stick and dropped on filter paper cards. Biotinidase activity (BA) from dried blood stains (DBS) was measured semi-quantitatively with a fluorescence-based assay, using biotinyl-6-aminoquinoline as an artificial substrate (Trimaris fluorometric biotinidase kit). Biotinidase activity levels equal to or greater than 65 motion reference units (MRU) were considered normal, while those with values less than 65 MRU were sent to repeat the measurement. In the repeated sample, patients with a BA value below 65 MRU were referred to the pediatric nutrition and metabolism outpatient clinic. Biotinidase enzyme activity level was studied with the colorimetric method from the patients whose screening test results were suspicious. At least two consecutive blood samples, taken on different days, were used for BA measurement of the patients. After the blood was centrifuged at 1500g for 10 minutes, the serum samples obtained were stored at -80°C until analysis. Enzyme activity measurements were performed semi-quantitatively by the colorimetric method (FOCUS Neonatal Biotinidase Assay) and the highest result of the patient results was included in the study. In the experiment, biotinyl-p-aminobenzoic acid (B-PABA) is used as the substrate for the reaction catalysed by biotinidase in serum and are products consisting of free biotin

and p-aminobenzoic acid (PABA). At the end of the reaction, PABA is used to obtain an azo compound spectrophotometrically measured at 570 nm (Thermo Fisher Scientific). Enzyme activity is expressed as % of average normal serum activity as released PABA. Low and high serum pools were used as quality control prior to each study. Results were determined by analysing after blank readings were taken.

For isolation of DNA from cases included in the study, 2 cc peripheral blood samples were taken into tubes with EDTA (ethylene diamine tetra acetic acid). DNA extraction of the patients was isolated, according to the kit protocol using a commercial kit (high pure pcr template preparation kit, Roche, Germany) from peripheral blood samples. The concentration and purity measurement of the DNA samples was carried out with the commercial kit (Qubit dsDNA HS Assay Kit, USA). As a result of qubit measurement, DNAs were diluted with nuclease-free water to 10 ng / µl. A library was created from the diluted DNA samples, using a commercial kit (IonAmpliseq Library Kit 2.0, USA). Consumables included in the commercial kit (AmpliSeq HiFi Mix, AmpliSeq Primer Pool, gDNA and nuclease-free Water) were used in the preparation of the PCR mix. Barcoding was done by the adapters (IonXpress Barcode Adapters) included in the kit. Amplification was performed with primer pairs containing exon regions of the BTD gene according to the kit protocol on PCR (NEXTflex™ DNA Sequencing Kits, Bioo Scientific Corporation, Texas, United States). Next-generation DNA sequencing data was evaluated using Mutation Surveyor programme.

The ratio of all genes for one allele in this study group was determined to calculate allele frequency. The sum of the wild type (normal) allele and mutant allele was calculated, in all patients. The number of alleles was proportioned to the total number of alleles for calculating the frequency of each allele. Allele frequencies were calculated, according to the genotype structures of the patients and determined as percentages (%).

RESULTS

Two hundred and nine patients, who were found to have low biotinidase enzyme activity in the neonatal screening programme and underwent genetic mutation analysis, were investigated retrospectively. One hundred and three (49.28%) patients were males and 106 (50.72%) were females. The authors observed that there were 20 different mutations when the BTD gene analysis results of patients with biotinidase deficiency were evaluated in the study population. The majority of the mutations were missense mutations; and it has been observed that they were especially located in the exon 4. The most frequent mutation was *c.1330 G>C (p.D444H)* with a rate of 44.86%, and the second most common mutation was *c.470 G>A (p.R157H)* with a rate of 29.22% (Table I).

Socio-demographic information, age at diagnosis, status of consanguinity, ethnicity, biotinidase enzyme activity, genetic results of 24 patients with clinical findings were summarised in Table II.

Table I: Mutations causing biotinidase deficiency and their features.

Nucleotide change	Protein change	Exon number	Allele number	Variant Type	Allele frequency (%)
c.1330 G>C	p.D444H	4	109	Missense	44.86
c.470 G>A	p.R157H	4	71	Missense	29.22
c.557 G>A	p.C186Y	4	3	Missense	1.23
c.100_103delGGCT	p.G34Vfs*35	4	7	Missense	2.88
c.38 G>T	p.C33F	4	7	Missense	2.88
c.641 A>G	p.N214S	4	1	Missense	0.41
c.104 G>C	p.C35S	4	7	Missense	2.88
c.1253 G>C	p.C418S	4	1	Missense	0.41
c.1361 A>G	p.Y454C	4	2	Missense	0.82
c.1595 C>T	p.T532M	4	1	Missense	0.41
c.1368 A>C	p.Q456H	4	10	Missense	4.12
c.171 T>G	p.Y57*	4	1	Nonsense	0.41
c.192_193insCATC	p.L69Hfs*24	4	2	Frame-shift	0.82
c.202_205dupATCC	p.Leu69Hisfs	2	1	Frameshift	0.41
c.235 C>T	p.R79C	2	12	Missense	4.94
c.1324 delG	p.V442Sfs*59	4	1	Frame-shift	0.41
c.1489 C>T	p.P497S	4	2	Missense	0.82
c.625 C>T	p.R209C	4	2	Missense	0.82
c.959 T>G	p.F320C	4	1	Missense	0.41
c. 98-104del7ins3	p.C33Ffs*36	2	2	Frame-shift	0.82

Eleven of the symptomatic patients had homozygous mutations, two had compound heterozygous, nine had heterozygous mutation, and two were normal. Although the results of genetic analysis were normal in two patients, enzyme activity was found to be partially low and have dermatological clinical findings. We observed that most of the patients with BTD mutations were asymptomatic (88.52%) and the symptoms were observed only in 24 (11.48%) patients. When symptomatic patients were evaluated, it was observed that most patients had dermatitis. In these patients, especially *p.D444H* and *p.R157H* mutations were found to accompany with dermatitis (Table II).

When the biotinidase enzyme activity was measured in patients whose neonatal screening test results were positive, enzyme activity was less than 10% in five (2.39%) patients, enzyme activity was between 10% and 30% in 85 (40.67%) patients. The rest of the patients had enzyme activity over 30%. When the genetic results of the patients were evaluated, it was observed that 82 (39.23%) patients have homozygous mutations, 42 (20.1%) have compound heterozygous mutations, 68 (32.54%) have mutations in a single allele, and 17 (8.13%) have not any mutations in the BTD gene.

DISCUSSION

Biotinidase deficiency is divided into two categories as serious and partial deficiency, according to enzyme activity. Serious biotinidase deficiency is characterised by less than 10% serum enzyme activity, partial biotinidase deficiency is characterised by 10-30% serum enzyme activity.¹⁰ It has been reported that *Q456H*, *A171T*, *D444H*, *D252G* mutations were common in the screening of the newborn in the United States by 52% of children with severe biotinidase deficiency.⁸ It was reported that the *p.D444H* was the most

common mutation in Europe.¹¹ Biotinidase deficiency is usually detected by newborn and family screening in Turkey. In a study involving a large number of patients with biotinidase deficiency, it was reported that *p.R157H*, *p.D444H*, *c.98-104del7ins3*, and *p.T532M* were common mutations in Turkey.¹² In this study, the authors determined that the most common mutation was *p.D444H* with a rate of 44.86%. Other common mutations were found to be *p.R157H*, *R79C* and *Q456H*, respectively. In Turkey, Karaca *et al.* reported a different result from this study; they reported that *p.R157H* mutation ratio was 32.2% in their study group.¹² In this study, the authors did not find *p.T196R*, *p.V199M*, *p.G310E*, *p.P368L*, *p.Y454C*, *R538C*, *p.D543H* mutations or 100 G>A 3' splice site mutations, which were previously reported another researches in Turkish patients.¹³⁻¹⁶

C418S mutation was identified for the first time in Hungary. It was first reported in Turkey in a patient with compound heterozygous mutation (*D444H/C418S*) by Karaca *et al.* It was observed that partial enzyme deficiency was accompanied by the patients with *C418S* mutation.^{12,17} In this study, this mutation in a patient with heterozygous form and the enzyme activity was found to be 30%. In addition, it was observed to cause frontal baldness clinically in the patient with *C418S/D444H* mutation. *D444H* allele could alleviate the clinical manifestations by reducing the effect of the *C418S* mutation.

In biotinidase deficiency, symptoms may appear early or late, depending on the degree of enzyme deficiency, and may sometimes not be seen throughout the life. Those with a biotinidase activity below zero or less than 1%, constitute a very high risk group in terms of symptom development. In most of these patients, symptoms begin to appear from the first weeks of life, and sometimes life-threatening symptoms may occur.

Table II: Clinical characteristics and laboratory findings of the symptomatic biotinidase- deficient patients.

Patient number	Sex	Age at diagnosis (months+days)	Consanguinity	Ethnicity	Clinical findings	Enzyme activity (%)	Nucleotide change	Protein change
1	M	0+27	No	T	Diaper dermatitis	32	Heterozygous c.1368 A>C	p. Q456H
54	F	0+21	Yes	T	Dry skin	28	Normal	-
57	M	5+16	Yes	T	Dermatitis	2.8	Homozygous c.1330 G>C	p. D444H
64	M	0+20	No	T	Hypotonia	30	Homozygous c.1330 G>C	p. D444H
70	M	6+17	No	T	Diaper dermatitis	28	Heterozygous c.470 G>A	p. R157H
81	F	0+22	Yes	T	Diaper dermatitis	38	Heterozygous c.470 G>A	p. R157H
88	F	1+19	No	T	Alopecia	22	Homozygous c.1330 G>C	p. D444H
93	M	22+6	No	T	Dermatitis	37	Homozygous c.470 G>A	p. R157H
99	M	10+0	Yes	T	Dermatitis	20	Heterozygous c.470 G>A	p. R157H
111	M	1+4	No	T	Dermatitis	12	Homozygous c.1368 A>C	p. Q456H
113	M	1+22	No	T	Dermatitis	12	Heterozygous c.235 C>T	p. R79C
117	M	23+27	No	T	Hyperkeratosis on the scalp	16	Homozygous c.470 G>A	p. R157H
123	M	0+23	Yes	T	Frontal baldness	30	Heterozygous c.1253 G>C	p. C418S
129	M	3+7	No	T	Hyperemia of the skin, Dermatitis	44	Heterozygous c.235C>T Heterozygous c.470 G>A	p. R79C p. R157H
136	M	2+12	No	T	Dermatitis	31	Normal	-
140	F	23+1	No	T	Neurological dysfunction	45	Homozygous c.470 G>A	p. R157H
143	F	2+19	No	T	Dermatitis	28	Heterozygous c.470 G>A	p. R157H
155	F	0+18	No	T	Dermatitis	18	Homozygous c.1330 G>C	p. D444H
157	F	3+0	Yes	S	Seborrheic dermatitis	18	Homozygous c.1330 G>C	p. D444H
158	M	0+22	Yes	T	Difficulty in walking	10	Homozygous c.1330 G>C	p. D444H
160	F	95+30	Yes	T	Hyperkeratosis on the scalp	18	Homozygous c.1330 G>C	p. D444H
171	F	2+15	Yes	T	Dermatitis	40	Heterozygous c.235 C>T Heterozygous c.1330 G>C	p. R79C p. D444H
172	M	0+29	No	T	Dermatitis	25	Heterozygous c.235 C>T	p. R79C
208	F	2+3	No	T	Dermatitis	35	Heterozygous c.192_193ins CATC	p. L69Hfs*24

People with enzyme activity between 1-10% also have the risk of being symptomatic.¹⁸ In some patients, only one symptom is occurred; while in others, more than one neurological and skin manifestations can be observed. Skin symptoms in patients with biotinidase deficiency include erythematous rash, seborrheic dermatitis and partial/total alopecia. Symptoms in infancy include signs of nutritional difficulties, respiratory system such as tachypnea and apnea. In fact, if treatment is not initiated early in the patients, restlessness, lethargy and coma may develop and even result in death.^{19,20} In a study of 31 Turkish patients

with deep biotinidase deficiency and symptomatic by Pomponio *et al.*, it was stated that 98G:del7ins3 mutation was common in patients with clinics in Turkish populations similar to the United States.¹⁴ In this study, it is noteworthy that this mutation was not observed in symptomatic patients. On the other hand, R79C mutation was reported to be seen common in Turkish pediatric patients detected by both clinical and newborn screening by Pomponio *et al.*¹⁴ In this study, it was observed that this mutation was the third most frequent mutation in patients with clinical findings.

In a study conducted in America, the most common mutation detected in children with symptomatic findings was reported to be c.98-104del7ins3 with a rate of 35%. In the present study, the most frequent mutation was found to be D444H and R157H with a rate of 66.66% in symptomatic patients. Although the D444H mutation has been reported as mostly asymptomatic in the literature, this mutation in the present study was found to be one of the most common variants, mostly in symptomatic patients. This suggests that this allele may change from a clinically asymptomatic to a symptomatic state, and this may be due to underlying stress and additional diseases. Therefore, it should be kept in mind

that this mutation may cause changes in enzyme activity in the presence of underlying factors. R157H mutation was reported with a slightly lower frequency (27%)²¹ or as a rare mutation in several reports.^{13,22} In the present study, the R157H (29.22%) was the second most common mutation.

Dermatological manifestations were observed most frequently among clinical findings in this study. The most common cutaneous findings were dermatitis, alopecia, dry skin, frontal baldness, hyperkeratosis. The mutation frequently accompanying dermatological findings was observed to be p.R157H and p.D444H mutations. In the

study conducted by Canda *et al.*, different from this study results, it was reported that dermatological findings were detected more rarely and that these individuals had homozygous *p.D444H* mutation.²³ On the other hand, when the authors evaluated the patients with clinical findings in the present study by origin, one patient with seborrheic dermatitis complaint was of Syrian origin and had homozygous *p.D444H* mutation with partial enzyme deficiency.

In this study, it was determined that only three patients diagnosed with neurological findings included walking difficulties, hypotonia and mental retardation findings. Homozygous *p.D444H* mutation was detected in two patients with hypotonia and walking difficulties, and one patient with mental retardation had homozygous *p.R157H* mutation. In the study of Canda *et al.*, neurological findings were reported to be observed with *p.D444H* mutation.²³ Furthermore in the study of Karaca *et al.*, only neurological findings were observed in symptomatic patients and these patients were reported to have mostly homozygous *c.98-104del7ins3* mutation.¹² In this study, this mutation was observed as heterozygous in only two patients, and the patients were asymptomatic. Furthermore, in this study, neurological findings were found less frequently than other studies. In 2008, together with the receipt of the newborn screening programme, biotinidase activity has increased the number of patients detected in Turkey.²⁴ In this way, contrary to what is stated in other studies, neurological findings were found to be rare and dermatological findings were less mild in this study.

Consanguineous marriage is usually observed at a high rate in this region. Accordingly, the incidence of diseases with autosomal recessive inheritance is high in our society. In this study, children were mostly homozygous due to the increasing incidence of kinship in Middle Eastern countries as in the Jordan example.²⁵ The parents of the patients of this study, with mutations, were tested for the carrier status and detailed information and genetic counselling were given about the risk of diseases in their next pregnancies.

CONCLUSION

As a result, the most common mutations in patients with biotinidase deficiency in our region were identified and the reflection of these mutations to the clinic was described in this study. In addition, it is thought that different clinical findings may occur in individuals with the same mutation and also, when the socio-economic status of the patients with the phenotype and genotype incompatibility was considered. It is necessary to be closely followed and informed by metabolic specialists and genetic counsellor.

CONFLICT OF INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

OO, MK, NA, AG, ME: Principal investigations, study conception and design, sample collection and molecular analysis, patient management and interpretation of clinic data.

All authors were involved in drafting the article and / or revising it critically for important intellectual content.

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