Preface

Neural tube defects (NTDs) are a complex developmental trait in which several genes, interacting with environmental factors, create the phenotype. In the United States, the rate of NTDs has been reported to range from 4 to 10 per 10,000 live births. Currently, in the United States, the two most common types of NTDs, anencephaly and spina bifida, which occurs in varying degrees of severity, affect approximately 4000 pregnancies *per annum*, resulting in the birth of 2500 to 3000 children manifesting one or the other of these conditions. Nevertheless, it has been proposed that 50–70% of the defects comprising this constellation of conditions could be prevented with daily intake of 400 μg of folic acid throughout the periconceptional period. In this regard, The Centers for Disease Control and Prevention (CDC) recommended, in 1991, that women who have experienced a pregnancy affected by NTDs who are planning a new pregnancy consume 400 μg of folic acid daily beginning at least one month prior to conception and continuing through the first three months of pregnancy. In addition, in 1992, the US Public Health Service (USPHS) recommended that all women of reproductive age consume 400 μg of folic acid *per diem*. In 1996, the US Food and Drug Administration authorized and then required (1998) that enriched grain products be supplemented with folate. Also in 1998, the Institute of Medicine (IOM) recommended that all women of childbearing potential consume 400 μg of synthetic folic acid per day from fortified foods and/or a supplement in addition folate obtained from a varied diet.

These actions resulted in a significant decrease in the incidence of NTDs despite the 1998 report that only 29% of US women complied with the USPHS and IOM recommendations. Thus, in women receiving prenatal care, the rate decreased more than 19% (from 3.78/10,000 live births to 3.05/10,000) while in women receiving prenatal care only during the third trimester or none at all, the rate decreased approximately 13% (from 5.34/10,000 live births to 4.65/10,000).

The greatest need for folate occurs during pregnancy. During development, as the number of rapidly dividing cells increases, the requirement for folate increases. The situation is complicated by decreased absorption and increased clearance of folate during pregnancy. By the third trimester, the requirement for folate has almost doubled. In addition to NTDs, periconceptional folic acid supplementation reduces the occurrence of several human congenital malformations including craniofacial and heart defects. In the United States, because normal diets seldom supply the 400 μg *per diem* of folate required during pregnancy, 20–25% of otherwise normal pregnancies are associated with low-serum folate levels. During July and August 1998, a survey was conducted to assess knowledge of the benefits of adequate folic acid consumption among women of childbearing age in the United States. The results were compared to those obtained from a similar survey conducted in 1995. The 1998 findings revealed that only 7% of women knew that folic acid should be taken prior to pregnancy to reduce the risk of NTDs. It is to be noted that, although recommendations regarding folic acid consumption were issued by health authorities in a number of countries in the early 1990s, assessment of periconceptional intake of folic acid revealed a disappointingly low level of compliance. Therefore, it appears that, regardless of food fortification policies, continued promotion of the benefits of folic acid supplementation to optimize the folate status of women of child-bearing age will be required in most countries. Indeed, although periconceptional folate supplementation has been encouraged in the United Kingdom since the early 1990s, no concurrent decline in NTD pregnancies has been observed by regional congenital anomaly registries. Conceivably, additional nutritional inadequacies also may be involved. However, these have not been extensively researched. In this regard, it is of interest to note that, in California between 1989 and 1991, the interaction between maternal preconceptional dietary and supplemental zinc intake and the risk of NTDs was investigated in a population-based case-control study. Four hundred and thirty (430) NTD-affected fetuses/infants and 429 randomly selected, non-malformed infants comprised the case and control populations. The preconceptional use of vitamins, minerals, and food supplements was reported by the mothers who completed a 98-item food frequency questionnaire. Phytate intake, a dietary constituent known to interfere with zinc absorption, appeared to negatively impact the zinc/NTD association. It was observed that increased servings of animal products, which are the most bioavailable food source of zinc, were associated with a reduced risk for NTDs. Risk estimates for zinc intake changed little after controlling for multiple

sociodemographic factors and total folate intake, but were attenuated after controlling for nutrients highly correlated with dietary sources of zinc. The analyses indicated that risk of NTDs in fetuses and infants decreased with increasing maternal preconceptional zinc intake. However, it remains unclear whether increased zinc intake or other nutrients or combinations of nutrients that may be highly correlated with dietary zinc intake are causally associated with reduced NTD risk.

Homocysteine status also appears to play a role in NTDs. Homocysteine, a sulfur-containing amino acid is generated through the demethylation of methionine. It is metabolized via three principal routes. The predominant pathway is selected by physiological need. Thus, homocysteine can be metabolized to cysteine by transsulfuration or remethylated to methionine or hydrolyzed to α-ketobutyrate, ammonia, and H_2S. Regulation of the plasma level of homocysteine is dependent on nutrient uptake, especially uptake of folate and vitamins B_6 (pyridoxine) and B_{12} (cobalamin). In addition, its metabolism is affected by genetic individuality. Excess levels of homocysteine are thrombophilic and damage the vascular endothelium. In adult populations, total plasma homocysteine (tHcy) is an established clinical risk factor for coronary artery disease as well as other arterial and vasoocclusive diseases. These vascular effects appear to be related to its role as a teratogen in the pathogenesis of NTDs and other developmental defects since genetic variants resulting in hyperhomocysteinemia are associated with NTDs. Thus, genetic variation in folate metabolic genes is expected to contribute to the risk of NTDs.

The observation that homocysteine and vitamin B_{12} levels are independent predictors of NTD risk suggested that the gene encoding methionine synthase might play a role in the induction of NTDs. Methionine synthase catalyzes the vitamin B_{12}-dependent conversion of homocysteine and 5-methyltetrahydrofolate to methionine and tetrahydrofolate. However, tests of an association between specific methionine synthase alleles and NTDs indicated that inherited variations in the gene do not contribute to NTD risk, at least not in the population studied. However, impairment of folate and vitamin B_{12} metabolism has been observed in families with NTDs. Therefore, it is conceivable that genetic variants/mutants of enzymes in the homocysteine remethylation pathway might act as predisposing factors contributing to NTD risk. The first polymorphism discovered that was associated with increased NTD risk was the 677C–>T mutation (A222V) in methylenetetrahydrofolate reductase (MTHFR). It is to be noted that this variant also has been associated with increased risk of nonsyndromic orofacial clefts. A polymorphism, 66A–>G (I22M), in

the gene that encodes the enzyme, methionine synthase reductase (MTRR), that activates vitamin B_{12}-dependent methionine synthase, also has been reported. This mutation has an allelic frequency of 0.51 and increases the risk of NTDs when vitamin B_{12} status is low. In addition, in the presence of the 677C–>T mutant MTHFR genotype, the 66A–>G (I22M) MTRR mutant increases the risk of NTDs under conditions of adequate vitamin B_{12}. When the genotype and B_{12} status of 56 children with spina bifida and 58 mothers of spina bifida children were compared to control groups consisting of 97 children and 89 mothers, the spina bifida cases and associated mothers were approximately twice as likely to possess the homozygous 66A–>G (I22M) MTRR mutant genotype than the control groups. But, the difference was not statistically significant. However, the risk for homozygous 66A–>G (I22M) MTRR mutant genotype mothers with low B_{12} status to deliver a child with spina bifida increased approximately fivefold [odds ratio (OR) = 4.8, 95%; CI = 1.5–15.8], while the OR for spina bifida in children with this combination was 2.5 (95%; CI = 0.63–9.7). On a background of combined MTHFR and MTRR homozygous mutant genotypes, children had a fourfold increase in risk (OR = 4.1, 95%; CI = 1.0–16.4) of manifesting spina bifida while mothers had a threefold increase in risk (OR = 2.9, 95%; CI = 0.58–14.8) in delivering a child with this condition. Clearly, the interaction between vitamin B_{12} deficiency and the mutant MTHFR and MTRR genotypes indicates a multifactorial induction of NTDs. However, the mechanism is complex and unresolved. Furthermore, the MTHFR 677C–>T and MTRR 66A–>G mutations are each associated with increased risk of Down syndrome. In the presence of both mutations, the risk is even greater.

It is well known that drugs and other chemicals can induce birth defects in humans. For example, pharmaceuticals such as valproic acid and other antiepileptic drugs that interfere with folate metabolism can induce NTDs. Therefore, a question worth considering is: Is low folate status during pregnancy a factor that increases or contributes to an increase in the risk of induction of NTDs and other birth defects from exposure to ambient levels of environmental xenobiotics? Unfortunately, this is a public health issue for which we have little information. Therefore, to gain further insight into the etiology of NTDs as well as all other birth defects that affect so many for their lifetime, continued research on the role of folate and other nutrients is imperative. The editors believe that the research data gathered by the contributors to this work are a step in the right direction.

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