A Novel Two-Nucleotide Deletion of *MMADHC* Gene Causing cblD Disease in a Chinese Family

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To the Editor: Vitamin B12 (cobalamin, Cbl) is essential for normal growth and development in humans. It can be converted into two important active coenzymes, namely adenosyl cobalamin (AdoCbl) and methyl cobalamin (MeCbl), which play crucial roles in mitochondria and cytoplasm, respectively. The disorders of intracellular cobalamin metabolism that are inherited in an autosomal recessive manner result from the deficient synthesis of AdoCbl and MeCbl derived from Vitamin B12; the types of disorders includes: cblA, cblB, cblC, cblD, etc., depending on the pathogenic genes.[1,2] The cblD disease (MIM# 277410) caused by mutations in the *MMADHC* gene contains three subtypes: cblD-isolated methylmalonic acidemia (MMA), cblD-isolated homocystinuria (HC) and cblD-MMA/HC (combined MMA and HC).[1] We herein reported the Chinese patient with cblD disease attributable to a novel *MMADHC* mutation related to translation reinitiation.

A 5-year-old Han Chinese boy was admitted to Tianjin Children's Hospital due to vomiting and hematemesis. Apart from bilateral rough breath sound and scattered full abdomen tenderness, physical examination showed no obvious abnormalities. Most of the laboratory tests demonstrated no obvious abnormalities; however, and blood gas analysis revealed decompensated metabolic acidosis (pH: 7.13; $PaCO_2$: 13 mmHg [1 mmHg = 0.133 kPa]; and BEb: –22.7 mmol/L). The boy was initially diagnosed with acute hemorrhagic gastritis and metabolic acidosis (decompensatory stage) and given symptomatic treatment. In subsequent laboratory tests, liquid chromatography-tandem mass spectrometry showed elevated blood level of propionyl carnitine (C3: 7.927 μmol/L; normal range: 0–5.140 μmol/L) and elevated C3/acetylcarnitine (C2) ratio (0.739; normal range: 0.020–0.290). Serum homocysteine concentration was normal (4.9 μmol/L; normal range: 0–15.0 μmol/L). Urine gas chromatography-mass spectrometry revealed a highly elevated ratio of methylmalonic acid (199.3; normal range: <1.0) and methyl citrate (33.6; normal range: <1.0; Figure 1a). The boy's parents were healthy and denied the history of heredofamilial disease. His two sisters were healthy, but his brother was admitted to hospital because of vomiting and eventually died at the age of 3. Given probable isolated MMA, intramuscular injection of Vitamin B12 and oral L-carnitine was added to the treatment regime. After treatment for 5 days, results of the blood and urine tests significantly improved. To further confirm the cause of the disease, all the exons and flanking sequence of *MUT*, *MMAA*, *MMAB,* and *MMADHC* genes were amplified successively by polymerase chain reaction (PCR), followed by direct Sanger sequencing and comparison with the reference sequence. A novel homozygous mutation c.24-25delAG (p.R8SfsX15) in exon 3 of the *MMADHC* gene was finally found in the patient. The parents were heterozygous for the same mutation; one of his sisters also carried the mutation, and the other two were normal [Figure 1b]. This deletion was not reported in the 1000 Genome database (http://www.1000genomes.org/home); the Exome Aggregation Consortium browser (http://exac.broadinstitute.org); and the National Heart, Lung, and Blood Institute Exomes (http://evs.gs.washington.edu/EVS/). Further inquiries revealed relatively distant consanguinity between the parents, which explained why the parents carried exactly the same mutation.

Previous research suggested that the N-terminal of the MMADHC protein contains a mitochondrial guide sequence, and the N-terminal and C-terminal domains of the MMADHC protein play important roles in mitochondria and cytoplasm, respectively. Loci and nature of the mutations in *MMADHC* determine the subtype of cblD disease.[3] Mutation c.24-25delAG at the 5' end of the *MMADHC* coding sequence is a frameshift mutation that can produce premature termination codons. On the basis of classical theory, this kind of mutation may lead to a complete the loss of gene function. However, reinitiation of translation occurs in patients with cblD-MMA. In this phenomenon, Met 62 or Met 116 acts as a second start codon. When a premature termination codon arises in the upstream sequence, the downstream sequence of Met62 or Met116 can escape nonsense-mediated mRNA decay and translate

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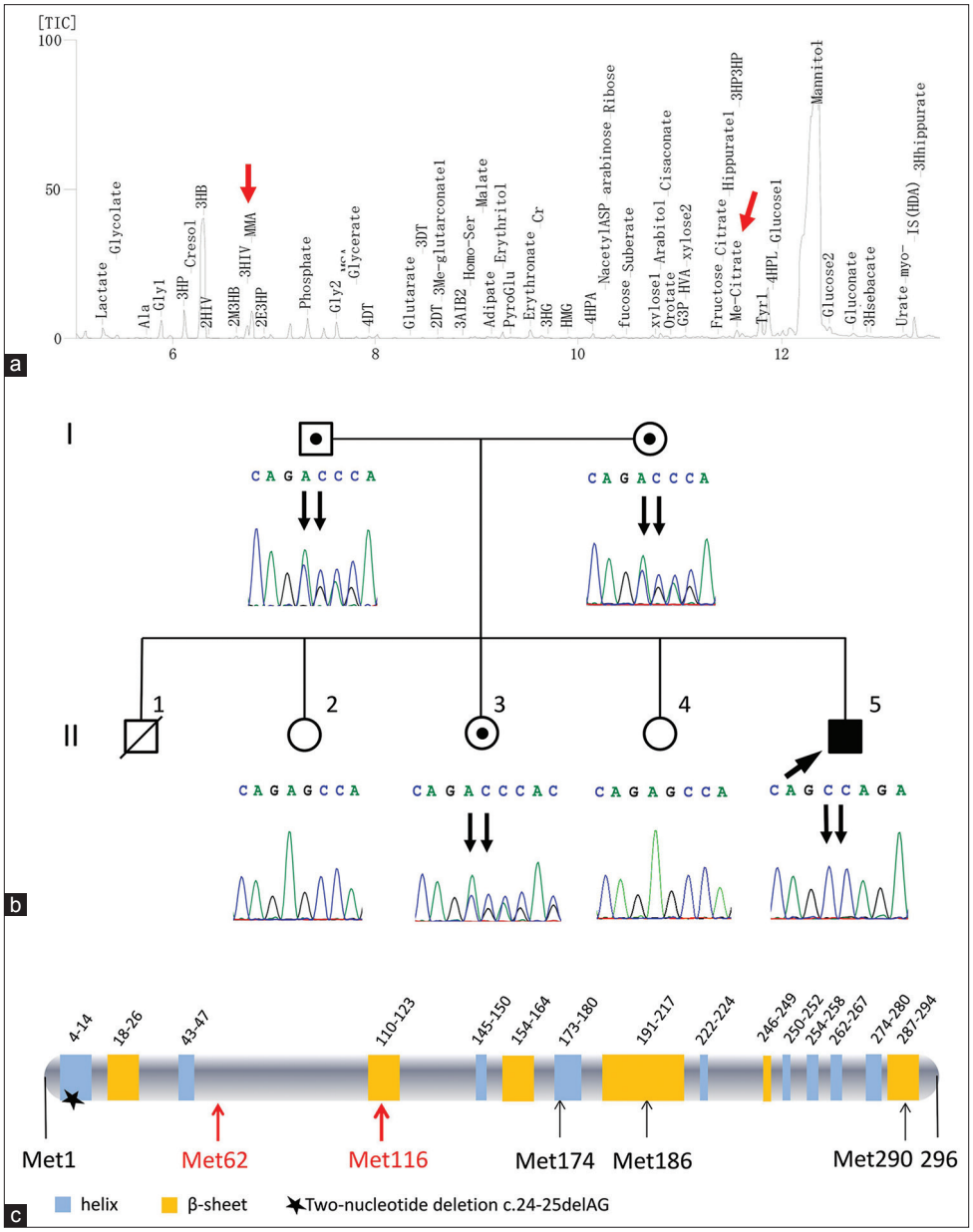

Figure 1: (a) Urine gas chromatography-mass spectrometry chromatogram of a 5-year-old Han Chinese boy with cblD disease. Red arrows indicating significantly elevated levels of methylmalonic acid and methyl-citrate. (b) Pedigree of a Chinese family with cblD disease and chromatograms of the *MMADHC* mutation c.24-25delAG (p.Arg8SerfsX15). Double black arrows indicating the deleted site of the *MMADHC* gene. The patient is homozygous and his parents and one sister are heterozygous for the mutation. (c) Reinitiation sites and predicted secondary structure (http://www.predictprotein.org) of the MMADHC protein. The reported reinitiation sites are marked with a red arrow. Light blue boxes and yellow boxes representing α-helices and β-sheets, respectively. Black star indicating the location of the two-nucleotide deletion.

into protein with residual function [Figure 1c], resulting in blockade of the metabolism of AdoCbl in mitochondria. Meanwhile, the metabolism of MeCbl in the cytoplasm is unaffected and eventually leads to isolated MMA.

Previously reported manifestations of cblD disease included developmental delay, dystonia, epilepsy, seizures, hyperammonemia, metabolic dysequilibrium, etc.[1] The patient in this study was similar to one patient in the report of Miousse *et al*.;[4] both of patients were admitted to hospital due to vomiting and suffered from metabolic acidosis before the diagnosis of cblD disease, and they were both responsive to Vitamin B12. These similarities further validated its genotype-phenotype correlation, which is helpful for clinical diagnosis and management.

The cblD disease is extremely rare, and only about 20 cases have been reported worldwide till now. This case reported a novel mutation of *MMADHC* gene causing clbD disease in a Chinese family. The recognition of clbD disease has important implications since it is one of the few treatable inherited diseases.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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