Two novel BTD mutations causing profound biotinidase deficiency in a Chinese patient

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Abstract

Background: Biotinidase deficiency (OMIM 253260) is an autosomal recessively inherited disorder affecting about 1/60,000 people worldwide. The absence or deficiency of biotinidase impairs free biotin recycling and affects biotin-dependent carboxylase functions.

Methods: A Chinese patient with spontaneous recurrent epilepsy, an eczema-like rash, hair loss, hypotonia, and hearing loss began at three months of age. Her biotinidase activity was 1.0 nmol/ml/min, 9.5% of the mean control activity, which confirmed profound biotinidase deficiency.

Results: Compound heterozygous for c.250-1G > C and c.878dupT variants in the BTD gene were identified in this patient. These two variants were novel and absent in the population matched controls and any databases.

Conclusions: This study expanded the mutation spectrum of alterations of the BTD gene. Our patient also emphasized the critical role of biotinidase activity measurement combined with mutation analysis in early diagnosis of biotinidase deficiency.

KEYWORDS
biotinidase deficiency, genetic diagnosis

1 | INTRODUCTION

Biotinidase deficiency (OMIM 253260) is an autosomal recessively inherited disorder characterized by neurological and cutaneous symptoms. The incidence of biotinidase deficiency varies from 1/9,000 in Brazil (Neto et al., 2004) to 1/150,000 in East Asians (Yamaguchi, 2008), affecting an estimated 1/60,000 people worldwide (Wolf, 1991). Biotin is a coenzyme of the carboxylase enzymes involved in the fatty acid synthesis, amino acid catabolism, and gluconeogenesis (Hymes & Wolf, 1996). Biotinidase cleaves biotin and lysine from foods and biocytin or biotinyl peptides, which are carboxylase degradation products. The deficiency of biotinidase impairs free biotin formation and affects biotin-dependent carboxylase functions.

Clinically, biotinidase deficiency is classified as profound (residual activity <10%) or partial (residual activity 10~30%)(Strovel et al., 2017). Patients with profound
biotinidase deficiency usually have severe cutaneous symptoms at an early age, including dermatitis, conjunctivitis, and alopecia, and neurological symptoms such as hypotonia, seizures, developmental delay, and optic atrophy (Salbert et al., 1993; Wolf, 2011). About 76% of profound biotinidase deficiency patients exhibit sensorineural hearing loss (SNHL) of differing severity, typically with bilateral moderate to profound SNHL with a down-sloping audiogram (Genc et al., 2007; Wolf et al., 2002). If untreated, profound biotinidase deficiency patients may develop metabolic abnormalities, coma, or even death (Wolf et al., 1983). Patients with partial biotinidase deficiency usually have milder clinical features in adulthood (Oizumi et al., 1987). Fortunately, biotinidase deficiency can be prevented and treated with oral biotin, but the neurological symptoms, such as hearing loss are usually irreversible once they occur (Weber et al., 2004).

The human BTD gene (OMIM 609019) for biotinidase, located on chromosome 3p25, was first identified in 1994 (Cole et al., 1994). More than 200 pathogenic variants of the BTD gene have been reported, with 22 identified in Chinese patients (Hsu et al., 2019; Liu et al., 2018; Yang et al., 2003; Ye et al., 2009). We present the clinical and molecular characteristics of a Chinese patient diagnosed with biotinidase deficiency. Two novel pathogenic variants of the BTD gene were identified in this patient, expanding the mutation spectrum of biotinidase deficiency.

2 | MATERIALS AND METHODS

2.1 | Clinical evaluation

The patient was from Hubei Province in South central, China, and was diagnosed with biotinidase deficiency. The medical history included onset age, clinical manifestations of biotinidase deficiency, clinical tests, degree of hearing loss, noise exposure, and ototoxic drug exposure. A family medical history was obtained. The degree of hearing loss was defined according to pure-tone averages (PTA) based on 500, 1 k, and 2 kHz frequencies. The study protocol was approved by the Institutional Review Board of Southwest Hospital at the Army Medical University of China. Informed consent was obtained from the guardians on behalf of the patient before their participation.

2.2 | Biotinidase activity measurement

Biotinidase activity was determined using a modified colorimetric method described previously (Blau et al., 2008). The mean activity of 139 healthful children detected in the laboratory was 10.52 ± 2.05 nmol/ml/min.

2.3 | Panel sequencing

About 5 ml of peripheral blood was collected from the patient and family members. DNA was isolated using the AxyPrep-96 Blood Genomic DNA Kit (Axygen BioScience, Union City, CA, USA). Exons of 158 known hearing-loss genes, including the BTD, were captured and enriched using the Agilent SureSelect Target Enrichment Kit (Agilent Technologies, Santa Clara, CA, USA), and then sequenced on Illumina HiSeq 2000 (Illumina, San Diego, CA, USA). In-house variants calling workflow was developed based on the GATK best practice. Minor allele frequencies (MAF) were annotated by wANNOVAR (update: May 9, 2019) using NCBI, dbSNP, 1000 Genomes, Genome Aggregation Database (gnomAD), and the in-house control cohort, which involved 7205 Chinese adults unaffected by SNHL or biotinidase deficiency. Variants were hard filtered for quality (QD > 2, DP > 10, and MAF >1%). Variant pathogenicity was interpreted according to the guidelines recommend of the American College of Medical Genetics and Genomics and Association for Molecular Pathology (Richards et al., 2015).

2.4 | Sanger sequencing

Sanger sequencing was used for mutation screening. Primers were designed for the BTD gene variants using Primer3 online (http:// primer3.ut.ee/). A pair of exon three primers (Forward, 5’-TGGTTGCCCCAAAAGAATGAACAG-3’ and Reverse, 5’-CTGTGTGACCCTACGCTACT-3’) over intron–exon boundaries and a pair of exon four primers (Forward, 5’-GATGAACCAGCTCCCACTCT-3’ and Reverse, 5’-GTCCGTTTACCTGTTGCAT-3’) were used to span the sequences. Genomic DNA (100 ng) was used as the template in a total reaction volume of 15 μl (10× buffer 2 μl, dNTP mixture 10 mmol/L 1 μl, primer Forward 10 μmol/L 0.25 μl, primer Reverse 10 μmol/L 0.25 μl, TaKaRa Taq 0.15 μl, and dH2O to 15 μl). PCR was performed with an initial 5-min denaturation at 95°C, followed by 35 cycles of 94°C for 45 s, 60°C for 40 s, and 72°C for 1 min, and a final 10-min extension at 72°C. Direct DNA sequencing was performed in both directions by Sangon Biotech (Chengdu, China). The reference sequence of BTD used was GenBank NM_001281723.3.

3 | RESULTS

3.1 | Clinical manifestation

The patient was a 17-year-old female. She developed spontaneous recurrent epilepsy with no response to
standard anti-epileptics at three months. She gradually exhibited an eczema-like rash, hair loss, hypotonia, and hearing loss. Subsequently, she underwent high-performance liquid chromatography, which revealed very high alanine levels (1028.4 μmol/L, normal control: 171–576 μmol/L), and urine organic acid gas chromatography-mass spectrometry showed no abnormal or elevated organic acids and no amino acid metabolites. Diagnosis of biotinidase deficiency was ignored until she turned to other medications at the age of 6 months, and then biotin treatment was initiated. One week after of oral biotin treatment (32 mg/day), the patient’s seizures stopped, muscle tone and activity increased, hair loss ceased, and the skin lesions resolved, while improvement was little in hearing impairment. Then, the biotin was reduced to 15 mg/day orally. She had motor retardation and delayed walking at the age of 3 years. At the age 15 years, after withdrawal of biotin for one week with an unclear reason, no indicative symptoms were observed except hair loss. After insistence on biotin therapy for many years, she has the ability of self-care and attend the general senior high school with normal intelligence. She still suffers from hearing loss and hearing aids improved her hearing status.

3.2 Imaging and hearing test

At the time of epilepsy occurred, brain magnetic resonance imaging (MRI) revealed brain atrophy, enlarged ventricles, and a long lamellar T2 signal in symmetrical white matter; magnetic resonance spectrometry showed a decreased N-acetylaspartate-to-choline ratio and the presence of a lactate signal. However, she reexamined brain MRI recently which revealed no abnormalities, and pure-tone averages audiometry showed moderate to severe bilateral hearing loss with a down-sloping audiogram (Figure 1).

3.3 Biotinidase activity measurement and molecular analysis

Biotinidase activity of the patient was 1.0 nmol/ml/min, which was 9.5% of the control mean value. Two heterozygous variants, c.250-1G>C and c.878dupT, were identified in the BTD gene in this patient. These two variants were novel and absent in the unrelated Chinese control alleles, as well as in NCBI, 1000 Genomes, Genome Aggregation Database (gnomAD). Mutation c.878dupT was inherited from her mother, while c.250-1G>T was inherited from her father, and her younger sister carried neither of these variants (Figures 1 and 2a). Mutation c.878dupT is located in the fourth exon of the BTD gene, leading to a predicted truncating mutation p.H294Tfs*11 in protein, while c.250-1G>T is located one base pair before exon 3 and might influence the normal splicing of exon 3 (Figure 2b).

4 DISCUSSION

Biotinidase deficiency is an autosomal recessively inherited. The profound type is associated with neurological and cutaneous consequences if untreated (Wolf et al., 1983). Here, we described a female patient with biotinidase deficiency who harbored compound heterozygous pathogenic variants in BTD.
To date, 203 BTD gene mutations have been identified and most are missense mutations (Procter et al., 2013). We identified two novel heterozygous mutations in one patient: c.250-1G > C and c.878dupT mutations. The c.878dupT mutation leads to a predicted truncating mutation p.H294Tfs*11 in the protein (Figure 2b), while c.250-1G > T might influence normal splicing of exon three. These two novel variants are pathogenic because (1) they are null variants in a loss of function gene, (2) absent from gnomAD and 7205 controls, and (3) the patient's phenotype was compatible with biotinidase deficiency.

Patients with profound biotinidase deficiency usually develop neurological symptoms within 1 week to 10 years after birth, with an average age of onset of about 3.5 months (Wolf, 2011), similar to our patient's onset age. The patient was diagnosed with profound hearing loss at the age of 6 months. The hearing loss was irreversible despite biotin treatment. A retrospective survey study found that 76% of untreated children with profound biotinidase deficiency suffer from hearing loss and the mean age of symptom onset in the group with hearing loss was 10 (range 0.5–60) months (Wolf et al., 2002). The difference in the onset age of hearing loss could be related to the kind of mutation, as a study of untreated children with profound biotinidase deficiency reported that children with null mutations were more likely to develop hearing loss than those with missense mutations without treatment (Sivri et al., 2007). Biotin therapy within an effective time window may prevent hearing loss. In one report, two siblings were diagnosed with profound biotinidase deficiency and had the same BTD variants, but different symptoms; one had apnea and hearing loss, while the other one did not, because treatment had been started at different ages (Hsu et al., 2019).

For the purpose of early diagnosis of biotinidase deficiency and timely biotin treatment, the first government-wide biochemical newborn screening for biotinidase activity was performed in Virginia, USA, in 1984 (Wolf et al., 1985), and then it had been widely undertaken in many countries. China has not launched biotinidase activity newborn screening yet, though 25 biotinidase deficiency patients have been reported (Hsu et al., 2019; Liu et al., 2018; Yang et al., 2007; Yang et al., 2003; Ye et al., 2009) (Table S1). All the symptomatic children reported in China had motor delay characterized by hypotonia; almost 72.0% (16/22) had different kinds of dermatitis; 59.0% (13/22) had seizures; 31.8% (7/22) had irreversible hearing loss; 27.3% (6/22) suffered hair loss and 22.7% (5/22) had optic atrophy, so it could be misdiagnosed merely by phenotype. Considering the serious consequences can effectively be prevented with early biotin treatment and the methods of newborn screening are inexpensive with appropriate specificity (97%) and sensitivity (100%) for identifying enzyme deficient individuals (Işeri-Erten et al., 2016), we recommend adding biotinidase activity to newborn genetic metabolic disease screening panel, and combining genome sequencing to curate false-positive results and to

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**FIGURE 2**  Mutation analysis of the patient. (a) Mutation spectrum of BTD gene. This configuration represents schematically the two novel mutations identified from our patient. c.250-1G > C mutation located in the splicing site of exon3, while c.878dupT located in exon4 of BTD gene. The patient performed c.250-1G > C and c.878dupT mutations from Sanger sequencing. (b) Protein schematic diagram of wide-type and c.878dupT (p.His294ThrfsTer11) mutation. It represents c.878dupT mutation would truncate the protein in the 294th amino acid and terminated after 11 wrong translated amino acids.
accelerate the update of “biotinidase deficiency and BTD” database (http://www.arup.utah.edu/database/BTD/BTD_welcome.php).

In conclusion, we report a biotinidase deficiency patient with two novel mutations (c.250-1G > C and c.878dupT) on the BTD gene, expanding the mutation spectrum. Early diagnosis of biotinidase deficiency and timely biotin treatment is needed to avoid permanent neurological deficits, such as hearing loss.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest.

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AUTHOR CONTRIBUTIONS

YS and WYX collected clinical data of the patient. JG, MJZ, YZ, QLZ, YIZ, and ZWB carried out the molecular genetic studies and the bioinformatic analysis of the sequencing data. JG drafted the paper. JC, YL, and HJY given final approval of the version to be published. YL, and HJY participated in its design and coordination. All authors have read the final paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the Supporting Information section.

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