American Journal of Medical Genetics 71:384–390 (1997)

Functional Methionine Synthase Deficiency Due to cblG Disorder: A Report of Two Patients and a Review

Cary O. Harding,[1]* Georgianne Arnold,[3] Lewis A. Barness,[1] Jon A. Wolff,[1,2] and David S. Rosenblatt[4]

[1]*Department of Pediatrics, University of Wisconsin Medical School, Madison*
[2]*Departments of Medical Genetics and Neurology, University of Wisconsin Medical School, Madison*
[3]*Department of Pediatrics, University of Rochester School of Medicine and Dentistry, Rochester, New York*
[4]*Departments of Medicine, Pediatrics, and Human Genetics, McGill University, Montréal, Canada*

Functional methionine synthase deficiency due to abnormal methylcobalamin metabolism causes megaloblastic anemia, moderate to severe developmental delay, lethargy, and anorexia in association with homocystinuria. Patients with this disorder of cobalamin metabolism can be classified into two separate groups, cblE or cblG, primarily on the basis of complementation analysis with cultured skin fibroblasts. We describe two unrelated boys, ages 3 and 5 years, with the cblG defect in methylcobalamin synthesis. Both children presented with severe developmental delay, lethargy, anorexia, and megaloblastic anemia. The diagnosis of homocystinuria was delayed in each case due to difficulties with detection of small amounts of homocystine in physiologic samples. The clinical course of cblG disease is favorably altered by treatment with intramuscular hydroxycobalamin. Megaloblastosis in the presence of adequate supplies of cobalamin and folate in the blood must alert the clinician to the possibility of functional methionine synthase deficiency and should prompt a careful search for associated biochemical hallmarks, including homocystinuria/emia. Am. J. Med. Genet. 71:384–390, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: vitamin B_{12}; homocystinuria; megaloblastic anemia

INTRODUCTION

Methionine synthase (5-methyltetrahydrofolate:homocysteine methyltransferase (EC 2.1.1.13)) is a cytosolic folate and methylcobalamin-requiring enzyme which catalyzes the methylation of homocysteine to methionine (Fig. 1). Functional methionine synthase deficiency may be caused by deficiency of the 5-methyltetrahydrofolate (5-methylTHF) or methylcobalamin cofactors [Mudd et al., 1995]. Very limited data on the presentation and outcome of patients with defects in methylcobalamin (MeCbl) metabolism are available. All 12 previously reported patients with defects in MeCbl metabolism had megaloblastic anemia and homocystinuria/emia without other consistent phenotypic characteristics [Watkins and Rosenblatt, 1989]. Complementation analysis with skin fibroblasts demonstrated that these patients are separable into two complementation groups, cblE (McKusick No. 236270) (four patients) and cblG (McKusick No. 250940) (eight patients) [Watkins and Rosenblatt, 1989].

In this report, we describe two previously unreported, unrelated boys with functional methionine synthase deficiency secondary to the cblG defect in MeCbl synthesis in order to better define the clinical and laboratory presentation of this disorder. Pitfalls which may delay or obscure diagnosis and the value of treatment with intramuscular hydroxycobalamin will be discussed.

CASE REPORTS

Patient 1

This 5-year-old Caucasian male with spastic quadriplegia, generalized motor seizure disorder, and global developmental delays was admitted to the University of Wisconsin Children's Hospital for evaluation of severe lethargy and anorexia which had developed over the previous three months.

Partial complex seizures (staring, apnea) and infantile spasms had begun at 4 weeks of age after an uncomplicated pregnancy and term vaginal delivery to nonconsanguinous parents. Electroencephalogram showed multifocal spike and slow wave activity superimposed upon a slow background. Cranial CT scan

Dr. L.A. Barness is now at the Department of Pediatrics, University of South Florida, Tampa, FL.

*Correspondence to: Cary O. Harding, Department of Pediatrics, University of Wisconsin Medical School, Waisman Center, 1500 Highland Ave., Madison, WI 53705.

Received 8 February 1996; Accepted 21 January 1997

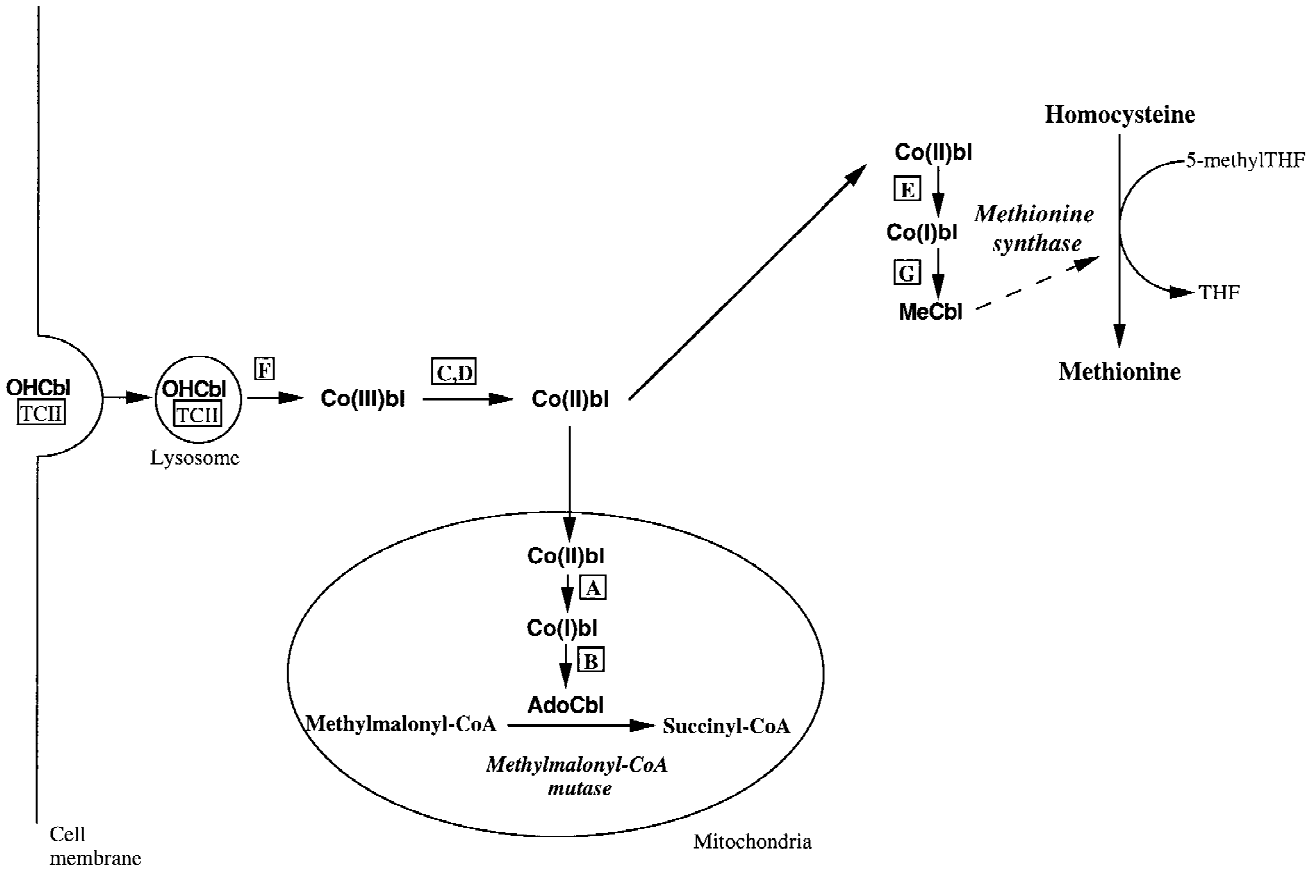

Fig. 1. Intracellular metabolism of cobalamin and associated inborn errors. Seven inborn errors of intracellular Cbl metabolism (complementation groups A through G) are illustrated with capital letters placed at the site of the known or proposed defect associated with each of the complementation groups. cblE—presumed failure of methionine synthase-associated Cbl reduction. cblG—presumed failure of methionine synthase-associated Cbl methylation.

showed poor gray-white matter differentiation and a possible sagittal sinus venous thrombosis. A repeat cranial CT scan at age 5 months revealed generalized cortical atrophy and ventriculomegaly. At 15 months, generalized motor seizures were occurring weekly despite oral phenobarbital therapy. Seizure control improved substantially with oral valproic acid.

Development was severely, globally delayed. He was severely hypotonic from early infancy, but hypertonia of the distal limbs appeared gradually during early childhood, while truncal tone remained diminished. At age 5 years, he could sit upright without support and could crawl, although his mobility was severely limited by spasticity in all limbs. He was completely dependent on others for personal care.

Just after his fifth birthday, the child began to deteriorate physically and cognitively over a period of several weeks. He became anorexic, would retch and vomit after meals, and lost 2.3 kg in weight. During this period, he was initially very irritable, but then became increasingly lethargic and inactive. He slept for extended periods, waking only when aroused by his parents for feedings. No overt seizure activity or focal neurologic changes were noted by the family.

On examination, he was a thin, cachectic-appearing boy who was lethargic and would sleep continuously if left undisturbed. He was afebrile, normotensive, and exhibited no respiratory distress. Weight, 17.2 kg (10th centile, down from 19.5 kg five months earlier); length, 109 cm (40th centile); head circumference, 49.5 cm (5th centile). Face was thin and wasted but no minor anomalies were noted. Lenses, corneas, and retinas were normal by direct ophthalmoscopy. Chest was long and thin with marked pectus excavatum and anterior flaring of the inferior rib cage. Limbs were long, thin, and wasted. Thoracolumbar scoliosis with concavity toward the left was present. He was lethargic and had central hypotonia and severe spasticity of all limbs.

A review of hematologic data over Patient 1's lifetime showed that macrocytosis was apparent by age 5 months (MCV, 99 fL/RBC) and persistent (MCV, 109 fl/RBC at age 5 years), but the Hgb concentration and Hct remained above 12 mg/dL and 35%, respectively, through the first five years of life. At age 5 7/12 years, severe macrocytic anemia (Hgb, 7.7 mg/dL; Hct, 23%; MCV, 117 fL/RBC; MCH, 39.8 pg/RBC; RDW, 20.4%) was detected. Serum iron, ferritin, total iron binding capacity, folate, and total cobalamin levels were normal. Reticulocyte count was 1.2% on admission. White blood cell count was 6,800 cells/μL with no nuclear hypersegmentation apparent. Qualitative screening of urine for cystine/homocystine using the cyanide-nitroprusside reagent [Shih et al., 1991] was trace positive. The presence of free homocystine in both plasma

and urine was confirmed by quantitative amino acid analysis (Table I) using a Beckman 6300 amino acid analyzer [Slocum and Cummings, 1991]. Using trimethylsilyl derivitization and GC/MS analysis of urine organic acids [Hoffmann et al., 1989], no methylmalonic acid was detected in urine.

Following the diagnosis of homocystinuria, nasogastric feedings were begun with whole protein restricted to 1 gm/kg/day. Later, a gastrostomy was placed. The child continued to show no interest in oral feedings but did not vomit and began to regain weight. He was treated empirically for homocystinuria with pyridoxine, 100 mg/day, and a daily multivitamin supplement containing 100 μg folic acid. Over several days, the child's mental status and activity level improved substantially. The reticulocyte index increased to 4.8% within one week, and the anemia gradually improved, although macrocytosis persisted.

Six weeks following discharge from the hospital, the child died after acute respiratory arrest following a feeding via the gastrostomy. Autopsy showed severe aspiration pneumonia. Neuropathologic examination of the brain showed astrogliosis of the cerebral cortex and pons, which may have been a primary manifestation of this child's disorder. Defective MeCbl metabolism due to the cblG defect was documented in cultured skin fibroblasts (Tables III and IV) by the laboratory of Dr. David S. Rosenblatt.

Patient 2

This Caucasian male child with global developmental delay and excessive sleepiness presented for evaluation of macrocytic anemia at age 4 years. He was born at 37 weeks of gestation following an uncomplicated pregnancy and delivery. The family history is unremarkable and without consanguinity. The neonatal period was uncomplicated, but the baby began to sleep for excessive periods (up to 18 hours per day) following surgical repair of bilateral inguinal hernias at age 6 weeks. Global developmental delay was apparent from early infancy; he rolled over at age 6 months, sat unsupported at 12 months, and began to walk and speak single words at 2 years. Clinical seizure activity was never noted, but an EEG during sleep revealed a complete lack of normal sleep spindles and asymmetric slowing of the background in the temporal regions, left greater than right. Cranial CT scan showed mild enlargement of the lateral ventricles and prominent sulci bilaterally, suggesting cortical atrophy.

TABLE I. Screening Metabolic Tests on Patient 1 Prior to Diagnosis*

	5 mo	4 yr, 9 mo	5 yr, 7 mo	Normal (age >1 yr)
Plasma methionine(μmol/L)	24	17	12	7–47
Plasma-free homocystine (μmol/L)	36.6[a]	ND	27	0
Urine homocystine (mmol/gm creatinine)	+[b]		0.77	0
Urine cyanide-nitroprusside test	Negative		Trace positive	Negative

*ND, none detected.
[a]Unidentified peak in original report. Concentration estimated retrospectively from peak height.
[b]Homocystine present but not quantitated on this sample. Urine studies not performed at 4 yr., 9 mo.

The child took formula feedings readily throughout infancy, but consistently refused solid foods. After weaning from infant formula at age 3.5 years, he continued to exhibit anorexia for nearly all solid foods, and has maintained adequate weight for his height only with significant intervention.

At age 4 years, his height was 96.1 cm (5th centile), weight 15 kg (20th centile), and head circumference 50.5 cm (50th centile). Physical findings were unremarkable; there were no minor facial anomalies nor skeletal abnormalities. He was mildly hyperreflexic with significantly increased muscle tone in the lower limbs. Motor strength was normal throughout. Plantar responses were extensor bilaterally but no ankle clonus was present. The child's gait was mildly wide-based, and his balance was impaired. Ophthalmologic findings were normal.

Laboratory evaluation demonstrated megaloblastosis and mild anemia. The hemoglobin and hematocrit were 11.4 g/dL and 33.4%, respectively. MCV, 120.4 fL; MCH, 41.0 pg; MCHC, 34.1 g/dL; and RDW, 19.8%. Serum cobalamin and folate levels were normal. Urine amino acid screening by one-dimensional paper chromatography and qualitative urine testing for cystine or homocystine in the laboratory of Dr. Stephen Goodman, University of Colorado, were both initially negative, but a repeat urine specimen exhibited a slightly positive cyanide-nitroprusside test. Quantitative amino acid analysis of blood and urine demonstrated hypomethioninemia, homocystinuria, and cystathionuria (Table II). Methylmalonic acid was not detected in urine by GC/MS. Impaired MeCbl synthesis was documented in cultured skin fibroblasts (Table IV), and the diagnosis of cblG defect was confirmed by fibroblast complementation in the laboratory of Dr. David S. Rosenblatt (Table III).

Treatment with weekly intramuscular injections of hydroxycobalamin (OHCbl) (1 mg) (Rugby Labs, West Hempstead, NY) resulted in dramatic improvement in alertness, but subsequently he developed severe oppositional behavior problems. His weight has been maintained with difficulty at approximately the 10th centile for age by adding a liquid nutritional supplement to his diet. His height continues at the 10th percentile. At age 7 years, he receives special education services and continues to have severe behavior problems. Formal developmental assessment at 5 years demonstrated global developmental delays with skills equivalent to 2–2½ years.

Approximately two weeks following the initiation of therapy with intramuscular OHCbl, the child's hemoglobin and hematocrit had increased to 13.3 g/dL and 37.8%, and his excessive sleepiness had improved substantially. The MCV decreased to 111.3 fL. After six months of therapy, the MCV had decreased to 85.6 fL.

TABLE II. Effect of Therapy on Amino Acid Concentrations in Plasma and Urine

	Patient 1[a]			Patient 2[b]		
	Before therapy	One week	Four weeks	Before therapy	One week	Six mo
Plasma methionine (μmol/L) (normal = 7–47)	12	12	14	13	32	43
Plasma homocystine (μmol/L) (normal = 0)	27	1	31	0	0	0
Urine homocystine (mmol/g creatinine) (normal = 0)	0.77	0.03	0.02	— (173)[c]	0.175 (29.7)[c]	0
Urine cyanide-nitroprusside test	Trace positive	Negative	Negative	Trace positive	Negative	

[a]Therapy for patient 1 included a protein-restricted diet and oral pyridoxine and folate supplements.
[b]Therapy for patient 2 included intramuscular OHCbl, oral folate, and betaine.
[c]Nmol/mL, creatinine not determined on urine sample obtained prior to therapy.

His current therapeutic regimen includes OHCbl (1 mg IM per week), betaine (2,100 mg orally per day), and folic acid (1 mg orally per day).

SUMMARY OF METABOLIC STUDIES

Screening Metabolic Testing

Sequential quantitative plasma and urine amino acid analyses and the results of urine screening tests on Patient 1 prior to diagnosis are presented in Table I. Quantitative amino acid analysis of plasma and urine samples obtained from Patient 1 at age 5 months demonstrated two small unidentified peaks which were interpreted as probable metabolites of anticonvulsant medications. In retrospect, one of these peaks was probably homocystine; the other peak remains unidentified. Urine metabolic screening of Patient 2 (Table II) initially failed to detect homocystine but the cyanide-nitroprusside was slightly positive on a second urine sample. Quantitative urine and plasma amino acid analyses documented homocystinuria and hypomethioninemia (Table II), but free homocystine was not detected in plasma.

Vitamin B_{12} Metabolism

Methylmalonyl-CoA mutase function in both patients as measured by uptake of radiolabeled propionate in cultured skin fibroblasts [Willard et al., 1978] was normal compared to a control cell line (data not shown). The incorporation of radiolabel from 5-methyl-THF into trichloroacetic acid (TCA)-precipitable material [Rosenblatt et al., 1984] was severely decreased in fibroblasts from both children (Table III) and did not recover with the addition of OHCbl to the culture medium (data not shown). The uptake of transcobalamin II-bound cyanocobalamin was normal, but cellular synthesis of MeCbl was very low, with adenosylcobalamin synthesis occurring normally (Table IV). These results demonstrate functional methionine synthase deficiency secondary to an inability to methylate cobalamin. This deficiency is completely corrected in skin fibroblasts from our patients through co-culture and polyethyleneglycol (PEG)-induced fusion with fibroblasts from previously investigated patients with the cblE defect in cobalamin metabolism [Watkins and Rosenblatt, 1988], but is not complemented by fibroblasts from patient's with the cblG defect (Table III). These results indicate that the two children described in this report have the cblG defect in functional methionine synthase activity.

TABLE III. Methionine Synthase Function and Complementation Studies in Cultured Skin Fibroblasts*

	Cellular 5-methyltetrahydrofolate uptake (pmol/mg protein/18 hours)				
	Patient 1	Patient 2	cblE	cblG	Control
No fusion	10	14	24	20	114
PEG fusion with cblE	152	101			
PEG fusion with cblG	17	18			

*5-methyltetrahydrofolate uptake in cultured skin fibroblasts from our patients, previously described patients with cblE or cblG defect, and a control cell line. 5-methylTHF uptake measurements were repeated following co-culture and polyethylene glycol (PEG)-induced fusion of fibroblasts from our patients with fibroblasts from previously described cases of cblE or cblG defect.

DISCUSSION

Cobalamin (vitamin B_{12}) is a required cofactor for the activity of two enzyme reactions in mammals, methionine synthase (5-methyltetrahydrofolate:homocysteine methyltransferase) and methylmalonyl-CoA mutase [Fenton and Rosenberg, 1995] (Fig. 1). Vitamin B_{12} circulates in the blood bound to transcobalamin II (TCII) and enters cells through endocytosis into lysosomes where it is reduced to co(I)balamin and converted into either MeCbl or adenosylcobalamin (AdoCbl). MeCbl is necessary for cytoplasmic methionine synthase activity, and cellular MeCbl deficiency leads to homocystinuria/emia. AdoCbl is a required cofactor for the mitochondrial enzyme methylmalonyl-CoA mutase, which catalyzes the conversion of methylmalonyl-CoA to succinyl-CoA in the metabolism of propionic acid; AdoCbl deficiency leads to methylmalonic aciduria. Previously reported patients with cobalamin utilization defects have been classified into seven different groups, cblA through cblG, on the basis of complementation analysis

TABLE IV. Cellular Cobalamin Uptake and Distribution in Cultured Skin Fibroblasts

	Total cobalamin uptake (picogram)	Distribution of cobalamin intermediates (% of total cobalamin)				
		OHCbl	CNCbl	AdoCbl	MeCbl	Other
Patient 1	11.5 ± 2	34.9	16.6	34.3	1.7	12.5
Patient 2	3.6 ± 0.1	47.9	6.1	29.2	2.7	14.1
Control	7.2 ± 3.4	11.1	11.3	16.6	51.5	9.5

employing cultured skin fibroblasts [Cooper and Rosenblatt, 1987]. In complementation classes A and B, synthesis of AdoCbl alone is impaired while metabolism of both AdoCbl and MeCbl are disturbed in the cblC, D, and F defects. The remaining patients (cblE and cblG) demonstrate cellular deficiency of MeCbl alone, resulting in functional methionine synthase deficiency and, therefore, they exhibit homocystinuria/emia and hypomethioninemia without methylmalonic aciduria. In cblE disease, the primary defect is probably impaired reduction of Co(III) in cobalamin to Co(I) [Rosenblatt et al., 1984], while problems with adenosylmethionine-dependent co(I)balamin methylation may be the cause of the cblG defect [Hall et al., 1989]. The clinical phenotypes of patients with MeCbl deficiency due to cblE or cblG defects appear identical, and differentiation of these two disorders requires biochemical evaluation and complementation analysis of cultured skin fibroblasts.

The separation of MeCbl synthetic defects into cblE and cblG complementation groups was first reported in 1988 [Watkins and Rosenblatt, 1988]. To date, including our two patients, a total of ten cblG cases have been published [Watkins and Rosenblatt, 1989]. Five previously reported patients diagnosed with cblE defect were reclassified as cblG on the basis of complementation analysis and in vitro methionine synthase activities [Hall et al., 1987; McKie et al., 1986; Morton et al., 1986; Rosenblatt et al., 1987; Shin et al., 1986]. Four other unreported cases of cblG disease are known to us, yielding a total of 14 patients worldwide with the cblG defect. With the exception of one adult presenting with megaloblastic anemia and neurologic symptoms [Carmel et al., 1988], all reported cases of cblG defect have been diagnosed in early childhood. Retrospectively, the adult cblG patient reported by Carmel et al. [1988] exhibited lethargy and poor feeding during infancy, symptoms which are common to other cblG patients.

The common manifestations and laboratory findings associated with the cblG defect are listed in Table V. The most common findings include red blood cell macrocytosis, developmental delay, seizures, hypotonia, anorexia, and feeding problems. RBC macrocytosis is ubiquitous among reported cases of cblG defect and is central to clinical suspicion of defects in cobalamin metabolism, but frank anemia is variably present. In the two children described here, anemia was mild in Patient 2 and developed only late in Patient 1, even though macrocytosis had been worsening since infancy. In Patient 1, severe physical and cognitive deterioration accompanied the onset of clinically significant anemia. Neither the factors which delayed the onset of anemia in Patient 1 nor which triggered clinical deterioration at age 5 years are understood.

Severe developmental delay and central hypotonia are major manifestations of cblG defect in previously reported cases and in the two children reported here. Diffuse cerebral atrophy, apparent on neuroimaging studies, demonstrates an anatomic correlate to the central nervous system dysfunction apparent clinically. No neuropathologic data are available on any of the previously reported cblG cases. Histologic changes associated with severe anoxia dominated the neuropathologic examination of Patient 1, but astrogliosis of the cortex and pons was also seen. This finding may be a primary manifestation of the cblG defect.

Anorexia was a persistent serious problem in both children in our report and has been a common symptom in other patients. The cause of the anorexia is obscure, particularly given that it has not responded to dietary protein restriction or to OHCbl supplementation.

Our patients and most previously reported cblG patients exhibited severe lethargy at presentation. The cause of the lethargy is poorly understood. In Patient 1, the development of lethargy appeared to coincide with the onset of clinically significant anemia, but Patient 2 was severely lethargic despite only mild anemia. In previously reported cblG patients, lethargy improved within 24–48 hours of intramuscular OH- or MeCbl injections, and this improvement is associated with a rapid response in homocystinuria and hypomethioninemia, but no acute change in megaloblastic anemia [Watkins and Rosenblatt, 1989].

Although not occurring in our patients, one previously reported child with cblG defect has had recurrent infections, including pneumonia and candidiasis [McKie et al., 1986] while onset of clinical symptoms of cblG defect was associated with varicella infection in another [Rosenblatt et al., 1987]. Thrombotic complications of homocystinemia, which are common in cystathionine β-synthase deficiency, have not been previ-

TABLE V. Clinical Characteristics of Published cblG Patients

	Patient 1	Patient 2	Published patients n = 8	Totals (%) n = 10
Age at diagnosis	5 yr	4 yr	11 weeks–27 yr	
Sex	Male	Male	5 females 3 males	5 females 5 males
Megaloblastosis	Yes	Yes	8/8	100
Developmental delay	Severe	Severe	8/8	100
Seizures	Yes	Yes	7/8	90
Hypotonia	Central	Central	7/8	80
Anorexia	Severe	Severe	6/8	80
Lethargy	Severe	Severe	5/8	70
Cerebral atrophy	Yes	Yes	4/8	70
Vision abnormalities	No	No	3/8	30
Skeletal abnormalities	Scoliosis Pectus excavatum	No	None	10

ously reported in cblG patients, although a single patient with cblE disease died of bilateral renal artery thrombosis [Watkins and Rosenblatt, 1989]. During infancy, a possible sagittal sinus thrombosis was detected by cranial CT scan in Patient 1, but there were no symptoms of thrombotic complications and no vascular abnormalities were detected at autopsy, including histologic examination of the brain.

Skeletal abnormalities have not been previously reported in cblG disease, but Patient 1 in this report exhibited pectus excavatum and scoliosis, reminiscent of bony abnormalities seen in more common forms of homocystinuria.

Previously reported patients with cblG defect have responded biochemically and clinically to parenteral cobalamin supplementation. Although a few cblG patients have been reported to benefit from injections with CNCbl, OHCbl (1 mg IM per day and reduced as tolerated to 2–3 times per week) is the drug of choice. Patient 2 in this report initially received several injections of CNCbl, which lead to respiratory depression and lethargy; these symptoms immediately improved when OHCbl was substituted. The definitive cause of the respiratory problems associated with CNCbl therapy is unknown but could be related to cyanide toxicity. As has been reported in other cblG patients, plasma and urine homocystine levels in Patient 2 decreased significantly with OHCbl therapy, and megaloblastic anemia completely resolved.

Several adjunct therapies may be used along with, but should not substitute for, OHCbl therapy. Betaine can act as a methyl donor in the conversion of homocysteine to methionine. Oral betaine (250 mg/kg/day) has been shown to further reduce plasma-free homocystine beyond the effect of parenteral cobalamin in patients with cblC defect [Bartholomew et al., 1988]. The restriction of dietary protein will reduce methionine consumption and should in turn decrease homocysteine production. Of course, this will also cause worsening of hypomethioninemia in these patients and could lead to hypocystinemia. The use of medical foods designed for the treatment of homocystinuria secondary to cystathionine β-synthase deficiency would allow dietary protein restriction without causing cystine deficiency but would not provide methionine supplementation. The effects of dietary therapy have not been examined rigorously in defects of cobalamin metabolism. High-dose pyridoxine supplementation (250–500 mg orally per day) might theoretically enhance homocysteine removal via its action as the cofactor for cystathionine β-synthase, but this possibility has not been formally studied in patients with cobalamin metabolism defects. The megaloblastic anemia associated with cobalamin deficiency has been shown to improve when folic acid supplements are given, even if treatment with cobalamin is not begun [Fenton and Rosenberg, 1995]. The reason for this effect has not been proven, but cobalamin deficiency and consequent functional methionine synthase deficiency may prevent the production of tetrahydrofolate from 5-methyltetrahydrofolate, leading to relative folate deficiency, the so-called "methyl-trap" hypothesis [Herbert and Zalusky, 1962]. Before the diagnosis of cblG defect was made, Patient 1 was treated empirically for homocystinuria with a combination of dietary protein restriction, folate, and pyridoxine supplements but did not receive cobalamin or betaine. On this regimen, reticulocytosis resumed and anemia resolved, but RBC macrocytosis did not improve and homocystinuria persisted. Patient 1 died before the initiation of planned therapeutic trials with oral betaine or intramuscular vitamin B_{12}.

Cobalamin therapy in previously reported patients has resulted in stabilization but not complete correction of neurologic deficits present at diagnosis. Previously reported children with the cblG defect have been cognitively delayed but have shown continued developmental progress [Watkins and Rosenblatt, 1989] when treated with cobalamin. A child (currently 10 years old) diagnosed prenatally with the cblE defect is cognitively normal on OHCbl therapy begun before and continued after birth [Rosenblatt et al., 1985], indicating that early treatment of MeCbl deficiency may prevent the major neurologic complications of these diseases.

The biochemical hallmark of cblE and cblG defects is homocystinuria/emia in the absence of methylmalonic aciduria or hypermethioninemia. However, homocystine in blood and urine is not always detectable by qualitative screening or by quantitative amino acid analysis. In inborn errors of homocysteine metabolism, most of the plasma homocysteine is bound to plasma proteins through disulfide linkages. Non-protein-bound excess homocysteine readily forms homocystine, the dimer of homocysteine. Traditional metabolic screening tests, such as the urine cyanide-nitroprusside test or quantitative amino acid analysis, detect free homocystine alone and therefore may fail to detect mild to moderate homocysteine elevations because total plasma homocysteine must be substantially elevated before detectable amounts of free homocystine are formed. This fact is reflected in our inability to reliably and consistently detect homocystinuria/emia in our patients using traditional screening methodologies. Laboratory methods for the sensitive and specific detection of total homocysteine and methylmalonic acid in plasma and urine are now available. We strongly recommend that these studies be performed on individuals with macrocytic anemia and normal serum folic acid and cobalamin concentrations, particularly when neurologic abnormalities are present. Qualitative urine screening or quantitative amino acid analysis of plasma or urine should not be relied upon to rule out abnormalities of homocysteine metabolism in this clinical situation.

We report two additional cases of functional methionine synthase deficiency due to the cblG defect in cobalamin metabolism. The clinical histories of these two boys illustrate the protean manifestations of this disorder and clearly demonstrate the difficulties in diagnosis. The key to making this diagnosis is the recognition of megaloblastic anemia in association with abnormal homocysteine metabolism and normal blood cobalamin and folate levels. Traditional methods of detecting free homocystine in plasma or urine lack adequate sensitivity to reliably rule out cobalamin metabolism defects; a sensitive measurement of total plasma homocysteine may be required to detect these

disorders. Definitive diagnosis is accomplished through functional assays and complementation analysis utilizing cultured skin fibroblasts. Treatment with intramuscular injections of OHCbl will alleviate megaloblastic anemia and stabilize neurologic deterioration in children with cblG defect but may not completely correct hypotonia and developmental delay nor improve the anorexia or poor weight gain associated with cblG disease.

NOTE ADDED IN PROOF

The human methionine synthase cDNA has recently been cloned and putative pathogenic mutations within the cDNA sequence have been detected in three cblG patients [Gulati et al., 1996; Leclerc et al., 1996].

REFERENCES

Bartholomew DW, Batshaw ML, Allen RH, Roe CR, Rosenblatt D, Valle DL, Francomano CA (1988): Therapeutic approaches to cobalamin-C methylmalonic acidemia and homocystinuria. J Pediatr 112:32–39.

Carmel R, Watkins D, Goodman SI, Rosenblatt DS (1988): Hereditary defect of cobalamin metabolism (cblG mutation) presenting as a neurologic disorder in adulthood. N Engl J Med 318:1738–1741.

Cooper BA, Rosenblatt DS (1987): Inherited defects of vitamin B_{12} metabolism. Ann Rev Nutr 7:291–320.

Fenton WA, Rosenberg LE (1995): Inherited disorders of cobalamin transport and metabolism. In Scriver CR, Beaudet AL, Sly WS, Valle D (eds): "The Metabolic and Molecular Bases of Inherited Disease." New York: McGraw-Hill, pp 3129–3149.

Gulati S, Baker P, Li YN, Fowler B, Kruger W, Brody LC, Banerjee R (1996): Defects in human methionine synthase in cblG patients. Hum Molec Genet 5:1859–1865.

Hall C, Arenson E, Chu R, Begley J (1987): Defective cobalamin methylation in cblG mutation. Blood 70:47a, abstract.

Hall CA, Lindenbaum RH, Arenson E, Begley JA, Chu RC (1989): The nature of the defect in cobalamin G mutation. Clin Invest Med 12:262–269.

Herbert V, Zalusky R (1962): Interrelations of vitamin B_{12} and folic acid metabolism: Folic acid clearance studies. J Clin Invest 41:1263–1276.

Hoffmann G, Aramaki S, Blum-Hoffmann E, Nyhan WL, Sweetman L (1989): Quantitative analysis for organic acids in biological samples: Batch isolation followed by gas chromatographic–mass spectrometric analysis. Clin Chem 35:587–595.

Leclerc D, Campeau E, Goyette P, Adjalla CE, Christensen B, Ross M, Eydoux P, Rosenblatt DS, Rozen R, Gravel RA (1996): Human methionine synthase: cDNA cloning and identification of mutations in patients of the *cblG* complementation group of folate/cobalamin disorders. Hum Molec Genet 5:1867–1874.

McKie VC, Roesal A, Hommes FA, Watkins D, Rosenblatt DS, Flannery DB (1986): Clinical findings in an infant with methylcobalamin deficiency (cblE variant). Am J Hum Genet 39:A71, abstract.

Morton DH, Levy HL, Bresnan MI, Hall CA, Watkins D, Rosenblatt DS (1986): Cobalamin (cbl) E mutation with developmental delay, myoclonic seizures and hypsarrhythmia: Therapy with methylcobalamin (MeCbl). Am J Hum Genet 39:A16, abstract.

Mudd S, Levy H, Skovby F (1995): Disorders of transsulfuration. In Scriver CR, Beaudet AL, Sly WS, Valle D (eds): "The Metabolic and Molecular Bases of Inherited Disease." New York: McGraw-Hill, pp 1285–1287.

Rosenblatt DS, Cooper BA, Pottier A, Lue-Shing H, Matiaszuk N, Grauer K (1984): Altered vitamin B_{12} metabolism in fibroblasts from a patient with megaloblastic anemia and homocystinuria due to a new defect in methionine biosynthesis. J Clin Invest 74:2149–2156.

Rosenblatt DS, Cooper BA, Schmutz SM, Zaleski WA, Casey RE (1985): Prenatal vitamin B_{12} therapy of a fetus with methylcobalamin deficiency (cobalamin E disease). Lancet 1:1127–1129.

Rosenblatt DS, Thomas IT, Watkins D, Cooper BA, Erbe RW (1987): Vitamin B_{12} responsive homocystinuria and megaloblastic anemia: Heterogeneity in methylcobalamin deficiency. Am J Med Genet 26:377–383.

Shih VE, Mandell R, Sheinhait I (1991): General metabolic screening tests. In Hommes FA (ed): "Techniques in Diagnostic Human Biochemical Genetics." New York: Wiley-Liss, pp 49–50.

Shin YS, Reiter S, Zelger O, Brunstler I, Rucker AV (1986): Orotic aciduria, homocystinuria, formiminoglutamic aciduria and megaloblastosis associated with the formiminotransferase/cyclodeaminase deficiency. In Nyhan WL, Thompson F, Watts RWE (eds): "Purine and Pyrimidine Metabolism in Man." New York: Plenum, pp 71–76.

Slocum RH, Cummings JG (1991): Amino acid analysis of physiological samples. In Hommes FA (ed): "Techniques in Diagnostic Human Biochemical Genetics." New York: Wiley-Liss, pp 87–126.

Watkins D, Rosenblatt DS (1988): Genetic heterogeneity among patients with methylcobalamin deficiency: Definition of two complementation groups, cblE and cblG. J Clin Invest 81:1690–1694.

Watkins D, Rosenblatt DS (1989): Functional methionine synthase deficiency (cblE and cblG): Clinical and biochemical heterogeneity. Am J Med Genet 34:427–434.

Willard HF, Mellman IS, Rosenberg LE (1978): Genetic complementation among inherited deficiencies in methylmalonylcoA mutase activity: Evidence for a new class of human cobalamin mutant. Am J Hum Genet 30:1–13.