Received: 6 July 2020 | Revised: 8 December 2020 | Accepted: 15 December 2020

DOI: 10.1002/mgg3.1591

Molecular Genetics & Genomic Medicine Open Access WILEY

ORIGINAL ARTICLE

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

Jia Geng[1,2] | Yi Sun[3] | Yi Zhao[2] | Wenyu Xiong[2] | Mingjun Zhong[2] | Yajuan Zhang[1] | Qiuling Zhao[1] | Zhongwei Bao[1] | Jing Cheng[2] | Yu Lu[2] | Huijun Yuan[1]

[1]Medical Genetics Center, Southwest Hospital, Army Medical University, Chongqing, China

[2]Institute of Rare Diseases, West China Hospital of Sichuan University, Chengdu, China

[3]Department of Otorhinolaryngology & Head-Neck Surgery, General Hospital of Central Theater Command, Wuhan, China

Correspondence
Huijun Yuan, Medical Genetics Center, Southwest Hospital, Army Medical University, Chongqing 400038, China.
Email: yuanhj301@163.com

Yu Lu, Institute of Rare Diseases, West China Hospital of Sichuan University, Chengdu 610041, China.
Email: samuelluyu@163.com

Funding information
National Natural Science Foundation of China, Grant/Award Number: 81530031

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

Jia Geng and, Yi Sun contributed equally to this work.

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′-TGGTTGCCAAAAGAATGAACAG-3′ and Reverse, 5′-CTGTGTACCCTCAGCCTACT-3′) over intron–exon boundaries and a pair of exon four primers (Forward, 5′-GATGAACCAGCTCCCACTCT-3′ and Reverse, 5′-GTCCGTTTCACCTGTTGCAT-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 dH_2O 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*.

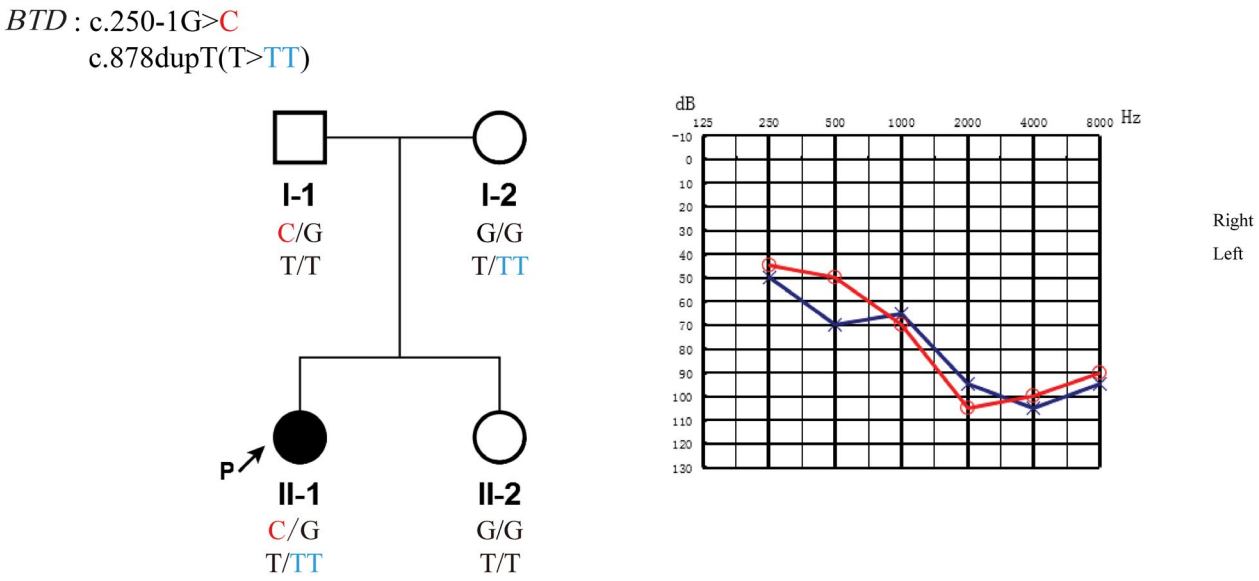

FIGURE 1 Pedigree of the patient with biotinidase deficiency and audiogram of the affected subject. Affected patient is denoted in black. Based on the audiograms of the patient, the severity of hearing impairment was bilateral severe sensorineural HL in pure-tone averages and profound in high frequency

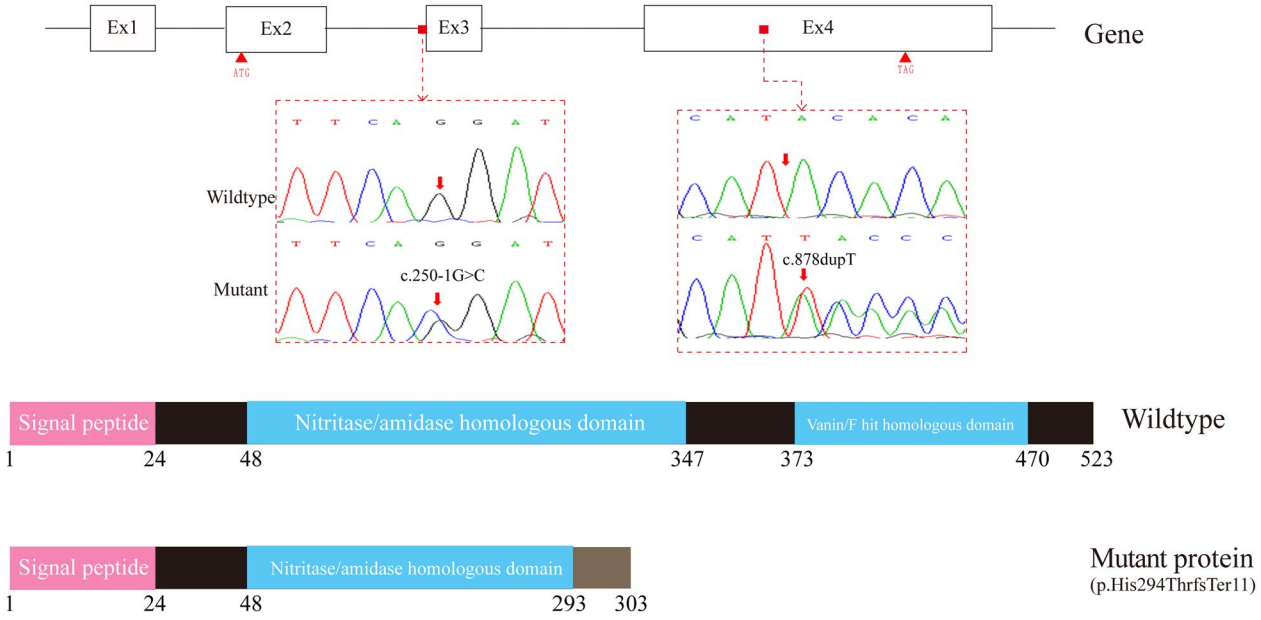

FIGURE 2 Mutation analysis of the patient. (a) Mutation spectrum of *BTD* gene. This configuration represents schematically the two novel mutations identified from our patient. c.250-1G > C mutation located in the splicing site of exon3, while c.878dupT located in exon4 of *BTD* gene. The patient performed 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

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

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.

ACKNOWLEDGMENTS

We sincerely thank the family for their participation and support in this study. This investigation was supported by the Key Project of National Natural Science Foundation of China [81530031].

AUTHOR CONTRIBUTIONS

YS and WYX collected clinical data of the patient. JG, MJZ, YZ, QLZ, YJZ, 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.

ORCID

Jia Geng https://orcid.org/0000-0001-8443-7231

REFERENCES

Blau, N., Duran, M., & Gibson, K. M. (2008). *Laboratory guide to the methods in biochemical genetics* (Vol. *253*). Springer.

Neto, E. C., Schulte, J., Rubim, R., Lewis, E., DeMari, J., Castilhos, C., Brites, A., Giugliani, R., Jensen, K. P., & Wolf, B. (2004). Newborn screening for biotinidase deficiency in Brazil: Biochemical and molecular characterizations. *Brazilian Journal of Medical and Biological Research*, *37*(3), 295–299. https://doi.org/10.1590/s0100-879x2004000300001

Cole, H., Reynolds, T. R., Lockyer, J. M., Buck, G. A., Denson, T., Spence, J. E., & Wolf, B. (1994). Human serum biotinidase. cDNA cloning, sequence, and characterization. *Journal of Biological Chemistry*, *269*(9), 6566–6570.

Genc, G. A., Sivri-Kalkanoğlu, H. S., Dursun, A., Aydın, H. I., Tokatlı, A., Sennaroglu, L., Belgin, E., Wolf, B., & Coşkun, T. (2007). Audiologic findings in children with biotinidase deficiency in Turkey. *International Journal of Pediatric Otorhinolaryngology*, *71*(2), 333–339. https://doi.org/10.1016/j.ijporl.2006.11.001

Hsu, R.-H., Chien, Y.-H., Hwu, W.-L., Chang, I.-F., Ho, H.-C., Chou, S.-P., Huang, T.-M., & Lee, N.-C. (2019). Genotypic and phenotypic correlations of biotinidase deficiency in the Chinese population. *Orphanet Journal of Rare Diseases*, *14*(1), 6. https://doi.org/10.1186/s13023-018-0992-2

Hymes, J., & Wolf, B. (1996). Biotinidase and its roles in biotin metabolism. *Clinica Chimica Acta*, *255*(1), 1–11. https://doi.org/10.1016/0009-8981(96)06396-6

Işeri-Erten, S., Dikmen, Z. G., & Ulusu, N. N. (2016). Comparison of Spectrophotometric and Fluorimetric Methods in Evaluation of Biotinidase Deficiency. *Journal of Medical Biochemistry*, *35*(2), 123–129. https://doi.org/10.1515/jomb-2016-0004

Liu, Z., Zhao, X., Sheng, H., Cai, Y., Yin, X., Chen, X., & Liu, L. (2018). Clinical features, BTD gene mutations, and their functional studies of eight symptomatic patients with biotinidase deficiency from Southern China. *American Journal of Medical Genetics. Part A*, *176*(3), 589–596. https://doi.org/10.1002/ajmg.a.38601

Oizumi, J., Hayakawa, K., Iinuma, K., Odajima, Y., & Iikura, Y. (1987). Partial deficiency of biotinidase activity. *The Journal of Pediatrics*, *110*(5), 818–819. https://doi.org/10.1016/s0022-3476(87)80038-0

Procter, M., Wolf, B., Crockett, D. K., & Mao, R. (2013). The biotinidase gene variants registry: A paradigm public database. *G3 (Bethesda)*, *3*(4), 727–731. https://doi.org/10.1534/g3.113.005835

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., & Rehm, H. L. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*, *17*(5), 405–424. https://doi.org/10.1038/gim.2015.30

Salbert, B. A., Astruc, J., & Wolf, B. (1993). Ophthalmologic findings in biotinidase deficiency. *Ophthalmologica*, *206*(4), 177–181. https://doi.org/10.1159/000310387

Sivri, H. S. K., Genç, G. A., Tokatlı, A., Dursun, A., Coşkun, T., Aydın, H. İ., Sennaroğlu, L., Belgin, E., Jensen, K., & Wolf, B. (2007). Hearing loss in biotinidase deficiency: Genotype-phenotype correlation. *The Journal of Pediatrics*, *150*(4), 439–442. https://doi.org/10.1016/j.jpeds.2007.01.036

Strovel, E. T., Cowan, T. M., Scott, A. I., & Wolf, B. (2017). Laboratory diagnosis of biotinidase deficiency, 2017 update: A technical standard and guideline of the American College of Medical Genetics and Genomics. *Genetics in Medicine*, *19*(10), https://doi.org/10.1038/gim.2017.84

Weber, P., Scholl, S., & Baumgartner, E. R. (2004). Outcome in patients with profound biotinidase deficiency: Relevance of newborn screening. *Developmental Medicine and Child Neurology*, *46*(7), 481–484. https://doi.org/10.1017/s0012162204000799

Wolf, B. (1991). Worldwide survey of neonatal screening for biotinidase deficiency. *Journal of Inherited Metabolic Disease*, *14*(6), 923–927. https://doi.org/10.1007/bf01800475

Wolf, B. (2011). The neurology of biotinidase deficiency. *Molecular Genetics and Metabolism*, *104*(1–2), 27–34. https://doi.org/10.1016/j.ymgme.2011.06.001

Wolf, B., Grier, R. E., Allen, R. J., Goodman, S. I., Kien, C. L., Parker, W. D., Howell, D. M., & Hurst, D. L. (1983). Phenotypic variation

in biotinidase deficiency. *The Journal of Pediatrics*, *103*(2), 233–237. https://doi.org/10.1016/s0022-3476(83)80351-5

Wolf, B., Heard, G. S., Jefferson, L. G., Proud, V. K., Nance, W. E., & Weissbecker, K. A. (1985). Clinical findings in four children with biotinidase deficiency detected through a statewide neonatal screening program. *New England Journal of Medicine*, *313*(1), 16–19. https://doi.org/10.1056/nejm198507043130104

Wolf, B., Spencer, R., & Gleason, T. (2002). Hearing loss is a common feature of symptomatic children with profound biotinidase deficiency. *The Journal of Pediatrics*, *140*(2), 242–246. https://doi.org/10.1067/mpd.2002.121938

Yamaguchi, S. (2008). Newborn screening in Japan: Restructuring for the new era. *Annals of the Academy of Medicine, Singapore*, *37*(12 Suppl), 13–15.

Yang, Y., Li, C., Qi, Z., Xiao, J., Zhang, Y., Yamaguchi, S., Hasegawa, Y., Tagami, Y., Jiang, Y., Xiong, H., Zhang, Y., Qin, J., & Wu, X.-R. (2007). Spinal cord demyelination associated with biotinidase deficiency in 3 Chinese patients. *Journal of Child Neurology*, *22*(2), 156–160. https://doi.org/10.1177/0883073807300307

Yang, Y. L., Yamaguchi, S., Tagami, Y., Zhang, Y. H., Xiong, H., Hasegawa, Y., & Wu, X. (2003). Diagnosis and treatment of biotinidase deficiency-clinical study of six patients. *Zhonghua Er Ke Za Zhi*, *41*(4), 249–251.

Ye, J., Wang, T., Han, L. S., Qiu, W. J., Zhang, H. W., Zhang, Y. F., Gao, X. L., Wang, Y., & Gu, X. F. (2009). Diagnosis, treatment, follow-up and gene mutation analysis in four Chinese children with biotinidase deficiency. *Journal of Inherited Metabolic Disease*, *32*(Suppl 1), S295–302. https://doi.org/10.1007/s10545-009-1238-1

SUPPORTING INFORMATION

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

How to cite this article: Geng J, Sun Y, Zhao Y, et al. Two novel *BTD* mutations causing profound biotinidase deficiency in a Chinese patient. *Mol Genet Genomic Med*. 2021;9:e1591. https://doi.org/10.1002/mgg3.1591