Cobalamin C defect: a patient of late-onset type with homozygous p.R132* mutation

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Methylmalonic aciduria and homocystinuria, cobalamin C (cblC) type, is the most frequent inborn error of vitamin B_{12} metabolism. The clinical phenotype includes systemic symptoms and neurological decompensation. Affected patients can be divided into two broad groups, as early-onset and late-onset. We present a Turkish patient who had neurological impairment at the age of four years as presented with late-onset cblC defect. Homozygous c.394C>T; p.R132* mutation in the MMACHC gene was detected. The patient was treated with hydroxocobalamin, betaine and folic acid combination with good clinical and biochemical response.

Key words: cobalamin C (cblC) type, late-onset form, hyperhomocysteinemia, methylmalonic aciduria and homocystinuria, vitamin B_{12}, MMACHC gene.

Cobalamin C (cblC, MIM 277400) defect is an error of intracellular cobalamin metabolism presenting with combined methylmalonic aciduria (MMA-uria) and hyperhomocysteinemia[1]. The incidence of this autosomal-recessive disease has been estimated as approximately 1:100,000 live births[2].

Cobalamin C defects present with heterogeneous clinical pictures. Based on the age at onset, two distinct clinical forms have been recognized. The early-onset (EO) form presents symptoms within the first year of life: multisystemic disease with feeding difficulties, failure to thrive, hypotonia, lethargy, hydrocephalus, progressive developmental delay, seizures, mild dysmorphic features, macrocytic anemia, hemolytic uremic syndrome, and ophthalmological abnormalities including maculopathy and retinal degeneration. The late-onset (LO) form, which is seen after four years of age, is less common and presents a milder clinical phenotype, characterized by progressive neurological symptoms and behavioral disturbances. The long-term outcome is usually poor, with frequent neurological impairment. LO patients have better survival and response to treatment and fewer neurological sequelae compared to EO patients. Treatment with hydroxocobalamin, betaine, carnitine, and folic acid decreases metabolite levels, although without complete normalization[3].

The diagnosis of cblC is usually first suspected in the presence of clinical findings along with the typical biochemical abnormalities of MMA-uria, homocystinuria, increased plasma total homocysteine, low-to-normal plasma methionine, and normal serum levels of vitamin B_{12}. Confirmation of the diagnosis has been based on complex *in vitro* complementation studies using cultured fibroblasts of the eight complementation groups[4].

Since among the nearly 500 patients reported with CblC defect, only approximately 10% were in the LO group[5-32], we present a LO patient who had good clinical and laboratory recovery after treatment.

Case Report

A $4^{4/12}$-year-old girl born to healthy parents of Turkish descent presented with loss of speech and inability to walk. She was the second child of consanguineous parents. Her six-month-old sister was in good health.

Four months prior to presentation, the patient started to be fearful of various noises and became less active. Over weeks, her speech decreased gradually to two words, and she developed stereotypic hand-clapping movements, ataxia, and difficulty swallowing solid food. On physical examination, she was a thin and weak child who could sit with help; she had little eye contact, marked hypotonia, and hypoactive deep tendon reflexes. Eye examination was normal (Fig. 1a). Initial laboratory investigations including electrolytes, urea, creatinine, aminotransferases, blood gases, glucose, and ammonia were normal. Complete blood count showed normochromic, macrocytic anemia (hemoglobin [Hb]: 10 g/dl, N: 10.5–14; mean corpuscular volume [MCV]: 83.9 fl, N: 70–74). Serum vitamin B_{12} level was 695 pg/ml (N: 197–866), serum lactate was normal, and pyruvate was mildly increased. Metabolic screening by gas chromatography-mass spectrometry (GC-MS) revealed significant MMA-uria (2080 μmol/mmol creatinine, normal: not detectable) and the presence of methylcitrate. Plasma acylcarnitine analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS) demonstrated significant elevation of propionyl carnitine to 6.13 μmol/L (N: 0.28–2.9). Quantitative methionine level was 5.4 μmol/L (normal range: 43–223), and homocysteine was 232 μmol/L (N: 5.5–17). Screening for MTHFR mutation was negative. The EEG was normal; cranial magnetic resonance imaging (MRI) showed bilateral signal intensity changes in the basal ganglia and cerebral white matter and diffuse atrophy of the corpus callosum and cerebrum. MR spectroscopy revealed lactate and myoinositol peaks in the basal ganglia. Subsequent fibroblast complementation studies confirmed the diagnosis of cblC defect, and molecular studies of the *MMACHC* gene revealed a homozygous c.394C>T mutation. Hydroxocobalamin injections were commenced daily for seven days followed by twice weekly injections (1 mg/day, i.m.). In addition, oral supplementation of folic acid (5 mg/day) and betaine (150 mg/kg/day) was given. The patient has been followed for one year. Plasma homocysteine levels decreased from the maximum level of 232 μmol/L to 32 μmol/L and urine MMA, from 2080 μmol/mmol creatinine to 195 μmol/L creatinine (normally not detectable) (Figs. 2a, b). Propionyl carnitine levels decreased from 6.13 μmol/L to 3.2 μmol/L (N: 0.28–2.9), and plasma methionine levels increased from 5.4 μmol/L to 47 μmol/L. Clinically, her perception, speech and walking improved. Her developmental level is around 3-3.5 years; she had a mild spastic ataxic gait and bilateral extensor plantar responses (Fig. 1b).

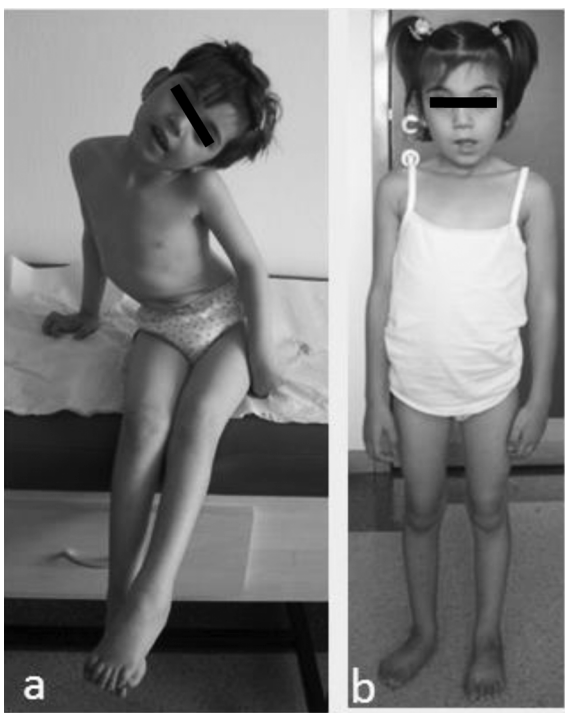

Fig. 1a. cblC patient (late-onset) at 4.5 years (before treatment); **1b.** cblC patient at 5.5 years (after treatment).

Discussion

Inherited disorders of cbl metabolism have been classified into eight complementation groups

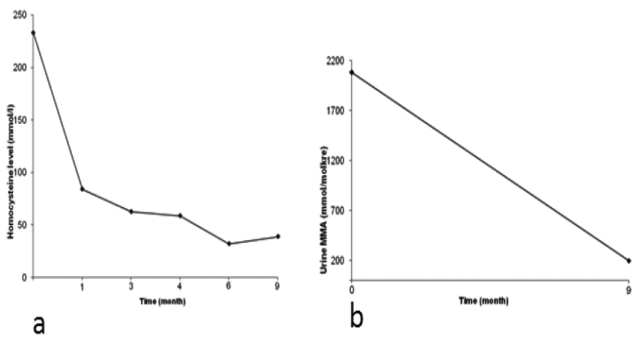

Fig. 2a. Plasma homocysteine levels before and following treatment; **2b.** Urine methymalonic acid (MMA) levels before and following treatment.

(cblA-H). Three of these disorders (cblC, cblD, cblF) affect the biosynthesis and/or actions of both adenosylcobalamin and methylcobalamin, and they are characterized biochemically by combined MMA-uria and homocystinuria. CblC defect is the most frequent form[1,2]. In the study of Rosenblatt et al.[3], the clinical data from 50 cblC (44 EO, 6 LO) patients suggested two distinct phenotypes defined by age of onset. LO patients had a more promising outcome. In their study, all of the six patients with LO survived with good neurological outcome and only mild or moderate impairment. Cognitive function improved shortly after treatment was started, and two cases showed clinical improvement before any biochemical changes were noted. Motor function, particularly in the lower limbs, did not improve as quickly. One patient, who presented at 14 years with personality changes and confusion, was last seen at the age of 25 years with persisting lower extremity weakness. Two patients showed complete recovery in blood homocysteine and urine MMA clearance, two patients showed incomplete recovery, and in two patients, biochemical responses were unknown. Another patient with LO was reported to be a good responder with recovery of the neuropsychiatric symptoms following cyanocobalamin (vitamin B_{12}) treatment[32].

In our patient, urine MMA excretion and blood homocysteine level decreased after treatment (Figs. 2a, b). Near complete recovery was achieved clinically except for mild spastic paraparesis and mild developmental delay. Myelopathy appears to be the typical sequela of the LO type.

The gene responsible for cblC, named *MMACHC*, catalyzes the reductive decyanation of cyanocobalamin (CNCbl)[6]. The recent cloning of the disease gene and identification of nearly 70 different mutations over 350 cblC patients permitted preliminary genotype-phenotype correlations and to relate specific gene variants to distinct ethnicities[5,7,8,]. Mutation screening studies revealed that the most common mutation is the c.271dupA, followed by c.394C>T[8]. Individuals with c.394C>T mutation tend to present with LO disease, whereas patients with c.271dupA, c.331C>T and c.609G>A tend to present in infancy[5,8,28]. Based on the genetic screening studies in different populations, *MMACHC* mutations cluster by ethnicity. Screening studies have shown that the c.331C>T mutation is seen in Cajun and French-Canadian patients, the c.394C>T mutation is common in the Asiatic-Indian/Pakistani/Middle Eastern populations, and the c.609G>A is the most prevalent mutation in Chinese patients[5,8,28]. There is no report on the frequency and distribution of *MMACHC* gene mutations in the Turkish population. In our patient, the commonest mutation for the LO form (c.394C>T) was detected homozygous.

Brain MRI findings are less specific and include periventricular white matter abnormalities (T2 and FLAIR hyperintensities in the cerebral white matter), cortical atrophy, and bilateral ventricular dilatation in LO disease[27]. Only one of the six previously reported LO patients had cerebral atrophy[7]. In our patient, cranial MRI showed bilateral signal intensity changes in the basal ganglia and cerebral white matter and diffuse atrophy of the corpus callosum and cerebrum. MR spectroscopy revealed lactate and myoinositol peaks in the basal ganglia. Although our patient was the LO form, lactate peak in basal ganglia, as seen in EO patients, was detected[33,34].

The presented patient was homozygous for the c.394C>T (p.R132*) mutation, which is common in the Asiatic-Indian/Pakistani/Middle Eastern populations. Although it presents a milder clinical phenotype and good response to the therapy, myelopathy appears to be the typical sequela of LO type.

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