Severe Combined Immunodeficiency Resulting From Mutations in \textit{MTHFD1}

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**KEY WORDS**
immunodeficiency, folate, cobalamin, megaloblast, hemolytic uremic syndrome

**ABBREVIATIONS**
ADA—adenosine deaminase
HUS—hemolytic-uremic syndrome
Ig—immunoglobulin
PCFT—proton-coupled folate transporter
PNP—purine nucleoside phosphorylase
SCID—severe combined immunodeficiency

Dr Keller was involved in care of the patient, wrote the manuscript, collected data, and produced the figures and tables; Dr Ganesh cared for the patient and wrote the manuscript; Dr Heltzer cared for the patient, collected data, and revised the manuscript; Dr Paessler collected data and revised the manuscript; Dr Bergqvist cared for the patient, collected data, and wrote the manuscript; Dr Baluarte cared for the patient and revised the manuscript; Dr Watkins analyzed data and wrote the manuscript; Dr Rosenblatt analyzed data, wrote the manuscript, and revised the figures; and Dr Orange designed the study, collected and analyzed data, and wrote and revised the manuscript.

www.pediatrics.org/cgi/doi/10.1542/peds.2012-0899
doi:10.1542/peds.2012-0899
Accepted for publication Sep 11, 2012

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PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275). Copyright © 2013 by the American Academy of Pediatrics

**FINANCIAL DISCLOSURE:** The authors have indicated they have no financial relationships relevant to this article to disclose.

**FUNDING:** No external funding.

**abstract**

Folate and vitamin B₁₂ metabolism are essential for de novo purine synthesis, and several defects in these pathways have been associated with immunodeficiency. Here we describe the occurrence of severe combined immunodeficiency (SCID) with megaloblastic anemia, leukopenia, atypical hemolytic uremic syndrome, and neurologic abnormalities in which hydroxocobalamin and folate therapy provided partial immune reconstitution. Whole exome sequencing identified compound heterozygous mutations in the \textit{MTHFD1} gene, which encodes a trifunctional protein essential for processing of single-carbon folate derivatives. We now report the immunologic details of this novel genetic cause of SCID and the response to targeted metabolic supplementation therapies. This finding expands the known metabolic causes of SCID and presents an important diagnostic consideration given the positive impact of therapy. \textit{Pediatrics} 2013;131: e629–e634
Folate and cobalamin (vitamin B₁₂) are essential cofactors for the transfer of single carbon units that are necessary for multiple cell processes, including de novo nucleotide synthesis and production of methionine. Several in-born errors in folate and cobalamin metabolism have been described that have a profound impact on many systems, including hematopoiesis and neuronal function. Inherited defects in vitamin B₁₂ absorption and metabolism can result in variable-onset megaloblastic anemia, neutropenia, metabolic derangements including homocystinuria, and neurologic abnormalities including seizures and developmental delay. Defects in folate uptake and metabolism are similarly rare and severe and include methylene tetrahydrofolate reductase deficiency, glutamate formiminotransferase-cyclodeaminase deficiency, and deficiencies in folate transporters.

Immunodeficiency of varying degrees has been associated with folate and cobalamin pathway defects. In transcobalamin II deficiency, neutropenia is thought to be the primary cause of recurrent bacterial infections, although infection with opportunistic agents such as *Pneumocystis jiroveci* pneumonia has also been described. A severe combined immunodeficiency (SCID)-like phenotype was associated with functional methionine synthase deficiency, with lymphopenia and severe bacterial and viral infections. Hereditary folate malabsorption resulting from deficiency in the proton-coupled folate transporter (PCFT) has similarly been linked with a SCID phenotype with severe opportunistic infections and was shown to be responsive to folate replacement therapy. Recently we described a case of SCID associated with megaloblastic anemia and neurologic disease that displayed partial immune reconstitution after vitamin B₁₂ and folate replacement.

Further investigation by exomic sequencing demonstrated heterozygous mutations in the trifunctional protein expressed by *MTHFD1*. Here we report the immunologic phenotype and response to therapy for this novel molecular defect.

**PATIENT PRESENTATION**

The patient was a full-term infant who developed poor feeding and pallor within weeks of birth. At 2 months of age, she was noted to be markedly anemic with hemoglobin of 4.9 g/dL and had a urinary tract infection with *Escherichia coli*. She was transfused with red blood cells for her anemia and treated with antibiotics. Less than 1 week later, she was hospitalized for respiratory distress and found to have pancytopenia and elevated lactate dehydrogenase (2550 U/L, reference 500–920 U/L). A silver stain from a

**TABLE 1** Initial Laboratory Values at 2.5 Months Old

<table>
<thead>
<tr>
<th>Value</th>
<th>Result</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cell</td>
<td>3.9</td>
<td>5–19.6 (× 10⁹)/mclL</td>
</tr>
<tr>
<td>Platelets</td>
<td>721</td>
<td>150–400 (× 10⁹)/mclL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>4.9</td>
<td>10–18 g/dL</td>
</tr>
<tr>
<td>Mean corpuscular vol</td>
<td>95</td>
<td>85–125 fl</td>
</tr>
<tr>
<td>Absolute neutrophil count</td>
<td>2178</td>
<td>1500–11 500/mclL</td>
</tr>
<tr>
<td>Absolute lymphocyte count</td>
<td>1408</td>
<td>2800–11 500/mclL</td>
</tr>
<tr>
<td>CD5+</td>
<td>199</td>
<td>2500–6500 cells/mclL</td>
</tr>
<tr>
<td>CD5+/CD4+</td>
<td>55</td>
<td>1500–5000 cells/mclL</td>
</tr>
<tr>
<td>CD5+/CD8+</td>
<td>142</td>
<td>500–1600 cells/mclL</td>
</tr>
<tr>
<td>CD16+/CD56+</td>
<td>17</td>
<td>100–1300 cells/mclL</td>
</tr>
<tr>
<td>CD20</td>
<td>59</td>
<td>100–479 cells/mclL</td>
</tr>
<tr>
<td>IgG</td>
<td>99</td>
<td>218–636 mg/dL</td>
</tr>
<tr>
<td>IgA</td>
<td>7</td>
<td>1–59 mg/dL</td>
</tr>
<tr>
<td>IgM</td>
<td>31</td>
<td>11–60 mg/dL</td>
</tr>
<tr>
<td>IgE</td>
<td>&lt;5</td>
<td>0–15 IU/mL</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>29.2</td>
<td>6–9 μmol/L</td>
</tr>
<tr>
<td>Methylmalonic acid</td>
<td>478</td>
<td>360–1500 nmol/L</td>
</tr>
<tr>
<td>Methionine</td>
<td>9.7</td>
<td>8–49 nmol/mL</td>
</tr>
<tr>
<td>B₁₂</td>
<td>362</td>
<td>228–1514 pg/mL</td>
</tr>
<tr>
<td>Folate</td>
<td>6.74</td>
<td>6.3–22.7 ng/mL</td>
</tr>
<tr>
<td>Uric acid</td>
<td>3.2</td>
<td>1.4–5.5 mg/dL</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>1387</td>
<td>470–1220 U/L</td>
</tr>
</tbody>
</table>

**FIGURE 1**

A, Bone marrow aspirate showing maturing myeloid precursors (promyelocytes and myelocytes) and a large erythroid precursor (orthochromatic) with nuclear to cytoplasmic dysynchrony (Wright-Giemsa, magnification ×1000). B, Hypersegmented neutrophils in a peripheral blood smear (hematoxylin and eosin, magnification ×1000).
bronchioalveolar lavage showed evidence of *Pneumocystis jiroveci*, and she was treated with trimethoprim/sulfamethoxazole and methylprednisolone.

She is the second child born to healthy, nonconsanguineous parents. Her siblings (an older sister and half-brother) are healthy. Family history was remarkable for a maternal uncle and great uncle who died in infancy of unknown causes. There is no known history of immunodeficiency in other family members.

Immunologic studies showed marked hypogammaglobulinemia and lymphopenia, with few T cells, B cells, or natural killer cells at 3 months old (Table 1). A bone marrow biopsy showed giant bands and nuclear-cytoplasmic dysynchrony (Fig 1A), and peripheral blood smear showed hypersegmented neutrophils (Fig 1B). There was no evidence of malignancy. Serum vitamin B₁₂ and folate levels were within the reference range. Her plasma biochemical profile was significant for elevated homocysteine and slightly low methionine.

On the basis of these findings, treatment with intramuscular hydroxocobalamin, oral folate, and betaine was initiated for a suspected defect in cobalamin/folate metabolism. Methylcobalamin production by patient fibroblasts was decreased compared with control cells, further supporting a defect in this pathway.⁶

She was treated with immunoglobulin replacement, and prophylactic trimethoprim/sulfamethoxazole was added after completion of her treatment of *Pneumocystis*. The patient’s

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**FIGURE 2**

Hematologic values before and after metabolic therapy with cobalamin and folate (Cb/F). A, Hemoglobin (Hgb) improved after therapy. B, Thrombocytopenia with periods of thrombocytosis were seen, which similarly improved after therapy. C, Absolute lymphocyte count (ALC) showed profound lymphopenia, which gradually improved after therapy. In each figure, the arrow depicts the initiation of metabolic therapy. Trimethoprim/sulfamethoxazole was discontinued at 12 months of age (*). Methylcobalamin and methylfolate were added at age 23 months (†), and the ketogenic diet was initiated at 38 months (§).
early hospitalization was further complicated by acute renal failure with microangiopathic hemolysis, which suggested hemolytic-uremic syndrome (HUS). She required temporary dialysis before recovery of renal function, and the HUS did not recur. Additional complications included a central line–associated infection with Candida albicans and a hyponatremic seizure in the setting of renal failure.

After initiation of folate and hydroxocobalamin therapy, the patient demonstrated improvement in hematopoiesis (Fig 2 A and B). There was also partial immune-reconstitution as demonstrated by improvements in lymphocyte counts (Fig 2C) and lymphocyte subsets (Fig 3), although they remained low for age. Because of ongoing neutropenia and seizures, methylfolate and methylcobalamin were later empirically added to her regimen at 23 months. Lymphocyte proliferation assays were obtained shortly after initiation of therapy (Fig 4, pre), and also showed improvement over time and with presumed optimization of metabolic therapy (Fig 4, post). Her biochemical parameters including homocysteine level normalized on hydroxocobalamin and folate replacement, and she was able to tolerate a diet unrestricted in protein.

Prophylactic trimethoprim/sulfamethoxazole was discontinued at 12 months of age because of concerns of suppression of hematopoiesis. Immunoglobulin replacement was continued until age 22 months. Although she maintained normal immunoglobulin levels during a trial without therapy, she did not show evidence of specific antibodies, immunoglobulin (Ig) replacement was resumed at 39 months of age. IgA and IgM levels remained within normal ranges. She was diagnosed with intermittent asthma at 18 months due to wheezing with respiratory infections and eczema at 6 months of age. She has had occasional virally induced asthma flares and has been treated with inhaled corticosteroids. As of age 6 years, there have been no additional opportunistic or severe bacterial infections.

Our patient’s neurologic phenotype includes mild bilateral sensorineural hearing loss, symptomatic partial epilepsy that remains refractory to multiple antiepileptic agents (as well as a ketogenic diet), and mild mental retardation. A brain MRI at age 11 months noted bilateral small hippocampi due to hypoplasia versus atrophy, which was unchanged on a repeat study at 4 years of age, although new T2/Flair signal changes were noted involving the medial temporal lobes and subinsular regions and high signal in the occipital lobes of unclear etiology.

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Cerebrospinal fluid amino acid and neurotransmitter profiles were normal at 11 months of age. She was diagnosed with pervasive developmental disorder and at age 6 years successfully attends a special education kindergarten with occupational, speech, and behavioral therapy.
Investigation of the causative defect was performed via whole exome sequencing of the patient and family as recently described. Compound heterozygous mutations in MTHFD1 were found and were unique to the patient. A c.517C→T substitution resulted in a missense mutation in exon 7 (p.R173C), and c.727+1G→A affected a splice site of intron 8 and was predicted to result in an early stop codon due to inclusion of the intron sequence.

**DISCUSSION**

SCID results from a multitude of rare genetic disorders that compromise lymphocyte development, function, or survival. Several metabolic diseases are well-known causes of SCID, including adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) deficiency. In ADA and PNP, accumulation of toxic deoxyribonucleotides contributes to substantial lymphocyte death, and studies have shown that deoxyadenosine compromises T-cell receptor activation at multiple levels. Furthermore, deoxyadenosine has been described to compromise transmethylation reactions via inhibition of S-adenosyl-homocysteine hydrolase, which may suggest a potential overlap with MTHFD1 deficiency.

Defects in cobalamin and folate metabolism are also associated with immunodeficiency of varying severity. The variability may relate to the degree of pathway disruption, as with transcobalamin II and PCFT deficiency in which cobalamin and folate uptake (respectively) are profoundly reduced. MTHFD1 encodes an essential trifunctional protein that interconverts several THF metabolites (Fig 5). De novo purine synthesis requires 10-formyl-THF, and 5, 10-methylene-THF is necessary for thymidine production. Thus there is likely to be immunologic overlap between MTHFD1 SCID and deficiencies of ADA or PNP. Poor nucleic acid production may account in part for the immunologic phenotype in our patient, because in vitro lymphocyte stimulation performed in the presence of excess deoxyribonucleotides in routine cell culture media yielded reasonable proliferative indices (Fig 4). Given the degree of the patient’s initial lymphopenia, it is not clear if MTHFD1 deficiency may also compromise lymphocyte survival. After replacement therapy, lymphocyte numbers and in vitro proliferation improved but did not normalize completely. Thymic function was unfortunately not studied directly in this case. Although immunoglobulin levels were largely maintained when replacement was held, it is unclear if specific antibody production normalizes with cobalamin and folate replacement.

The neurologic phenotype in this patient resembled those described in the vitamin B12 complementation defects which affect homocysteine remethylation. Early replacement therapy has also been found to prevent neurologic deterioration in other inborn errors of cobalamin and folate metabolism. Despite ongoing metabolic therapy, the patient’s seizure disorder remains refractory, and she has mild to moderate intellectual disability. Whether this is secondary to her initial illness, the seizure disorder, or a separate process is currently under investigation.

Acute renal failure with characteristics of HUS also occurred in this patient. Atypical HUS has been described in several vitamin B12 complementation defects. It has been theorized that hyperhomocysteinemia may be nephrotoxic, although in this case, the insult occurred in the setting of pneumonia with respiratory failure, making the exact cause difficult to discern.
Genetic defects in the cobalamin and folate pathways are rare causes of megaloblastic anemia, neurologic abnormalities, and variable immunodeficiency. We propose that deficiency of MTHFD1 represents a new form of SCID resulting from a defect in folate metabolism. Like PCFT, a beneficial response was seen with replacement of the depleted vitamins. Although uncommon, these defects collectively illustrate the range of metabolic causes of immunodeficiency and the importance of early treatment to minimize neurologic injury or opportunistic infections. Despite limited mechanistic data, the immunologic experience in our patient suggests the benefit of pursuing a diagnosis because the information gained through whole exomic sequence provided valuable data for targeted therapy. Thus, pursuing whole exomic sequence has the potential to directly improve patient management and should be considered to have benefits in addition to research value.

ACKNOWLEDGMENTS

We thank the patient’s family for their participation in this study. We also thank the clinical laboratory staff at both Children’s Hospital of Philadelphia and McGill University for their assistance.

REFERENCES

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Orange

Pediatrics 2013;131;e629; originally published online January 6, 2013;
DOI: 10.1542/peds.2012-0899

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