A Patient With an Inborn Error of Vitamin B\textsubscript{12} Metabolism (cblF) Detected by Newborn Screening

abstract

A neonate, who was found to have an elevated C3/C2 ratio and minimally elevated propionylcarnitine on newborn screening, was subsequently identified as having the rare cblF inborn error of vitamin B\textsubscript{12} (cobalamin) metabolism. This disorder is characterized by the retention of unmetabolized cobalamin in lysosomes such that it is not readily available for cellular metabolism. Although cultured fibroblasts from the patient did not show the expected functional abnormalities of the cobalamin-dependent enzymes, methylmalonyl-CoA mutase and methionine synthase, they did show reduced synthesis of the active cobalamin cofactors adenosylcobalamin and methylcobalamin. Mutation analysis of LMBRD1 established that the patient had the cblF disorder. Treatment was initiated promptly, and the patient showed a robust response to regular injections of cyanocobalamin, and she was later switched to hydroxocobalamin. Currently, at 3 years of age, the child is clinically well, with appropriate development. Adjusted newborn screening cutoffs in Ontario allowed detection of a deficiency that might not have otherwise been identified, allowing early treatment and perhaps preventing the adverse sequelae seen in some untreated patients. Pediatrics 2013;132:e257–e261

AUTHORS: Christine M. Armour, MD, Alison Brebner, BSc, David Watkins, PhD, Michael T. Geraghty, MD, Alicia Chan, MD, and David S. Rosenblatt, MD

ABBREVIATIONS

AdoCbl—adenosylcobalamin
CNCbl—cyanocobalamin
MeCbl—methylcobalamin
MMA—methylmalonic acid
NBS—newborn screening
OHcbl—hydroxocobalamin

Dr Armour cared for the patient, collected data, drafted the initial manuscript, and approved the final manuscript as submitted; Ms Brebner compiled the data, drafted the initial manuscript, and approved the final manuscript as submitted; Dr Watkins collected the data, analyzed the data, wrote the manuscript, and approved the final manuscript as submitted; Dr Geraghty contributed to the diagnosis and care of the patient and approved the final manuscript; Dr Chan contributed to the ongoing care of the patient, contributed clinical data, and approved the final manuscript; and Dr Rosenblatt designed the study, reviewed and revised the manuscript, and approved the final manuscript as submitted.
Derivatives of vitamin B$_{12}$ (cobalamin) are required for the function of 2 essential cellular enzymes. Adenosylcobalamin (AdoCbl) is required by the mitochondrial enzyme methylmalonyl-CoA mutase, which converts methylmalonyl-CoA to succinyl-CoA. Methylcobalamin (MeCbl) is required by the cytoplasmic enzyme methionine synthase in the conversion of homocysteine to methionine. Mutations in the genes encoding either of these enzymes, or encoding proteins required for the synthesis of either cobalamin cofactor, result in inborn errors of cobalamin metabolism. There are currently 9 known disorders caused by mutations in genes coding for proteins involved in the pathway: cblA-cblG, cblI, and mut. Affected patients typically have elevated serum and urine levels of the precursor metabolites for one or both of these reactions, including methylmalonic acid (MMA) and/or homocysteine, depending on where in the pathway the defect occurs.

The cblF inborn error (Online Mendelian Inheritance in Man database number 277380) is caused by mutations in the LMBRD1 gene on chromosome 6q13, which encodes the lysosomal membrane protein LMBD1. Endocytosis of transcobalamin-bound circulating cobalamin is mediated by the transcobalamin receptor, with dissociation of the transcobalamin-cobalamin complex and proteolysis of transcobalamin occurring in the lysosome. LMBD1 is thought to play a role in transporting cobalamin from the lysosome into the cytoplasm after its release from transcobalamin. Fibroblasts from patients with cblF accumulate free cobalamin, unbound to protein, within lysosomes, with decreased synthesis of both cobalamin cofactors and decreased function of both cobalamin-dependent enzymes. This results in increased levels of MMA and homocysteine in the blood and urine. To date there are 15 reported cblF patients.

Several have had decreased serum vitamin B$_{12}$ levels, apparently reflecting a role for the lysosome in intestinal uptake of ingested cobalamin; the Schilling test for cobalamin absorption has been reported to be abnormal in cblF patients. A second disorder with an identical biochemical and cellular phenotype, cblJ, caused by mutations in the gene which codes for a different lysosomal membrane protein, ABCD4, has recently been described in 3 patients.

There is a great deal of variability in clinical presentation among the small number of known cblF patients. Common clinical findings include failure to thrive, developmental delay, congenital heart defects, macrocytic anemia, neutropenia, thrombocytopenia, pancytopenia, and minor facial abnormalities such as pegged teeth. Patients have generally responded well to therapy with hydroxocobalamin (OHcbl) by intramuscular injection. When cultured patient fibroblasts are incubated in the presence of transcobalamin-bound labeled cyanocobalamin (CNcbl), they accumulate high levels of cobalamin, almost all of which is unmetabolized CNcbl localized to the lysosome. There is relatively little synthesis of AdoCbl or MeCbl and decreased function of both methylmalonyl-CoA mutase and methionine synthase.

### CLINICAL REPORT

The proband, the product of an unremarkable pregnancy, was a full-term female infant with a birth weight of 3175 g. There was no family history of metabolic disease or consanguinity. Her parents were of mixed white European descent, and she has a healthy older sister. She was exclusively breastfed and clinically well at the time of ascertainment. She was identified after a positive newborn screen showing a borderline elevation of C3 acylcarnitine and increased ratio of C3/C2 acylcarnitines (Table 1). Follow-up testing 12 days after birth demonstrated a continued elevation of C3 acylcarnitine, with increased excretion of MMA without methylcitric acid. Serum amino acids revealed an elevated homocysteine level.

### TABLE 1 Diagnostic Laboratory Data

<table>
<thead>
<tr>
<th>Age</th>
<th>Test</th>
<th>Patient Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>C3 acylcarnitine</td>
<td>5.75 μM</td>
<td>&lt;5.5 μM</td>
</tr>
<tr>
<td></td>
<td>C3/C2 ratio</td>
<td>0.27</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>12 d</td>
<td>Plasma C3 acylcarnitine</td>
<td>2.55 μM</td>
<td>&lt;0.65 μM</td>
</tr>
<tr>
<td></td>
<td>Serum total homocysteine</td>
<td>39 μM</td>
<td>4–15 μM</td>
</tr>
<tr>
<td></td>
<td>Hemoglobin</td>
<td>117</td>
<td>135–206</td>
</tr>
<tr>
<td></td>
<td>Serum cobalamin</td>
<td>62 pM</td>
<td>165–740 pM</td>
</tr>
<tr>
<td>26 d</td>
<td>Urine MMA</td>
<td>54 mmol/mol Cr</td>
<td>4.3 ± 2.3 mmol/mol Cr</td>
</tr>
<tr>
<td></td>
<td>Serum total homocysteine</td>
<td>34 μM</td>
<td>4–15 μM</td>
</tr>
<tr>
<td>34 d</td>
<td>Serum cobalamin</td>
<td>306 pM</td>
<td>165–740 pM</td>
</tr>
<tr>
<td></td>
<td>Serum total homocysteine</td>
<td>17 μM</td>
<td>4–15 μM</td>
</tr>
<tr>
<td>51 d</td>
<td>Urine MMA</td>
<td>25 mmol/mol Cr</td>
<td>4.3 ± 2.3 mmol/mol Cr</td>
</tr>
<tr>
<td></td>
<td>Plasma C3 acylcarnitine</td>
<td>1.11 μM</td>
<td>&lt;0.65 μM</td>
</tr>
<tr>
<td></td>
<td>Serum cobalamin</td>
<td>243 pM</td>
<td>165–740 pM</td>
</tr>
<tr>
<td></td>
<td>Serum total homocysteine</td>
<td>13 μM</td>
<td>4–15 μM</td>
</tr>
<tr>
<td>83 d</td>
<td>Plasma C3 acylcarnitine</td>
<td>0.97 μM</td>
<td>&lt;0.65 μM</td>
</tr>
<tr>
<td></td>
<td>Urine MMA</td>
<td>14 mmol/mol Cr</td>
<td>4.3 ± 2.3 mmol/mol Cr</td>
</tr>
<tr>
<td></td>
<td>Serum cobalamin</td>
<td>158 pM</td>
<td>165–740 pM</td>
</tr>
<tr>
<td></td>
<td>Mean corpuscular vol</td>
<td>83.2 fl</td>
<td>85–123 fl</td>
</tr>
<tr>
<td></td>
<td>Urine MMA</td>
<td>8.4 mmol/mol Cr</td>
<td>4.3 ± 2.3 mmol/mol Cr</td>
</tr>
<tr>
<td></td>
<td>Plasma C3 acylcarnitine</td>
<td>0.89 μM</td>
<td>&lt;0.65 μM</td>
</tr>
</tbody>
</table>

C3, propionyl carnitine; Cr, creatinine.

* Four days after 1 mg CNcbl intramuscular injection.

* Three weeks after 1 mg CNcbl intramuscular injection.
parameters subsequently normalized, and she was maintained on this schedule.
At 3 months of age, her length was 54.6 cm (3rd–10th percentile), weight 5.55 kg (50th percentile) and head circumference 39.2 cm (25th–50th percentile). Apart from mild diffuse, patchy eczema, she had a completely normal physical examination. She was reported to be colicky but slept 6 to 7 hours overnight. Echocardiogram was normal, with only a patent foramen ovale. There was no evidence of any neurologic abnormalities. At 3 years of age, her development is appropriate in all spheres, and there is no evidence of any developmental delay, or any ongoing health issues.

LABORATORY STUDIES AND FINDINGS

Incorporation of the radiolabeled substrates $[^{14}C]$propionate and $[^{14}C]$methyltetrahydrofolate into cellular macromolecules were measured both with and without the addition of 3.75 μM OHCbl to the media as previously reported. Both $[^{14}C]$propionate incorporation, a measure of methylmalonyl-CoA mutase function, and from $[^{14}C]$methyltetrahydrofolate incorporation, a measure of methionine synthase function, were within the reference range (Table 2). Incorporation of labeled propionate was stimulated by incubation with OHCbl, consistent with a defect in cobalamin metabolism. The distribution of the cobalamin cofactors in the patient’s fibroblasts was measured by incubating them for 96 h with $[^{57}Co]$CNCbl bound to transcobalamin, then performing a hot ethanol extraction and high performance liquid chromatography as previously described. There was decreased synthesis of AdoCbl and MeCbl and accumulation of CNCbl. Restriction endonuclease analysis and sequencing of the LMNDR1 gene in patient genomic DNA identified the common c.1056delG (p.L352fsX18) mutation and a novel c.1339-10G>T splice site mutation, confirming the diagnosis of the cblIF disorder.

DISCUSSION

Newborn screening by tandem mass spectrometry identified a small increase in blood C3 acylcarnitine concentration as well as an increase in the ratio of C3 to C2 acylcarnitine. The latter measure has been shown to offer a higher specificity and sensitivity than measurement of C3 acylcarnitine concentration alone and had only recently been incorporated into the newborn screening algorithm used for determining the cutoff between positive and negative values in Ontario. This combination of abnormal C3 acylcarnitine and C3/C2 ratio allowed the patient to be initially detected and referred for additional testing regarding her abnormal metabolic profile. The presence of elevated MMA and homocysteine levels suggested an inborn error of cobalamin metabolism. The mother was not cobalamin-deficient.

### TABLE 2 Studies of Patient Fibroblasts

<table>
<thead>
<tr>
<th>Assay</th>
<th>$[^{14}C]$-propionate Incorporation (nmol/mg protein/18 h)</th>
<th>$[^{14}C]$-methylTHF Incorporation (nmol/mg protein/18 h)</th>
<th>Cobalamin Distribution (% of total Cbl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−OHCbl</td>
<td>+OHCbl</td>
<td>−OHCbl</td>
</tr>
<tr>
<td>Patient</td>
<td>6.9 7.5</td>
<td>11.1 12.2</td>
<td>75 69</td>
</tr>
<tr>
<td>cblF patients (n = 11)</td>
<td>1.9 ± 1.8</td>
<td>7.2 ± 3.2</td>
<td>43 ± 11</td>
</tr>
<tr>
<td>Reference range</td>
<td>10.8 ± 3.7</td>
<td>10.9 ± 3.5</td>
<td>225 ± 185</td>
</tr>
</tbody>
</table>

Fibroblast cultures were incubated for 18 h in a medium containing labeled methyl tetrahydrofolate and propionate with and without OHCbl. Cellular macromolecules were precipitated in 3% trichloroacetic acid and radioactivity determined by liquid scintillation counting. The distribution of the cobalamin cofactors in the patient’s fibroblasts was measured by incubating them with $[^{57}Co]$CNCbl bound to transcobalamin, then performing a hot ethanol extraction and high performance liquid chromatography.
nor was the decreased serum cobalamin level in the baby the result of dietary deficiency.

Studies of cultured fibroblasts provided information about the metabolic block in this patient but did not allow definitive assignment to one of the known inborn errors of cobalamin metabolism. Analysis of uptake of labeled CNCbl and its conversion to the active cobalamin cofactor forms showed a pattern similar to that of other cblF patients, with accumulation of large amounts of unmetabolized CNCbl and decreased synthesis of AdoCbl and MeCbl (Table 2). However, indirect measures of methylmalonyl-CoA mutase and methionine synthase function gave values within the reference ranges, precluding assignment of the patient’s disorder by complementation analysis. Diagnosis of the cblF disorder was made by analysis of the LMBRD1 gene, which demonstrated the presence of a small amount of LMBRD1 mRNA of the normal size in addition to the misspliced product. It is possible that the presence of a “leaky” splice site mutation in combination with the common LMBRD1 mutation in this patient was responsible for her milder phenotype. However, another cblF patient heterozygous for the common mutation, and a different splice site mutation had a typical biochemical and clinical phenotype. The results of fibroblast studies indicate that measurement of cobalamin cofactor synthesis is more sensitive than indirect measures of cobalamin-dependent enzyme function in detecting inborn errors of metabolism. Similar findings have been reported in a patient with the cblJ disorder, who had abnormal cobalamin cofactor synthesis in the presence of normal methylmalonyl-CoA mutase and methionine synthase function.

Early treatment of the patient may have prevented the onset of adverse clinical symptoms typically seen in cblF patients because the patient is currently doing well at 3 years of age. Onset of symptoms has typically occurred in the first months of life in cblF patients and in patients with the phenotypically similar cblJ disorder, although diagnosis has occurred as late as 11 years of age in one cblF patient. Similarly, a patient with a biochemically milder form of the cblJ disorder came to medical attention at 8 years of age.

At the time that this patient was ascertained, cblF was the only known inborn error to cause accumulation of unmetabolized cobalamin in the lysosomes. Since this time, the cblJ disorder, which has an identical clinical and biochemical phenotype, has been identified, and if a patient similar to ours came to medical attention today, molecular analysis of both the LMBRD1 and ABCD4 genes would need to be undertaken.

**ACKNOWLEDGMENTS**

We thank Gail Dunbar for growth and storage of fibroblasts and Jocelyne Lavallée for the somatic cell clinical investigations. Thanks to Isabelle R. Miousse and Maria Galvez for the mutation analysis of the patient. Dr Rosenblatt is a member of the Research Institute of the McGill University Health Centre.

**REFERENCES**

10. Watkins D. Cobalamin metabolism in methionine-dependent human tumour and


A Patient With an Inborn Error of Vitamin B\textsubscript{12} Metabolism (cblF) Detected by Newborn Screening
Christine M. Armour, Alison Brebner, David Watkins, Michael T. Geraghty, Alicia Chan and David S. Rosenblatt

*Pediatrics* 2013;132;e257; originally published online June 17, 2013; DOI: 10.1542/peds.2013-0105

Updated Information & Services
including high resolution figures, can be found at:
/content/132/1/e257.full.html

References
This article cites 15 articles, 3 of which can be accessed free at:
/content/132/1/e257.full.html#ref-list-1

Subspecialty Collections
This article, along with others on similar topics, appears in the following collection(s):
Endocrinology
/cgi/collection/endocrinology_sub
Metabolic Disorders
/cgi/collection/metabolic_disorders_sub
Fetus/Newborn Infant
/cgi/collection/fetus:newborn_infant_sub
Neonatology
/cgi/collection/neonatology_sub

Permissions & Licensing
Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at:
/site/misc/Permissions.xhtml

Reprints
Information about ordering reprints can be found online:
/site/misc/reprints.xhtml

PEDIATRICS is the official journal of the American Academy of Pediatrics. A monthly publication, it has been published continuously since 1948. PEDIATRICS is owned, published, and trademarked by the American Academy of Pediatrics, 141 Northwest Point Boulevard, Elk Grove Village, Illinois, 60007. Copyright © 2013 by the American Academy of Pediatrics. All rights reserved. Print ISSN: 0031-4005. Online ISSN: 1098-4275.
A Patient With an Inborn Error of Vitamin B₄₂ Metabolism (cblF) Detected by Newborn Screening
Christine M. Armour, Alison Brebner, David Watkins, Michael T. Geraghty, Alicia Chan and David S. Rosenblatt

Pediatrics 2013;132;e257; originally published online June 17, 2013;
DOI: 10.1542/peds.2013-0105

The online version of this article, along with updated information and services, is located on the World Wide Web at:
/content/132/1/e257.full.html