Biallelic loss-of-function variations in BTD cause profound biotinidase deficiency in an Indian patient

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Abstract

Background

Biotinidase deficiency (BD) is a rare, autosomal recessive metabolic disorder characterized by neurocutaneous symptoms. This study investigates a case of profound BD in an Indian patient and the underlying genetic basis.

Methods

A 10-month-old male presenting with seizures, hypotonia, ataxia, visual impairments, and developmental delay underwent biochemical and genetic analysis. Biotinidase activity was measured using an ELISA kit. Sanger sequencing of the \textit{BTD} gene was performed to identify mutations. \textit{In silico} analysis was employed to assess the potential impact of the identified variants.

Results

The patient exhibited profound biotinidase deficiency. Biallelic loss-of-function variations (c.903G\textgreater{}A and c.946C\textgreater{}T) in the \textit{BTD} gene were identified, leading to premature stop codons and truncated, non-functional protein fragments. \textit{In silico} analysis supported the functional significance of these variations, demonstrating their location within a critical domain essential for enzyme activity.

Conclusion

This case expands our knowledge of BD genetic diversity and underscores the critical role of early diagnosis and newborn screening programs in managing this treatable condition.

Introduction

Biotinidase deficiency (BD) is a rare, autosomal recessive metabolic disorder characterized by insufficient biotin utilization. The global incidence of BD exhibits regional variations, affecting approximately 1 in 60,000 newborns [1–3]. Biotin, a water-soluble B vitamin, functions as a crucial coenzyme for carboxylases. These enzymes participate in a multitude of metabolic pathways, including amino acid catabolism, fatty acid synthesis, and gluconeogenesis [4, 5]. Biotinidase, an enzyme located within lysosomes and the endoplasmic reticulum, is responsible for liberating biotin from dietary sources and recycling endogenous biotin. Deficiency of biotinidase disrupts biotin homeostasis, consequently impacting the activity of biotin-dependent carboxylases [5–7].

Patients with BD primarily present with neurological and cutaneous manifestations. The phenotypic spectrum is diverse, suggesting multi-systemic involvement [2, 8]. Clinical classification of BD is based
on serum enzyme activity: profound deficiency (residual activity < 10%) and partial deficiency (residual activity 10–30%) [9]. Profound BD typically manifests with severe clinical symptoms, including alopecia, developmental delays, hearing loss, hypotonia, optic atrophy, and seizures. Sensorineural hearing loss, a frequent complication affecting up to 76% of symptomatic individuals with profound deficiency, is often irreversible [1, 10]. Patients with partial deficiency exhibit milder clinical presentations and often experience symptoms during periods of stress. Fortunately, oral biotin supplementation offers a well-established and effective therapy for BD. However, certain complications, such as hearing loss, remain irreversible once established [11, 12].

The human biotinidase (BTD) gene, located on chromosome 3p25, encodes the biotinidase enzyme. Cole et al. first identified this gene in 1994 [13]. Mutations within the BTD gene are responsible for BD, with over 300 pathogenic variants reported worldwide [14]. A strong correlation often exists between specific mutations and the phenotypic severity observed in patients. The prevalence of specific BTD gene mutations can vary considerably across different ethnicities [2, 15–18]. This knowledge can be instrumental in refining newborn screening programs and genetic counseling strategies for targeted populations at higher risk. This study delves into the case of an Indian patient diagnosed with profound BD. Our primary objective was to identify the underlying genetic cause by analyzing the BTD gene. By employing advanced genetic techniques, we successfully identified biallelic loss-of-function mutations in the BTD gene. This finding provides a definitive molecular basis for the observed enzymatic deficiency in this patient.

Materials and Methods

Patient and measurement of biotinidase activity

This study recruited a ten-month-old male patient, who presented with seizures, hypotonia, ataxia, visual impairments, and developmental delay. Following ethical approval (Ref. No: 003/11/2023/IEC/SMCH) from the Institutional Human Ethics Committee (IHEC) Saveetha Institute of Medical and Technical Sciences, Chennai, written informed consent was obtained from the patient's parents. A commercially available ELISA kit for human biotinidase (BTD) was utilized to measure biotinidase activity.

DNA sequencing

DNA was extracted from blood using a Purelink DNA Mini Spin Kit (K182001, Invitrogen, MA, USA) according to the manufacturer's instructions. The extracted DNA's quality and quantity were evaluated using a Nanodrop One instrument (Thermo Fisher, USA) and 0.8% agarose gel electrophoresis. All exons and intron-exon boundaries of the BTD gene were amplified using primer sequences that were previously published [16]. A 50 µL PCR reaction mixture was prepared containing 25 µL of 2X Emerald green PCR master mix (Takara, Tokyo, Japan), 0.5 µM each of the forward and reverse primers, 5 µL of the isolated DNA, and nuclease-free water (DDH2O) to reach the final volume. The PCR amplification protocol consisted of initial denaturation: 95°C for 5 minutes, 35 cycles of denaturation: 95°C for 45 seconds, annealing: 55°C-60°C for 30 seconds, extension: 72°C for 1 minute, and final extension: 72°C for 5
minutes. The MiniAmpPlus thermal cycler (Applied Biosystems by Thermofisher, USA) was used for amplification. The quality of the PCR product was confirmed by running it on a 2% agarose gel. Bidirectional Sanger sequencing was then performed using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems by Thermofisher, USA) and analyzed on a 3730XL Genetic Analyzer (Applied Biosystems by Thermofisher, USA). The reference sequence for the BTD gene (NM_001281723.3) was retrieved from the National Center for Biotechnology Information (NCBI) and used for comparative analysis.

In silico analysis

Following the identification of variations in the BTD gene, a comprehensive in silico analysis was conducted to assess its potential pathogenicity and functional impact. Public databases and computational tools were used to collect relevant information and predict the potential impact of variations in the BTD gene. The Single Nucleotide Polymorphism Database (dbSNP) (https://www.ncbi.nlm.nih.gov/SNP/) was queried to determine if the identified variations had been previously reported. The Genome Aggregation Database (gnomAD) browser (https://gnomad.broadinstitute.org/) [20] was utilized to assess the polymorphic status of the variations within a large cohort of unrelated individuals (> 130,000), providing insights into its potential prevalence in the general population. Mutation Taster (https://www.mutationtaster.org/) [20] was employed to predict the potential functional impact of variations on the encoded protein. This tool analyzes the variant's effect on amino acids and its potential for disrupting protein structure and function. Swiss-Model (https://swissmodel.expasy.org/) [21] served as a valuable resource for locating the precise amino acid position affected by variations within the three-dimensional structure of the BTD protein. This information is crucial for understanding how the mutation might alter protein folding and activity. Finally, MetaDome software (https://stuart.radboudumc.nl/metadome/dashboard) [22] was utilized to gain further insights into the mutation's tolerance. This tool integrates data from various sources and provides a more comprehensive evaluation of variants with unknown significance, aiding in a more informed interpretation of the identified variations. This comprehensive in silico approach aimed to elucidate the potential functional consequences of the BTD gene variations and its contribution to the observed clinical phenotype.

Results

This study involved a 10-month-old male infant with symptoms such as seizures, hypotonia, ataxia, visual impairments, and developmental delay. Due to the nature of these symptoms, we decided to prioritize screening the patient for biotinidase levels. Using an ELISA-based BTD deficiency screening kit, we discovered profound biotinidase deficiency in the patient (Fig. 1A).

Further analysis of the BTD gene identified two variations: c.903G > A and c.946C > T (Fig. 1B). These variations introduce premature stop codons at positions p.Trp301Ter (p.W301X) and p.Gln316Ter (p.Q316X) in the protein sequence. We employed various bioinformatics tools to assess the functional
significance of these variations, particularly their location within conserved regions. Data from the National Center for Biotechnology Information (NCBI) indicated that both p.W301X and p.Q316X reside within a crucial domain – the biotinidase-like and nitrolases superfamily (2A). Additionally, the presence of these amino acids in other species suggests a highly conserved region within the \textit{BTD} gene (Fig. 2B).

Structural analysis of the BTD protein revealed that p.W301X and p.Q316X are situated in a critical location (Fig. 3). Introducing premature stop codons at these positions could potentially disrupt the protein structure and impair its function. Additionally, MutationTaster analysis predicted both variants as disease-causing due to nonsense-mediated mRNA decay. Moreover, MetaDome software predicted that p.W301X variation is intolerant (Fig. 4).

\section{Discussion}

This study presents a unique case of a patient in India diagnosed with profound BD caused by novel biallelic loss-of-function variations in the \textit{BTD} gene. This report highlights the importance of early diagnosis and newborn screening programs for this treatable yet potentially devastating condition.

We identified the compound heterozygous variations (c.903G > A and c.946C > T) in the \textit{BTD} gene in an Indian patient with profound BD. These variations introduce premature stop codons, producing truncated, non-functional biotinidase protein fragments (p.W301X and p.Q316X). \textit{In silico} analysis supported these findings, demonstrating that both variations reside within a critical domain essential for enzyme activity. This ultimately leads to a breakdown in biotin metabolism, causing a deficiency in this vital vitamin.

Profound BD is a life-threatening condition if left untreated \cite{22}. Biotin is a crucial vitamin involved in various metabolic pathways and acts as a coenzyme for four carboxylases in the human body. Insufficient biotin metabolism directly impacts the carboxylase cycle, resulting in a range of symptoms \cite{23}. In patients with profound BD, the neurological system is often the most affected, with over 70\% of children exhibiting seizures, hypotonia, skin rash, or alopecia. Partial BD typically results in milder symptoms, which are often exacerbated by stress, such as prolonged fasting or infection \cite{14, 23, 24}.

BD is a genetic disorder, with the severity determined by the specific mutations in the \textit{BTD} gene. The complete absence of enzyme activity is usually due to deletions, insertions, or nonsense mutations, while missense mutations can have varied effects \cite{14, 24, 25}. For instance, a study by Iqbal found the nonsense variant c.1275T > G in Austrian patients, leading to a premature stop codon and profound deficiency, although the affected infants did not exhibit symptoms initially \cite{26}. Similarly, recent studies in Turkey identified nonsense variants such as c.171 T > G (p.Y57*) and compound heterozygous mutations c.499C > T (p.Pro167Ser) and c.572G > A (p.Arg191His), which severely impact BTD enzyme activity \cite{27, 28}.

While several case reports in India have documented BD in various states, the focus has primarily been on enzymatic activity levels and treatment response \cite{29–33}. Genetic analysis of \textit{BTD} variants remains limited. This study adds to the growing body of Indian cases with a detailed analysis of novel biallelic
variations (c.903G > A and c.946C > T) in the BTD gene. Our findings are similar to previous reports in India describing a case with a c.466-3T > G mutation causing profound BD and another with a c.133C > T (p.H447Y) mutation presenting as recurrent myelopathy [34, 35]. These cases highlight the importance of BTD gene analysis alongside enzymatic activity assessment for a more comprehensive understanding of the genetic basis and potential for future genetic counseling to prevent recurrence in families.

Bioinformatics tools play a crucial role in understanding the functional impact of genetic mutations. Studies by Carvalho et al. (2019), Wolf et al. (2005), and Swango et al. (2000) noted that BTD gene mutations resulting in truncated proteins due to nonsense mutations lead to profound BD [18, 36, 37]. The current study aligns with these findings, with the newly reported variations c.903G > A and c.946C > T causing premature stop codons and significantly impairing protein function.

Fortunately, profound BD is a treatable condition. Children diagnosed with this form typically receive lifelong oral biotin supplementation. While biotin treatment is demonstrably effective and safe, there remains some uncertainty regarding the optimal dosage for patients with partial BD. Studies suggest that lower doses, ranging from 5 to 10 milligrams per day, might be sufficient for managing partial BD in children [12, 38]. Early intervention is critical for achieving positive outcomes. Ideally, individuals with profound BD should be identified through newborn screening programs and promptly initiated on biotin therapy. This early intervention can significantly improve their chances of achieving normal physical and cognitive development [39, 40].

Newborn screening programs play a vital role in the early detection and management of biotinidase deficiency. These programs enable prompt diagnosis and initiation of treatment, potentially preventing severe complications. While several developed countries have implemented comprehensive newborn screening (NBS) programs, many developing nations, including India, lack such widespread access. In these regions, NBS facilities are often limited to private healthcare settings or concentrated in urban areas [29]. These programs are crucial for early detection, allowing for timely intervention with biotin supplementation. This case underscores the urgent need to expand NBS programs, especially in regions with a high prevalence of consanguineous marriages, a known risk factor for BTD deficiency.

Expanding newborn screening programs to encompass biotinidase deficiency, particularly in developing countries, is critical. Increased accessibility to these programs would ensure early detection and treatment for all infants, regardless of location or socioeconomic background. This expansion would significantly improve patient outcomes and quality of life for individuals with biotinidase deficiency.

**Conclusion**

The identification of novel variations in the BTD gene responsible for profound biotinidase deficiency in this Indian patient highlights the genetic heterogeneity of this metabolic disorder. Early diagnosis through newborn screening and prompt intervention with biotin supplementation are essential for preventing severe clinical manifestations. Expanding newborn screening programs to include biotinidase
deficiency, especially in developing countries, is a crucial step towards improving patient outcomes and overall well-being.

**Declarations**

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**Conflicts of Interest**
The authors declare that they have no conflicts of interest.

**Authors’ Contributions**
K. Balachander conducted the experiments, performed the analysis, and drafted the manuscript. Dr. Vijayashree Priyadharsini was responsible for formal analysis and proofreading the manuscript. Dr. A. Paramasivam contributed to the concept, formal analysis, result interpretation, and proofreading of the final manuscript. Dr. Hephzibah KN and Dr. Lal DV were involved in the clinical analysis of patients. All authors reviewed and approved the final version of the manuscript.

**Ethical Statement**
Ethical approval for this study was obtained from the Institutional Human Ethics Committee (IHEC) of Saveetha Institute of Medical and Technical Sciences, Chennai (Ref. No: 003/11/2023/IEC/SMCH). Written informed consent was obtained from the patient’s parents.

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Figures
Figure 1

**Biotinidase Activity and BTD Gene Mutations in the Patient.** A) Pedigree: Graph depicting 10 moth old male affected profound deficiency. B) BTD Gene Variations: Sequencing chromatograms showcasing the identified variations in the BTDgene: reference control sequence (upper panel), c.903G>A and c.946C>T variations (lower panel).
Figure 2

**Localization of Variations within the BTD Gene**  A) Schematic Representation of the BTD Protein: A diagram illustrating the functional domains of the BTD protein. The location of variations identified in this study (p.W301X and p.Q316X) is highlighted within a critical domain. B) Protein Sequence Alignment: A multiple sequence alignment demonstrating the conservation of the amino acids affected by variations (p.W301 and p.Q316) across various species. This signifies a highly conserved region within the *BTD* gene.
Figure 3

**Structural Impact of Variations on the BTD Protein.** (A-C) Three-dimensional structure of the BTD protein with the locations of the identified variations (p.W301X and p.Q316X) marked. The potential impact of these variations on the protein structure and function is highlighted.
**Figure 4**

*In Silico Prediction of Mutation Significance.* Peak graphs depicting the results of *in silico* analysis for the identified mutations amino acids effects (p.W301X and p.Q316X) using software MetaDome. MetaDome shows p.W301X intolerance level (A) and p.Q316X are highly tolerance (B) location in the BTD protein.