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 B₁₂) 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;
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.

**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
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).

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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 & 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 homocysteine 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 haematologic 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 (MTRFR) deficiency (see below) and those belonging to complementation groups cblE and cblG have a defect in methionine synthesis. The latter 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 homocysteine 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 OHChbl 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(1)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 academia 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 MMAB 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 acidic 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 megaloblastic anaemia. Folate, Cbl and tHcy levels are normal. Patients do not have complications. Some late-onset cases have occurred in children who avoided protein in their diet. Patients do not have megaloblastic anaemia or methylmalonic aciduria. The gene has been extensively characterised and a dozen mutations described (Leclerc et al., 1998; Wilson et al., 1999a).

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 folic 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 (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 methylenetetrahydrofolate cyclohydrolase deficiency. These lack sufficient definition at the present time (Rosenblatt & Fenton, 2001; Morel & Rosenblatt, 2006).

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Glutamim 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 (Gueant-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 (GCPPII) (1561C → T) (Relton et al., 2003, 2004), the trifunctional enzyme (MTHFDI) (1958G → A) (Brody et al., 2002); and cystathionine beta-synthase (CBS) (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 omphalocoe (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).

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