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The Role of Folic Acid in the Prevention of Neural Tube Defects

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1. Introduction

1.1 What is folic acid?

Folate is a water-soluble B vitamin that occurs naturally in food. Folic acid is the synthetic form of folate that is found in supplements and added to fortified foods. Folate gets its name from the Latin word "folium" for leaf. A key observation of researcher Lucy Wills nearly 70 years ago led to the identification of folate as the nutrient needed to prevent the anemia of pregnancy. Dr. Wills demonstrated that the anemia could be corrected by a yeast extract. Folate was identified as the corrective substance in yeast extract in the late 1930s, and was extracted from spinach leaves in 1941. Folate helps produce and maintain new cells. This is especially important during periods of rapid cell division and growth such as infancy and pregnancy. Folate is needed to make DNA and RNA, the building blocks of cells. It also helps prevent changes to DNA that may lead to cancer. Both adults and children need folate to make normal red blood cells and prevent anemia. Folate is also essential for the metabolism of homocysteine, and helps maintain normal levels of this amino acid (NIH, n.d).

Folic acid (pteroylmonoglutamic acid), which is the most oxidized and stable form of folate, occurs rarely in food but is the form used in vitamin supplements and in fortified food products. Folic acid consists of a *p*-aminobenzoic acid molecule linked at one end to a pteridine ring and at the other end to one glutamic acid molecule. Most naturally occurring folates, called *food folate* in some reports, are pteroylpolyglutamates, which contain one to six additional glutamate molecules joined in a peptide linkage to the γ-carboxyl of glutamate (IOM, 1998). Mammals are able to synthesize the pteridine ring but are unable to couple it to other compounds and are thus dependent on either dietary intake or bacterial synthesis within the intestine (Birn, 2006).

Dietary folates are a complex mixture of pteroylglutamates of various chain lengths and with a variety of substitutions on the pteridine ring. More than 90% of dietary folates exist as pteroylpolyglutamates, while the remaining is pteroymonoglutamate. The process of folate absorption requires a process involving hydrolysis to convert it to the monoglutamate form in the gut before absorption. An intestinal brush border pteroylpolyglutamate

hydrolase (BB-PPH) has been identified in human and pig jejunum (Halsted, 1989). The pteroylmonoglutamyl folate form is absorbed along the entire length of the small intestine, although the jejunum is the primary site for its absorption. Pteroylglutamic acid is rapidly absorbed from the duodenum and jejunum by an active carrier-mediated transport mechanism involving the reduced folate carrier, and also by passive diffusion (Matherly & Goldman, 2003). Folate is not absorbed from the large intestine (otherwise, folate-producing colonic bacteria would be able to supply the body with considerable amounts of the vitamin). Enterohepatic circulation of folate occurs. A rise in blood folate level occurs as soon as 15 minutes after an oral dose (Butterworth, 1968; Olinger et al., 1973). The dominant folate form in serum is 5-methyl-tetrahydrofolate (5-MTHF) present either free, bound to high-affinity folate-binding protein (FBP), or loosely associated with other serum proteins including albumin (Matherly & Goldman, 2003).

Folates are widely distributed in tissues, most of them as polyglutamate derivatives. The main storage organ is the liver, which contains about half of the body's stores and represents 5 to 15 mg/kg of liver weight (Higdon, 2003). Total body content of folate has been estimated to be 38–96 mg (86–165 micromol) (Birn, 2006). A small amount is excreted in the feces and urine but the additional amounts are presumed to be metabolized and also lost by cells coming off in scales from body surfaces. Folate can be found in human milk. Folate is mainly required by organs or systems involved on the rapid proliferation of new cells. Folates are essential for normal cell division and growth. There are three stages of folic acid deficiency and high index of suspicion is needed because at the beginning may be subtle. After four to five months of continuing deficient intake of folates the clinical stage of folic megaloblastic acid anemia can be established. Aside of the bone marrow tissue, the immune system, mucous membranes, hair and fingernails may be affected as well (Shills, et al., 2006). Folate deficiency manifest primarily as anemia and physical symptoms that are considered to be classic include anemia, pallor, generalized weakness, mouth ulcers, an inflamed and sore tongue, peptic ulcers, a general numbness or tingling sensation in the hands and the feet, problems like indigestion and diarrhea, persistent depression, constant irritability and neurologic syndromes. In infants and children folate deficiency can be associated with slow overall growth rate. Folate deficiency during pregnancy increases the risk of prematurity and low birth weight (Herbert, 1999).

The folates cannot be significantly stored in the body and its replacement then requires a constant supply of the vitamin for preventing deficiency.The most common cause of folate deficiency is a low daily intake due to lack of ingestion of folate containing food, chronic alcoholism or total parenteral nutrition. Other causes can be related to impaired absorption, inadequate utilization, increased demand, and or increased excretion, or a combination of them. Impaired absorption includes Celiac disease (Sprue), gastric diseases that cause low stomach acid, congenital or acquired folate malabsorption and certain medications such as phenytoin, primidone and barbiturates. Numerous drugs are also known to inhibit the body’s ability to utilize folate, including aspirin, cholesterol lowering drugs, oral birth control pills, antacids, and methotrexate when used for rheumatoid arthritis. Other nutrient deficiencies (zinc, riboflavin, niacin and vitamin B_{12}) may affect folates absorption and metabolism. Inadequate utilization may be seen in congenital or acquired enzyme deficiency and alcoholism. Alcohol interferes with folate metabolism and increases folate breakdown. Deficiency may be seen in the presence of an increased demand during pregnancy, lactation, infancy, increased metabolism as seen in

paraneoplastic syndrome and in hemolytic anemia. Increased excretion can be secondary to renal dialysis (Johnson LE et al., 2007; Shills et al., 2006).

The primary indicator selected to determine folate adequacy is erythrocyte folate. Because folate is taken up only by the developing erythrocyte in the bone marrow and not by the circulating mature erythrocyte during its 120-day lifespan, erythrocyte folate concentration is an indicator of long-term status (IOM, 1998). If serum folate is < 3 ng/mL (< 7 nmol/L), deficiency is likely. Serum folate reflects folate status unless intake has recently increased or decreased. If intake has changed, erythrocyte (RBC) folate level better reflects tissue stores. A level of < 140 ng/mL (< 305 nmol/L) indicates inadequate status (Johnson LE et al., 2007).

1.2 Folate metabolism

Folate coenzymes play an important role in the metabolism of several amino acids, which are the building blocks of proteins. Tetrahydrofolic acid is involved in the formation of purines (adenine and guanine) and pyrimidines (thymidine) which are essential for the synthesis of the nucleic acids (DNA and RNA), responsible to carry the genetic instructions used in the development and functioning of all known living organisms. The 5-methyl THFA participates as a folate cofactor with vitamin B_{12} in the methylation cycle where the methyl group is transferred to homocysteine to produce the aminoacid methionine. Methionine reacts with adenosine-5'-triphosphate (ATP) to produce S-adenosylmethionine which is the key methyl group donor. The methylation cycle is then essential to regulate deoxyribonucleic acid (DNA) gene expression, post-translational modification in proteins formation and synthesis of lipid synthesis. Methylation is also important step in the metabolism of neurotransmitters and detoxification of xenobiotics. Deficiency of 5-MeTHF causes accumulation of possible toxic metabolites such as homocysteine and, consequently, the inhibition of methyltransferases affecting gene expression, protein function and lipid and neurotransmitter metabolism (Blom et al., 2006; Gentili et al., 2009).

Folate in the 5-methyl THFA form is a cosubstrate required by methionine synthase when it converts homocysteine to methionine. As a result, in the scenario of folate deficiency, homocysteine accumulates (Gentili et al., 2009). High homocysteine levels are associated with an increased risk for atherosclerotic diseases, which has been linked with the risk of arterial disease, dementia and Alzheimer's disease. Accumulation of possibly toxic levels of homocysteine and impairment of methylation reactions involved in the regulation of gene expression also increase the neoplastic risks. Homocysteine and cysteine are associated with oxidative damage and metabolic disorders, which may lead to carcinogenesis (Eikelboom et al., 1999; Lin et al., 2010; Ray, 1998; Sedhadri et al., 2002).

1.3 Sources of folic acid in food

Natural foods like leafy green vegetables, spinach, brussel sprouts, turnip greens, potatoes, wheat germ, yeast, dried beans, legumes, fruits (such as citrus fruits and juices), and organ foods such as liver are rich sources of folate. Most dietary folates exist as polyglutamates, which are converted to the monoglutamate form and absorbed in the proximal small intestine. However, the body absorbs only about 50% of food folate. This problem is compounded by cooking practices such as prolonged stewing, processing, and storage, which can destroy some of the folate in natural foods (Talaulikar & Arulkumaran, 2011).

Red blood cell folate, plasma homocysteine and folate levels are used to determine the Recommended Dietary Allowance (RDA) of folate which reflects how much of this vitamin should be consumed daily. This recommended value can vary depending on age, gender, health status and other metabolic conditions. The synthetic folic acid has a greater bioavailability and the absorption is approximately 1.7 times higher from supplements and fortified foods compared with natural sources of folate. The USA Food and Nutrition Board of the Institute of Medicine recommended intakes for individuals as Daily Reference Intakes (DRI). The World Health Organization and several countries have their own set of DRIs recommendations. The 1998 USA Dietary Reference Intakes express the new Recommended Dietary Allowances for folate in dietary folate equivalents, which adjust for the nearly 50 percent of differences in the absorption of naturally occurring food folate and the more bioavailable synthetic folic acid: 1 μg of dietary folate equivalent = 0.6 μg of folic acid from fortified food or as a supplement taken with meals = 1 μg of food folate = 0.5 μg of a supplement taken on an empty stomach. (de Bree, 1997; IOM, 1998; Suitor & Bailey, 2000).

1.4 Relation of folic acid and neural tube defects

During the last decades, major interest has been devoted to preventable causes of central nervous system malformations such as neural tube defects (NTD). This term is applied to a variety of malformations resulting from incomplete to total absence of closure of the neural tube between 17 and 30 postconceptional days (Siebert et al., 1990; Volpe, 1994). The neural tube defects may present with different phenotypes depending on the affected region. The most vulnerable areas of the neural tube are the anterior and posterior neuropores because they are the last to close. Failure to close the anterior neural tube region results in anencephaly. Anencephaly is the most severe form of neural tube defect and is considered a lethal malformation (AAP & AHA, 2010).

Neural tube defects are a major cause of mortality in newborns and have been estimated to affect 0.5 to 8 per 1000 live births. Anencephaly and spina bifida are the most common manifestations of the spectrum (Gilbert, 2000; Siebert et al., 1990). Neural tube defects are considered multifactorial in origin with a combination of genetic and environmental influences predisposing its occurrence. It can be seen along with chromosomal abnormalities (trisomies 13 and 18), and other rare syndromes, and is associated to uncertain modes of inheritance (Hoyme, 1990; Saitoh et al., 2005; Volpe, 1994). Medications such as phenytoin, valproic acid, cotrimazole, aminopterin, thalidomide, carbamazepine, acetyl salicylic acid and efavirenz have been associated with an increased risk of NTD (Gilbert, 2000; Holmes et al., 1976; Hoyme, 1990; Volpe, 1994). Other factors such as maternal hyperthermia, maternal health status and metabolic disorders, ethnic variations, genetic predisposition, plasma vitamin levels, plasma folate levels, smoking, alcohol consumption and differences in folate metabolism have been implicated (Holmes ,1976; Larroche & Encha-Razavi, 1991; Saitoh et al., 2005; Sandford et al., 1992; Volpe, 1994).

In 1952, Thiersch reported the association of neural tube defects with the use of 4-aminopteroglutamic acid (Aminopterin), a folic acid antagonist, during early pregnancy. Edwards (1958) and Stein & Susser (1976) reported the association between dietary deficiencies and neural tube defects. The teratogenic effect of compromised nutrient intakes was confirmed in animal models (Miller, 1963; Seller, 1983). Folic acid deficiency was identified as cause of NTD and other birth defects in 1965 by Hibbard & Smithells, other studies by the later

suggested that its supplementation could greatly reduce the incidence of these central nervous system malformations (Smithells, 1959, 1983). Since then, multiple research investigations have validated the role of folic acid in preventing neural tube defects (Watkins, 1998). Folate metabolism evaluation revealed that it is an important co-factor necessary in the conversion of homocysteine to methionine. Steegers-Theunissen et al. (1994) and Mills et al . (1995) reported elevated homocysteine levels in mothers of children with neural tube defects. Homocysteine is a sulfur amino acid formed by the demethylation of methionine and remethylated to conserve methionine. The methylation hypothesis points that the reason for failure of neural tube closure may be a relative shortage of methionine (methylation capacity) at a crucial stage of fetal development (Mills et al., 1995). Methionine is required for neural tube closure.

2. Mutations in folate-related enzymes

2.1 Methylenetetrahydrofolatereductase (MTHFR)

MTHFR catalyzes the conversion of of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (Figure 1).This reaction is required for conversion of homocysteine to methionine by the enzyme methionine synthase. This conversion of homocysteine to methionine can also be catalyzed by the folate-independent enzyme betaine-homocysteinemethyltransferase (BHMT). 5,10-methylenetetrahydrofolate is used to convert dUMP to dTMP for *de novo* thymidine synthesis.MTHFR contains a bound flavin cofactor and uses NAD(P)H as the reducing agent. Because of the genetic complexity of folate metabolism, MTHFR alleles may be expected to interact with other folate-related genes and with folate consumption (van der Linden et al., 2006a).

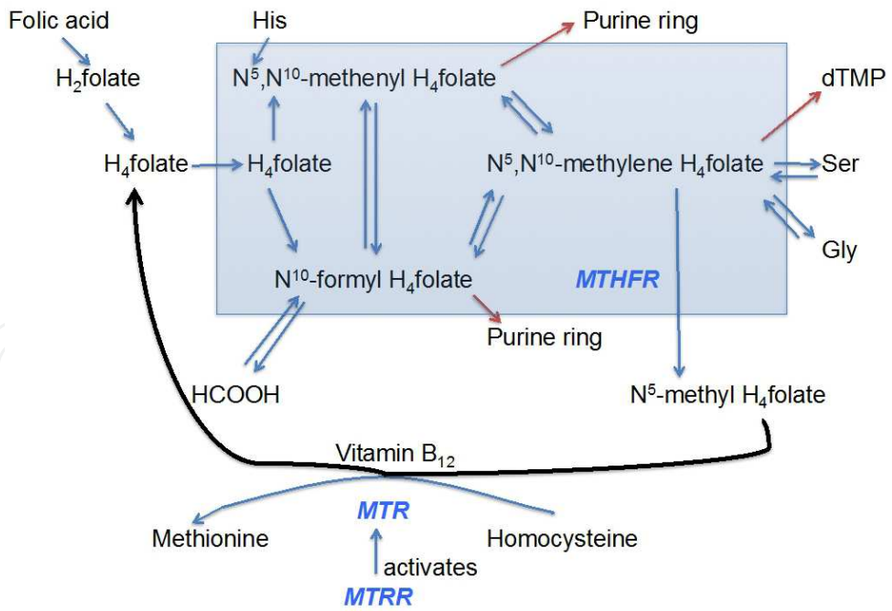

Fig. 1. One carbon metabolism. Interconversions of folic acid and its derivatives are indicated by blue arrows. Red arrows indicate pathways which depend exclusively on folate. A black arrow indicates the important B12-dependent reaction converting N^5-methyl tetrahydrofolate (H_4folate) back to H_4folate. The various one carbon derivatives of H_4folate are enclosed in the blue box overlay.

2.1.1 C677T

Among folate-related genes, the gene coding for 5,10-methylenetetrahydrofolate reductase (MTHFR) has been the principal focus of attention (Greene et al., 2009). A common polymorphism (677C-T, Ala222Val, rs1801133) was first identified as an important genetic risk factor in vascular disease (Frosst et al., 1995).This mutation creates a Hinf I site, which allows for easy screening of this missense mutation by PCR-RFLP. When lymphocyte extracts from individuals heterozygous or homozygous for this mutation were evaluated, reduced enzyme activity and increased thermolability was observed. In vitro expression of a cDNA bearing this missense mutation confirmed the thermolability of the mutant MTHFR. Thethermolabile enzyme with the 677C-T mutation is stabilized by folate (Frosst et al., 1995). When serum folate levels are greater than 15.4 nM, the effects of 677C-T mutations are neutralized (Jacques et al., 1996). In addition, individuals who are homozygous for the mutation have significantly elevated plasma homocysteine levels (Frosst et al., 1995). The first report that the 677C-T polymorphism was associated with increased risk of spina bifida (van der Put et al., 1995) was published shortly after, followed quickly by similar studies that included other NTDs (Botto & Yang, 2000a; Narasimhamurthy et al. 2010; Naushad et al, 2010; Possey et al., 1996; Shaw et al., 2009; van der Put et al., 1997; Whitehead et al., 1995). Homozygosity for 677C-T was associated with a 7.2 fold increased risk for NTDs (95% confidence interval: 1.8-30.3; p value: 0.001) (Ou, etal., 1996). Many subsequent studies had similar findings (García-Fragoso et al., 2002; Martínez de Villareal et al., 2001; Richter et al., 2001).

The 677C-T mutation was early on reported to be found more frequently among Caucasians than in African Americans (McAndrew, 1996). Among 151 consecutively born white infants in South Carolina, 20 were homozygous and 65 were heterozygous for the 677 T allele; among consecutive black newborns, none of 146 were homozygous, and 31 were heterozygous (Stevenson et al., 1997). The estimated allele frequency of the mutation was 0.35 among white newborns and 0.11 among black newborns. Subsequently, the 677C-T polymorphism was found in relatively high frequency throughout the world (for example, Schneider et al., 1998; Relton et al., 2004), even in admixed populations like that in the island of Puerto Rico (García-Fragoso et al., 2010). In Mexico, the proportion of CC (17.6%), CT (47.6%), and TT (34. 8%) genotypes were found to be high, with gene frequencies of 0.414 and 0.586% for the C and T alleles, respectively (Mutchinick et al., 1999). The 677T allele was, associated with one haplotype, G-T-A-C, in white and Japanese homozygotes (Rosenberg et al., 2002). Among the African individuals, analysis of maximum likelihood disclosed an association with the G-T-A-C haplotype, although none of the 174 subjects examined was homozygous for the 677C-T polymorphism. These results suggested that the 677C-T alteration occurred on a founder haplotype that may have had a selective advantage.

In contrast, other studies have found either no association of 677C-T with increased risk of NTD (for example, Boyles et al., 2006; Dávalos et al., 2000; Erdogan et al., 2010; Johnson WE et al., 1999; Stegmann et al., 1999,) or even a protective effect (Doudney et al., 2009 and Relton et al., 2003) of the 677C-T polymorphism. Yet, other studies suggested that additional candidate genes other than MTHFR may be responsible for an increased risk to NTD in some American Caucasian families (Rampersaud et al., 2003). A meta-analysis that included results from 27 studies concluded that the 677TT genotype confers an overall 1.9 times increase in risk of NTD (Blom et al., 2006). A more recent study included 37 different European populations from 32 studies and a total of 3,530 cases and 6,296 controls, where

data was stratified according to geographical region and ethnicity, produced two separated meta-analyses for non-Latin European and Latin European descent populations (Amorim et al., 2007). No association was demonstrated for the 677TT genotype in Latin European populations (1.16; 0.95-1.43), while the non-Latin European meta-analyses (1.62; 1.38-1.90) indicated an association of the TT genotype and NTDs. The examination of non-Latin European studies revealed that the association of TT genotype with NTD has only been proven for Irish populations, both by case-control studies, and by family-based tests, such as the allele transmission disequilibrium test (TDT) (Amorim et al, 2007).

Genomic DNA methylation directly correlates with folate status and inversely with plasma homocysteine (tHcy) levels ($P < 0.01$) (Friso et al., 2002). The 677TT genotypes had a diminished levelof DNA methylation compared with those with the 677CC wild-type (32.23 vs.62.24 ng 5-methylcytosine/μg DNA, $P < 0.0001$). When analyzed according to folate status, however, only the 677TT subjects with low levels of folate accounted for the diminished DNA methylation ($P < 0.0001$). Moreover, in 677TT subjects, DNA methylationstatus correlated with the methylated proportion of red blood cell folate and was inversely related to the formylated proportion of red blood cell folates ($P < 0.03$), that is known to be solely represented in those individuals (Friso et al., 2002). These results indicate that the MTHFR C677T polymorphism influences DNA methylation status through an interaction with folate status.

It is clear from the many studies that have evaluated the MTHFR gene 677C-T polymorphism that it may elevate the risk of NTD in many populations, that the magnitude of the risk conferred by this mutation depends on nutritional factors and may range from close to 2-fold and possibly higher, depending on the ethnic group examined. Of all the folate gene polymorphisms, the 677C-T appears to be the most consistently present genetic factor conferring risk for NTDs.

2.1.2 A1298C

A second common mutation in the methylenetetrahydrofolatereductase gene (1298 A→C, glu429-to-ala (E429A, rs1801131) was reported in 1998 by van der Put et al. 1998 and Weisberg et al., 1998). The mutation destroys an MboII recognition site and had an allele frequency of 0.33 in Canadian subjects tested (van der Put, et al., 1998). This polymorphism was associated with decreased enzyme activity; homozygotes had approximately 60% of control activity in lymphocytes. Heterozygotes for both the C677T and the A1298C mutation, which accounted for approximately 15% of individuals in the Canadian study (Weisberg et al., 1998), had 50-60% of control activity, a value that was lower than that seen in single heterozygotes for the C677T variant. These results suggested that a combined heterozygosity for the two *MTHFR* common mutations may account for a proportion of folate-related neural tube defects. While the 677C-T transition occurs within the predicted catalytic domain of the MTHFR enzyme, the 1298A-C polymorphism is located in the presumed regulatory domain. Van der Put, et al., (1998) found that combined heterozygosity at the 2 polymorphic sites was associated with reduced MTHFR-specific activity, higher Hcy, and decreased plasma folate levels. This combined heterozygosity was observed in 28% of the neural tube defect (NTD) patients compared with 20% among controls, resulting in an odds ratio of 2.04. In NTD families in Italy and Turkey, the *MTHFR*A1298C polymorphism was found to be a genetic determinant for NTD risk

(Boduroğlu et al., 2005; De Marco et al., 2002) but there are conflicting studies in spina bifida occulta patients in Turkey (Eser et al., 2010). Other studies have failed to find an association between the 1298A-C polymorphism and risk of NTDs (Parle-McDermott et al., 2003).

Most 677T and 1298C alleles appear to be associated with 1298A and 677C alleles, respectively. There may be an increased frequency of the very rare cis 677T/1298C haplotype in some parts of the United Kingdom and Canada, possibly due to a founder effect (Ogino & Wilson, 2003). A Canadian study demonstrated that 677T and 1298C alleles could occur in both cis and trans configurations (Isotalo et al., 2000). Combined 677CT/1298CC and 677TT/1298CC genotypes, which contain three and four mutant alleles, respectively, were not observed in the neonatal group (P=.0402). This suggests decreased viability among fetuses carrying these mutations and a possible selection disadvantage among fetuses with increased numbers of mutant MTHFR alleles. Vaughn et al (2004) determined in 362 women 20–30 yrs of age that plasma homocysteine was inversely ($P < 0.0001$) associated with serum folate and plasma vitamin B-12 regardless of genotype. Plasma homocysteine was higher ($P < 0.05$) for women with the MTHFR 677 TT/1298 AA genotype combination compared with the CC/AA, CC/AC, and CT/AA genotypes.

2.2 Methionine synthase (MTR)

5-methyltetrahydrofolate-homocysteine S-methyltransferase (MTR), also known as methionine synthase, catalyzes the remethylation of homocysteine to form methionine. This remethylation reaction takes place in all cells, except erythrocytes. The MTR enzyme requires vitamin B_{12} as a cofactor, and the MTR-cobalamin(I) complex then binds the methyl group of 5-methyl H_4folate to form methyl-cobalamin(III)MTR. When the methyl group is transferred to homocysteine, the cobalamin(I)MTR complex is reformed and available for another methyl donation step by 5-methyl H_4folate. Loss of function mutations in the *MTR* gene cause increased levels of plasma homocysteine. In liver and kidneys, homocysteine remethylation is carried out by another enzyme system, the betaine-homocysteinemethyltransferase enzyme, which is responsible for 50% of the homocysteine remethylation.

2.2.1 A2756G

In 1996 Leclerc et al. identified a missense mutation and a 3 bp deletion in patients of the cobalamin (cblG) complementation group of inherited homocysteine/folate disorders by SSCP and DNA sequence analysis, as well as an amino acid substitution present in high frequency in the general population (2756A-G), which changes an aspartic acid residue to a glycine. This mutation is associated with relatively elevated homocysteine and relatively low vitamin B12 and red blood cell folate levels. In a study of 56 patients with spina bifida, 62 mothers of patients, 97 children without NTDs (controls), and 90 mothers of controls, the 2756A-G MTR polymorphism was associated with a decreased O.R. (O.R.); none of the cases and only 10% of controls were homozygous for this variant (Christensen et al., 1999). Doolin et al., (2002) studied the genetics of spina bifida in families (n = 209) that included at least one affected (i.e., with meningocele, meningomyelocele, or myelocele) member who were ascertained through several sources. Samples were obtained from the family member(s) affected with spina bifida (i.e., the proband) and his or her (their) parents, sibs, and maternal grandparents. Doolin et al (2002) assessed associations between maternal and offspring MTR and MTRR genotypes and spina bifida using the two-step TDT and using a log-linear

approach. They determined that the risk of having a child with spina bifida appears to increase with the number of high-risk alleles in the maternal genotype for MTR (R1=2.16, 95% CI 0.92-5.06; R2=6.58, 95% CI 0.87-49.67). In contrast, Al Farra (2010) tested for both the 2756A-G and the 2758C-G and found no association between the two examined polymorphisms and the increase in maternal risk for giving birth to NTD children.

2.3 Methionine synthase reductase (MTRR)

The product of the methionine synthase reductase (MTRR) gene is required for the regeneration of functional methionine synthase by reductive methylation through a reaction catalyzed by MTRR gene in which the methyl donor used is S-adenosyltmethionine. Methionine synthase uses cobalamin(I) cofactor, which becomes oxidized to cobalamin (II), thus rendering the MTR enzyme inactive (Leclerc et al., 1998). Patients who are defective in the reductive activation of methionine synthase exhibit megaloblastic anemia, developmental delay, hyperhomocysteinemia and hypomethioninemia (Wilson et al., 1999a).

2.3.1 A66G

A common MTRR polymorphism, i.e. a 66A-G substitution that results in an isoleucine to methionine substitution (I22M), was identified in a Canadian study, where this mutation has an allele frequency of 0.51 and increases NTD risk when cobalamin status is low or when the *MTHFR* mutant genotype is present (Wilson et al., 1999b). When a study population of 601 Northern-Irish men, aged 30-49, for which biochemical and genetic data relevant to folate/homocysteine metabolism had already been acquired, the 66AA genotype had a frequency of 29%.There was a significant influence of MTRR genotype on total Homocysteine ranking(tHcy)(P=0.004) and the 66AA genotype contributes to a moderate increase in tHcy levels across the distribution [OR 1.59 (95% CI: 1.10--2.25) for the 66AA genotype to be in the upper half of the tHcy distribution, P=0.03] (Gaughan et al., 2001). Doolin et al., (2002) also assessed the 66A-G mutation in their spina bifida study and determined that for the risk of having a child with spina bifida appears to increase with the number of high-risk alleles in the maternal MTR genotype as well as the MTRR genotype mentioned above (R1 p 2.16, 95% CI 0.92–5.06; R2 p 6.58, 95% CI 0.87–49.67) and MTRR (R1 p 2.05, 95% CI 1.05–3.99; R2 p 3.15, 95% CI 0.92–10.85). These findings highlight the importance of considering both the maternal and embryonic genotype when evaluating putative spina bifida susceptibility loci.

Vaughn and colleagues (2004) concluded from assaying for common genetic variants (MTHFR 677C3T, MTHFR 1298A3C, and MTRR 66A3G), folate, and vitamin B-12 status on plasma homocysteine in women (20–30 yrs old; n = 362) that coexistence of the MTHFR 677 TT genotype with the MTRR 66A3G polymorphism may exacerbate the effect of the MTHFR variant alone and that the potential negative effect of combined polymorphisms of the MTHFR and MTRR genes on plasma homocysteine in at-risk population groups with low folate and/or vitamin B-12 status, such as women of reproductive potential, deserves further investigation. Furthermore, women with the MTHFR 677 TT/MTRR 66 AG genotype had higher ($P < 0.05$) plasma homocysteine than all other genotype combinations except the TT/AA and TT/GG genotypes (Vaughn et al., 2004).Conflicting results were obtained in a case-control study by van der Linden and colleagues (2006b), where they studied the association between the MTRR 66A-Gpolymorphism and spina bifida risk in 121

mothers, 109 spina bifida patients, 292 control women, and 234 pediatric controls and found that the MTRR66A-G polymorphism had no influence on spina bifida risk.

Since the MTR and MTRR genes have been much less studied as genes conferring risk to NTD, the conflictive evidence still needs to be clarified with further studies that evaluate the metabolic as well as genetic factors that contribute to these developmental defects.

3. Folic acid supplementation

Epidemiological studies that associate folate supplementation with a decreased risk of NTDs date back to the 1960s. The most definitive research addressing the benefits of folic acid supplementation in decreasing the risk of NTDs was the multicentre, randomized, double-blind trial by the Medical Research Council in the United Kingdom (MRC Vitamin Study Research Group, 1991). The aim of this trial was to evaluate the efficacy of 4-mg doses of folic acid in preventing recurrent NTDs in women who had previously delivered children with NTDs. The trial showed that women randomized to take folic acid supplementation had a 1.0% chance of having children with NTDs (relative risk [RR] 0.28, 95% confidence interval [CI] 0.12 to 0.71), but women in the unsupplemented group did not show a decrease in the risk of NTDs (3.49%) (RR 0.8, 95% CI 0.37 to 1.72) (Czeizel & Dudas, 1992; MRC Vitamin Study Research Group, 1991). Overall, supplementation with folic acid reduced the rate of recurrence of NTDs by 72% (MRC Vitamin Study Research Group, 1991).

A second key trial evaluating folic acid–fortified multivitamin supplementation during pregnancy was a double-blind, randomized controlled trial, in which women were randomized to take a multivitamin supplement containing 0.8 mg of folic acid or a multivitamin containing trace-element supplementation (Czeizel & Dudas 1992). Five thousand women were randomized in each group; no NTDs were observed in babies from the folic acid–fortified group, whereas 6 NTDs were found in those from the trace-element group. A recent meta-analysis observed that use of multivitamin supplements provided consistent protection against neural tube defects with an odds ratio (OR) of 0.67 (95% CI 0.58 to 0.77) in case-control studies and an OR 0.52 (95% CI 0.39 to 0.69) in cohort and randomized controlled studies. An OR of 0.67 means 0.33 (or 33%) protective effect; an OR of 0.52 means 0.48 (or 48%) protective effect (Goh et al., 2006). A study investigating the relationship between serum and red blood folate concentrations and the risk of NTDs found an inverse relationship between maternal red blood cell folate and the risk of NTD (Daly LE et al., 1995). Daly et al showed that women receiving less than 150 μg and more than 400 μg of folic acid had a 6.6/1000 and 0.8/1000 chance of having children with NTDs, respectively. Supplementation at doses of 100 μg, 200 μg, and 400 μg of folic acid resulted in a 22%, 41%, and 47% decreased risk of NTDs, respectively (Daly S et al., 1997).

3.1 Recommendations for women without a previous pregnancy affected by NTD

In 1991, the British Medical Research Council Vitamin Study reported that folic acid supplements reduced the recurrence neural tube defects (spina bifida or anencephaly) by 71% (MRC Vitamin Study Research Group, 1991). Preliminary results from the Hungarian randomized controlled trial of multivitamin/mineral supplementation (including 0.8 mg of folic acid) among women who had not had a prior NTD-affected pregnancy were reported in 1989. This trial was stopped in May 1992 on the advice of an ad hoc scientific advisory

committee because of evidence of an NTD-protective effect of the multivitamin/mineral preparation relative to the study placebo preparation (CDC, 1992). In September 1992, the United States Department of Health and Human Services Public Health Service Centers for Disease Control recommended that all women of childbearing age in the United States who are capable of becoming pregnant and without a previous pregnancy affected by NTD should consume 0.4 mg of folic acid per day for the purpose of reducing their risk of having a pregnancy affected with spina bifida or anencephaly (CDC, 1992).

3.2 Recommendations for women with a previous pregnancy affected by NTD

Among US couples who have had a child with an NTD, the recurrence risk is 2% to 3% in subsequent pregnancies. The Medical Research Council (MRC) Vitamin Study Group reported the results of a trial of folic acid supplementation for the prevention of NTDs in pregnancies of women who had a previous child with an NTD and the CDC published its recommendations (CDC, 1991; MRC Vitamin Study Research Group, 1991). The guideline called for the consumption of a 4.0- mg daily dose of folic acid, from at least 1 month before conception through the first 3 months of pregnancy. The guideline did not specifically address the issue of folic acid consumption among these women during the times when they are not planning to become pregnant. Women who have had an NTD-affected pregnancy should consume 0.4 mg of folic acid per day, unless they are planning a pregnancy. When these women are planning to become pregnant, they can follow the guideline and consult their physicians about the desirability of using 4.0 mg of folic acid per day. Because 4.0 mg of folic acid per day is a very high dose, there may be risks associated with these levels. Although it appears that a lower dose, such as 0.4 mg, may have as great a beneficial effect as 4.0 mg, women who are at very high risk of having an NTD-affected pregnancy may choose to follow the guideline because it is based on data from the most rigorous study directly pertinent to their risk of NTDs, and because their risk of having an NTD-affected pregnancy may outweigh any risk that may occur as the result of the use of 4.0 mg of folic acid (CDC, 1991). The South Carolina NTD prevention program has reported great success in preventing the recurrence of isolated NTDs by providing counseling and vitamins to women who have had a previous NTD-affected pregnancy. Over the 6 years of surveillance (1992-1998) there were no NTD recurrences in 113 subsequent pregnancies to mothers of infants with isolated NTDs who took periconceptional folic acid (Stevenson et al., 2000).

3.3 Folic acid campaigns

Despite folic acid's clear link with NTD prevention, folic acid education campaigns worldwide have had mixed results in terms of knowledge about the benefits and sources of folic acid, and especially in terms of understanding the correct, periconceptional timing of folic acid intake. Official health education initiatives have promoted folic acid supplementation and a diet rich in folates. Campaigns range from media communications and information kits, to free product samples and discount vouchers, to improved labeling and in-store displays promoting dietary sources of folic acid. A review of 38 scientific publications about folic acid campaigns showed that awareness of folic acid improved post-campaign, with the percentage improvement between pre- and post-campaigns ranging from 6 to 41%. Although knowledge regarding appropriate sources of folic acid (foods or supplements) improved post-campaign, in most studies <50% of women were able to

provide details about supplements that contained folic acid. In general, folic acid consumption rose between 12.4 % and 25.3% after public health campaigns. Nevertheless, the percentage of women taking periconceptional folic acid as prescribed ranged from 13 to 57%. This suggests >43% of women were not taking folic acid as prescribed after campaigns ceased (Rofail, 2011).

Folate unawareness not only exists among general population but also among nurses, pharmacists and health professionals. A survey conducted among student pharmacists concluded that 94% knew folic acid supplements prevent birth defects, 74% knew supplementation should begin before pregnancy but only 55% knew the recommended levels or good folate sources (50%). A telephone survey among obstetricians in Delhi, India reported that although all of them were aware of folic acid, only 63% knew that it prevents birth defects and 30% knew that it should be given before pregnancy. None of them prescribed periconceptional folic acid or folate rich diet through child-bearing age. Interestingly, 80% of those surveyed were not aware of the preventive dose of folic acid (Gupta & Gupta, 2004).

Increased periconceptional consumption of dietary folates or supplements have had limited impact on decreasing the incidence of neural tube defects. In addition to unplanned pregnancy, factors such as adolescent pregnancies, ethnicity, low socio-economic status, maternal education, and access to preventive healthcare influence the consumption of folic acid supplements. Sustained educational health campaigns should continue but also they should address barriers and limitations for adequate preconceptional and reproductive health. These campaigns are needed to reassess the message sent and the ability people have to change their behaviors according to their own circumstances. Due to limited success of the folic acid campaigns in increasing the supplementation of folic acid, the primary prevention of these birth defects then relies on folic acid fortification (Gupta & Gupta, 2004; Ray et al., 2004; Rofail et al., 2011; Eichholzer et al., 2006).

4. Fortification of food with folic acid

4.1 Recommendations

In March 1996, the US Food and Drug Administration (FDA) mandated that enriched cereal-grain products be fortified with folic acid. The FDA determined that fortification of cereal-grain products with folic acid, along with fortification of ready-to-eat breakfast cereals and dietary supplements, would provide increased intakes of folate for women in their childbearing years, while keeping daily intakes for the non-target population within the recommended safe limit. Food fortification had the advantage of reaching a great number of women in the target population before conception and during early pregnancy. It also had the advantage of providing folic acid in a continuous and passive manner and, thus, represented a potentially effective means for improving the folate nutriture of women throughout their childbearing years (FDA, 1996). Many countries have not chosen mandatory folic acid fortification, in part because expected additional health benefits are not yet scientifically proven in clinical trials, in part because of feared health risks, and because of the issue of freedom of choice. In these countries, additional creative public-health approaches need to be developed to prevent neural tube defects and improve the folate status of the general population (Eichholzer et al., 2006).

4.2 Fortification results

The mandatory fortification of cereal grain products in the US resulted in a substantial increase in blood folate concentrations and a concomitant decrease in NTD prevalence (CDC, 2010). The prevalence estimates of low serum and RBC folate concentrations declined in women of childbearing age from 21% before to <1% after fortification. For RBC folate concentrations there was a decline from 38% to 5% (Pfeiffer et al., 2007). NTD prevalence decreased by 36% after fortification, from 10.8 per 10,000 population during 1995--1996 to 6.9 at the end of 2006 (CDC, 2010).

A similar trend has been seen in other countries. In Chile, mandatory wheat flour fortification started in 2000. A study was designed to measure the effect of such policy in a group of women of childbearing age in Santiago. Folate levels, as well as RBC folate concentrations, increased significantly, 2.8 and 1.4-fold, respectively. It is important to notice that none of the participants took folic acid supplements since there was no public health policy regarding their use in Chile (Hertrampt et al., 2003). During 2001-2002 the NTD rate in Chile was significantly reduced by 43% from 17.1/10,000 to 9.7/10,000 births (Hertrampt et al., 2008). In Argentina, fortification started in 2003. Hospital discharge statistics showed a decrease of 54% for anencephaly, 33% for encephalocele, and 45% for spina bifida between 2000 and 2005 (Calvo & Biglieri, 2008). Canada has also seen a 50% reduction in spina bifida (Godwin et al., 2008). Another fortification strategy was studied in New Zealand, where daily consumption of folic acid fortified milk in women of childbearing age for 12 weeks resulted in an increase in plasma folate and RBC folate concentrations (Green et al., 2005).

Improved survival of children with NTD has also been reported after fortification. A retrospective cohort study was conducted and included 2841 infants with spina bifida and 638 infants with encephalocele who were born between 1995 and 2001 and were registered in any of 16 participating birth defects monitoring programs in the United States. First-year survival rates for both spina bifida and encephalocele cohorts were measured and factors associated with improved chances of first-year survival, including birth before or during folic acid fortification, were measured with regression analysis. Infants with spina bifida experienced a significantly improved first-year survival rate of 92.1% (adjusted hazard ratio: 0.68; 95% confidence interval: 0.50-0.91) during the period of mandatory folic acid fortification, compared with a 90.3% survival rate for those born before fortification. Infants with encephalocele had a statistically nonsignificant increase in survival rates, ie, 79.1% (adjusted hazard ratio: 0.76; 95% confidence interval: 0.51-1.13) with folic acid fortification, compared with 75.7% for earlier births. It is possible that folic acid plays a role not only in preventing NTDs but also in ameliorating the severity of spina bifida among live-born infants. One possible mechanism for reduction of the severity of spina bifida is that folic acid may move the location of the lesion caudally along the developing spine (Bol et al., 2006). A study in Atlanta demonstrated lower survival rates for infants with high lesions (cervical or thoracic), compared with infants with low lesions (lumbar or sacral). In multivariable analysis, factors associated with increased mortality were low birthweight (<2500 g) and high lesions (Bol et al., 2006, Wong & Paulozzi, 2001).

4.3 Concerns

Before the mandatory fortification of grains, the average consumption of folic acid was estimated to be 0.25mg/day and the fortification is estimated to add 0.1 mg/day, leaving

many women of childbearing age under the recommended levels (Chacko et al., 2003). A study by Boushey and colleagues (2001) showed that 61% of women in childbearing age after fortification had intakes of folic acid below the recommended levels and those who achieved the guidelines were those who consumed supplements. Brent and Oakley (2005) have suggested that there is a need to increase the amount of folic acid in the fortification of grains. The Food and Drug Administration disagreed since fortification is nonspecific and must be safe for all groups (Rader & Schneeman, 2006). With fortification, the possibility remains that certain segments of the population may benefit less and may even experience some adverse effects from increased folic acid intakes.

The main concern has been the potential masking of vitamin B_{12} deficiency, a condition that affects 10–15% of the population over age 60 years. Increased folic acid intake may correct the hematologic signs of vitamin B_{12} deficiency, thus delaying diagnosis and treatment of the condition while its neurologic manifestations progress The Public Health Service fortification recommendation cautioned against daily intakes of folate above 1 mg (Liu et al., 2004). The agency noted that even with supplement use, 95th percentile intakes by adults 51+ years of age could reach 0.84 to 0.86 mg/day if enriched cereal-grain products are fortified. . While the agency recognized that this level approached the recommended safe upper limit and did not take into account likely underreporting biases regarding food intakes and underestimation of folate content of foods, it tentatively concluded that fortification of cereal-grain products with 140 g folic acid /100 g was the most appropriate fortification level of three levels analyzed (FDA, 1996). A study in Canada showed that the average dietary intake of folic acid due to fortification was 0.7 mg/day in women aged 19–44 years and 0.74 mg/day in seniors. Among seniors, there were no significant changes in indices typical of vitamin B_{12} deficiencies, and no evidence of improved folate status masking hematological manifestations of vitamin B_{12} deficiency (Liu et al, 2004).

Another concern related to folic acid supplementation is an increase in the rate of twins. The United Services Preventive Services Task Force (USPSTF) found no clear, consistent evidence that preconceptional folic acid use results in an increased rate of twinning. Many studies with methodological problems posit an association between folic acid and twinning; however, most of these studies did not appropriately adjust for fertility interventions, an important confounder. A study examined the association between risk for twinning in 176,042 women and exposure to a multivitamin or folic acid supplementation before or during pregnancy. After adjustment for age and parity, the authors reported an OR of 1.59 (CI, 1.41 to 1.78) for twin delivery after preconceptional folic acid supplementation. After accounting for the underreporting of folic acid use and in vitro fertilization, the OR for twin delivery after preconceptional supplementation decreased to 1.02 (CI, 0.85 to 1.24) and was no longer statistically significantly greater than the risk for women who did not take folic acid (USPSTF, 2009).

4.4 Health disparities

After mandatory fortification, prevalence of NTD in the US declined 30%--40% among the three largest racial and ethnic groups. Nevertheless, Hispanic women continue to be at significantly greater risk for having a baby affected by an NTD than non-Hispanic white women. Non-Hispanic black women have consistently had lower NTD prevalence than Hispanic women and non-Hispanic white women, despite having the lowest folate levels

before and after mandatory fortification. Factors suggested to be contributing to the inconsistency include genetic differences in folate metabolism, maternal diabetes, and obesity, which are known to vary by race and ethnicity; and intake of nutrients other than folic acid, such as Vitamin B12 (CDC, 2010).

Canfield and colleagues (1996) studied a group of women in Texas with the primary etiologic question of whether increased NTD risk in Hispanics is explained by maternal diabetes or by other factors (e.g., maternal birthplace, prenatal care, reproductive history, age, socioeconomic status). They found that having a Hispanic mother was a risk factor for anencephaly among infants born to women with early prenatal care but not for those born to latecomers. Earlier prenatal care seemed "protective" for non-Hispanics but not for Hispanics. After adjustment in multivariate analysis, having a Hispanic (versus non-Hispanic) mother remained a strong risk factor for both anencephaly and spina bifida. Hispanic mother was the only study variable significantly associated with spina bifida in multivariate analysis. An increased risk of NTDs among Hispanics remained after controlling for other factors. For anencephaly, this risk might be partially explained by economic and cultural differences between Hispanics and non-Hispanics, and the effect of these factors on rates of prenatal diagnosis and elective pregnancy termination (Canfield et al., 1996). They suggested that cultural differences between Hispanic and non-Hispanic groups, including access to and use of folic acid-fortified food products, may contribute to the increased prevalence of NTDs. These factors may in turn affect rates of prenatal diagnosis and elective pregnancy termination, yielding births with more severe NTDs and poorer prognoses (Bol et al., 2006; Canfield et al., 1996).

Data from the US National Birth Defects Prevention Study for expected delivery dates from October 1997 through 2003 was used to determine whether the increased risk in anencephaly and spina bifida in Hispanics was explained by selected sociodemographic, acculturation, and other maternal characteristics. Hispanic mothers who reported the highest level of income were 80% less likely to deliver babies with spina bifida. In addition, highly educated Hispanic and white mothers had 76 and 35% lower risk, respectively. Other factors showing differing effects for spina bifida in Hispanics included maternal age, parity, and gestational diabetes. For spina bifida there was no significant elevated risk for U.S.-born Hispanics, relative to whites, but for anencephaly, corresponding ORs ranged from 1.9 to 2.3. The highest risk for spina bifida was observed for recent Hispanic immigrant parents from Mexico or Central America residing in the United States <5 years (Canfield et al., 2009).

In 2008 we did a study in Puerto Rico and found consumption of folic acid supplements to be 30% with 21% reporting use at least 4 times per week, and only 14% consuming it the day before the survey. Knowledge about the recommendation for women to consume folic acid was reported by 97% of the participants. Knowledge about the role of folic acid in preventing birth defects was reported by 90% of the participants. Awareness did not translate into practice. The use of folic acid was lower among women of lower education and lower social class. Women with higher education were 8.3 times more likely to consume folic acid (Garcia-Fragoso et al., 2008).

In 2009, the results of a study to evaluate the associations between neural tube defects and maternal folic acid intake among pregnancies conceived after fortification were published. This was a multicenter, case-control study using data from the National Birth Defects Prevention Study, 1998-2003. Among controls, 35.6% of non-Hispanic white women

compared with 63.3% of non-Hispanic black women and 71.3% of Hispanic women reported no use of supplement from 3 months before pregnancy through the first month of pregnancy. Only 7.2% of Hispanic controls and 14.9% of non-Hispanic black controls reported use of a supplement compared with 36.3% for non-Hispanic white controls. Case women were more likely than controls to be Hispanic, less likely to report educational levels beyond high school, and less likely to report household incomes at or above $50,000 (Mosley et al., 2009). Consideration of ways to enhance the intake of folic acid among Hispanics is a high priority (CDC, 2010). These include educational strategies as well as consideration of expanding the foods currently fortified. Prue and coworkers (2010) conducted Hispanics targeted folic acid promotion efforts in several major cities in the US. Efforts included paid and unpaid placements of Spanish language public service announcements and community-level education. Women who reported awareness of folic acid had greater folic acid knowledge and use of vitamins containing folic acid. Pregnancy waiters were most likely to use vitamins containing folic acid daily. For this group, however, awareness did not play as large a role in whether they reported consuming a vitamin containing folic acid or not, as it did for pregnancy waiters and avoiders (Prue et al., 2010). The possibility of selectively fortifying foods not included in the current fortification regulation that are staples in Hispanic communities, such as corn tortillas or other products made from corn masa flour, is being considered (CDC, 2010). Hamner et al. (2009) analyzed this possibility and found that with corn masa flour fortification, Mexican American women aged 15-44 y could increase their total usual daily folic acid intake by 19.9% and non-Hispanic white women by 4.2%.

5. Can folic acid reduce other births defects?

5.1 Down syndrome

Down syndrome (DS) is a complex genetic disease resulting from the presence and expression of 3 copies of the genes located on chromosome 21. In most cases, the extra chromosome stems from the failure of normal chromosomal segregation during meiosis (meiotic nondisjunction). Studies have shown that genomic DNA hypomethylation is associated with chromosomal instability and abnormal segregation. MTHFR acts at a critical metabolic juncture in the regulation of cellular methylation reactions (James et al., 1999). James and colleagues showed that a polymorphism of the MTHFR gene may be a direct genetic risk factor for meiotic non-disjunction such as Down's syndrome. A significant increase in plasma homocysteine concentrations and lymphocyte methotrexate cytotoxicity was observed in the mothers of children with Down syndrome, consistent with abnormal folate and methyl metabolism. Mothers with the 677CT polymorphism had a 2.6-fold higher risk of having a child with DS than did mothers without the T substitution. That study stimulated considerable investigation into the possible role of folate/homocysteine metabolism in the risk of having a DS child and several studies have been performed to better address this issue. Studies indicate that an impaired folate/homocysteine metabolism can result in chromosome 21 nondisjunction; however, the birth of a DS child seems to be the result of the interplay of several factors making it difficult to discriminate the single contribution of each of them (Coppedè, 2009).

If elevated homocysteine levels are associated with an increase risk of DS and folate can lower homocysteine levels, folic acid supplementation might be expected to decrease the incidence of DS. It has been possible to examine the incidence of births of children with DS

both prior to and after supplementation was initiated, and to evaluate its effectiveness by measuring folate levels in individuals pre- and post initiation of supplementation. An elevation of serum and red blood cell folate is observed post supplementation. However, there is no evidence of a decreased incidence of births of children with Down syndrome and some studies actually provided evidence for a slight increase in the incidence (Patterson, 2008). In Puerto Rico, we have seen an increase in the 2001-2007 DS incidence despite folate fortification of cereal-grain products and an active folic acid campaign (Puerto Rico Health Department, 2010).

5.2 Congenital heart disease

In 1995, Shaw and colleagues (1995) reported that women who take multivitamins from one month before until two months after conception have 30% to 35% lower risk of delivering offspring with conotruncal defects. Botto and coworkers (2000b) suggested that approximately one in four major cardiac defects could be prevented by periconceptional multivitamins use. Junker and colleagues (2001) showed that the embryonal MTHFR 677TT genotype was significantly associated with the development of structural congenital heart malformations during early pregnancy. Another study in the Netherlands reported that the maternal MTHFR 677CT and TT genotypes in combination with no use of periconceptional folate supplements were associated with a three-fold and six-fold increased risk for conotruncal heart defects in offspring (van Beynum et al., 2006). A study performed in a Hispanic population in Puerto Rico by these authors (Garcia-Fragoso et al., 2010) showed the prevalence of the TT polymorphism to be higher in mothers of children with congenital heart disease (CHD) than in controls. Compound heterozygosity for the 677TT and 1298AC polymorphisms was 3.7 times more common in children with CHD than in the newborn controls. Mothers of children with CHD were more likely to be compound heterozygotes. The higher prevalence of C677T polymorphism in mothers of children with CHD and of compound heterozygozity for both polymorphisms suggested the possible role of folic acid in the prevention of CHD. The American Academy of Pediatrics endorsed a statement by the American Heart Association Council on Cardiovascular Disease in the Young emphasizing that periconceptional intake of multivitamin supplements that contain folic acid may reduce the risk of congenital cardiovascular defects in offspring, similar to the known risk reduction for neural tube defects that is seen with folic acid intake (AAP, 2007). In 2009, investigators from Canada reported a 6% decrease per year in the rates of severe congenital heart defects after folic acid fortification of grain products (Lonescu-lttu et al., 2009).

5.3 Cleft lip and palate (CL/P)

Epidemiologic and family studies suggest that nonsyndromic cleft lip with or without cleft palate is a complex disorder caused by both genetic and environmental factors. The neural tube and the craniofacial regions both arise from neural crest cells, leading to the hypothesis that folic acid deficiency may also contribute to nonsyndromic clefting Perturbation of any part of the interacting folate metabolism pathways could result in folate deficiency with the effect of disrupting important biologic processes, such as craniofacial development. High levels of homocysteine may also affect developmental activities such as neural crest cell motility and migration, which are important in early development (Blanton et al., 2011). A study by Hernández and co-workers (2000) evaluated data on exposure to folic acid

antagonists, finding a correlation with cardiovascular defects and oral clefts. A national population-based study showed that folic acid supplementation during early pregnancy was associated with a reduced risk of isolated cleft lip with or without cleft palate. Independent of supplements, diets rich in fruits, vegetables, and other high folate containing foods reduced the risk somewhat. The lowest risk of cleft lip was among women with folate rich diets who also took folic acid supplements and multivitamins. Folic acid provided no protection against cleft palate alone (Wilcox et al., 2007).

In 2001, Martinelli and coworkers reported a significantly higher MTHFR C677T mutation frequency detected in mothers of patients with CL/P compared to controls supporting the involvement of the folate pathway in the etiology of CL/P, and indicating an effect of the maternal genotype, rather than influence of the embryo's genotype. In 2008, Boyles and coworkers analyzed multiple genes involved in folate and vitamin A metabolisms. Despite strong evidence for genetic causes of oral facial clefts and the protective effects of maternal vitamins, they found no convincing indication that polymorphisms in these vitamin metabolism genes play an etiologic role.

6. Summary

There is strong evidence that consumption of folic acid by women in childbearing age can prevent the occurrence of NTD in their children. Recommendations for supplementation and fortification of food products have been established in many countries. Mutations in folic related enzymes continue to be studied in an effort to explain the role of folic acid in the occurrence of NTD and other congenital anomalies.

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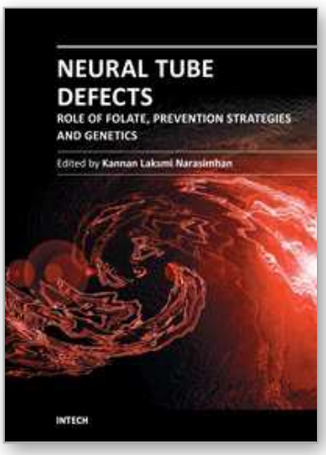

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