Acquired and inherited disorders of cobalamin and folate in children

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Summary

Cobalamin deficiency in the newborn usually results from cobalamin deficiency in the mother. Megaloblastic anaemia, pancytopenia and failure to thrive can be present, accompanied by neurological deficits if the diagnosis is delayed. Most cases of spina bifida and other neural tube defects result from maternal folate and/or cobalamin insufficiency in the periconceptual period. Polymorphisms in a number of genes involved in folate and cobalamin metabolism exacerbate the risk. Inborn errors of cobalamin metabolism affect its absorption, (intrinsic factor deficiency, Imerslund-Gräsbeck syndrome) and transport (transcobalamin deficiency) as well as its intracellular metabolism affecting adenosylcobalamin synthesis (*cblA* and *cblB*), methionine synthase function (*cblE* and *cblG*) or both (*cblC*, *cblD* and *cblF*). Inborn errors of folate metabolism include congenital folate malabsorption, severe methylenetetrahydrofolate reductase deficiency and formiminotransferase deficiency. The identification of disease-causing mutations in specific genes has improved our ability to diagnose many of these conditions, both before and after birth.

Keywords: cobalamin, folate, homocysteine, methylmalonic acid, megaloblastic anaemia.

Cobalamin (Cbl, vitamin B_{12}) and folic acid (folate) deficiencies in newborns and children classically present with megaloblastic anaemia. Neurological signs may be present as well. There has been increasing recognition during the past few decades that milder deficiencies or insufficiencies of these vitamins can result in health problems as well, often without manifesting classical clinical features. Detection of elevated circulating and urinary levels of methylmalonic acid (MMA) and of total homocysteine (tHcy) has advanced our ability to recognise subclinical derangements of Cbl and folate metabolism, since these abnormalities commonly denote insufficiency of one or the other vitamin (Allen *et al*, 1990). A number of rare and not so rare inborn errors of Cbl and folate absorption and metabolism have been delineated and disease-causing mutations identified. The current review will address the spectrum of conditions resulting from acquired insufficiencies and inherited disorders of Cbl and folate in newborns and children, focusing on clinical and diagnostic aspects of these conditions. The reader is referred to recent reviews addressing the relations of folate and Cbl insufficiency and elevated homocysteine levels to cardiovascular disease (Lentz, 2005; Lewis *et al*, 2005), to malignant disease (Ulrich, 2005) and to neuropsychiatric disorders (Hultberg *et al*, 2001; Quadri *et al*, 2004), topics that will not be addressed here.

Dietary deficiency of cobalamin and folate

Cobalamin deficiency due to maternal cobalamin deficiency

An earlier review focused on Cbl deficiency in newborns, most often the result of deficiency in the mother (Rosenblatt & Whitehead, 1999). Mothers following vegetarian, vegan, macrobiotic and other special diets were at particular risk. Occult maternal pernicious anaemia was and continues to be reported (Emery *et al*, 1997; Korenke *et al*, 2004). Not surprisingly, poverty and malnutrition continue to cause Cbl deficiency in mothers and children in many parts of the world (Casterline *et al*, 1997; Rogers *et al*, 2003; Guerra-Shinohara *et al*, 2004; Garcia-Casal *et al*, 2005).

In the newborn period, clinical signs of Cbl deficiency include irritability, failure to thrive, apathy and anorexia, refusal of solid foods and developmental regression. Later, infants may present with megaloblastic anaemia, and neurological deficits. There are reports of a self-limited 'infantile tremor syndrome', arising during Cbl therapy (Emery *et al*, 1997; Goraya, 1998).

Additional case reports have continued to appear, several emphasising the persistence of neurological deficits despite Cbl therapy [Emery *et al*, 1997; Goraya, 1998; Centers for Disease Control and Prevention (CDC), 2003; Korenke *et al*, 2004; Şimşek *et al*, 2004; Weiss *et al*, 2004; Codazzi *et al*, 2005;

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Reghu *et al*, 2005; Roschitz *et al*, 2005]. Cranial magnetic resonance imaging (MRI) studies have shown severe cerebral atrophy and retarded myelination (Lövblad *et al*, 1997) with persistence of neurological deficits even after complete resolution of all MRI abnormalities (von Schenck *et al*, 1997).

Up to 20% of normal pregnancies are associated with lower than normal serum Cbl levels in mothers. These levels return to normal after delivery (Lowenstein *et al*, 1960). In a recent study, low maternal Cbl levels were accompanied by elevated tHcy and MMA levels and were the principal predictors for low Cbl and raised tHcy and MMA levels in the newborn. These abnormalities persisted for 6 weeks in the newborns. The authors concluded that the Cbl status in the neonatal period was strongly associated with maternal Cbl status and parity (Bjørke Monsen *et al*, 2001).

Cobalamin deficiency was identified in a series of infants of families eating a macrobiotic diet and parents were persuaded to liberalise the family diets. A decade later, adolescents from these families were found to have persisting Cbl deficiency with low Cbl and raised MMA and tHcy levels. These adolescents had impaired cognitive function compared with controls (van Dusseldorp *et al*, 1999; Louwman *et al*, 2000). Together with results showing that parity increases the risk of low-Cbl status in mothers (Bjørke Monsen *et al*, 2001), these findings indicate that diet alone may be insufficient to replenish Cbl stores and that Cbl supplements may be needed during pregnancy and after.

Healthy pregnant Korean women were less likely to have folate deficiency and more likely to have Cbl deficiency than non-pregnant women (Park *et al*, 2004). At first antenatal visit, 61% of Nepali women had elevated serum MMA, and 49% had low serum Cbl values (Bondevik *et al*, 2001). These authors recommended Cbl supplements for this population. Worldwide Cbl deficiency has been the subject of a detailed review (Stabler & Allen, 2004).

Several reports have emphasised the permanence of neurological deficits induced by Cbl deficiency in newborns despite Cbl therapy (von Schenck *et al*, 1997; CDC, 2003; Korenke *et al*, 2004; Weiss *et al*, 2004; Codazzi *et al*, 2005). The consistency of such reports and the serious neurological sequelae they document suggest the need to add Cbl to folate supplements before and during pregnancy. Added Cbl would reduce both the occurrence of neural tube defects (NTDs), since the lack of Cbl has been implicated in their aetiology (Kirke *et al*, 1993) and nutritional Cbl deficiency in newborns, with its risk of long-term neurological deficits.

Folate deficiency and neural tube defects

Levels of plasma and red cell folate fell and plasma tHcy levels increased during pregnancy in 404 healthy Danish mothers (Milman *et al*, 2006). Despite this finding, folate deficiency in the infant due to folate deficiency in the mother is unusual. The major problem with folate insufficiency in children occurs before rather than after birth. It has been well established that maternal folate deficiency in the periconceptual period is the cause of more than 50% of cases of NTDs, including spina bifida, meningomyelocele and anencephaly (Medical Research Council, MRC; MRC Vitamin Study Research Group, 1991; Czeizel & Dudas, 1992; Botto *et al*, 1999). Intrauterine growth retardation has also been linked to reduced maternal folate levels and increased tHcy levels (Lindblad *et al*, 2005). Efforts to promote folate sufficiency through consumption of a healthy balanced diet have not been successful. Since January 1998, folic acid fortification of all enriched cereal-grain products has been mandated in the USA (Food and Drug Administration, 1996), in Canada and in parts of South America. Current levels of folate fortification are 140, 150 and 220 μg per 100 g of flour in the USA, Canada and Chile respectively (Oakley *et al*, 2004). As a result, the incidence of NTD-affected pregnancies has decreased by 26% in the USA and by 48% in Canada (Ray *et al*, 2002; Centers for Disease Control and Prevention, 2004). Serum and red cell folate levels are also higher. However, <10% of women of childbearing age have reached the recommended red cell folate level in excess of 906 nmol/l (Dietrich *et al*, 2005; Pfeiffer *et al*, 2005). This level has been shown to reduce the risk of NTDs substantially (Brown *et al*, 1997). There have been repeated pleas to increase the level of folic acid fortification (Brent *et al*, 2000; Oakley *et al*, 2004; Brent & Oakley, 2005). Adding folate to food seems not to have 'masked' the anaemia of pernicious anaemia (Mills *et al*, 2003).

Elsewhere in the world, food fortification remains a controversial question (Botto *et al*, 2005). It seems ironic that those countries that proved the benefit of folate in preventing spina bifida 15 years ago have yet to mandate food fortification with folic acid to benefit their own citizens.

Because 50% of pregnancies are unplanned, it is still recommended that a 0·4 mg folic acid daily supplement be taken by women during the childbearing years to ensure adequate folate during the initial 30 d following conception (Berry *et al*, 1999).

Inherited disorders of cobalamin and folate

Inborn errors of cobalamin absorption and transport

Hereditary Cbl deficiency results from defects in Cbl absorption and transport and in its intracellular processing (see below) (Morel & Rosenblatt, 2006). Mutations affecting the *GIF* gene for intrinsic factor (IF), the *CUBN* gene for cubilin, the *AMN* gene for amnionless and the *TCN* gene for transcobalamin (TC) affect binding of IF to Cbl in the intestine, absorption of the IF–Cbl complex in the terminal ileum and transport of Cbl into cells respectively (Fig 1). Long-term parenteral Cbl treatment is required for these conditions.

Congenital pernicious anaemia is a rare disorder characterised by a lack of IF, the gastric Cbl-binding protein that facilitates absorption of Cbl in the terminal ileum. Patients have normal acid secretion and mucosal cytology (Gordon
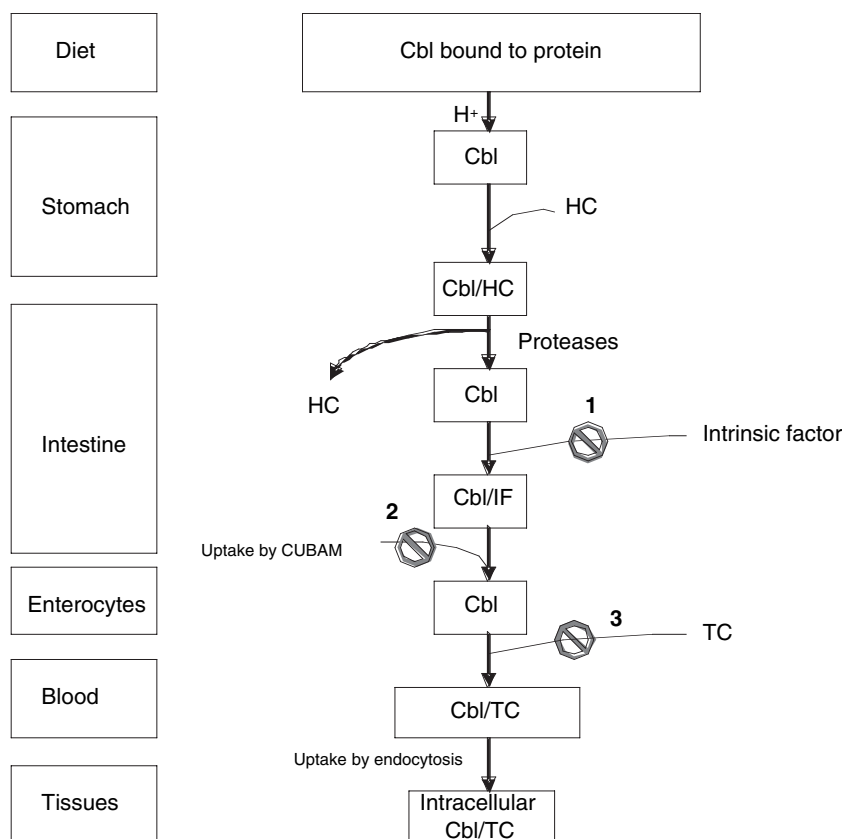
Fig 1. Summary of cobalamin absorption, transport and cellular uptake. Cbl, cobalamin; Cbl/HC, cobalamin haptocorrin complex; Cbl/IF, cobalamin intrinsic factor complex; Cbl/TC, cobalamin transcobalamin complex; CUBAM, ileal receptors made up of cubilin and amnionless proteins; HC, haptocorrin; TC, transcobalamin; 1, intrinsic factor deficiency, 2, Imerslund-Gräsbeck syndrome; 3, transcobalamin deficiency Adapted from: Morel and Rosenblatt (2006) with permission of The McGraw-Hill Companies.

et al, 2004; Yassin *et al*, 2004; Tanner *et al*, 2005). Fewer than 100 patients with this condition have been reported. Patients have a low serum Cbl level with megaloblastic anaemia. They do not have autoantibodies against IF or parietal cells as occurs in the acquired form of pernicious anaemia. The disorder usually presents between the first and fifth year of life, but patients have been identified in the first and third decades. Mutations have been identified in the *GIF* gene, together with a polymorphism (68A → G) which may be a marker for this inheritance (Gordon *et al*, 2004). A study to characterise the 'third' locus in seven families diagnosed with MGA1 (Tanner *et al*, 2005) (see below) revealed homozygous *GIF* mutations that predicted a complete loss of function in six families and possibly only reduced IF function in one sibship. These included a four-base deletion in one family of African ancestry, the second such reported, which may be common in some African populations (Yassin *et al*, 2004).

Megaloblastic anaemia 1 (MGA1, Imerslund-Gräsbeck syndrome) is a potentially fatal condition of childhood caused by the malabsorption of Cbl. Over 250 cases have been diagnosed worldwide, with clusters of reports in Finland, Norway and several Middle Eastern countries. Children usually present between the ages of three and 10 with pallor, fatigue, anorexia

and failure to thrive. There may be infection and bruising, reflecting pancytopenia. The bone marrow shows megaloblastic changes and neurological deficits may be present as well. Cbl treatment is effective and required indefinitely.

Children with MGA1 have normal production of IF. Many patients also have significant Cbl-resistant proteinuria. Forty years after MGA1 was first described, cubilin, the 640-kDa protein product of the human gene *CUBN*, was identified as the high-affinity intestinal receptor for the IF–Cbl complex (Aminoff *et al*, 1999; Kristiansen *et al*, 2000). Cubilin has a unique structural organisation of extracellular protein modules, comprising eight epidermal growth factor repeats followed by 27 CUB domains (CUB is an abbreviation of complement subcomponents C1r/C1s, Uegf and bone morphogenic protein-1). The binding site for the IF–Cbl complex is located on CUB modules 5–8. A single mutation, Pro1297Leu, in CUB module 8 accounted for 31 of 34 disease chromosomes in Finnish patients and resulted in decreased affinity of cubilin for IF–Cbl (Kristiansen *et al*, 2000). A total of six mutations in cubilin have been identified to date.

Cubilin lacks the transmembrane and cytosolic components required for endosomal transport of IF–Cbl. Mutations of a 50-kDa transmembrane protein of polarised epithelia,

amnionless, product of the *AMN* gene, have been shown to cause MGA1 as well (Fyfe *et al*, 2004; He *et al*, 2005). Amnionless binds tightly to cubilin and appears essential for production of mature cubilin and its transport to the apical surface brush border. The complex of cubilin and amnionless is believed to constitute the intestinal IF–Cbl receptor and has been named CUBAM (Fig 1). At least six disease-causing mutations have been identified in amnionless in Norwegian patients to date (Tanner *et al*, 2004). Mutations in a third gene causing MGA1 proved to be affecting the *GIF* gene, leading to re-classification of these families as suffering from IF deficiency (Tanner *et al*, 2005).

Megalin is a very large protein that shares tissue expression and cell localisation with cubilin. Both proteins are present in the ileum and renal proximal tubule and transport a large variety of protein-bound nutrients, including vitamins, hormones, drugs and proteins (Moestrup & Verroust, 2001). Megalin is not currently believed to play a role in IF–Cbl absorption from the intestine, but is required for renal re-absorption of the TC–Cbl complex (Birn *et al*, 2002).

About 80% of Cbl transported in plasma is bound to haptocorrin (HC) and 20% is bound to TC. TC collects Cbl from the basolateral surface of terminal ileal mucosal cells and mediates its uptake by cells throughout the body (Fig 1). In contrast, HC-bound Cbl is not taken up by cells with the exception of the hepatocyte. Recent improved antibody-based methods facilitate measurement of TC-bound Cbl (holo-TC), providing a more physiological measure of Cbl available to cells (Hvas & Nexø, 2005).

Congenital deficiency of TC presents in early infancy with megaloblastic anaemia, failure to thrive and, not infrequently, neurological complications. Fewer than 50 patients have been reported. Since most of the circulating Cbl is bound to HC, these infants usually have a normal serum Cbl level.

Several phenotypic variants of congenital TC disorders have been described. The commonest is the absence of TC, measured by Cbl-binding and immunoassay. Others include an immunoreactive TC that does not bind Cbl and a TC that binds Cbl, but the complex apparently does not bind to the cell membrane receptor for TC–Cbl (Rosenblatt & Fenton, 2001). A variance in RNA editing is the likely mechanism for multiple mutations discovered in TC cDNA, which were not found in genomic TC DNA (Qian *et al*, 2002). A point mutation in the intron-3 splice site of the TC gene, found in three sisters, activates a cryptic splice site in exon 3 and generates a transcript with an in-frame deletion of 81 nucleotides. The resulting truncated protein is unstable and is not secreted by the cells (Namour *et al*, 2003).

Inborn errors of cobalamin metabolism

Cobalamin is an essential co-factor for two enzymes, methionine synthase (MTR) and methylmalonyl-CoA mutase (MCM). The conversion of the vitamin to its coenzymes, methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl) requires a series of biochemical modifications for which several genetic diseases are known, comprising eight complementation groups (*cblA* − *cblH*) (Fig 2). Mutations in specific genes have been described for five of these complementation groups: *MMAA* (*cblA*), *MMAB* (*cblB*), *MMACHC* (*cblC*), *MTRR* (*cblE*) and *MTR* (*cblG*). Lifelong parenteral hydroxyCbl (OHCbl) treatment is required for most of these conditions (Morel & Rosenblatt, 2006). Many of these diseases can be diagnosed antenatally (Huemer *et al*, 2005; Morel *et al*, 2005).
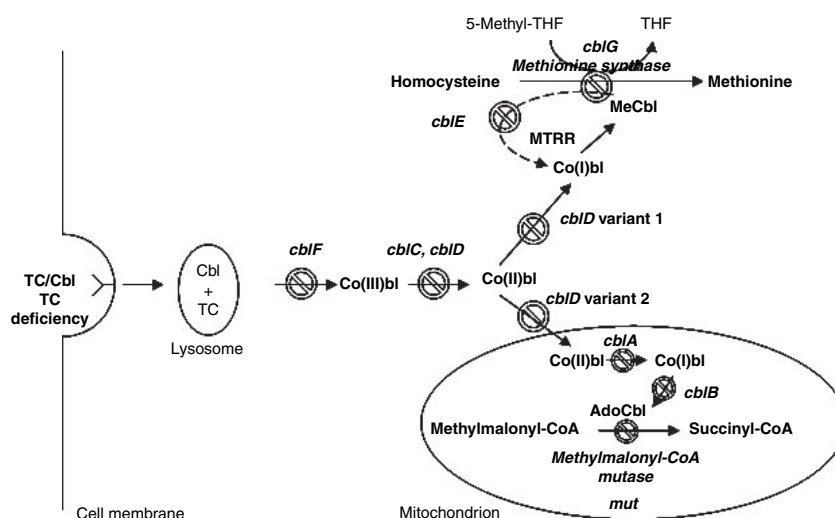

Fig 2. Summary of inborn errors of Cbl metabolism, including TC deficiency and mutase deficiency. AdoCbl, adenosylcobalamin; Cbl, cobalamin; MeCbl, methylcobalamin; MTRR, methionine synthase reductase; mut, methylmalonyl-CoA mutase deficiency; TC/Cbl, transcobalamin–cobalamin complex; TC, transcobalamin; THF, tetrahydrofolate. Adapted from: Morel and Rosenblatt (2006) with permission of The McGraw-Hill Companies.

Patients with complementation groups *cblC*, some *cblD* and *cblF* have lesions affecting both AdoCbl and MeCbl metabolism (Fig 2). They have megaloblastic anaemia and increased levels of MMA and tHcy in plasma and MMA and homocystine in urine.

The *cblF* complementation group is a very rare disorder of intracellular Cbl metabolism described in only two siblings and seven other unrelated patients. The defect appears to be due to trapping of unmetabolised Cbl in the lysosome after degradation of TC (Fig 2). This Cbl is not available for conversion to AdoCbl or MeCbl. In addition, *cblF* patients fail to absorb oral Cbl, suggesting that the lysosomal defect affects ileal Cbl absorption as well. Patients are usually seen within the first year of life. Some have low-birth weight. Findings include megaloblastic anaemia, stomatitis, feeding difficulties, developmental delay and hypotonia. Patients respond well to OHCbl injections. Betaine has also been used, but experience in the treatment of this condition is limited (Rosenblatt & Fenton, 2001).

Methylmalonic aciduria and homocystinuria, *cblC* type, is the most common inborn error of Cbl metabolism, with about 300 known cases. The gene responsible for *cblC* has recently been identified and designated *MMACHC* for methylmalonic aciduria *cblC* type with homocystinuria. It has been suggested that the *MMACHC* gene product plays a role in the removal of the upper axial ligand of Cbl, the reduction of Cbl, or both. Mutations have been identified in 173 of 204 patients. One mutation, 271dupA, accounted for 40% of all disease alleles. Common mutations were linked to early-onset and late-onset disease (Lerner-Ellis *et al*, 2006a).

Affected individuals have developmental, haematological, neurological, metabolic, ophthalmologic and dermatologic abnormalities. In a series of 50 patients, two clinical phenotypes were identified, which differed in age of onset, presence of systemic symptoms, type of neurological symptoms and outcome after diagnosis and treatment. Forty-four presented in the first year of life. Feeding difficulties, neurological dysfunction (hypotonia, seizures and developmental delay), and ophthalmologic and haematological abnormalities characterised their clinical picture. One-quarter of these patients died. Survival was associated with neurological impairment (Rosenblatt *et al*, 1997).

In contrast, onset later in childhood was associated with less severe haematological abnormalities confined to the red cell series. Extrapyramidal signs, dementia, delirium or psychosis characterised the neurological findings. Survival, with mild to moderate disability in some, was typical in patients with later onset. Treatment in both groups included OHCbl, betaine and carnitine. Complete normalisation of biochemical parameters was rare. Case reports have described newborns, without acute metabolic derangements, who were initially diagnosed and treated for sepsis, based on lethargy, hypotonia, dehydration and decreased oral intake. Although considered a disease of infancy or childhood, some individuals develop symptoms in adulthood (Rosenblatt *et al*, 1997).

Until recently, the complementation group for combined methylmalonic aciduria and homocystinuria, *cblD*-type, had been assigned to two siblings described over 30 years ago. Enzyme activities and Cbl metabolism in *cblD* fibroblasts from these patients revealed similar but less severe abnormalities compared with those found in *cblC* cells. Recently, a report has described three new unrelated patients showing considerable biochemical and clinical heterogeneity within the *cblD* complementation group (Fig 2). Two had isolated homocystinuria while the third had isolated methylmalonic aciduria (Suormala *et al*, 2004).

The two patients with isolated homocystinuria presented at 3 months and at 6 years of age. The younger had severe hypotonia, nystagmus, dystonic movements and seizures, which were difficult to control with anticonvulsants. Megaloblastic anaemia was present, with elevated plasma and red cell folate and decreased Cbl levels. The older child presented with global developmental delay, severe learning difficulties, spastic ataxia, absence of ankle jerks and rapid deterioration of gait. He had no vocal skills, and made no eye contact. There was macrocytosis, but normal haemoglobin and Cbl levels. Cranial MRI scans of both revealed reduced myelination and cerebral and cerebellar atrophy. Both had elevated tHcy and reduced methionine. MMA was not elevated in urine.

The third patient was born at 32 weeks with grade II respiratory distress syndrome, a cranial haemorrhage, necrotising enterocolitis and neonatal convulsions. MMA and methylcitrate were markedly elevated in the urine. Plasma tHcy and blood counts were normal.

Patients with severe methylenetetrahydrofolate reductase (MTHFR) deficiency (see below) and those belonging to complementation groups *cblE* and *cblG* have a defect in methionine synthesis. The latter two are distinguished by demonstrating an increase in MTR activity in the presence of a reducing system in *cblE* but not in *cblG* cells (Fig 2).

The clinical and biochemical disturbances associated with *cblE* and *cblG* complementation groups are virtually identical. Both these disorders present with a range of clinical abnormalities including megaloblastic anaemia, severe developmental delay, ataxia, cerebral atrophy, neonatal seizures and blindness. They have increased tHcy in blood and homocystine in urine, and normal or reduced blood methionine and S-adenosyl methionine levels. They do not have methylmalonic aciduria. Most patients present in the first months of life, but they may present as adults with milder features. Treatment is with OHCbl 1–2 mg by injection daily at first, then once or twice weekly. Folate, betaine and L-methionine have also been given. This results in rapid disappearance of biochemical and haematological abnormalities. However, neurological symptoms are slower to respond to treatment and severe neurological deficits often persist.

The *cblG* disorder is caused by mutations in the MTR gene (Leclerc *et al*, 1996). At least 33 patients make up the *cblG* complementation group. A single mutation, 3518C → T

(Pro1173Leu), was identified in 16 of 24 patients, which may facilitate diagnosis (Watkins *et al*, 2002).

At least 27 patients form the *cblE* complementation group. Over time, the cob(I)alamin cofactor of MTR becomes oxidised to cob(II)alamin, rendering the enzyme inactive. The gene involved, *MTRR*, encodes methionine synthase reductase, an enzyme required for the reductive reactivation of MTR, thereby restoring its folate/Cbl-dependent ability to remethylate homocysteine to methionine. The gene has been extensively characterised and a dozen mutations described (Leclerc *et al*, 1998; Wilson *et al*, 1999a).

Methylmalonic acidemia is a rare disorder caused by decreased activity of mitochondrial MCM, due to mutations affecting either the enzyme itself (the *MUT* gene) (Worgan *et al*, 2006) or genes involved in the synthesis of AdoCbl (*cblA*, *cblB* and *cblH*). MCM catalyses the AdoCbl-dependent rearrangement of L-methylmalonyl-CoA to succinyl-CoA. This is an important intermediary step in the catabolism of branched-chain amino acids and odd chain fatty acids.

Patients in the *cblA*, *cblB*, some *cblD* and *cblH* complementation groups have disorders that affect AdoCbl, but not MeCbl synthesis (Fig 2). The genes responsible for *cblA* (*MMAA*) and *cblB* (*MMAB*) have been cloned. The product of *MMAA* may function in aiding translocation of Cbl into mitochondria, in maintaining the stability of MCM, or in intramitochondrial reduction reactions. The product of *MMAB* is the enzyme ATP:cobalamin adenosyltransferase, which catalyses the synthesis of AdoCbl (Dobson *et al*, 2002a,b; Lerner-Ellis *et al*, 2004, 2006b).

Patients affected with *cblA* and *cblB* have similar clinical and biochemical features. Most present with an acidotic crisis in the first year of life, many in the neonatal period. Symptoms include vomiting, dehydration, tachypnea, lethargy, failure to thrive, developmental delay, hypotonia and encephalopathy. They have elevated MMA levels in the blood and urine. Toxic levels of MMA can be associated with anaemia, leucopenia and thrombocytopenia. Chronic renal failure may be a long-term complication. Some late-onset cases have occurred in children who avoided protein in their diet. Patients do not have megaloblastic anaemia. Folate, Cbl and tHcy levels are normal. Treatment consists of dietary restriction of protein and oral or injected OHCbl. In addition, carnitine supplementation is essential. Biochemical monitoring is necessary to ensure dietary balance and the success of therapy.

At least 60 patients with *cblA* and about 40 mutations in *MMAA* have been described. One mutation, 433C → T, was identified in 21/37 patients. To date, there are fewer than 50 patients with *cblB*. Nineteen mutations in *MMAB* have been documented. The *cblB* disorder generally has a more severe clinical course and is less responsive to therapy than the *cblA* disorder. This has been attributed to the relative 'leakiness' of the *cblA* mutations (Rosenblatt & Fenton, 2001).

A single patient belonging to the cblD complementation group excreted MMA, consistent with functional MCM deficiency, without any impairment of MTR function

(Suormala *et al*, 2004) (see above). A further patient with *cblA*-type MCM deficiency has been designated as *cblH*, based on the ability of his cells to complement other known *cblA* and *cblB* cells (Watkins *et al*, 2000).

Inborn errors of folate transport and metabolism

The rare and sometimes life-threatening inborn errors of folate metabolism consist of hereditary (or congenital) folate malabsorption (HFM), MTHFR deficiency, glutamate formiminotransferase deficiency and functional MTR deficiency (see *cblC–G* above). A number of additional defects in folate metabolism have been described, including cellular folate uptake defects, dihydrofolate reductase deficiency and methenyltetrahydrofolate cyclohydrolase deficiency. These lack sufficient definition at the present time (Rosenblatt & Fenton, 2001; Morel & Rosenblatt, 2006).

The intestinal absorption of folate is less complex than that of Cbl. Dietary folate polyglutamates are hydrolysed to simple folates. These are absorbed in the duodenum and upper small intestine and transported to the liver, becoming 5-methyltetrahydrofolate, the principle circulating folate form. The existence of patients with HFM is the best evidence available for a specific intestinal folate transporter, one that seems to be shared by the choroid plexus.

Hereditary folate malabsorption has been described in about 30 patients in a dozen families (Geller *et al*, 2002). Newborns have presented at 2–6 months of age with megaloblastic anaemia, mucositis, diarrhoea, failure to thrive, recurrent infections and neurological deficits including seizures. They are unable to absorb folic acid, 5-methyltetrahydrofolate or 5-formyltetrahydrofolate (folinic acid). Consanguinity has been described, as well as deaths in siblings, suggestive of recessive inheritance. Both the serum and spinal fluid folate levels are extremely low. There is loss of the normal spinal fluid to serum folate ratio of 3:1, indicative of failure to transport folates across the choroid plexus. Treatment with daily folinic acid by injection to maintain the spinal fluid folate level near the normal range can be associated with normal development. In the latest reported family, the first five children died and the last three all had HFM (Jebnoun *et al*, 2001).

Severe MTHFR deficiency is a rare autosomal recessive disorder with severe hyperhomocysteinaemia and homocystinuria, together with low or low normal plasma methionine. Patients show a range of neurological and vascular complications, including developmental delay, mental retardation, seizures, motor and gait abnormalities and thromboses. They do not have megaloblastic anaemia or methylmalonic aciduria. The severity of the clinical course correlates reasonably well with age of onset and residual enzyme activity. Over 100 patients and more than 50 disease-causing mutations have been reported.

The disease is very resistant to treatment, but betaine has improved the overall prognosis. In addition, treatment with folate, Cbl, methionine, pyridoxine, riboflavin and carnitine

has been tried. Criteria for improvement have included reduction of tHcy, increase in methionine and the clinical picture. Early diagnosis and treatment may modify the poor prognosis associated with neurological involvement (Rosenblatt & Fenton, 2001). A case report describes the fatal outcome of nitrous oxide anaesthesia in a patient with this disorder (Selzer *et al*, 2003)

Glutamate formiminotransferase-cyclodeaminase (FTCD) deficiency is a rare autosomal recessive disorder that has been described in fewer than twenty patients. Two clinical phenotypes have been delineated: a severe form, found in five patients in Japan, and a mild form, identified in patients from a variety of ethnic origins. Severe cases had mental and physical retardation, a raised serum folate level and increased formiminoglutamate (FIGLU) in the urine after administration of L-histidine. Liver biopsies revealed enzyme activity of 33% and 30–50% in two patients. In mild cases, FIGLU excretion has been far higher, without an L-histidine load, the serum folate was normal and there was slight developmental delay. Mutations in the formiminotransferase-cyclodeaminase (*FTCD*) gene have now been identified in three patients with the mild phenotype (Hilton *et al*, 2003). Expression of the mutant enzymes revealed activity levels of 57% and 61% of wild-type. What distinguishes these two phenotypes remains unclear.

It is not clear whether reducing FIGLU excretion is of any clinical value. Folic acid treatment reduced FIGLU excretion in two patients in one family, but had no effect in six other unrelated patients. Methionine and pyridoxine have also been tried.

Gene polymorphisms and neural tube defects

There is much evidence to suggest a genetic basis of NTDs (Botto *et al*, 1999). Homozygosity (TT genotype) for the 677C → T (Ala222Val) mutation in the *MTHFR* gene is associated with a 70% lower enzyme activity, whereas heterozygosity (CT genotype) is associated with a 30% decrease in activity. The *MTHFR* 677C → T polymorphism was the first and remains the clearest and most extensively studied genetic risk factor for NTDs (Whitehead *et al*, 1995; van der Put *et al*, 1995). A meta-analysis was conducted of case–control studies of the risk for an NTD-affected fetus or baby in relation to the 677C → T genotype in fetuses or patients (21 studies), in mothers (16 studies) and in fathers (nine studies). Comparing the TT with the CC genotype, the estimated relative risk for an NTD-affected pregnancy or child was 1·76 [95% confidence interval (CI) 1·45–2·14], 1·92 (95% CI 1·51–2·45) and 1·27 (95% CI 0·91–1·77) when the genotype was carried by the infant, mother and father respectively. The corresponding relative risk estimates comparing the CT with CC genotype were 1·26 (95% CI 1·09–1·45), 1·24 (95% CI 1·04–1·47) and 1·21 (95% CI 0·97–1·50) respectively (Vollset & Botto, 2004).

The risk is highest in those whose folate levels are reduced (Christensen *et al*, 1999) and is lowered in those taking folate supplements. While many studies have confirmed this finding, the *MTHFR* 677C → T polymorphism accounts for only part of the genetic risk. Other polymorphisms have therefore been examined for their impact on NTD incidence. The homozygous 1298A → C (Glu429Ala) *MTHFR* polymorphism reduces enzyme activity to about sixty per cent (Weisberg *et al*, 1998). It was found to be a risk factor for NTDs in Italy (De Marco *et al*, 2002), but not in Ireland (Parle-McDermott *et al*, 2003).

By means of the two-step transmission-disequilibrium test, it has been shown that mutations in both the *MTR* (2756A → G) and the *MTRR* (66A → G) genes influence the risk of NTDs through the maternal more than through the embryonic genotype (Doolin *et al*, 2002). A polymorphism of *MTR* (2756A → G) alone and of *MTRR* (66A → G) and *TCN* (776C → G) each in combination with *MTHFR* 677C → T increase the risk of spina bifida (Guéant-Rodriguez *et al*, 2003). The *TCN* 776C → G polymorphism has been linked to reduced serum holo-TC concentrations and elevated plasma tHcy and may prove to be a risk factor for NTDs (von Castel-Dunwoody *et al*, 2005). The *MTR* (2756A → G) polymorphism has been associated particularly with NTDs affecting the lumbosacral region (Pietrzyk *et al*, 2003). These genes encode proteins involved in Cbl transport and Cbl-dependent synthesis of methionine, providing a genetic basis for the involvement of Cbl insufficiency in the causation of spina bifida (Kirke *et al*, 1993; Steen *et al*, 1998; Wilson *et al*, 1999b; Groenen *et al*, 2004).

Gene polymorphisms of other folate-related enzymes or transporters implicated in the aetiology of NTDs include the reduced folate carrier (*RFC1*) (80G → A) (De Marco *et al*, 2003; Morin *et al*, 2003; Relton *et al*, 2003; Pei *et al*, 2005); thymidylate synthase (*TYMS*) (28-bp tandem repeat) in non-Hispanic whites (Volcik *et al*, 2003a); glutamate carboxypeptidase II (*GCPII*) (1561C → T) (Relton *et al*, 2003, 2004), the trifunctional enzyme (*MTHFD1*) (1958G → A) (Brody *et al*, 2002); and cystathionine beta-synthase (*CβS*) (844ins68) (Relton *et al*, 2004). Conflicting results have been reported for a number of these polymorphisms, both folate- and Cbl-related (Parle-McDermott *et al*, 2003; Wilding *et al*, 2004; O'Leary *et al*, 2005, 2006). A polymorphism in cytoplasmic serine hydroxymethyltransferase (*cSHMT*) (1420C → T) has been linked to the risk of cardiovascular disease, but has not yet been studied in relation to NTDs (Lim *et al*, 2005).

Gene polymorphisms and down syndrome

Of considerable interest are reports linking folate insufficiency and/or polymorphisms with other birth defects, in particular Down syndrome (James, 2004; Eskes, 2006). Raised tHcy is a risk factor for Down syndrome (Bosco *et al*, 2003; Sheth & Sheth, 2003; Takamura *et al*, 2004; da Silva *et al*, 2005). The *MTRR* 66A → G, the *MTR* 2756A → G and the *MTHFR* 677C → T polymorphisms in association with increased tHcy or decreased folate or Cbl increase the maternal risk of

having a child with Down syndrome (James *et al*, 1999; Hobbs *et al*, 2000; O'Leary *et al*, 2002; Bosco *et al*, 2003). There is a significant increase in Down syndrome frequency in mothers who have had a child with spina bifida and vice versa (Barkai *et al*, 2003). Fortification of food with folate has not decreased the incidence of Down syndrome as yet, probably because a much higher level of folate is needed to prevent genomic instability associated with marginal folate deficiency (James, 2004).

Food folate fortification and other malformations

A survey conducted in the USA following food fortification has found modest, yet statistically significant reductions in transposition of the great arteries (12%), cleft palate only (12%), pyloric stenosis (5%), upper limb reduction defects (11%) and omphalocele (21%). More substantial subgroup decreases were observed for renal agenesis among programs that conduct prenatal surveillance (28%), for common truncus among Hispanics (45%), and for upper limb reduction defects among Hispanics (44%) (Canfield *et al*, 2005).

Acknowledgements

References

Allen, R.H., Stabler, S.P., Savage, D.G. & Lindenbaum, J. (1990) Diagnosis of cobalamin deficiency I: usefulness of serum methylmalonic acid and total homocysteine concentrations. *American Journal of Hematology*, **34**, 90–98.

Aminoff, M., Carter, J.E., Chadwick, R.B., Johnson, C., Gräsbeck, R., Abdelaal, M.A., Broch, H., Jenner, L.B., Verroust, P.J., Moestrup, S.K., de la Chapelle, A. & Krahe, R. (1999) Mutations in CUBN, encoding the intrinsic factor-vitamin B12 receptor, cubilin, cause hereditary megaloblastic anaemia 1. *Nature Genetics*, **21**, 309–313.

Barkai, G., Arbuzova, S., Berkenstadt, M., Heifetz, S. & Cuckle, H. (2003) Frequency of Down's syndrome and neural-tube defects in the same family. *The Lancet*, **361**, 1331–1335.

Berry, R.J., Li, Z., Erickson, J.D., Li, S., Moore, C.A., Wang, H., Mulinare, J., Zhao, P., Wong, L.Y., Gindler, J., Hong, S.X. & Correa, A. (1999) Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. *New England Journal of Medicine*, **341**, 1485–1490.

Birn, H., Willnow, T.E., Nielsen, R., Norden, A.G., Bönsch, C., Moestrup, S.K., Nexø, E. & Christensen, E.I. (2002) Megalin is essential for renal proximal tubule reabsorption and accumulation of transcobalamin-B(12). *American Journal of Physiology – Renal Physiology*, **282**, F408–F416.

Bjørke Monsen, A.L., Ueland, P.M., Vollset, S.E., Guttormsen, A.B., Markestad, T., Solheim, E. & Refsum, H. (2001) Determinants of cobalamin status in newborns. *Pediatrics*, **108**, 624–630.

Bondevik, G.T., Schneede, J., Refsum, H., Lie, R.T., Ulstein, M. & Kvåle, G. (2001) Homocysteine and methylmalonic acid levels in pregnant Nepali women. Should cobalamin supplementation be considered? *European Journal of Clinical Nutrition*, **55**, 856–864.

Bosco, P., Guéant-Rodriguez, R.M., Anello, G., Barone, C., Namour, F., Caraci, F., Romano, A., Romano, C. & Guéant, J.L. (2003) Methionine synthase (MTR) 2756 (A → G) polymorphism, double heterozygosity methionine synthase 2756 AG/methionine synthase reductase (MTRR) 66 AG, and elevated homocysteinemia are three risk factors for having a child with Down syndrome. *American Journal of Medical Genetics A*, **121**, 219–224.

Botto, L.D., Moore, C.A., Khoury, M.J. & Erickson, J.D. (1999) Neural-tube defects. *New England Journal of Medicine*, **341**, 1509–1519.

Botto, L.D., Lisi, A., Robert-Gnansia, E., Erickson, J.D., Vollset, S.E., Mastroiacovo, P., Botting, B., Cocchi, G., de Vigan, C., de Walle, H., Feijoo, M., Irgens, L.M., McDonnell, B., Merlob, P., Ritvanen, A., Scarano, G., Siffel, C., Metneki, J., Stoll, C., Smithells, R. & Goujard, J. (2005) International retrospective cohort study of neural tube defects in relation to folic acid recommendations: are the recommendations working? *British Medical Journal*, **330**, 571–576.

Brent, R.L. & Oakley, Jr, G.P. (2005) The food and drug administration must require the addition of more folic acid in 'enriched' flour and other grains. *Pediatrics*, **116**, 753–755.

Brent, R.L., Oakley, Jr, G.P. & Mattison, D.R. (2000) The unnecessary epidemic of folic acid-preventable spina bifida and anencephaly. *Pediatrics*, **106**, 825–827.

Brody, L.C., Conley, M., Cox, C., Kirke, P.N., McKeever, M.P., Mills, J.L., Molloy, A.M., O'Leary, V.B., Parle-McDermott, A., Scott, J.M. & Swanson, D.A. (2002) A polymorphism, R653Q, in the trifunctional enzyme methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase/formyltetrahydrofolate synthetase is a maternal genetic risk factor for neural tube defects: report of the Birth Defects Research Group. *American Journal of Human Genetics*, **71**, 1207–1215.

Brown, J.E., Jacobs, Jr, D.R., Hartman, T.J., Barosso, G.M., Stang, J.S., Gross, M.D. & Zeuske, M.A. (1997) Predictors of red cell folate level in women attempting pregnancy. *Journal of the American Medical Association*, **277**, 548–552.

Canfield, M.A., Collins, J.S., Botto, L.D., Williams, L.J., Mai, C.T., Kirby, R.S., Pearson, K., Devine, O., Mulinare, J. & National Birth Defects Prevention Network (2005) Changes in the birth prevalence of selected birth defects after grain fortification with folic acid in the United States: findings from a multi-state population-based study. *Birth Defects Research A Clinical and Molecular Teratology*, **73**, 679–689.

von Castel-Dunwoody, K.M., Kauwell, G.P., Shelnutt, K.P., Vaughn, J.D., Griffin, E.R., Maneval, D.R., Theriaque, D.W. & Bailey, L.B. (2005) Transcobalamin 776C → G polymorphism negatively affects vitamin B-12 metabolism. *American Journal of Clinical Nutrition*, **81**, 1436–1441.

Casterline, J.E., Allen, L.H. & Ruel, M.T. (1997) Vitamin B-12 deficiency is very prevalent in lactating Guatemalan women and their infants at three months postpartum. *Journal of Nutrition*, **127**, 1966–1972.

Centers for Disease Control and Prevention (CDC) (2003) Neurologic impairment in children associated with maternal dietary deficiency of cobalamin – Georgia, 2001. *Morbidity and Mortality Weekly Report*, **52**, 61–64.

Centers for Disease Control and Prevention (2004) Spina bifida and anencephaly before and after folic acid mandate – United States, 1995–1996 and 1999–2000. *Morbidity and Mortality Weekly Report*, **53**, 362–365.

Christensen, B., Arbour, L., Tran, P., Leclerc, D., Sabbaghian, N., Platt, R., Gilfix, B.M., Rosenblatt, D.S., Gravel, R.A., Forbes, P. & Rozen, R. (1999) Genetic polymorphisms in methylenetetrahydrofolate reductase and methionine synthase, folate levels in red blood cells, and risk of neural tube defects. *American Journal of Medical Genetics*, **84**, 151–157.

Codazzi, D., Sala, F., Parini, R. & Langer, M. (2005) Coma and respiratory failure in a child with severe vitamin B(12) deficiency. *Pediatric Critical Care Medicine*, **6**, 483–485.

Czeizel, A.E. & Dudas, I. (1992) Prevention of the first occurrence of neural tube defects by periconceptual vitamin supplementation. *New England Journal of Medicine*, **327**, 1832–1835.

De Marco, P., Calevo, M.G., Moroni, A., Arata, L., Merello, E., Finnell, R.H., Zhu, H., Andreussi, L., Cama, A. & Capra, V. (2002) Study of MTHFR and MS polymorphisms as risk factors for NTD in the Italian population. *Journal of Human Genetics*, **47**, 319–324.

De Marco, P., Calevo, M.G., Moroni, A., Merello, E., Raso, A., Finnell, R.H., Zhu, H., Andreussi, L., Cama, A. & Capra, V. (2003) Reduced folate carrier polymorphism (80A → G) and neural tube defects. *European Journal of Human Genetics*, **11**, 245–252.

Dietrich, M., Brown, C.J. & Block, G. (2005) The effect of folate fortification of cereal-grain products on blood folate status, dietary folate intake, and dietary folate sources among adult non-supplement users in the United States. *Journal of the American College of Nutrition*, **24**, 266–274.

Dobson, C.M., Wai, T., Leclerc, D., Wilson, A., Wu, X., Doré, C., Hudson, T., Rosenblatt, D.S. & Gravel, R.A. (2002a) Identification of the gene responsible for the cblA complementation group of vitamin B12-responsive methylmalonic acidemia based on analysis of prokaryotic gene arrangements. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 15554–15559.

Dobson, C.M., Wai, T., Leclerc, D., Kadir, H., Narang, M., Lerner-Ellis, J.P., Hudson, T.J., Rosenblatt, D.S. & Gravel, R.A. (2002b) Identification of the gene responsible for the cblB complementation group of vitamin B12-dependent methylmalonic aciduria. *Human Molecular Genetics*, **11**, 3361–3369.

Doolin, M.T., Barbaux, S., McDonnell, M., Hoess, K., Whitehead, A.S. & Mitchell, L.E. (2002) Maternal genetic effects, exerted by genes involved in homocysteine remethylation, influence the risk of spina bifida. *American Journal of Human Genetics*, **71**, 1222–1226.

van Dusseldorp, M., Schneede, J., Refsum, H., Ueland, P.M., Thomas, C.M., de Boer, E. & van Staveren, W.A. (1999) Risk of persistent cobalamin deficiency in adolescents fed a macrobiotic diet in early life. *American Journal of Clinical Nutrition*, **69**, 664–671.

Emery, E.S., Homans, A.C. & Colletti, R.B. (1997) Vitamin B12 deficiency: a cause of abnormal movements in infants. *Pediatrics*, **99**, 255–256.

Eskes, T.K. (2006) Abnormal folate metabolism in mothers with Down syndrome offspring: review of the literature. *European Journal of Obstetrics, Gynecology and Reproductive Biology*, **124**, 130–133.

Food and Drug Administration (1996) Food standards: amendment of standards of identity for enriched grain products to require addition of folic acid. *Federal Register*, **61**, 8781–8797.

Fyfe, J.C., Madsen, M., Højrup, P., Christensen, E.I., Tanner, S.M., de la Chapelle, A., He, Q. & Moestrup, S.K. (2004) The functional cobalamin (vitamin B12)-intrinsic factor receptor is a novel complex of cubilin and amnionless. *Blood*, **103**, 1573–1579.

Garcia-Casal, M.N., Osorio, C., Landaeta, M., Leets, I., Matus, P., Fazzino, F. & Marcos, E. (2005) High prevalence of folic acid and vitamin B12 deficiencies in infants, children, adolescents and pregnant women in Venezuela. *European Journal of Clinical Nutrition*, **59**, 1064–1070.

Geller, J., Kronn, D., Jayabose, S. & Sandoval, C. (2002) Hereditary folate malabsorption: family report and review of the literature. *Medicine (Baltimore)*, **81**, 51–68.

Goraya, J.S. (1998) Persistence of neurological damage induced by dietary vitamin B-12 deficiency in infancy. *Archives of Disease in Childhood*, **78**, 395.

Gordon, M.M., Brada, N., Remacha, A., Badell, I., del Río, E., Baiget, M., Santer, R., Quadros, E.V., Rothenberg, S.P. & Alpers, D.H. (2004) A genetic polymorphism in the coding region of the gastric intrinsic factor gene (GIF) is associated with congenital intrinsic factor deficiency. *Human Mutation*, **23**, 85–91.

Groenen, P.M., van Rooij, I.A., Peer, P.G., Gooskens, R.H., Zielhuis, G.A. & Steegers-Theunissen, R.P. (2004) Marginal maternal vitamin B12 status increases the risk of offspring with spina bifida. *American Journal of Obstetrics and Gynecology*, **191**, 11–17.

Guéant-Rodriguez, R.M., Rendeli, C., Namour, B., Venuti, L., Romano, A., Anello, G., Bosco, P., Debard, R., Gérard, P., Viola, M., Salvaggio, E. & Guéant, J.L. (2003) Transcobalamin and methionine synthase reductase mutated polymorphisms aggravate the risk of neural tube defects in humans. *Neuroscience Letters*, **344**, 189–192.

Guerra-Shinohara, E.M., Morita, O.E., Peres, S., Pagliusi, R.A., Sampaio Neto, L.F., D'Almeida, V., Irazusta, S.P., Allen, R.H. & Stabler, S.P. (2004) Low ratio of S-adenosylmethionine to S-adenosylhomocysteine is associated with vitamin deficiency in Brazilian pregnant women and newborns. *American Journal of Clinical Nutrition*, **80**, 1312–1321.

He, Q., Madsen, M., Kilkenney, A., Gregory, B., Christensen, E.I., Vorum, H., Højrup, P., Schäffer, A.A., Kirkness, E.F., Tanner, S.M., de la Chapelle, A., Giger, U., Moestrup, S.K. & Fyfe, J.C. (2005) Amnionless function is required for cubilin brush-border expression and intrinsic factor-cobalamin (vitamin B12) absorption in vivo. *Blood*, **106**, 1447–1453.

Hilton, J.F., Christensen, K.E., Watkins, D., Raby, B.A., Renaud, Y., de la Luna, S., Estivill, X., MacKenzie, R.E., Hudson, T.J. & Rosenblatt, D.S. (2003) The molecular basis of glutamate formiminotransferase deficiency. *Human Mutation*, **22**, 67–73.

Hobbs, C.A., Sherman, S.L., Yi, P., Hopkins, S.E., Torfs, C.P., Hine, R.J., Pogribna, M., Rozen, R. & James, S.J. (2000) Polymorphisms in genes involved in folate metabolism as maternal risk factors for Down syndrome. *American Journal of Human Genetics*, **67**, 623–630.

Huemer, M., Simma, B., Fowler, B., Suormala, T., Bodamer, O.A. & Sass, J.O. (2005) Prenatal and postnatal treatment in cobalamin C defect. *Journal of Pediatrics*, **147**, 469–472.

Hultberg, B., Isaksson, A., Nilsson, K. & Gustafson, L. (2001) Markers for the functional availability of cobalamin/folate and their association with neuropsychiatric symptoms in the elderly. *International Journal of Geriatric Psychiatry*, **16**, 873–878.

Hvas, A.M. & Nexø, E. (2005) Holotranscobalamin – a first choice assay for diagnosing early vitamin B deficiency? *Journal of Internal Medicine*, **257**, 289–298.

James, S.J. (2004) Maternal metabolic phenotype and risk of Down syndrome: beyond genetics. *American Journal of Medical Genetics A*, **127**, 1–4.

James, S.J., Pogribna, M., Pogribny, I.P., Melnyk, S., Hine, R.J., Gibson, J.B., Yi, P., Tafoya, D.L., Swenson, D.H., Wilson, V.L. & Gaylor, D.W. (1999) Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down syndrome. *American Journal of Clinical Nutrition*, **70**, 495–501.

Jebnoun, S., Kacem, S., Mokrani, C.H., Chabchoub, A., Khrouf, N. & Zittoun, J. (2001) A family study of congenital malabsorption of folate. *Journal of Inherited Metabolic Diseases*, **24**, 749–750.

Kirke, P.N., Molloy, A.M., Daly, L.E., Burke, H., Weir, D.G. & Scott, J.M. (1993) Maternal plasma folate and vitamin B12 are independent risk factors for neural tube defects. *Quarterly Journal of Medicine*, **86**, 703–708.

Korenke, G.C., Hunneman, D.H., Eber, S. & Hanefeld, F. (2004) Severe encephalopathy with epilepsy in an infant caused by subclinical maternal pernicious anaemia: case report and review of the literature. *European Journal of Pediatrics*, **163**, 196–201.

Kristiansen, M., Aminoff, M., Jacobsen, C., de la Chapelle, A., Krahe, R., Verroust, P.J. & Moestrup, S.K. (2000) Cubilin P1297L mutation associated with hereditary megaloblastic anemia 1 causes impaired recognition of intrinsic factor-vitamin B(12) by cubilin. *Blood*, **96**, 405–409.

Leclerc, D., Campeau, E., Goyette, P., Adjalla, C.E., Christensen, B., Ross, M., Eydoux, P., Rosenblatt, D.S., Rozen, R. & Gravel, R.A. (1996) Human methionine synthase: cDNA cloning and identification of mutations in patients of the cblG complementation group of folate/cobalamin disorders. *Human Molecular Genetics*, **5**, 1867–1874.

Leclerc, D., Wilson, A., Dumas, R., Gafuik, C., Song, D., Watkins, D., Heng, H.H., Rommens, J.M., Scherer, S.W., Rosenblatt, D.S. & Gravel, R.A. (1998) Cloning and mapping of a cDNA for methionine synthase reductase, a flavoprotein defective in patients with homocystinuria. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 3059–3064.

Lentz, S.R. (2005) Mechanisms of homocysteine-induced atherothrombosis. *Journal of Thrombosis and Haemostasis*, **3**, 1646–1654.

Lerner-Ellis, J.P., Dobson, C.M., Wai, T., Watkins, D., Tirone, J.C., Leclerc, D., Doré, C., Lepage, P., Gravel, R.A. & Rosenblatt, D.S. (2004) Mutations in the MMAA gene in patients with the cblA disorder of vitamin B12 metabolism. *Human Mutation*, **24**, 509–516.

Lerner-Ellis, J.P., Tirone, J.C., Pawelek, P.D., Doré, C., Atkinson, J.L., Watkins, D., Morel, C.F., Fujiwara, T.M., Moras, E., Hosack, A.R., Dunbar, G.V., Antonicka, H., Forgetta, V., Dobson, C.M., Leclerc, D., Gravel, R.A., Shoubridge, E.A., Coulton, J.W., Lepage, P., Rommens, J.M., Morgan, K. & Rosenblatt, D.S. (2006a) Identifica-
tion of the gene responsible for methylmalonic aciduria and homocystinuria, cblC type. *Nature Genetics*, **38**, 93–100.

Lerner-Ellis, J.P., Gradinger, A.B., Watkins, D., Tirone, J.C., Villeneuve, A., Dobson, C.M., Montpetit, A., Lepage, P., Gravel, R.A. & Rosenblatt, D.S. (2006b) Mutation and biochemical analysis of patients belonging to the cblB complementation class of vitamin B12-dependent methylmalonic aciduria. *Molecular Genetics and Metabolism*, **87**, 219–225.

Lewis, S.J., Ebrahim, S. & Davey Smith, G. (2005) Meta-analysis of MTHFR 677C->T polymorphism and coronary heart disease: does totality of evidence support causal role for homocysteine and preventive potential of folate? *British Medical Journal*, **331**, 1053–1058.

Lim, U., Peng, K., Shane, B., Stover, P.J., Litonjua, A.A., Weiss, S.T., Gaziano, J.M., Strawderman, R.L., Raiszadeh, F., Selhub, J., Tucker, K.L. & Cassano, P.A. (2005) Polymorphisms in cytoplasmic serine hydroxymethyltransferase and methylenetetrahydrofolate reductase affect the risk of cardiovascular disease in men. *Journal of Nutrition*, **135**, 1989–1994.

Lindblad, B., Zaman, S., Malik, A., Martin, H., Ekström, A.M., Amu, S., Holmgren, A. & Norman, M. (2005) Folate, vitamin B12, and homocysteine levels in South Asian women with growth-retarded fetuses. *Acta Obstetrica et Gynecologica Scandinavica*, **84**, 1055–61.

Louwman, M.W., van Dusseldorp, M., van de Vijver, F.J., Thomas, C.M., Schneede, J., Ueland, P.M., Refsum, H. & van Staveren, W.A. (2000) Signs of impaired cognitive function in adolescents with marginal cobalamin status. *American Journal of Clinical Nutrition*, **72**, 762–769.

Lövblad, K., Ramelli, G., Remonda, L., Nirkko, A.C., Ozdoba, C. & Schroth, G. (1997) Retardation of myelination due to dietary vitamin B12 deficiency: cranial MRI findings. *Pediatric Radiology*, **27**, 155–158.

Lowenstein, L., Lalonde, M., Deschenes, E.B. & Shapiro, L. (1960) Vitamin B12 in pregnancy and the puerperium. *American Journal of Clinical Nutrition*, **8**, 265–275.

Mills, J.L., Von Kohorn, I., Conley, M.R., Zeller, J.A., Cox, C., Williamson, R.E. & Dufour, D.R. (2003) Low vitamin B-12 concentrations in patients without anemia: the effect of folic acid fortification of grain. *American Journal of Clinical Nutrition*, **77**, 1474–1477.

Milman, N., Byg, K.E., Hvas, A.M., Bergholt, T. & Eriksen, L. (2006) Erythrocyte folate, plasma folate and plasma homocysteine during normal pregnancy and postpartum: a longitudinal study comprising 404 Danish women. *European Journal of Haematology*, **76**, 200–205.

Moestrup, S.K. & Verroust, P.J. (2001) Megalin- and cubilin-mediated endocytosis of protein-bound vitamins, lipids, and hormones in polarized epithelia. *Annual Review of Nutrition*, **21**, 407–428.

Morel, C. & Rosenblatt, D. (2006) Inborn errors of folate and cobalamin transport and metabolism. In: *Essential Pediatric Endocrinology and Metabolism, 1st edn.* (ed. by K. Sarafoglou, G. Hoffmann & T. Moran), McGraw Hill, New York (in press).

Morel, C.F., Watkins, D., Scott, P., Rinaldo, P. & Rosenblatt, D.S. (2005) Prenatal diagnosis for methylmalonic acidemia and inborn errors of vitamin B(12) metabolism and transport. *Molecular Genetics and Metabolism*, **86**, 160–171.

Morin, I., Devlin, A.M., Leclerc, D., Sabbaghian, N., Halsted, C.H., Finnell, R. & Rozen, R. (2003) Evaluation of genetic variants in the reduced folate carrier and in glutamate carboxypeptidase II for spina bifida risk. *Molecular Genetics and Metabolism*, **79**, 197–200.

MRC Vitamin Study Research Group (1991) Prevention of neural tube defects: results of the Medical Research Council Vitamin study. *The Lancet*, **338**, 131–137.

Namour, F., Helfer, A.C., Quadros, E.V., Alberto, J.M., Bibi, H.M., Orning, L., Rosenblatt, D.S. & Jean-Louis, G. (2003) Transcobalamin deficiency due to activation of an intra exonic cryptic splice site. *British Journal of Haematology*, **123,** 915–20.

O'Leary, V.B., Parle-McDermott, A., Molloy, A.M., Kirke, P.N., Johnson, Z., Conley, M., Scott, J.M. & Mills, J.L. (2002) MTRR and MTHFR polymorphism: link to Down syndrome? *American Journal of Medical Genetics*, **107,** 151–155.

O'Leary, V.B., Mills, J.L., Pangilinan, F., Kirke, P.N., Cox, C., Conley, M., Weiler, A., Peng, K., Shane, B., Scott, J.M., Parle-McDermott, A., Molloy, A.M., Brody, L.C. & Members of the Birth Defects Research Group (2005) Analysis of methionine synthase reductase polymorphisms for neural tube defects risk association. *Molecular Genetics and Metabolism*, **85,** 220–227.

O'Leary, V.B., Pangilinan, F., Cox, C., Parle-McDermott, A., Conley, M., Molloy, A.M., Kirke, P.N., Mills, J.L., Brody, L.C., Scott, J.M. & Members of the Birth Defects Research Group (2006) Reduced folate carrier polymorphisms and neural tube defect risk. *Molecular Genetics and Metabolism*, **87,** 364–369.

Oakley, Jr, G.P., Bell, K.N. & Weber, M.B. (2004) Recommendations for accelerating global action to prevent folic acid-preventable birth defects and other folate-deficiency diseases: meeting of experts on preventing folic acid-preventable neural tube defects. *Birth Defects Research (Part A) Clinical and Molecular Teratology*, **70,** 835–837.

Park, H., Kim, Y.J., Ha, E.H., Kim, K.N. & Chang, N. (2004) The risk of folate and vitamin B(12) deficiencies associated with hyperhomocysteinemia among pregnant women. *American Journal of Perinatology*, **21,** 469–475.

Parle-McDermott, A., Mills, J.L., Kirke, P.N., O'Leary, V.B., Swanson, D.A., Pangilinan, F., Conley, M., Molloy, A.M., Cox, C., Scott, J.M. & Brody, L.C. (2003) Analysis of the MTHFR 1298A → C and 677C → T polymorphisms as risk factors for neural tube defects. *Journal of Human Genetics*, **48,** 190–193.

Pei, L., Zhu, H., Ren, A., Li, Z., Hao, L., Finnell, R.H. & Li, Z. (2005) Reduced folate carrier gene is a risk factor for neural tube defects in a Chinese population. *Birth Defects Research (Part A) Clinical and Molecular Teratology*, **73,** 430–433.

Pfeiffer, C.M., Caudill, S.P., Gunter, E.W., Osterloh, J. & Sampson, E.J. (2005) Biochemical indicators of B vitamin status in the US population after folic acid fortification: results from the National Health and Nutrition Examination Survey 1999–2000. *American Journal of Clinical Nutrition*, **82,** 442–450.

Pietrzyk, J.J., Bik-Multanowski, M., Sanak, M. & Twardowska, M. (2003) Polymorphisms of the 5,10-methylenetetrahydrofolate and the methionine synthase reductase genes as independent risk factors for spina bifida. *Journal of Applied Genetics*, **44,** 111–113.

van der Put, N.M., Steegers-Theunissen, R.P., Frosst, P., Trijbels, F.J., Eskes, T.K., van den Heuvel, L.P., Mariman, E.C., den Heyer, M., Rozen, R. & Blom, H.J. (1995) Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. *The Lancet*, **346,** 1070–1071.

Qian, L., Quadros, E.V., Regec, A., Zittoun, J. & Rothenberg, S.P. (2002) Congenital transcobalamin II deficiency due to errors in RNA editing. *Blood Cells, Molecules and Diseases*, **28,** 134–142.

Quadri, P., Fragiacomo, C., Pezzati, R., Zanda, E., Forloni, G., Tettamanti, M. & Lucca, U. (2004) Homocysteine, folate, and vitamin B-12 in mild cognitive impairment, Alzheimer disease, and vascular dementia. *American Journal of Clinical Nutrition*, **80,** 114–122.

Ray, J.G., Meier, C., Vermeulen, M.J., Boss, S., Wyatt, P.R. & Cole, D.E. (2002) Association of neural tube defects and folic acid food fortification in Canada. *The Lancet*, **360,** 2047–2048.

Reghu, A., Hosdurga, S., Sandhu, B. & Spray, C. (2005) Vitamin B12 deficiency presenting as oedema in infants of vegetarian mothers. *European Journal of Pediatrics*, **164,** 257–258.

Relton, C.L., Wilding, C.S., Jonas, P.A., Lynch, S.A., Tawn, E.J. & Burn, J. (2003) Genetic susceptibility to neural tube defect pregnancy varies with offspring phenotype. *Clinical Genetics*, **64,** 424–428.

Relton, C.L., Wilding, C.S., Pearce, M.S., Laffling, A.J., Jonas, P.A., Lynch, S.A., Tawn, E.J. & Burn, J. (2004) Gene-gene interaction in folate-related genes and risk of neural tube defects in a UK population. *Journal of Medical Genetics*, **41,** 256–260.

Rogers, L.M., Boy, E., Miller, J.W., Green, R., Sabel, J.C. & Allen, L.H. (2003) High prevalence of cobalamin deficiency in Guatemalan school children: associations with low plasma holotranscobalamin II and elevated serum methylmalonic acid and plasma homocysteine concentrations. *American Journal of Clinical Nutrition*, **77,** 433–440.

Roschitz, B., Plecko, B., Huemer, M., Biebl, A., Foerster, H. & Sperl, W. (2005) Nutritional infantile vitamin B12 deficiency: pathobiochemical considerations in seven patients. *Archives of Diseases of Childhood Fetal and Neonatal Edition*, **90,** F281–F282.

Rosenblatt, D.S. & Fenton, W.A. (2001) Inherited disorders of folate and cobalamin transport and metabolism. In: *The Metabolic and Molecular Bases of Inherited Metabolic Disease*, 8th edn. (ed. by C.R. Scriver, A.L. Beaudet, W.S. Sly & D. Valle), (co-ed. by B. Childs, K.W. Kinzler & B. Vogelstein), pp. 3897–3933. McGraw-Hill, New York.

Rosenblatt, D.S. & Whitehead, V.M. (1999) Cobalamin and folate deficiency: acquired and hereditary disorders in children. *Seminars in Hematology*, **36,** 19–34.

Rosenblatt, D.S., Aspler, A.L., Shevell, M.I., Pletcher, B.A., Fenton, W.A. & Seashore, M.R. (1997) Clinical heterogeneity and prognosis in combined methylmalonic aciduria and homocystinuria (cblC). *Journal of Inherited Metabolic Diseases*, **20,** 528–538.

von Schenck, U., Bender-Götze, C. & Koletzko, B. (1997) Persistence of neurological damage induced by dietary vitamin B-12 deficiency in infancy. *Archives of Diseases of Childhood*, **77,** 137–139.

Selzer, R.R., Rosenblatt, D.S., Laxova, R. & Hogan, K. (2003) Adverse effect of nitrous oxide in a child with 5,10-methylenetetrahydrofolate reductase deficiency. *New England Journal of Medicine*, **349,** 45–50.

Sheth, J.J. & Sheth, F.J. (2003) Gene polymorphism and folate metabolism: a maternal risk factor for Down syndrome. *Indian Pediatrics*, **40,** 115–123.

da Silva, L.R., Vergani, N., de Galdieri, L.C., Ribeiro Porto, M.P., Longhitano, S.B., Brunoni, D., D'Almeida, V. & Alvarez Perez, A.B. (2005) Relationship between polymorphisms in genes involved in homocysteine metabolism and maternal risk for Down syndrome in Brazil. *American Journal of Medical Genetics A*, **135,** 263–267.

Şimşek, Ö.P., Gonç, N., Gümrük, F. & Çetin, M. (2004) A child with vitamin B12 deficiency presenting with pancytopenia and hyperpigmentation. *Journal of Pediatric Hematology and Oncology*, **26,** 834–836.

Stabler, S.P. & Allen, R.H. (2004) Vitamin B12 deficiency as a worldwide problem. *Annual Review of Nutrition*, **24,** 299–326.

Steen, M.T., Boddie, A.M., Fisher, A.J., Macmahon, W., Saxe, D., Sullivan, K.M., Dembure, P.P. & Elsas, L.J. (1998) Neural-tube defects are associated with low concentrations of cobalamin (vitamin B12) in amniotic fluid. *Prenatal Diagnosis*, **18,** 545–555.

Suormala, T., Baumgartner, M.R., Coelho, D., Zavadakova, P., Kožich, V., Koch, H.G., Berghäuser, M., Wraith, J.E., Burlina, A., Sewell, A., Herwig, J. & Fowler, B. (2004) The cblD defect causes either isolated or combined deficiency of methylcobalamin and adenosylcobalamin synthesis. *Journal of Biological Chemistry*, **279,** 42742–42749.

Takamura, N., Kondoh, T., Ohgi, S., Arisawa, K., Mine, M., Yamashita, S. & Aoyagi, K. (2004) Abnormal folic acid-homocysteine metabolism as maternal risk factors for Down syndrome in Japan. *European Journal of Nutrition*, **43,** 285–287.

Tanner, S.M., Li, Z., Bisson, R., Acar, C., Öner, C., Öner, R., Çetin, M., Abdelaal, M.A., Ismail, E.A., Lissens, W., Krahe, R., Broch, H., Gräsbeck, R. & de la Chapelle, A. (2004) Genetically heterogeneous selective intestinal malabsorption of vitamin B12: founder effects, consanguinity, and high clinical awareness explain aggregations in Scandinavia and the Middle East. *Human Mutation*, **23,** 327–333.

Tanner, S.M., Li, Z., Perko, J.D., Öner, C., Çetin, M., Altay, C., Yurtsever, Z., David, K.L., Faivre, L., Ismail, E.A., Gräsbeck, R. & de la Chapelle, A. (2005) Hereditary juvenile cobalamin deficiency caused by mutations in the intrinsic factor gene. *Proceedings of the National Academy of Sciences of the United States of America*, **102,** 4130–4133.

Ulrich, C.M. (2005) Nutrigenetics in cancer research – folate metabolism and colorectal cancer. *Journal of Nutrition*, **135,** 2698–2702.

Volcik, K.A., Shaw, G.M., Zhu, H., Lammer, E.J., Laurent, C. & Finnell, R.H. (2003a) Associations between polymorphisms within the thymidylate synthase gene and spina bifida. *Birth Defects Research (Part A) Clinical and Molecular Teratology*, **67,** 924–928.

Vollset, S.E. & Botto, L.D. (2004) Neural tube defects, other congenital malformations and single nucleotide polymorphisms in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene: a meta-analysis. In: *MTHFR Polymorphisms and Disease* (ed. by P.M. Ueland & R. Rozen), pp. 126–144. Landes Bioscience/Eurekah.com, Georgetown, TX.

Watkins, D., Matiaszuk, N. & Rosenblatt, D.S. (2000) Complementation studies in the cblA class of inborn error of cobalamin metabolism: evidence for interallelic complementation and for a new complementation class (cblH). *Journal of Medical Genetics*, **37,** 510–513.

Watkins, D., Ru, M., Hwang, H.Y., Kim, C.D., Murray, A., Philip, N.S., Kim, W., Legakis, H., Wai, T., Hilton, J.F., Ge, B., Doré, C., Hosack, A., Wilson, A., Gravel, R.A., Shane, B., Hudson, T.J. & Rosenblatt, D.S. (2002) Hyperhomocysteinemia due to methionine synthase deficiency, cblG: structure of the MTR gene, genotype diversity, and recognition of a common mutation, P1173L. *American Journal of Human Genetics*, **71,** 143–153.

Weisberg, I., Tran, P., Christensen, B., Sibani, S. & Rozen, R. (1998) A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. *Molecular Genetics and Metabolism*, **64,** 169–172.

Weiss, R., Fogelman, Y. & Bennett, M. (2004) Severe vitamin B12 deficiency in an infant associated with a maternal deficiency and a strict vegetarian diet. *Journal of Pediatric Hematology and Oncology*, **26,** 270–271.

Whitehead, A.S., Gallagher, P., Mills, J.L., Kirke, P.N., Burke, H., Molloy, A.M., Weir, D.G., Shields, D.C. & Scott, J.M. (1995) A genetic defect in 5,10 methylenetetrahydrofolate reductase in neural tube defects. *Quarterly Journal of Medicine*, **88,** 763–766.

Wilding, C.S., Relton, C.L., Sutton, M.J., Jonas, P.A., Lynch, S.A., Tawn, E.J. & Burn, J. (2004) Thymidylate synthase repeat polymorphisms and risk of neural tube defects in a population from the northern United Kingdom. *Birth Defects Research (Part A) Clinical and Molecular Teratology*, **70,** 483–485.

Wilson, A., Leclerc, D., Rosenblatt, D.S. & Gravel, R.A. (1999a) Molecular basis for methionine synthase reductase deficiency in patients belonging to the cblE complementation group of disorders in folate/cobalamin metabolism. *Human Molecular Genetics*, **8,** 2009–2016.

Wilson, A., Platt, R., Wu, Q., Leclerc, D., Christensen, B., Yang, H., Gravel, R.A. & Rozen, R. (1999b) A common variant in methionine synthase reductase combined with low cobalamin (vitamin B12) increases risk for spina bifida. *Molecular Genetics and Metabolism*, **67,** 317–323.

Worgan, L.C., Niles, K., Tirone, J.C., Hofmann, A., Verner, A., Sammak, A., Kucic, T., Lepage, P. & Rosenblatt, D.S. (2006) Spectrum of mutations in mut methylmalonic acidemia and identification of a common Hispanic mutation and haplotype. *Human Mutation*, **27,** 31–43.

Yassin, F., Rothenberg, S.P., Rao, S., Gordon, M.M., Alpers, D.H. & Quadros, E.V. (2004) Identification of a 4-base deletion in the gene in inherited intrinsic factor deficiency. *Blood*, **103,** 1515–1517.