Case report

The first reported HLCS gene mutation causing holocarboxylase synthetase deficiency in a Vietnamese patient

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Background: **Holocarboxylase synthetase deficiency is an inborn error of biotin metabolism leading to multiple carboxylase deficiency which is often biotin responsive. This disease is believed to be relatively common among the Asian population.**

Methods: **A 6-year-old Vietnamese boy presented with recurrent episodes of severe metabolic acidosis precipitated by intercurrent illnesses. An extensive skin rash was present since the onset of his illness. Multiple carboxylase deficiency was considered a likely diagnosis based on the history and the characteristic skin rash.**

Results: **This diagnosis was later confirmed by urine organic acid and molecular genetic studies. Urine organic acid showed characteristic excretion of glycine conjugates. Serum biotinidase activity was normal. Sequencing of the holocarboxylase synthetase gene revealed the patient being homozygous for a common mutation R508W. The patient showed a dramatic response to biotin within days of its administration.**

Conclusion: **This case illustrates a potential highly treatable inborn error of metabolism that can be recognized on clinical grounds and its favorable response to biotin treatment.**

World J Pediatr 2012;8(3):278-280

Key words: **biotin;**
biotinidase deficiency;
HLCS gene;
holocarboxylase synthetase deficiency;
inborn error of metabolism;
multiple carboxylase deficiency;
rash

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doi: 10.1007/s12519-011-0301-9

Introduction

Holocarboxylase synthetase (HLCS; EC 6.3.4.10)[1] is an enzyme which is responsible for biotinylation of several biotin-dependent carboxylases in the mitochondria, including pyruvate carboxylase, propionyl-CoA carboxylase and methylcrotonyl-CoA carboxylase. An autosomal recessive inherited deficiency of HLCS leads to multiple carboxylase deficiency (MCD, MIM: 253270).[2] As a result of defective biotinylation of carboxylase enzymes, there is increased excretion of metabolites like 3-methylcrotonylglycine, 3-hydroxyisovaleric and 3 hydroxypropionic acids in the urine. Other than HLCS deficiency, biotinidase deficiency is another enzyme defect that can cause multiple carboxylase deficiency leading to the same abnormal organic acid profile as that in HLCS deficiency. Both enzyme defects (HLCS and biotinidase) can be treated with high dose of biotin and the response to treatment is usually good.

Here we report the first case of Vietnamese origin which has been worked up with molecular genetic analysis. This information should be useful for the mutation analysis of future similar patients.

Case report

A 6-year-old Vietnamese boy was referred to our unit for investigation of a suspected metabolic condition. He was the second child of consanguineous ethnic Chinese parents who were first cousins. Since 18 months of age, he has had recurrent episodes (on average 2-3 episodes per year) of acute decompensation requiring hospital admissions in Vietnam. These admissions were precipitated by upper respiratory tract infections with cough, runny nose, and fever. Ranging from a few hours to two days after the onset of these symptoms, the patient would develop rapid breathing and repeated vomiting. During hospitalization in Vietnam, he was found to have severe metabolic acidosis with ketonemia. He usually responded to treatment with intravenous fluid and bicarbonate infusions and would

be discharged from hospital a few days later. In between hospital admissions, he was an active child with normal development. Fructose 1, 6 diphosphatase deficiency was initially suspected. He was advised on a fructose free diet which did not improve his symptoms.

On presentation to our unit, the patient's general condition was stable. A generalized skin rash involving his trunk, limbs and face, particularly the mouth corners and lower eyelids were affected. The rash was slightly itchy and there were different areas of erythema, hyperpigmentation and desquamation noted over the involved areas.[3] There was no alopecia. The rash was initially diagnosed as eczema and treated as such but it did not respond to the topical treatment prescribed. There was no direct correlation between skin exacerbation and the metabolic decompensations.

Initial blood investigations showed a compensated metabolic acidosis with pH 7.37, bicarbonate 12 mmol/L and a base excess of -10. Plasma lactate and ammonia were elevated at 8.6 mmol/L (reference range: <2.2 mmol/L) and 94 μmol/L (reference range: <48 μmol/L) respectively. Serum free carnitine was low at 3.9 μmol/L (reference range: 19.3-53.9 μmol/L) and propionylcarnitine was increased to 6.4 μmol/L (reference range: <1.3 μmol/L). The level of plasma alanine was increased to 1013 μmol/L (reference range: 152-547 μmol/L) while the rest of the amino acids were within normal ranges. The above abnormal findings were compatible with early metabolic decompensation in patients with underlying organic acidurias. Together with the characteristic skin rash, multiple carboxylase deficiency secondary to either HLCS or biotinidase deficiencies was strongly suspected. Urine organic acids later confirmed a profile compatible with the diagnosis of multiple carboxylase deficiency. There were elevated lactic, 3-OH isovaleric, 3-OH propionic, methylcitric and 2-methyl-3-OH butyric acids, 3-methylcrotonyl-, propionyl-, tiglyl-, isovaleryl-, isobutyryl- and 2-methylbutyryl-glycines. Serum biotinidase activity (at 37°C) was 16.2 nmol pABA/min per mL (normal: >4.4 nmol pABA/min per mL) and this excluded the diagnosis of biotinidase deficiency.

After excluding biotinidase deficiency, we proceeded to DNA studies to confirm the diagnosis of HLCS deficiency. DNA material was extracted from blood samples with genomic DNA preparation kit (G.E., USA). The protocol of mutation analysis using genomic DNA had been reported by us previously.[4] The holocarboxylase synthetase was encoded by the HLCS gene starting from exon 6 to exon 14. Primers were designed to cover the entire encoding regions. All exons were amplified by a single PCR except for exon 7, which was amplified as three PCR products. Mutation detection was performed by direct sequencing of PCR products in both directions using BigDye kit (Applied Biosystems, CA, USA). To establish the carrier frequency of R508W, 60 healthy control blood samples were screened for the mutation by restriction enzyme, FokI, digestion, in which a restriction site was created by R508W mutation.

Molecular genetic testing confirmed that our patient was homozygous for the R508W mutation (ARG-> TRP at codon 508) (Fig.). This is a known disease causing mutation identified in patients with a late onset phenotype and higher residual HLCS activity.

Immediately after the necessary blood and urine specimens for diagnosis were collected, our patient was started empirically on oral biotin 10 mg daily, L-carnitine 100 mg/kg per day in 2 divided doses and sodium bicarbonate 75 mg/kg per day in 2 divided doses as for the treatment of multiple carboxylase deficiency with early metabolic decompensation. Two days after treatment, our patient's metabolic acidosis resolved and on day 5 of treatment, his skin rash completely resolved. Plasma ammonia and lactate also returned to normal. Upon follow up, the skin rash did not recur and he had no further episodes of decompensation. Serial urine organic acid analysis after treatment revealed no

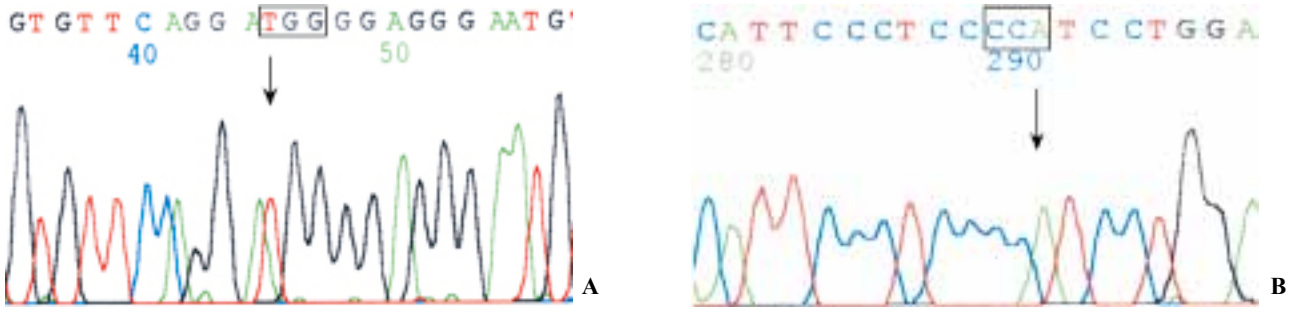

Fig. Sequencing of HLCS exon 11 which reveals a homozygote of R508W mutation. **A:** the sequencing by forward primer covering intron 10 and exon 11. The codon 508 is marked by a box. The wild type sequence CGG was mutated to TGG and the patient was found to be a homozygote. **B:** the sequence revealed by reverse primer which confirmed the results in **A**.

abnormal metabolites.

Discussion

Holocarboxylase synthetase (HLCS; EC 6.3.4.10) is an enzyme that catalyses biotin incorporation into biotin-dependent carboxylases in the mitochondria.[1] An autosomal recessive inherited deficiency of HLCS leads to multiple carboxylase deficiency (MCD, MIM: 253270).[2] While HLCS is responsible for covalently linking biotin to the carboxylase enzymes, biotinidase is involved in the salvage of biotin. Deficiencies in either HLCS or biotinidase result in multiple carboxylase deficiency leading to disruption of critical biochemical pathways involved in fatty acid synthesis, gluconeogenesis and amino acid catabolism. The diagnosis of MCD can be made by urine organic acid analysis which shows increased excretion of several metabolites, including 3-methylcrotonylglycine, 3-hydroxyisovaleric and 3 hydroxypropionic acids. For definitive diagnosis, biotinidase enzyme activity can be measured directly in blood. Previously HLCS deficiency was diagnosed by enzyme measurement in cultured skin fibroblasts. This has largely been taken over by molecular genetic testing now.[4] Both HLCS and biotinidase deficiency can potentially be treated effectively with high dose of biotin. Most patients with HLCS deficiency present in the newborn period. A subgroup of patients may present later in life.[4,5] This late-onset form of HLCS deficiency was thought to be due to mutations with substantial residual enzyme activities.[6]

In conclusion, holocarboxylase synthetase deficiency is a potential highly treatable inborn error of metabolism which can be recognized on clinical grounds by its characteristic rash. A high index of suspicion remains the crucial step towards its diagnosis in patients with recurrent metabolic decompensations and a characteristic skin rash.

Funding: None.
Ethical approval: Not needed.
Competing interest: None declared.
Contributors: Hui J wrote the first draft of this paper. All authors contributed to the intellectual content and approved the final version. Tang N is the guarantor.

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Received February 22, 2010
Accepted after revision August 11, 2010