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CASE REPORT

Cobalamin C Disease Missed by Newborn Screening in a Patient with Low Carnitine Level

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Abstract Cobalamin C (CblC) disease is the most common inherited disorder of intracellular cobalamin metabolism. It is a multisystemic disorder mainly affecting the eye and brain and characterized biochemically by methylmalonic aciduria, low methionine level, and homocystinuria. We report a patient found to have CblC disease who initially presented with low carnitine and normal propionylcarnitine (C3) levels on newborn screen. Newborn screening likely failed to detect CblC in this patient because of both his low carnitine level and the presence of a mild phenotype.

Introduction

Cobalamin C (CblC) disease is the most common inborn error of intracellular cobalamin metabolism. The disorder is caused by mutations in *MMACHC*. Pathogenesis is due to an inability to convert cobalamin to the active forms, methylcobalamin and adenosylcobalamin. Methylcobalamin is required for the conversion of homocysteine to methionine by methionine synthase. Adenosylcobalamin is required for the conversion of methylmalonyl-CoA to succinyl-CoA by methylmalonyl-CoA mutase (Martinelli et al. 2011). Therefore, CblC disease is characterized by elevated levels of homocysteine, low methionine level, and elevated methylmalonic acid (MMA).

CblC disease has a spectrum of severity with two distinct phenotypic forms: early and late onset. Early-onset patients present with intrauterine growth retardation (IUGR), microcephaly, failure to thrive, developmental delay, hypotonia, progressive retinopathy, and maculopathy (Fischer et al. 2014). Early-onset patients usually present in the first months of life and have an unfavorable prognosis (Carrillo-Carrasco et al. 2011). Late-onset CblC patients usually present with extrapyramidal and neuropsychiatric symptoms in any decade of life (Rosenblatt et al. 1997). Late-onset patients are less likely to have ocular involvement (Gerth et al. 2008).

Tandem mass spectrometry-based newborn screening (NBS) aims to detect patients with inborn errors of metabolism prior to the onset of symptoms. Based on the results of 5 years of expanded newborn screening in New York State, the estimated prevalence of CblC is 1:100,000 (Weisfeld-Adams et al. 2010). Elevated levels of propionylcarnitine (C3) and a high C3/C2 (acetyl) ratio could suggest a variety of disorders of propionate metabolism such as methylmalonic acidemia, propionic acidemia, and congenital cobalamin defects including CblC disease. These results require emergent evaluation by a metabolic specialist. Rapid diagnosis and therapy is crucial in the prevention of long-term complications of these disorders.

Here we present a case of CblC that was originally missed by NBS. However, NBS did indicate a low carnitine level, which in turn prompted further investigation leading to diagnosis of CblC.

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Clinical Report

The male patient was a full-term infant born to a 19-year-old G1P0 mother via normal spontaneous vaginal delivery after an uneventful pregnancy. He had no complications in the neonatal period and was discharged home on the third day of life. His parents were healthy, nonconsanguineous, and of Mexican descent. There was no known family history of metabolic disease. His NBS collected on his second day of life demonstrated an inconclusive result for a low carnitine (C0) level (9 μmol/L, normal >10.46) and normal C3 level. A repeat NBS on his fourteenth day again reported a normal C3 level and a low C0 level (10.26 μmol/L, normal >10.46) (Table 1).

Given the two low carnitine levels, he was referred to an outside hospital for evaluation of low carnitine at 7 weeks of life. His physical and development examinations were normal at this time. Diagnostic testing at 7–8 weeks of life confirmed the low carnitine level (20 μmol/L, normal 32–62). The metabolic work-up (Table 1) also revealed significantly elevated blood C3 acylcarnitine, total and free homocysteine, and urine and blood MMA. His vitamin B12 level was normal. These findings are consistent with a cobalamin defect. Interestingly, he had a normal methionine level. Methionine is often low in patients with cobalamin defects because they are unable to convert homocysteine to methionine. He was started on carnitine therapy and referred to our center for further evaluation and management.

His diagnosis of CblC was confirmed with fibroblast complementation assays. Gene sequencing of *MMACHC* demonstrated two mutations: c.482G>A (p.R161Q) and c.608G>A (p.W203X).

Therapy was initiated with hydroxycobalamin (1 mg IM three times per week) and levocarnitine (50 mg/kg/day). His biochemical markers of disease improved substantially after initiation of hydroxycobalamin therapy (Table 1). Within five days of his first injection, he had a roughly fivefold reduction in total homocysteine and urine and blood MMA levels. The mainstay of therapy for patients with CblC is parental hydroxycobalamin; this is often combined with oral betaine, carnitine, folate, and methionine as needed. He was also initially started on a low-protein diet that was subsequently liberalized. Hydroxycobalamin, rather than dietary therapy, is the definitive treatment for CblC (Martinelli et al. 2011).

Given his excellent response to hydroxycobalamin, he has not required therapy with betaine. His last exam was at 30 months of age; he has continued on 1 mg/week of IM hydroxycobalamin and has remained in good metabolic control (Table 1) with no episodes of acute metabolic decompensation.

At his 30-month visit, he had normal growth parameters. His examination demonstrated pseudostrabismus and a

Table 1 Metabolic laboratory values

Metabolite	Newborn screen #1 2 days of life	Newborn screen #2 14 days of life	Confirmatory testing 2 months of life	Response to OH-cobalamin 5 days after first injection	Follow-up values 30 months of life
C0	9 μmol/L (borderline <10.46)	10.26 μmol/L (borderline <10.46)			
C3	2.44 μmol/L (borderline >5.94)	2.74 μmol/L (borderline >5.94)	6.14 μmol/L (<0.94)	5.13 μmol/L (0–1.60)	1.99 μmol/L (0–1.60)
C3/C2	0.21 (borderline >0.2, positive >0.26)	0.4 (borderline >0.2, positive >0.26)			
Total carnitine			20 μmol/L (32–62)		58.5 μmol/L (25–69)
Total homocysteine			70.43 μmol/L (<15)	14.75 μmol/L (<15)	15.04 μmol/L (<15)
Free homocystine			3.3 μmol/L (0)		<2 μmol/L (0)
Methionine			30.3 μmol/L (8–49)		41.1 μmol/L (8-49)
Blood MMA			15.8 μmol/L (0.1–0.37)	3.4 μmol/L (0.1–0.37)	4.8 μmol/L (0.1–0.37)
Urine MMA			387.8 mmol MMA/mol Cr (<4)	61.3 mmol MMA/mol Cr (<4)	Not detected

speech delay. Ophthalmologic exam demonstrated myopic astigmatism but no other abnormalities.

Discussion

On newborn screening, elevations in C3 and the C3/C2 ratio are markers for disorders of propionate metabolism including CblC disease. Confirmatory testing including acylcarnitine profile, measurements of homocysteine and MMA, and urine organic acids can help identify an individual's specific diagnosis. Given the wide spectrum of clinical presentations of CblC disease, it is possible that cases could be missed on newborn screening. In a recent review of missed newborn screening cases in New South Wales, Australia, 11/15 missed cases could be attributed to mild phenotypes. Two of these cases were CblC patients (Estrella et al. 2014).

This is a unique case of a missed CblC disease because the patient was ultimately diagnosed through evaluation of a low carnitine level on newborn screen. Low carnitine may lead to a lower C3 acylcarnitine level. Therefore, depending on screening cutoffs, CblC patients with late-onset, mild mutations and low total carnitine levels could be missed on newborn screening.

Genotype-phenotype correlation of *MMACHC* mutations has been reported. While more than 50 mutations have been described, three common mutations exist, which demonstrate genotype-specific age of onset. The c.271dupA and c.331C>T mutations are associated with early-onset disease, while the c.394C>T mutation typically leads to late-onset disease (Lerner-Ellis et al. 2005). Genotypic differences in age of onset are likely due to mRNA stability and transcript levels. Individual variability in residual protein levels and activity results in variable levels of detectable diagnostic metabolites.

Our patient had two previously reported *MMACHC* mutations, c.482G>A (p.R161Q) and c.608G>A (p.W203X). The missense c.482G>A mutation is reported to be a late-onset mutation, with patients generally presenting in second or third decade (Lerner-Ellis et al. 2005, 2009; Morel et al. 2006; Thauvin-Robinet et al. 2007). A case series of five patients identified through NBS with homozygous c.482G>A mutations demonstrated that all were clinically well at age 2.5 months (Lin et al. 2009). Only one of the five patients had elevated blood MMA and homocysteine levels. Compound heterozygous patients carrying the c.482G>A mutation with an early-onset mutation (e.g., c.271dupA) have a milder phenotype than patients with homozygous early-onset mutations (Morel et al. 2006; Carrillo-Carrasco et al. 2011).

The nonsense c.608G>A (p.W203X) mutation found in our case has been previously described in patients of Hispanic origin with early-onset form of CblC (Lerner-Ellis et al. 2005, 2009; Weisfeld-Adams et al. 2010). A similar nonsense mutation, c.609G>A, is the most common mutation in patients of Chinese descent. Severity of disease and age of onset in compound heterozygotes carrying c.609G>A depend on the severity of the other mutation (Wang et al. 2010). Given our patient's compound heterozygosity for a late- and an early-onset mutation, he would be predicted to have a mild clinical presentation.

In addition to a mild mutation, low total carnitine likely masked this patient's diagnosis of CblC disease. Low carnitine can be due to primary carnitine disease or a secondary cause, such as prematurity, (Honzík et al. 2005) or low maternal carnitine levels since carnitine is transferred across the placenta (Stanley 2004). Unfortunately, we were unable to obtain a carnitine sample from our patient's mother. Low carnitine can also be seen in a number of fatty acid oxidation defects and organic acidemias, as free carnitine is consumed by excessive accumulating acyl-CoA species. Therefore this could contribute to missed newborn screens in patients with a variety of inborn errors of metabolism.

To increase the sensitivity of newborn screening for CblC disease, the threshold for labeling C3 as abnormal could be lowered. However, this would greatly increase the rate of false-positive tests. The C3/C2 ratio can also be used as a marker for CblC (McHugh et al. 2011). In retrospective analysis, this ratio was actually elevated in our patient (Table 1). However, in the state where the NBS was performed, the C3/C2 ratio is only reported if the C3 level is elevated. This case suggests that the C3/C2 ratio (and perhaps other ratios such as C3/C0) should be used as a second tier testing marker for disorders of propionate metabolism when the total carnitine level is low.

Some researchers have suggested that NBS reports additional markers, such as methionine and homocysteine, in samples with elevated C3. This could help increase the specificity of screening for cobalaminopathies (Chace et al. 2001; Tortorelli et al. 2010) and differentiate C3 elevations due to cobalaminopathies from methylmalonic acidemia or propionic acidemia. However, it is unclear that the addition of these markers would help increase the sensitivity of the NBS for CblC given that in mild cases these markers, like C3, could be normal.

Given that two NBS failed to detect CblC in our patient, metabolic evaluation and definitive therapy were delayed. Early diagnosis and treatment can improve outcomes but cannot prevent all complications of CblC disease (Rosenblatt et al. 1997; Andersson et al. 1999; Boxer et al. 2005; Smith et al. 2006; Thauvin-Robinet et al. 2007; Martinelli et al. 2011; Aleman et al. 2014). Early therapy may be particularly beneficial for late-onset patients, as it can be started prior to the development of any organ damage (Huemer et al. 2014). However, recent studies have

demonstrated residual neurodevelopmental delays and progression of ocular disease in patients identified by newborn screening and treated since birth (Weisfeld-Adams et al. 2013).

In our case, ordering plasma acylcarnitine profile and urine organic acids (UOA) in addition to blood and urine carnitine levels helped to detect CblC disease. Although the current recommended work-up is to measure blood and urine carnitine levels to rule out primary and nutritional carnitine deficiency in babies with low carnitine level on newborn screen, this case suggests the benefits of adding plasma acylcarnitine and UOA.

In conclusion, newborn screening may not be able to detect all patients with CblC disease. This case report about a newborn with CblC suggests that medical providers should consider CblC in older patients with multisystemic symptoms of unclear etiology such as cognitive decline, neuropsychiatric disease, and unexpected thrombosis.

Synopsis

A CblC patient may be missed on newborn screening if he carries a late-onset, mild mutation and has low total carnitine levels.

Compliance with Ethics Guidelines

Conflict of Interest

Rebecca Ahrens-Nicklas declares she has no conflicts of interest.

Esra Serdaroglu declares she has no conflicts of interest.

Colleen Muraresku declares she has no conflicts of interest.

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This article does not contain any studies with human or animal subjects performed by any of the authors.

Contributions

Concept/design: RA, ES, CM, CF

Drafting of article: RA, ES

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References

Aleman TS, Brodie F, Garvin C et al (2014) Retinal structure in cobalamin c disease: mechanistic and therapeutic implications. Ophthalmic Genet 2014:1–10. doi:10.3109/13816810.2014.885059

Andersson HC, Marble M, Shapira E (1999) Long-term outcome in treated combined methylmalonic acidemia and homocystinemia. Genet Med 1:146–150. doi:10.1097/00125817-199905000-00006

Boxer AL, Kramer JH, Johnston K et al (2005) Executive dysfunction in hyperhomocystinemia responds to homocysteine-lowering treatment. Neurology 64:1431–1434. doi:10.1212/01.WNL.0000158476.74580.A8

Carrillo-Carrasco N, Chandler RJ, Venditti CP (2011) Combined methylmalonic acidemia and homocystinuria, cblC type. I. Clinical presentations, diagnosis and management. J Inherit Metab Dis 35:91–102. doi:10.1007/s10545-011-9364-y

Chace DH, DiPerna JC, Kalas TA et al (2001) Rapid diagnosis of methylmalonic and propionic acidemias quantitative tandem mass spectrometric analysis of propionylcarnitine in filter-paper blood specimens obtained from newborns. Clin Chem 47:2040–2044

Estrella J, Wilcken B, Carpenter K et al (2014) Expanded newborn screening in New South Wales: missed cases. J Inherit Metab Dis 37:881–887. doi:10.1007/s10545-014-9727-2

Fischer S, Huemer M, Baumgartner M et al (2014) Clinical presentation and outcome in a series of 88 patients with the cblC defect. J Inherit Metab Dis 37:831–840. doi:10.1007/s10545-014-9687-6

Gerth C, Morel CF, Feigenbaum A, Levin AV (2008) Ocular phenotype in patients with methylmalonic aciduria and homocystinuria, cobalamin C type. YMPA 12:591–596. doi:10.1016/j.jaapos.2008.06.008

Honzík T, Chrastina R, HansíkovÄ H et al (2005) Carnitine concentrations in term and preterm newborns at birth and during the first days of life. Prague Med Rep 106:297–306

Huemer M, Scholl-Bürgi S, Hadaya K et al (2014) Three new cases of late-onset cblC defect and review of the literature illustrating when to consider inborn errors of metabolism beyond infancy. Orphanet J Rare Dis 9:161. doi:10.1186/s13023-014-0161-1

Lerner-Ellis JP, Anastasio N, Liu J et al (2009) Spectrum of mutations in MMACHC, allelic expression, and evidence for genotype-phenotype correlations. Hum Mutat 30:1072–1081. doi:10.1002/humu.21001

Lerner-Ellis JP, Tirone JC, Pawelek PD et al (2005) Identification of the gene responsible for methylmalonic aciduria and homocystinuria, cblC type. Nat Genet 38:93–100. doi:10.1038/ng1683

Lin HJ, Neidich JA, Salazar D et al (2009) Asymptomatic maternal combined homocystinuria and methylmalonic aciduria (cblC) detected through low carnitine levels on newborn screening. J Pediatr 155:924–927. doi:10.1016/j.jpeds.2009.06.046

Martinelli D, Deodato F, Dionisi-Vici C (2011) Cobalamin C defect: natural history, pathophysiology, and treatment. J Inherit Metab Dis 34:127–135. doi:10.1007/s10545-010-9161-z

McHugh DMS, Cameron CA, Abdenur JE et al (2011) Clinical validation of cutoff target ranges in newborn screening of metabolic disorders by tandem mass spectrometry: a worldwide collaborative project. Genet Med 13:230–254. doi:10.1097/GIM.0b013e31820d5e67

Morel CF, Lerner-Ellis JP, Rosenblatt DS (2006) Combined methylmalonic aciduria and homocystinuria (cblC): Phenotype–genotype correlations and ethnic-specific observations. Mol Genet Metab 88:315–321. doi:10.1016/j.ymgme.2006.04.001

Rosenblatt DS, Aspler AL, Shevell MI et al (1997) Clinical heterogeneity and prognosis in combined methylmalonic aciduria and homocystinuria (cblC). J Inherit Metab Dis 20:528–538. doi:10.1023/A:1005353530303

Smith SE, Kinney HC, Swoboda KJ, Levy HL (2006) Subacute combined degeneration of the spinal cord in cblC disorder

despite treatment with B12. Mol Genet Metab 88:138–145. doi:10.1016/j.ymgme.2006.02.007

Stanley CA (2004) Carnitine deficiency disorders in children. Ann N Y Acad Sci 1033:42–51. doi:10.1196/annals.1320.004

Thauvin-Robinet C, Roze E, Couvreur G et al (2007) The adolescent and adult form of cobalamin C disease: clinical and molecular spectrum. J Neurol Neurosurg Psychiatry 79:725–728. doi:10.1136/jnnp.2007.133025

Tortorelli S, Turgeon CT, Lim JS et al (2010) Two-tier approach to the newborn screening of methylenetetrahydrofolate reductase deficiency and other remethylation disorders with tandem mass spectrometry. J Pediatr 157:271–275. doi:10.1016/j.jpeds.2010.02.027

Wang F, Han L, Yang Y et al (2010) Clinical, biochemical, and molecular analysis of combined methylmalonic acidemia and hyperhomocysteinemia (cblC type) in China. J Inherit Metab Dis 33:435–442. doi:10.1007/s10545-010-9217-0

Weisfeld-Adams JD, Bender HA, Miley-Åkerstedt A et al (2013) Neurologic and neurodevelopmental phenotypes in young children with early-treated combined methylmalonic acidemia and homocystinuria, cobalamin C type. Mol Genet Metab 110:241–247. doi:10.1016/j.ymgme.2013.07.018

Weisfeld-Adams JD, Morrissey MA, Kirmse BM et al (2010) Molecular genetics and metabolism. Mol Genet Metab 99:116–123. doi:10.1016/j.ymgme.2009.09.008