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RESEARCH REPORT

Cobalamin D Deficiency Identified Through Newborn Screening

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Abstract Cobalamin D deficiency (cblD) is one of the least common cobalamin metabolism disorders. It may result in isolated homocystinuria, isolated methylmalonic aciduria, or combined methylmalonic aciduria and homocystinuria (cblD-combined). Only seven cases of the combined cblD form have been reported to date. Due to the rarity of this disorder, the presentation and symptoms are not well described. We present an eighth case of the cblD-combined subtype, who had a positive newborn screen (NBS) on day of life 3. She was symptomatic and developed lethargy and poor oral intake at 8 days of life. She was treated with 10% dextrose, folinic acid, intramuscular hydroxocobalamin, and betaine. Despite the early initiation of treatment, she developed complications of the disease and was found to have abnormal brain imaging findings at 17 days of age and macular atrophy at 3 months of age and has global developmental delay. We provide detailed description of her presentation, her treatment, and her complications to aid in the understanding of this rare disorder, which is very similar to the more common cobalamin C disorder (cblC).

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Introduction

Vitamin B12 (cobalamin) is metabolized to 5′-deoxyadenosylcobalamin (AdoCbl), a required cofactor for the mitochondrial enzyme methylmalonyl-CoA mutase and methylcobalamin (MeCbl), a required cofactor for the cytoplasmic enzyme methionine synthase (Watkins and Rosenblatt 2011). Methylmalonyl-CoA mutase is required for the conversion of methylmalonyl-CoA into succinyl-CoA, and methionine synthase is required for the remethylation of homocysteine to methionine (Watkins and Rosenblatt 2011). Remethylation disorders affect the remethylation process of homocysteine to methionine, resulting in accumulation of homocysteine (Huemer et al. 2017). Acquired or inherited disturbances of cobalamin metabolism may result in elevations of homocysteine and/or methylmalonic acid in plasma and urine.

Cobalamin D (cblD) deficiency is one of the rarest cobalamin metabolism disorders (Atkinson et al. 2014). CblD results in isolated homocystinuria (cblD-homocystinuria) due to missense mutations located in the C-terminal part of the protein, isolated methylmalonic aciduria (cblD-methylmalonic aciduria) due to mutations in the N-terminal part of the protein, or combined methylmalonic aciduria and homocystinuria (cblD-combined) due to nonsense, splice site, or truncating variants toward the C-terminal part of the protein (Coelho et al. 2008). Fewer than 20 patients with cblD deficiency have been described in the literature, only seven of these had cblD-combined (Stucki et al. 2012; Miousse et al. 2009; Suormala et al. 2004; Parini et al. 2013; Soylu Ustkoyuncu et al. 2018).

We report an eighth case of cblD-combined who presented in the neonatal period symptomatically after an abnormal newborn screen. Despite early treatment, she has developmental delay and bilateral maculopathy. We

describe her clinical, biochemical, and radiographic findings, the progression over the first months of her life, and her current status.

Case Report

The patient had a positive newborn screen, and follow-up testing was pending when she developed poor feeding and decreased alertness as an 8-day-old infant. She was born at term to consanguineous parents who are second cousins once removed (maternal grandfather and father are first cousins); she has an older brother who is healthy. The newborn screen, collected at 22 h of life, was positive for low methionine, 4 μmol/L (cutoff >8 μmol/L), and was reported on day of life (DOL) 3. C3 acylcarnitine was not flagged as the value was 5.5 μmol/L (cutoff <6.3 μmol/L); the C3/C2 ratio was elevated at 0.4 (cutoff <0.3), but this was not flagged per the California newborn screening protocol. Homocysteine and other follow-up testing were recommended after the positive newborn screening result. Homocysteine was collected on DOL 5 but had not been resulted at the time that she developed clinical symptoms. The patient presented on DOL 8 to her PCP with poor feeding and jaundice and was admitted to an outside hospital on DOL 9. The follow-up newborn screen test results were not yet available. The metabolic service was contacted on DOL 10, and urgent homocysteine and other laboratories were requested. At that time, ammonia, lactate, and pH were normal, and urine ketones were negative. The infant was receiving dextrose only due to the concern for an inborn error. Reinitiation of breast milk and the administration of intramuscular hydroxocobalamin (after the collection of laboratories) were recommended. The outside hospital homocysteine was resulted on DOL 11, along with that previously obtained, both were markedly elevated. The homocysteine collected on DOL 5 was the peak value at 299.5 μmol/L (ref. <10.4 μmol/L) and from DOL 11 was elevated at 208 μmol/L (ref. 3–10 μmol/L). On DOL 12, she was transferred to our hospital for further evaluation and management. Due to a suspected diagnosis of cblC disease, she was empirically initiated on intravenous 10% dextrose solution at 80 mL/kg/day, intramuscular hydroxocobalamin (1 mg/day), betaine (250 mg/kg/day), and folinic acid (5 mg/day). After the prolonged protein restriction at the outside hospital prior to our management, protein intake was recommended to be at DRI (dietary reference intake) of 2.0–2.2 g/kg/day temporarily during the initial presentation and neonatal crisis, while the DOL 5 and 12 methylmalonic acid levels were pending, due to a possible markedly elevated methylmalonic acid level. Diet was not restricted in protein after the initial period, as current guidelines recommend against dietary protein restriction in combined remethylation disorders. Methylmalonic acid level on admission on DOL 12 was 38 μmol/L, ref. <0.3 μmol/L, and on DOL 5 had been 97.2 μmol/L, ref. <0.318 μmol/L. Hematologic parameters included a platelet count of 24 (ref. 140–450 × $10e^9$/L), hemoglobin of 13.6 g/dL (ref. 13.5–21.5 g/dL), and white blood count of 4.7 (ref. 5–21 × $10e^9$/L) on DOL 12. At the time of admission to our hospital, she continued to be encephalopathic. Methionine on admission was 0 μmol/L (ref. 9–42 nmol/mL). After 5 days on the above treatment (betaine dose was increased to 500 mg/kg/day on DOL 14), her mental status and her tone had improved, her methionine improved to 33 nmol/mL (ref. 10–60 nmol/mL), total homocysteine came down to 53 μmol/L (ref. 3–10 μmol/L), and MMA came down to 10.24 μmol/L (ref. <0.3 μmol/L).

Ophthalmological examination including a dilated fundus examination at 18 days of age was normal with a normal macular appearance. At 3 months of age, she had developed mild nystagmus, and fundus examination showed bilateral macular atrophy. A full-field electroretinography (ERG) was performed to the International Society for Clinical Electrophysiology of Vision (ISCEV) standards (McCulloch et al. 2015) using Burian-Allen contact lens electrodes under brief general anesthesia at 7 months of age and showed normal responses. Fundus examination at that time showed bilateral macular atrophy with a bull's eye appearance that had progressed since the examination at 3 months of age (Fig. 1). The peripheral retina was relatively normal in appearance.

Current Treatment: She continues on daily hydroxocobalamin 1 mL IM (100 mcg/kg/day once daily), betaine (Cystadane®) 300 mg/kg/day divided in three daily doses, carnitine 15 mg/kg/day divided in three daily doses, and leucovorin (folinic acid) 10 mg daily, with no dietary restrictions. Her most recent levels (at 21 months of age): MMA is 41.39 μmol/L (ref. 0.0–0.4 μmol/L), total plasma homocysteine is 45 μmol/L (ref. 3–8 μmol/L), and methionine is 23 nmol/mL (ref. 9–42 nmol/mL).

Developmental History

Despite the above therapy since less than 2 weeks of age, our patient has global developmental delay and has required early intervention services. For gross motor milestones: she sat with support at 8 months, she sat unsupported at 10 months, she crawled at 10 months, and started to walk at 16 months of age. Regarding her social and language development, at 6 months she was smiling, she was babbling and saying "mama, baba" at 10 months of age, at 14 months she was imitating words, and at 21 months of age she was saying few other words in addition to "mama, baba." She requires physical therapy, occupational therapy, oral speech and language therapy, and vision therapy.

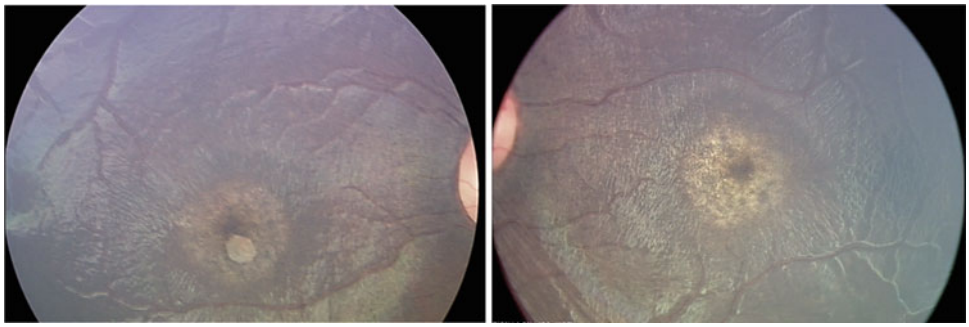

Fig. 1 Color fundus photography at 7 months of age showing bilateral macular atrophy with retinal pigment epithelium (RPE) hyperplasia beneath the anatomic fovea in each eye. There were refractile features in the region of macular RPE depigmentation, and a well-demarcated region of RPE atrophy was present inferior to the fovea in the right eye

Genetic Tests

SNP microarray identified the *MMADHC* gene to be contained within a region of homozygosity. DNA panel testing identified a homozygous pathogenic *MMADHC* mutation c.472C>T (p.Arg158Ter), confirming the diagnosis of cblD deficiency. This nonsense variant in the *MMADHC* gene is predicted to result in the loss of expression of the encoded protein. This variant was not identified in the approximately 6,500 participants in the NHLBI exome project, and gnomAD reports an allele frequency of 1/2,460,765 or 0.000004064. To our knowledge, this variant was not previously reported in other patients with cblD deficiency. Sequencing and deletion/duplication analysis of *MMACHC*, which can also cause combined methylmalonic aciduria and homocystinuria, was normal.

Imaging Studies

Brain magnetic resonance imaging at 17 days of age showed symmetric regions of abnormal T2 hyperintensity in the frontal and parietal subcortical white matter bilaterally and in the frontal periventricular white matter bilaterally. There was also delayed sulcation for age and thinning of the corpus callosum (Fig. 2). Echocardiogram at 2 weeks of age was performed due to suspected cobalamin C deficiency (cblC) and was essentially normal. Abdominal ultrasound was not performed. Upper GI with small bowel follow-through showed no evidence of malrotation. A swallow study performed at 10 months of age identified aspiration with thin liquids and penetration of nectar-thick liquids.

Discussion

Seven other patients have been reported in the literature with cblD-combined; two of these are siblings (Goodman et al. 1970). The age of presentation varied from the first 22 days of life (Coelho et al. 2008) to 14 years of age as published in the original report in 1970 (Goodman et al. 1970). The current report presents the first reported patient to have been identified through a newborn screening program, and this is the earliest reported symptomatic presentation of the described patients with cblD-combined type. This patient's mutation and her biochemical findings were consistent with the combined MMA/HC cblD diagnosis.

Overall, the combined disorders of remethylation that lead to both homocystinuria and methylmalonic aciduria may present in the neonatal period or infancy, as the reported patient presented when she was 8 days old. Usual neonatal presentations are similar to the patient's presentation; they may have lethargy, decreased oral intake, hypotonia, in addition to anemia, and thrombocytopenia.

Abnormal neurological findings have been observed in the combined remethylation disorders as well, such as microcephaly, seizures, hypotonia, and global developmental delay (Huemer et al. 2017). The current manuscript reports a patient with developmental delay who requires physical and speech therapy.

Ophthalmological findings are common in cblC disease and include macular atrophy, nystagmus, strabismus, and optic nerve atrophy (Bonafede et al. 2015; Weisfeld-Adams et al. 2015; Brooks et al. 2016). The macular changes are usually progressive and associated with abnormal rod and cone function on ERG (Brooks et al. 2016). Similar manifestations might be expected in cblD patients, but given the rarity of this disorder, the eye findings have not been well described. Ophthalmologic findings were not described in the previously reported patients with cblD-combined type. The current study presents a patient with an abnormal eye exam at 3 months of age who subsequently developed nystagmus and bilateral macular atrophy. The ocular findings in the patient described in this manuscript were similar to those that have been described in patients with cblC disease (Brooks et al. 2016; Bonafede et al. 2015; Aleman et al. 2015; Bacci et al. 2017; Traboulsi et al. 1992); the patient displayed macular atrophy with a bull's

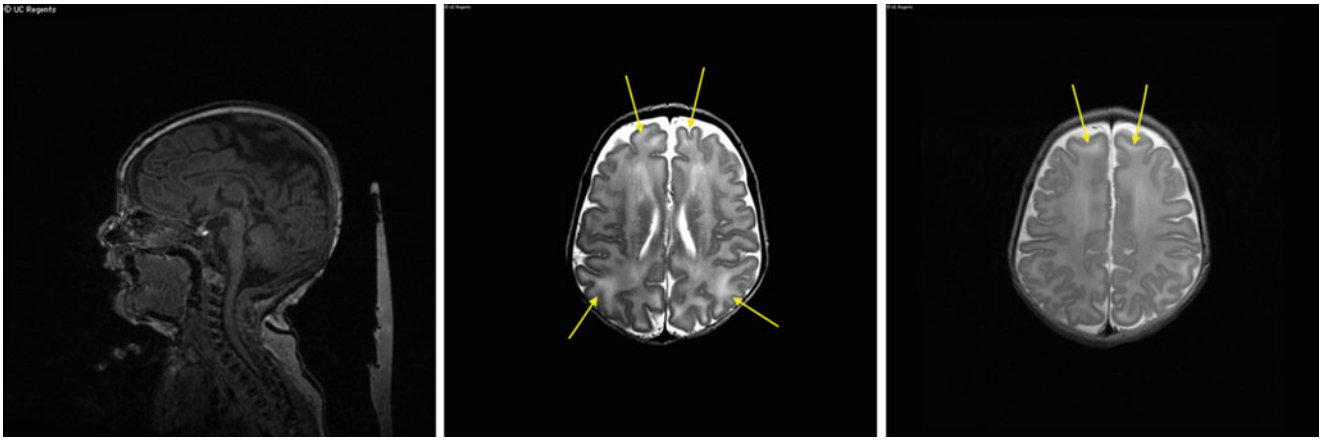

Fig. 2 Brain MRI at 17 days of life; left, midline sagittal T1 showing thin corpus callosum; middle: axial T2 showing bilateral symmetric subcortical white matter T2 hyperintensity in the frontal and parietal lobes; right, axial T2 at a slightly higher level showing bilateral symmetric subcortical frontal white matter T2 hyperintensity

eye appearance. The peripheral retina was relatively normal in appearance, and the full-field ERG demonstrated normal diffuse outer retinal function at 7 months of age; however, full-field ERG responses may become abnormal with time as progressive cone dysfunction has been reported in patients with cblC deficiency. The patient has the characteristic macular findings seen in cblC deficiency in infants, but it is possible that she will develop peripheral pigmentary retinopathy at a later stage as usually occurs in cblC deficiency (Brooks et al. 2016).

Cardiomyopathy has been the main cardiac disease reported in patients with remethylation disorders (Huemer et al. 2017). The current study reports a patient with a normal echocardiogram performed at 2 weeks of age and no cardiac symptoms at age 21 months.

Renal complications may include thrombotic microangiopathy that may lead to atypical hemolytic-uremic syndrome with its associated complications (Huemer et al. 2017). There was no evidence of renal disease, with normal BUN/creatinine at 2 months of age and negative urine analysis on multiple occasions, but no renal imaging was performed in the current study.

Conclusion

CblD-MMA/HC can be identified on newborn screening for remethylation defects by flagging low methionine, which is not done in all states in the United States, and may be missed by newborn screening by elevated C3 acylcarnitine alone, as illustrated here. Early identification may decrease the morbidity and the mortality associated with this condition. Early hospitalization might have been avoided with a more rapid turnaround time of the newborn screen follow-up laboratories. Developmental delay, macular degeneration, and other complications may develop despite early treatment and compliance with therapy, though the progression of the disease and its complications may progress more slowly with the appropriate therapy. Careful monitoring and close follow-up are warranted.

One Sentence Summary

We report a case of cobalamin D combined subtype that was identified by the newborn screening program, with a detailed description of the patient's maculopathy and brain imaging findings.

Details of Contributions of Authors

Dr. Aya Abu-El-Haija: Wrote the manuscript, reviewed the literature, and participated in the clinical care and clinical diagnosis of the patient.

Dr. Bryce Mendelsohn: Revised the manuscript, participated in the biochemical and molecular diagnosis of the patient, and participated in the clinical care of the patient.

Dr. Anthony Moore: Revised the manuscript and participated in the evaluation of the ophthalmologic findings of the patient.

Kara Weisiger: Revised the manuscript and participated in the clinical diagnosis and clinical care of the patient.

Dr. Jacque L. Duncan: Revised the manuscript, performed and interpreted the ERG, took the ophthalmic

images, and participated in the evaluation of the ophthalmologic findings of the patient.

Dr. Orit A. Glenn: Revised the manuscript and participated in the evaluation of the radiologic findings of the brain imaging of the patient.

Dr. Renata Gallagher: Revised the manuscript, participated in the biochemical and molecular diagnosis of the patient, and participated in the clinical care of the patient.

Compliance with Ethics Guidelines

Conflict of Interest

Aya Abu-El-Haija, Bryce A. Mendelsohn, Anthony T. Moore, Jacque L. Duncan, Orit A. Glenn, Kara Weisiger, and Renata C. Gallagher declare that they have no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was not obtained from the patient given that this is de-identified patient information and is a single case report.

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