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A Novel Double Homozygous *BTD* Gene Mutation in a Case of Profound Biotinidase Deficiency

Ağır Biyotinidaz Eksikliği Olgusunda Yeni Çift Homozigot *BTD* Gen Mutasyonu

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ABSTRACT

Biotinidase deficiency is a rare autosomal recessive inherited metabolic disorder. If not treated in the early neonatal period, profound biotinidase deficiency can cause serious neurological defects, metabolic abnormalities, coma and death. Screening for biotinidase deficiency in newborns and early treatment with free biotin supplementation can prevent all symptoms from occurring. The biotinidase enzyme is encoded by the *BTD* gene. More than 165 mutations have been identified in the *BTD* gene. In this case report; a rare case with homozygous double mutation in the *BTD* gene is presented; and a new allelic variant and genotype is defined. Especially in societies where consanguineous marriages are common; it should be kept in mind that apart from common mutations, different genetic variants may also be seen.

Key Words: Inborn errors of metabolism, Biotinidase deficiency, Newborn screening

ÖZ

Biyotinidaz eksikliği, nadir görülen otozomal çekinik olarak kalıtılan bir hastalıktır. Erken yenidoğan döneminde tedavi edilmezse ciddi nörolojik kusurlara, metabolik bozukluklara, komaya ve ölüme neden olabilir. Yenidoğanlarda biyotinidaz eksikliği taraması ve biyotin takviyesi ile erken tedavi, semptomların çoğunun ortaya çıkması engellenebilir. Biyotinidaz enzimi, *BTD* geni tarafından kodlanır. *BTD* geninde 165'ten fazla mutasyon tanımlanmıştır. Bu olgu bildiriminde Ulusal Yenidoğan Tarama programında tespit edilen, *BTD* geninde homozigot çift mutasyon saptanan nadir bir tablo sunulmuş olup yeni bir allelik varyant ve genotip bildirilmiştir. Özellikle akraba evliliklerinin sık rastlanıldığı toplumlarda; yaygın görülen mutasyonlar haricinde farklı genetik tabloların da görülebileceği akılda tutulmalıdır.

Anahtar Kelimeler: Doğumsal metabolik hastalıklar, Biyotinidaz eksikliği, Yenidoğan taraması

INTRODUCTION

Biotinidase is the enzyme that separates vitamin biotin from its biocytin and sources bound to dietary proteins, thereby recycling biotin. Free biotin can enter the biotin pool directly and is used to convert four human carboxylase enzymes from apocarboxylases to active holocarboxylase forms (1). Biotin-dependent carboxylases catalyze the fixation of bicarbonate in organic acids and are involved in fatty acid, amino acid and glucose metabolism. Carboxylase activities are significantly reduced in biotin deficiency resulting from biotinidase deficiency (2).

Biotinidase deficiency is a rare autosomal recessive inherited disease. The incidence of biotinidase deficiency has been reported to be approximately 1/60 000 in the world. In a study conducted in Turkey in 1998, the incidence of biotinidase deficiency was reported as 1/1100 (3). Clinical manifestations include sensorineural hearing loss, lactic acidosis, and neurological (acute

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metabolic encephalopathy, neurodevelopmental delay, refractory epilepsy, myelopathy, hypotonia, myelopathy), dermatological (eczematous skin rash, seborrheic dermatitis, alopecia), immunological (T cell abnormalities) and ophthalmological (infections, optic neuropathies and visual disturbances, motility disturbances, retinal pigment changes and pupillary findings) abnormalities (4) .

If biotinidase deficiency is not treated in the early neonatal period, it can cause serious neurological defects,coma and death (5). Screening for biotinidase deficiency in newborns and early treatment with biotin supplementation can prevent symptoms from occurring (6). The diagnosis of biotinidase deficiency is made by measuring biotinidase activity in plasma (7). In profound biotinidase deficiency, enzyme activity is considered to be less than 10% of the laboratory standard. In partial biotinidase deficiency, enzyme activity is between 10% and 30%. Enzyme activity may also be temporarily low due to indirect hyperbilirubinemia or prematurity (8, 9). Enzymatic assay for biotinidase activity measurement is usually sufficient to determine whether a child has profound biotinidase deficiency. However, enzymatic assays may not always be sufficient to distinguish whether a child has partial deficiency or is a carrier for profound deficiency (9). Therefore, DNA sequencing analysis is important to confirm the diagnosis (10).

The biotinidase enzyme is encoded by the *BTD* gene, which is located on chromosome 3p25 and contains four exons. More than 165 mutations have been identified in the *BTD* gene (11). Biallelic pathogenic variants in *BTD* gene (especially deletion, insertion, or nonsense mutations) usually cause profound or near- profound loss of biotinidase activity. In this case report, a case of biotinidase deficiency detected via the National Newborn Screening Program, who had a novel genotype with a homozygous double mutation in *BTD* gene is presented.

CASE REPORT

A 17-day-old male was referred to our center with a preliminary diagnosis of biotinidase deficiency with the National Neonatal Screening program. He was born as the first child of consanguineous parents, from an 18-year-old mother via spontaneous vaginal delivery at 40 weeks of gestation. Perinatal history was uneventful, and the family history was otherwise unremarkable. The biotinidase activity in the capillary blood sample taken on the second and fourth postnatal days were 1.68 MRU, and 8.31 MRU, respectively (Normal: >65 MRU). The patient's family was alerted by the primary health care center and was referred to the pediatric metabolic diseases department, and he was diagnosed with profound biotinidase deficiency, since the plasma biotinidase activity was 0.28 U/L (3.9% of the laboratory standard) by the spectrophotometric measurement and the enzyme activity was not detectable by the colorimetric method. Free biotin treatment was started at a dose of 10 mg/day. In the clinical follow-up of the patient, who used the treatment regularly, his examination findings, growth and development were normal, and his routine follow-up visits were continued.

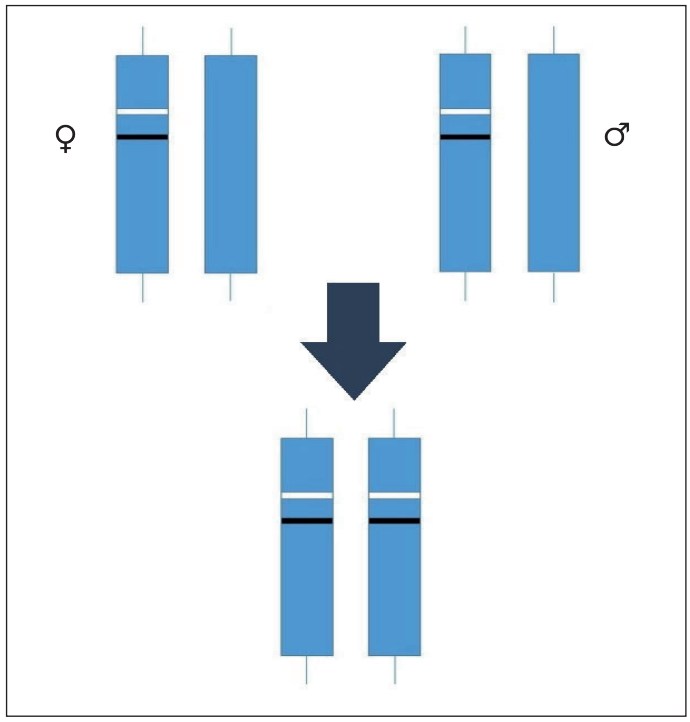

Figure 1: Segregation analysis of the *BTD* gene in the family. Both parents were found heterozygous for .c.499C>T;p.Pro167Ser and c.572 G>A;p.Arg191His mutations (in cis position).

Within the scope of genotyping studies; c.499C>T; p.Pro167Ser and c.572G>A;p.Arg191His mutations in *BTD* gene (RefSeq NM_001370658.1) were determined as homozygous "double mutation" in the patient by Sanger DNA sequencing (Genotype: c.(499C>T;572G>A);(499C>T;572G>A)). Paternal biotinidase activity was 4.12 U/L (58.0%) whereas maternal biotinidase activity was 4.22U/L (59.4%), both consistent with carrier status. Segregation analysis was performed to determine the "cis-trans" positions of nucleotide changes detected in family members. In the segregation analysis, both c.499C>T;p.Pro167Ser and c.572G>A;p.Arg191His mutations were found to be heterozygous for *BTD* gene in both parents; Since biotinidase activities were compatible with carrier status, it was thought that these mutations in the mother and father were in the cis position on the same allele. Segregation analysis is shown in figure 1.

DISCUSSION

Many different point mutations detected in the *BTD* gene to date have been associated with biotinidase deficiency. What makes this patient different is the genetic defect reported at two different

points in the same allele in the mother and father. Literature review did not reveal any reported cases with c.559C>T;p.Pro187Ser and c.572G>A;p.Arg191His mutations detected in the "cis position" on the same allele of the *BTD* gene. However, considering the relevant mutations, it was observed that the c.559C>T; p.Pro187Ser homozygous mutation in the *BTD* gene previously associated with profound biotinidase deficiency (12). Biallelic c.572 G>A; p.Arg191His variant in the *BTD* gene have been reported in ClinVar database, but there are not any case presesantations in the literature correlating pathogenicity of this variant. This variant was evaluated as "probably pathogenic" according to the ACMG 2015 criteria. When this variant was examined with the in silico analysis program ("UniProt"), it was observed that it disrupted the three-dimensional structure of the enzyme and was classified as likely pathogenic. Other allelic variations harboring double mutations have also been reported in patients with biotinidase deficiency. The most well-known of these is double homozygosity of the p.Ala171Thr; p.Asp444His allele. This double homozygous genotype (p.(Ala 171Thr;Asp444His);(Ala171Thr;Asp444His)) was also reported in six patients in a study conducted in our center. Individuals who are homozygous for the p.Asp444His pathogenic variant are expected to have approximately 45%-50% of mean normal serum biotinidase enzyme activity (which is similar to the activity of heterozygotes for profound biotinidase deficiency) and do not require biotin therapy (13). A double homozygous mutation of p.Phe403Val and p.Asp444His in the *BTD* gene was also reported in a patient from the United Arab Emirates (14).

As a result, considering that consanguineous marriages are common in the Middle East and our region, it is not an extraordinary situation to encounter diverse genetic variations. High rate of consanguineous marriages in a society leads to a rise in the allele frequency of ancestrally inherited disease-causing genotypes and pathogenic alleles in the common gene pool, increasing the chance of co-occurrence of these mutations in future generations. Double mutations can cause synergistic effects on the enzyme that may be more or less severe than the effects caused by either mutation separately. In this study, a rare case homozygous for an allele with two mutations is presented, and a new allelic variant and genotype has been reported. The most important limitations of this case report is lack of functional studies showing the pathogenicity of homozygous c.572 G>A;p.Arg191His variant. Although the presence this double mutation has been associated with the clinical phenotype of profound biotinidase deficiency in this patient, functional studies are required to reveal the individual or combined contributions of the variants to this phenotype. Screening only for common mutations and not sequencing all the coding exons and exon-intron junctions may cause similar situations to be missed. In biotinidase deficiency, in which double-mutated alleles are reported, analysis of the whole coding sequence is important for accurate genotyping.

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