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Case Report

Biotinidase deficiency diagnosed by metabolomic analysis despite inconclusive genomic findings

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ABSTRACT

Purpose of the study was to report a case of profound biotinidase deficiency diagnosed through biochemical and enzymatic testing despite inconclusive genomic results, emphasizing the complementary role of metabolomic approaches in diagnosing treatable metabolic disorders. A 4-year-old girl with suspected metabolic disorder underwent comprehensive diagnostic evaluation including whole exome sequencing, blood acylcarnitine profiling by liquid chromatography-tandem mass spectrometry (LC-MS/MS), urine organic acid analysis, and biotinidase enzyme activity measurement. Whole exome sequencing identified a variant of uncertain significance in ACTL6B but revealed no definitive pathogenic variants in the BTD gene. Blood acylcarnitine analysis demonstrated markedly elevated hydroxyisovalerylcarnitine with C5-OH/C8 ratio. Urine organic acid analysis revealed elevated 3-hydroxyisovalerate, 3-methylcrotonylglycine, and multiple metabolites consistent with multiple carboxylase deficiency. Biotinidase enzyme activity was profoundly reduced at 0.80 nmol/ml/min (reference: >2.4 nmol/ml/min), confirming profound biotinidase deficiency. Biotinidase deficiency can be definitively diagnosed through metabolomic and enzymatic testing when genomic analysis is inconclusive, underscoring the critical complementary role of biochemical approaches alongside molecular diagnostics in treatable metabolic disorders.

Keywords: Biotinidase deficiency, LC-MS/MS, GC-MS, Metabolomics, Whole exome sequencing, Acylcarnitine profile, Enzyme assay

INTRODUCTION

Biotinidase deficiency (OMIM #253260) is an autosomal recessive neurocutaneous metabolic disorder caused by mutations in the BTD gene, characterized by impaired recycling of biotin (vitamin B7), leading to multiple carboxylase deficiency. The worldwide incidence varies by population, with combined profound and partial deficiency affecting approximately 1 in 61,067 live births.[1]

Biotin serves as an essential co factor for four carboxylases: 3-methylcrotonyl-CoA carboxylase (MCC), propionyl-CoA carboxylase (PCC), pyruvate carboxylase (PC), and acetyl-CoA carboxylases (ACC). These enzymes are critical for amino acid catabolism, fatty acid synthesis, and gluconeogenesis. The biotinidase enzyme cleaves biocytin to release free biotin for re-utilization. In biotinidase deficiency, impaired biotin recycling results in secondary deficiency of all biotin-dependent carboxylases, leading to accumulation of toxic metabolites and subsequent neurological and cutaneous manifestations.

Clinical features typically emerge after the first weeks to months of life in previously normal-appearing newborns. Manifestations include hypotonia, ataxia, seizures, developmental delay, alopecia, seborrheic dermatitis, conjunctivitis, hearing loss, optic nerve atrophy, and metabolic acidosis. Untreated severe cases can progress

to coma and death. Fortunately, early diagnosis and lifelong oral biotin supplementation can prevent or ameliorate these complications.

Diagnosis traditionally relies on enzyme activity assays using colorimetric methods, which remain the gold standard for newborn screening worldwide.[2] According to American College of Medical Genetics and Genomics (ACMG) guidelines, confirmation requires both enzymatic assay and molecular testing.[3] However, blood acylcarnitines profiling by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) and urine organic acid analysis by Gas Chromatography Mass Spectrometry (GC-MS), while not primary screening tools, provide valuable complementary diagnostic information and help differentiate biotinidase deficiency from other metabolic disorders.[4]

This report presents a case of profound biotinidase deficiency diagnosed definitively through biochemical and enzymatic studies, where whole exome sequencing yielded inconclusive findings. This case illustrates the continuing importance of metabolomic approaches in the genomic era.

CASE REPORT

Presenting complaint and history

A 4-year-old female child was admitted at hospital for complaining of progressive breathlessness which was initially managed by oxygen supplementation. She was born via LSCS and was the product of consanguineous marriage with unremarkable antenatal history. The symptoms were aggravated by exposure to dust and smoke without diurnal variation. Physical examination revealed tachypnea, and arterial blood gas analysis showed persistent metabolic acidosis with low bicarbonate (HCO_3) and elevated lactate levels.

Past medical history

The patient had a significant history of recurrent hospitalizations. She had been admitted four times previously with similar complaints of respiratory distress and tachypnea. At 1 year of age, she required blood transfusion during one hospitalization (indication: severe anemia secondary to metabolic crisis). She had been diagnosed as asthma and was on treatment with inhalers Budesonide and Levosalbutamol with partial symptomatic relief.

Additional concerning features included developmental delay, particularly in language development, and gait instability with ataxia that worsened during acute illness. The child also experienced progressive alopecia, recurrent seborrheic dermatitis, frequent episodes of fever, cough, and cold, irritability, and tactile hallucinations with sensations of insects crawling on her skin. Neurological examination revealed hypotonia and ataxia, while dermatological examination showed areas of alopecia and eczematous skin lesions.

Investigations

Brain MRI-Normal (no structural abnormalities), ABG-Metabolic acidosis (low HCO_3, elevated lactate), complete blood count: Previous anemia requiring transfusion and routine biochemistry showed within normal limits during stable period.

Whole exome sequencing

Given the developmental delay and recurrent hospitalizations with unclear etiology, whole exome sequencing was performed. The report identified a variant in the ACTL6B gene: Gene-ACTL6B (chromosome 7), variant was c.920G>T (p.Gly307Val) in exon 7, genomic position at chr7:g.100648576G>T, sequencing depth-75x, inheritance pattern-autosomal dominant, interpretation was variant of uncertain significance and clinical correlation-"Intellectual developmental disorder with severe speech and ambulation defect (OMIM # 618470).

The WES report did not identify definitive pathogenic variants in the BTD gene, and the ACTL6B variant could not fully explain the metabolic and dermatological features.

Metabolomic testing

Blood acylcarnitine profile (LC-MS/MS)

Following the inconclusive genetic findings and considering the clinical phenotype, blood acylcarnitine analysis was performed on a Waters tandem mass spectrometry system with two-level quality controls.

Key findings

Hydroxyisovalerylcarnitine (C5-OH): 5.16 µmol/L (Reference range: 0.10-0.45 µmol/L), C5-OH/C8 ratio: 2,064 (Reference cutoff: <8) and other acylcarnitines: Within normal limits.

The marked elevation of C5-OH carnitine suggested potential diagnosis: Biotinidase deficiency, 3-Methylcrotonyl-CoA carboxylase (3-MCC) deficiency and 3-Hydroxy-3-methylglutaric aciduria (HMG-CoA lyase deficiency).

Urine organic acid analysis (GC-MS)

Urine sample analyzed by Agilent gas chromatography - mass spectrometry revealed multiple abnormal metabolites (Figure 1).

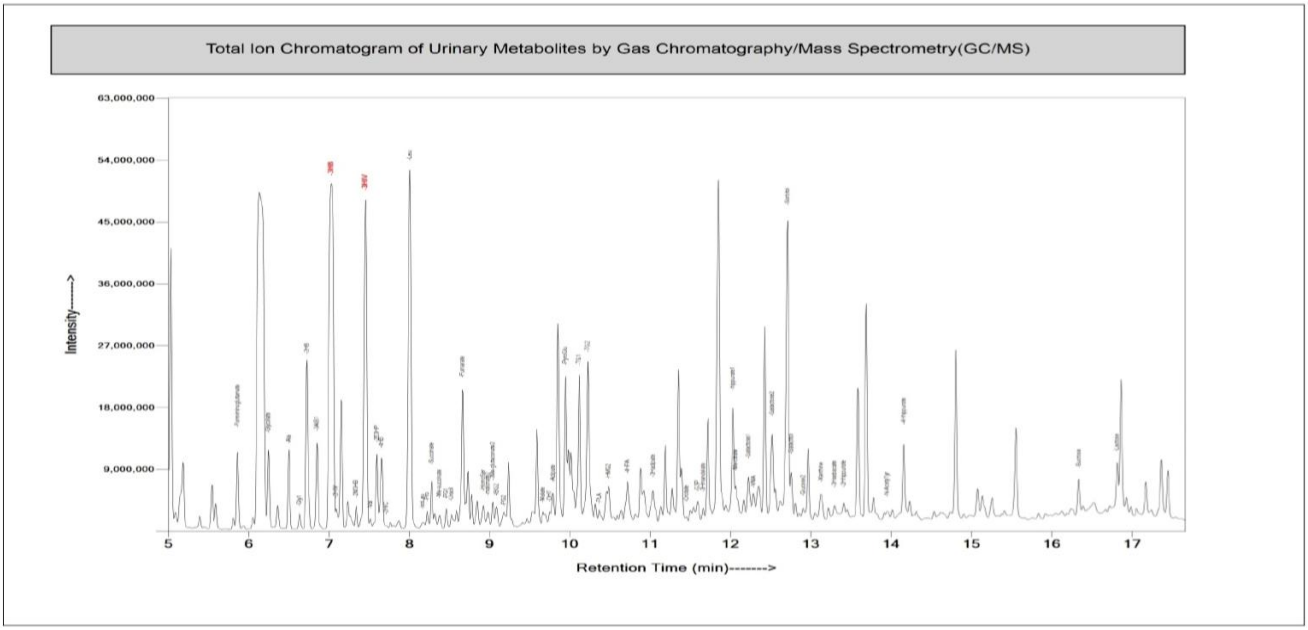


Figure 1: Total ion chromatogram of urinary metabolites analyzed by GC-MS showing elevated organic acids consistent with multiple carboxylase deficiency.

Elevated organic acids (ratio to creatinine >1 magnitude; reference: <1): Lactate (↑↑), 3-Hydroxyisovalerate (3-HIV) (↑↑↑), 3-Hydroxypropionate (3-HP) (↑↑), 3-Methylcrotonylglycine (3-MCG) (↑↑↑), Methylcitrate (↑↑), 3-Hydroxybutyrate (3-HB) (↑), Tiglylglycine isomers (1 and 2) (↑↑) and Propionylglycine isomers (1 and 2) (↑↑).

This pattern is consistent with multiple carboxylase deficiency affecting MCC, PCC, and PC pathways.

Biotinidase enzyme activity assay

Definitive enzyme testing was performed on dried blood spots using the colorimetric method: Results (duplicate analysis): Run 1-0.84 nmol/ml/min, run 2-0.76 nmol/ml/min, mean-0.80 nmol/ml/min, reference range->2.4 nmol/ml/min and classification: Profound biotinidase deficiency (<10% of normal activity).

Diagnosis

Based on the clinical presentation, characteristic metabolic profile, and profoundly reduced biotinidase enzyme activity, the diagnosis of severe biotinidase deficiency was established.

DISCUSSION

Biotinidase deficiency demonstrates considerable phenotypic variability, with presentations ranging from severe early-onset symptoms to milder manifestations in late childhood or adulthood. Early diagnosis through newborn screening is crucial for initiating biotin supplementation and preventing irreversible neurological damage. Worldwide, broad mutational analysis in the BTD gene has been reported, but treatment decisions are primarily based on enzyme analysis by biochemical colorimetric assay.[5] Profound deficiency is typically detected by mutational analysis when common BTD mutations are present, whereas partial deficiency requires enzyme analysis to confirm diagnosis and guide treatment. The genotype-phenotype correlation remains unclear, as mutational studies have shown deletions, insertions, and missense mutations that result in complete loss of enzyme activity.[1]

In the absence of neonatal screening and treatment, affected children develop symptoms that may resemble other metabolic disorders.[6] In the present case, although the patient presented with characteristic dermatological features, the predominant symptoms of tachypnea and breathlessness initially led to an asthma workup. Additional clinical indicators including developmental delay, frequent hospitalizations, and normal brain MRI prompted whole exome sequencing.

WES identified a variant of uncertain significance in the ACTL6B gene, associated with neurodevelopmental disorders, but this finding could not explain the metabolic acidosis, characteristic organic aciduria, or dermatological features observed. Critically, WES did not identify definitive pathogenic variants in the BTD gene-possibly due to inadequate coverage, deep intronic variants not captured by exome sequencing, large deletions/duplications not detected by standard pipelines,[7] regulatory region variants, or technical filtering artifacts.[8]

Given the persistent clinical suspicion for a metabolic disorder based on the combination of dermatological findings, metabolic acidosis, and multisystem

involvement that remained unexplained by WES, metabolite analysis was pursued. This decision was further supported by the recognition that biotinidase deficiency can present with variable genetic findings while showing characteristic biochemical abnormalities. The metabolomic approach using mass spectrometry - based technologies, coupled with enzyme analysis, ultimately provided the definitive diagnosis despite inconclusive mutational analysis.

This case underscores that WES, despite its diagnostic power, is not infallible and should not preclude biochemical testing when clinical suspicion is high. The complementary role of metabolomic testing proved essential in confirming biotinidase deficiency and differentiating it from other metabolic and dermatological conditions.

When diagnosed early, the prognosis is excellent, with most patients leading normal lives with lifelong biotin supplementation. However, delayed diagnosis can lead to permanent developmental delays, seizures, and other irreversible sequelae. This case emphasizes that early intervention and accurate diagnosis using multiple diagnostic platforms-particularly when genetic testing is inconclusive-remains critical in ensuring optimal outcomes.

CONCLUSION

This case reports profound biotinidase deficiency diagnosed through metabolomic analysis and enzyme assay despite inconclusive whole exome sequencing. This emphasizes that genomic testing, while powerful, has inherent limitations and traditional biochemical approaches remain essential diagnostic tools. The complementary use of multiple diagnostic platforms-genomics, metabolomics, and enzymatic assays-provides the most comprehensive approach to diagnosing inherited metabolic disorders. Early diagnosis through newborn screening and prompt biotin supplementation can prevent significant morbidity. In regions without established screening programs, clinicians must maintain high clinical suspicion for treatable metabolic disorders. A fundamental principle emerges: the critical question is whether the enzyme functions, not which genes are mutated-functional assays should guide diagnosis and therapy.

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